UNIVERSITÉ DU QUÉBEC À MONTRÉAL

NITRATE ISOTOPE ANOMALIES AS INDICATOR OF N_2 FIXATION IN THE AZORES FRONT REGION (SUBTROPICAL N-E ATLANTIC)

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ANOMALIES ISOTOPIQUES DU NITRATE COMME INDICATION DE LA FIXATION D'AZOTE DANS LA RÉGION DU FRONT DES AÇORES (ATLANTIQUE NORD-EST SUBTROPICAL)

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AVANT-PROPOS

Ce mémoire de maîtrise est présenté sous la forme d'un article dont une version abrégée sera soumise pour publication dans la revue anglophone *Journal of Geophysical Research, Oceans*. L'article a donc été rédigé en anglais et selon les exigences de l'éditeur lors de la soumission du manuscrit. Par conséquent, les références, les légendes des figures, les tableaux et les figures se trouvent à la fin de l'article. Le titre original de l'article est : *Nitrate isotope anomalies as indicator of N_2 fixation in the Azores Front region (subtropical N-E Atlantic)*. Le résumé a été traduit de l'anglais et se trouve à la suite de la table des matières. L'article a été écrit en étroite collaboration avec mon directeur de maîtrise, le professeur Moritz F. Lehmann et la professeure Joanna J. Waniek (Institut für Ostseeforschung, Warnemünde, Allemagne).

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RÉSUMÉ

Afin de mieux comprendre les sources et flux de nitrate dans la zone euphotique et le rôle potentiel de la fixation de l'azote atmosphérique dans la région du front des Açores (Atlantique nord-est subtropical), nous avons étudié la distribution du $\delta^{15}N$ et $\delta^{18}O$ du nitrate à six stations. Nous avons également mesuré le $\delta^{15}N$ de l'azote organique dissous (AOD) de l'eau de surface, ainsi que la composition isotopique de l'azote particulaire (AP) exporté de 2003 à 2005 à la station KIEL276 (2000 m de profondeur). Nous observons une diminution du $\delta^{15}N\text{-}NO_3^-$ et une augmentation du $\delta^{18}O\text{-}NO_3^-$ à mesure que la concentration de nitrate diminue dans l'eau de surface pour la plupart des stations. Étant donné que l'assimilation du nitrate par le phytoplancton produit un enrichissement égal du $\delta^{15}N$ et $\delta^{18}O$ du nitrate, les anomalies des isotopes du nitrate dans l'eau de surface ($\Delta(15;18)$ jusqu'à ~-6‰) indiquent que l'assimilation du nitrate n'est pas le seul processus qui contrôle la composition isotopique du nitrate dans la zone photique et suggère qu'il y a reminéralisation d'azote atmosphérique nouvellement fixé dans les eaux de surface et sous surface. La concentration et le $\delta^{15}N$ de l'AOD de l'eau de surface sont spatialement invariables avec des valeurs moyennes respectives de 4,7±0,5 µmol/L et 2,6±0,4‰ (n=35), ce qui est en accord avec l'idée d'un réservoir océanique d'AOD majoritairement récalcitrant. La moyenne pondérée du $\delta^{15}N$ de l'AP exporté (1,8±0,8‰, n=33) pendant les deux années d'échantillonnage est faible par rapport à la valeur du $\delta^{15}N$ du nitrate dans la thermocline. Le faible $\delta^{15}N$ de l'AP exporté, avec les anomalies isotopiques du N et O du nitrate et l'observation de ratios élevés des concentrations de nitrate par rapport au phosphate dans les eaux de surface et sous surface suggèrent fortement que la fixation de l'azote atmosphérique, étant un apport de matière organique ayant un faible $\delta^{15}N$, représente une composante majeure du cycle de l'azote dans l'Atlantique nord-est subtropical. Des bilans isotopiques simplifiés (pour le N et O du nitrate) indiquent que la fixation de l'azote, à un taux de 56-75 mmol N m^{-2} an^{-1}, pourrait représenter jusqu'à ~40% de la production primaire exportée et être ainsi aussi importante que la diffusion verticale du nitrate à travers la thermocline pour soutenir la production nouvelle.

Mots clés : Fixation de l'azote, Front des Açores, Atlantique nord-est subtropical, $\delta^{15}N$ et $\delta^{18}O$ du nitrate, $\delta^{15}N$ de l'azote organique dissous, $\delta^{15}N$ de l'azote particulaire exporté.

INTRODUCTION GÉNÉRALE

Ce travail porte sur le cycle de l'azote dans la région du front des Açores (Atlantique nord-est subtropical). Le front des Açores (~34,4°N), associé avec le courant des Açores, sépare les eaux chaudes et salées venant de la Mer des Sargasses des masses d'eaux froides et plus productives situées au nord. De ce fait, à cause des différentes concentrations d'azote fixé (biodisponible) de part et d'autres du front, cet environnement est très pertinent pour l'étude des processus impliquant l'azote, qui jouent un rôle primordial dans la séquestration du CO_2 atmosphérique dans les régions de basses latitudes. De plus, l'observation d'une production exportée (Jenkins, 1982) beaucoup plus grande que la diffusion de nitrate à travers la thermocline (Dietze, Oschlies et Kähler, 2004; Lewis *et al.*, 1986; Oschlies, 2002), qui est généralement la majeure source d'azote dans la zone euphotique, suggère fortement l'apport d'azote provenant d'autres sources dans l'Atlantique nord-est subtropical. La fixation de l'azote atmosphérique pourrait être une source importante d'azote dans les eaux de surface (Gruber et Sarmiento, 1997; Hansell, Bates et Olson, 2004; Mahaffey *et al.*, 2003). Par contre, les connaissances actuelles du cycle de l'azote dans cette région sont limitées, la plupart des études se concentrant dans la partie ouest de l'océan Atlantique nord subtropical (Altabet, 1988; Capone *et al.*, 2005; Knapp, Sigman et Lipschultz, 2005; Lipschultz, 2001; Michaels *et al.*, 1996). Une étude plus approfondie du cycle de l'azote dans cette région océanique s'avérait donc essentielle.

i.1 L'azote dans l'océan

L'azote est un macronutriment essentiel pour les organismes vivants et il est un élément limitant la productivité primaire dans les milieux marins oligotrophes tropicaux et subtropicaux. De ce fait, l'étude du cycle de l'azote océanique est primordiale afin de

mieux comprendre les échanges de CO_2 avec l'atmosphère dans le passé (durant les cycles glaciaires/interglaciaires) et de nos jours, dans une perspective de changements climatiques. En effet, la séquestration du carbone atmosphérique par le phytoplancton lors de la photosynthèse (pompe biologique) dépend de la distribution et de la concentration moyenne du nitrate et des autres éléments limitants et/ou co-limitants la production primaire océanique, tels le phosphore, le silicium, les métaux traces (par exemple, Fe, Mo) et la luminosité. Du point de vue de l'échelle géologique, le cycle de l'azote océanique est dynamique, l'azote ayant un temps de résidence inférieur à ~3000 ans (Brandes et Devol, 2002; Gruber et Sarmiento, 1997). De ce fait, plusieurs scénarios impliquant l'azote ont été proposés afin d'expliquer les changements dans la concentration de CO_2 atmosphérique durant les cycles glaciaires et interglaciaires. Un changement dans les sources et pertes d'azote causerait un excès d'azote fixé durant les périodes glaciaires et l'inverse pendant les périodes interglaciaires (McElroy, 1983). Par exemple, l'excès d'azote fixé pendant les périodes glaciaires proviendrait soit de l'augmentation de la fixation d'azote dans les régions de basses latitudes (Broecker et Henderson, 1998; Falkowski, 1997) ou d'une réduction de la dénitrification (Ganeshram *et al.*, 2000; Altabet, Higginson et Murray, 2002). Michaels, Karl et Capone (2001) et Karl *et al.* (2002) ont proposé un scénario plus complexe impliquant des rétroactions. De façon spécifique, une augmentation de la fixation de l'azote causerait une diminution de la concentration de CO_2 atmosphérique (et vice-versa) et une subséquente modification du climat, qui, à son tour, entraînerait des rétroactions négatives (ou positives) en diminuant (ou augmentant) la fixation d'azote atmosphérique notamment en diminuant (ou augmentant) l'apport de poussières atmosphériques (donc de fer, qui est un élément limitant la fixation de l'azote) dans l'océan. Pour sa part, Gruber (2004) a suggéré un fort couplage entre la dénitrification et la fixation de l'azote durant les périodes glaciaires et interglaciaires ne permettant pas de changements significatifs dans la concentration de CO_2 atmosphérique.

i.2 Le cycle de l'azote océanique

La fixation de l'azote atmosphérique, qui se produit majoritairement dans l'eau de surface des régions de basses latitudes, est considérée comme une source majeure d'azote et se fait par une grande diversité d'organismes procaryotes, le plus étudié étant la cyanobactérie *Trichodesmium* (Capone *et al.*, 1997, 2005; Carpenter, Subramaniam et Capone, 2004; Carpenter *et al.*, 1997, Karl *et al.*, 2002). L'azote fixé est ultimement transformé en nitrate, qui est la forme d'azote fixé la plus abondante dans l'océan, après la reminéralisation de la matière organique et la nitrification. La dénitrification, qui contrebalance l'ajout d'azote par la fixation de l'azote, se produit en conditions suboxiques (O_2<~5 µmol/L) et transforme les nitrates en N_2 gazeux, NO_2^-, NO et N_2O étant des intermédiaires. La colonne d'eau de l'océan actuel étant bien oxygénée, la dénitrification se produit essentiellement dans les sédiments (Hammond *et al.*, 1999; Jahnke et Jahnke, 2000) ou dans la colonne d'eau de quelques zones isolées, notamment la mer d'Arabie (Burkill, Mantoura et Owens, 1993; Howell *et al.*, 1997; Naqvi *et al.*, 1990) et l'est de l'océan Pacifique (Deutsch *et al.*, 2001; Sigman *et al.*, 2005). Le nitrate peut également être transformé en NH_4^+ par réduction dissimilatrice. La réaction anammox (oxidation anaérobique de l'ammonium), qui se produit dans les sédiments (Thamdrup et Dalsgaard, 2002) et dans la colonne d'eau de zones anoxiques, notamment la Mer Noire et une Baie côtière profonde du Costa-Rica (Dalsgaard *et al.*, 2003; Kuypers *et al.*, 2003), oxyde le NH_4^+ et réduit le NO_2^-, simultanément, en N_2. Le cycle interne de l'azote comprend l'assimilation de l'ammonium et du nitrate par les producteurs primaires (plantes et bactéries) dans les eaux de surface et la reminéralisation (ammonification et nitrification) qui se produit à la base de la zone euphotique (≤~100 m de profondeur) (Bianchi *et al.*, 1997; Dore et Karl, 1996; Ward, 2005; Ward *et al.*, 1989) ou à de plus grandes profondeurs (fig. i-1).

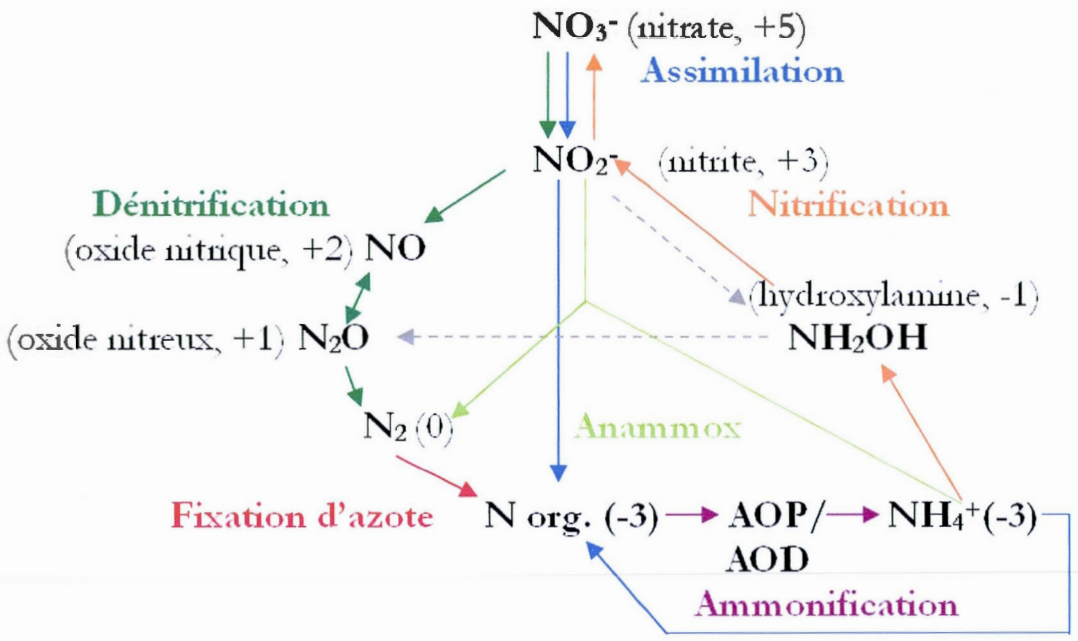

Figure i.1 : Cycle océanique de l'azote simplifié (modifié de Codispoti *et al.*, 2001). La couleur des flèches correspond aux processus associés (en caractère gras). N org. : azote organique. AOP : azote organique particulaire et AOD : azote organique dissous. L'état d'oxydation de l'azote est indiqué.

Aujourd'hui encore, nous ne savons pas si le cycle de l'azote océanique actuel est en balance ou pas, c'est-à-dire si la perte d'azote fixé équivaut à l'ajout d'azote, à cause des grandes incertitudes reliées aux estimations des sources et puits d'azote dans l'océan (tabl. i.1) (Codispoti *et al.*, 2001; Codispoti, 2007; Gruber, 2004; Gruber et Sarmiento, 2002). Selon les plus récentes estimations, la fixation de l'azote ajouterait globalement 100-200 Tg/an d'azote au réservoir océanique (Capone *et al.*, 1997; Falkowski, 1997; Gruber et Sarmiento, 1997; Gruber et Sarmiento, 2002; Karl *et al.*, 2002) tandis que la dénitrification en enlèverait jusqu'à plus de 400 Tg/an (Codispoti *et al.*, 2001; Codispoti, 2007). Par contre, la contribution provenant de la fixation de l'azote serait grandement sous-estimée selon certains (Brandes et Devol, 2002; Capone, 2001; Codispoti, 2007; Zehr *et al.*, 2001). Les estimations quantitatives de la fixation d'azote atmosphérique comportent beaucoup d'incertitudes causées par l'hétérogénéité spatiale et temporelle de ce processus, aux limites d'échantillonnage et à la sous-estimation de la fixation de l'azote

par d'autres organismes que *Trichodesmium* (Zehr *et al.*, 2001). Des estimations plus précises de la fixation de l'azote peuvent être obtenues en étudiant les concentrations de nitrate par rapport au phosphate, ce qui permet une intégration sur une plus longue période de temps et dans l'espace (Deutsch *et al.* 2001; Deutsch *et al.* 2007; Gruber et Sarmiento, 1997). Étant donné que la fixation de l'azote ajoute seulement de l'azote et pas de phosphore, l'observation de valeurs positives de N* (qui est une mesure de la déviation du ratio N/P par rapport au ratio Redfield de 16 :1) dans l'océan Atlantique Nord a été attribuée à la fixation de l'azote. À l'aide de données sur la circulation océanique et le temps de résidence des masses d'eaux, un taux de fixation d'azote variant entre 4-90 Tg N an^{-1} a pu être calculé pour l'Atlantique Nord à partir de la distribution de N* (Gruber et Sarmiento, 1997; Hansell, Bates et Olson, 2004; Micheals *et al.*, 1996). Par contre, la reminéralisation préférentielle du phosphore par rapport à l'azote dans l'Atlantique Nord tropical et l'advection subséquente de la matière organique dissoute résiduelle ayant un ratio N/P élévé vers le nord le long des isopycnes où elle est remineralisée pourrait être la cause d'un excès de nitrate dans les subtropiques, causant ainsi une surestimation de la fixation de l'azote basée sur l'approche N* (Monteiro et Follows, 2006).

Tableau i.1. Estimation des sources et pertes d'azote fixé (en Tg N an^{-1}) dans l'océan (tiré de Gruber, 2004)

Processus	Codispoti *et al.* (2001)	Gruber (2004)
Fixation N_2 pélagique	117	120 ± 50
Fixation N_2 benthique	15	15 ± 10
DON (rivières)	34	35 ± 10
PON (rivières)	42	45 ± 10
Dépositions atmosphériques	86	50 ± 20
Total des sources	**294**	**265 ± 55**
Export de N organique	1	1
Dénitrification benthique	300	180 ± 50
Dénitrification (colonne d'eau)	150	65 ± 20
Enfouissement dans les sédiments	25	25 ± 10
Pertes de N_2O dans l'atmosphère	6	4 ± 2
Total des pertes	**482**	**275 ± 55**

i.3 Étude des réactions du cycle de l'azote en utilisant les isotopes stables (N et O) du nitrate

Depuis les travaux de Cline et Kaplan (1975), la signature isotopique du nitrate s'est avérée comme étant un outil indispensable dans l'étude du cycle de l'azote océanique. Durant les transformations biologiques, les organismes utilisent préférentiellement les composés qui contiennent les isotopes les plus légers (par exemple ^{14}N et ^{16}O) créant ainsi un enrichissement du substrat résiduel en isotopes lourds (^{15}N et ^{18}O). De plus, les composés provenant de sources différentes possèdent des signatures isotopiques distinctes. Le facteur de séparation isotopique (ε, en ‰) lors des réactions consommant le nitrate peut être estimé par une approximation de l'équation de Rayleigh (éq. i.1) :

$$\delta^{15}N \text{ ou } \delta^{18}O\text{-}NO_3^- = \delta^{15}N \text{ ou } \delta^{18}O\text{-}NO_{3\,initial}^- - \varepsilon \times \ln([NO_3^-]_{final}/[NO_3^-]_{initial}) \quad (i.1)$$

où le $\delta^{15}N$ et $\delta^{18}O$ du nitrate sont exprimés en unités ‰ (éq. i.2), R représentant le ratio des rapports $^{15}N/^{14}N$ ou $^{18}O/^{16}O$ de l'échantillon et d'un standard international (air pour N et Vienna Standard Mean Ocean Water (V-SMOW) pour O) :

$$\delta^{15}N \text{ ou } \delta^{18}O\text{-}NO_3^- = ((R_{échantillon}/R_{standard}) - 1) \times 1000 \quad (i.2)$$

Lors de la fixation de l'azote, le fractionnement isotopique est minime et le $\delta^{15}N$ du nouveau N ajouté varie entre ~-2 et 0‰ (Carpenter *et al.*, 1997; Sach et Repeta, 1999) comparativement à 0‰ pour le N_2 atmosphérique. La reminéralisation et subséquente nitrification de l'azote fixé peut donc ajouter un faible $\delta^{15}N\text{-}NO_3^-$ dans les eaux de surface et sous-surface. Par contre, le fractionnement isotopique lors des processus qui consomment le nitrate (assimilation et dénitrification) cause une augmentation du $\delta^{15}N$ du nitrate résiduel (Bandes *et al.*, 1998; Cline et Kaplan, 1975; Lehmann *et al.*, 2003; Sigman *et al.*, 2005; Voss, Dippner et Montoya., 2001). De ce fait, les processus qui

produisent du nitrate (fixation de N_2) ne peuvent pas être séparés quantitativement des processus qui consomment le nitrate (assimilation et dénitrification) lorsqu'ils se produisent simultanément en utilisant seulement la relation entre le $\delta^{15}N$ et la concentration de nitrate.

La mesure combinée du $\delta^{15}N$ et $\delta^{18}O$ du nitrate s'avère donc essentielle afin de pouvoir identifier l'occurrence simultanée de différents processus océaniques qui ont des effets opposés sur le $\delta^{15}N\text{-}NO_3^-$ et N* (par exemple la fixation de N_2 atmosphérique et la dénitrification). Durant l'assimilation et la dénitrification dans l'océan et en laboratoire, la relation $\delta^{18}O$ en fonction de $\delta^{15}N$ est linéaire avec une pente de ratio 1 (Casciotti *et al.*, 2002; Granger *et al.*, 2004; Sigman *et al.*, 2003), ε étant ~5‰ lors de l'assimilation du nitrate (Altabet, 2001; Sigman *et al.*, 1999) et ~25‰ lors de la dénitrification dans la colonne d'eau (Brandes *et al.*, 1998; Voss, Dippner et Montoya, 2001). Durant la production de nitrate par le processus de nitrification, le $\delta^{15}N$ et $\delta^{18}O$ du nitrate sont affectés différemment. La valeur du $\delta^{15}N\text{-}NO_3^-$ produit dépend du $\delta^{15}N$ de la matière organique qui est reminéralisée (Sigman et Casciotti, 2001), mais aussi du fractionnement relatif entre la nitrification et l'assimilation de l'ammonium régénéré, mais rapidement et complètement recyclé (Wankel *et al.*, 2007). Si tout l'ammonium régénéré était complètement réassimilé par le phytoplancton ou nitrifié, ou si les ε associés avec la nitrification et l'assimilation du NH_4^+ étaient égaux, il n'y aurait pas, selon le bilan massique, de changement net dans le $\delta^{15}N\text{-}NO_3^-$. Par contre, si la nitrification et la réassimilation du NH_4^+ se produisaient simultanément dans la zone photique, la nitrification pourrait ajouter un faible $\delta^{15}N$ au compartiment de nitrate étant donné que le ε associé au processus de nitrification (14 à 19‰ pour des bactéries nitrifiantes marines; Casciotti, Sigman et Ward, 2003) est généralement plus élevé que le ε associé à l'assimilation du NH_4^+ par le phytoplancton lors d'études sur le terrain (6.5 à 9‰; Cifuentes *et al.*, 1989; Montoya, Korrigan et McCarthy, 1991). Concernant l'atome d'oxygène, la transformation de l'ammonium en nitrate (en passant par le nitrite) représente une source absolue. Durant la nitrification, l'atome d'oxygène incorporé dans

le nitrate provient majoritairement de l'eau ($\delta^{18}O$-H_2O étant égal à 0‰ dans l'océan) (Dispirito et Hooper, 1986; Casciotti, 2002), ce qui donne un $\delta^{18}O$-NO_3^- dans les fonds marins de ~3‰ (Sigman *et al.*, 2005, Wankel *et al.*, 2007). De ce fait, le $\delta^{18}O$ du nitrate nouvellement produit ne dépend pas de l'origine de la matière organique reminéralisée, par exemple des organismes qui fixent l'azote ou qui assimilent complètement ou partiellement le nitrate dans l'eau de surface. La différence fondamentale entre les isotopes N et O du nitrate permettrait donc la séparation de processus de consommation ou de production de l'azote dans les milieux où ils se font simultanément (Sigman *et al.*, 2005).

i.4 Intérêts et buts de l'étude

Tel que mentionné ci-dessus, une motivation majeure pour l'étude du cycle de l'azote dans l'atlantique nord-est subtropical est de comprendre la différence entre un faible flux vertical de nitrate dans la zone euphotique de 50-100 mmol N m^{-2} an^{-1} (Lewis *et al.*, 1986; Dietze, Oschlies et Kähler, 2004) et la relativement grande production exportée de 630 mmol N m^{-2} an^{-1} basée sur la mesure du taux d'utilisation de l'oxygène dans les eaux de sous surface (Jenkins, 1982). À l'état stationnaire, et en l'absence de d'autres sources d'azote, par exemple les dépositions atmosphériques et la fixation de l'azote, le flux d'azote particulaire hors de la zone euphotique devrait être égal à la diffusion et l'advection du nitrate dans les eaux de surface (Eppley et Peterson, 1979). Malgré le fait que les estimations de la fixation de l'azote dans la région du front des Açores en particulier, et dans l'Atlantique nord-est subtropical en général, sont rares, la mesure de ratios N/P élevés (Gruber et Sarmiento, 1997; Hansell, Bates et Olson, 2004) ainsi qu'un faible $\delta^{15}N$ (2.25‰) de l'azote organique particulaire en suspension dans l'eau de surface (Mahaffey *et al.*, 2003) suggèrent un rôle important pour la fixation de l'azote dans cette région. De ce fait, la région du front des Açores s'avérait un environnement idéal pour étudier les effets combinés de la fixation de l'azote et de l'assimilation du

nitrate sur la composition du $\delta^{15}N$ et $\delta^{18}O$ du nitrate dans les eaux de surface et sous surface.

De plus, l'absence de nitrate dans les eaux de surface de l'Atlantique nord-est subtropical permettait de mesurer pour la première fois le $\delta^{15}N$ de l'azote organique dissous (AOD) océanique dans cette région et d'étudier le rôle de l'AOD dans le bilan isotopique de l'azote. Malgré le fait que l'AOD représente une composante importante de la boucle microbienne et est souvent le réservoir principal d'azote fixé dans l'eau de surface des régions oligotrophes (Doval, Alvarez-Salgado et Pérez, 2001; Knapp, Sigman et Lipschultz, 2005), le rôle exact de l'AOD dans le budget isotopique de l'azote est encore mal connu. L'AOD provenant de la fixation de l'azote atmosphérique pourrait représenter un flux important de faible $\delta^{15}N$ dans les eaux de surface et sous surface (Glibert et Bronk, 1994; Karl *et al.*, 1992). Par contre, l'invariabilité temporelle des concentrations d'AOD et du $\delta^{15}N$ de l'AOD au site BATS (Bermuda Atlantic Time-series Study) suggère un compartiment d'AOD récalcitrant qui ne participe pas activement, à court terme, au cycle dynamique de l'azote, du moins dans la mer des Sargasses (Knapp, Sigman et Lipschultz, 2005).

Le but de cette étude était d'utiliser les compositions isotopiques combinées du N et O du nitrate et de d'autres paramètres géochimiques afin de déterminer l'importance et d'estimer quantitativement la fixation de l'azote dans l'eau de surface de l'Atlantique nord-est subtropical. Nous avons également évalué la variabilité spatiale du $\delta^{15}N$ de l'AOD dans l'eau de surface afin de comprendre le rôle de l'AOD dans le cycle de l'azote du front des Açores, particulièrement par rapport à la fixation de l'azote. Enfin, nous avons analysé la composition isotopique de la matière particulaire exportée à l'aide de trappes à sédiments à 2000 m de profondeur à la station KIEL276 et établi un budget isotopique de l'azote pour la région du front des Açores afin de vérifier si ce dernier requiert une source d'azote ayant un faible $\delta^{15}N$ tel qu'ajouté lors de la fixation de l'azote.

CHAPITRE I

NITRATE ISOTOPE ANOMALIES AS INDICATOR OF N_2 FIXATION IN THE AZORES FRONT REGION (SUBTROPICAL N-E ATLANTIC)

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Abstract

The subtropical Northeast Atlantic has previously been identified as a marine environment with an apparent imbalance between low nitrate supply to the surface and concurrent high export production. To better constrain the sources and fluxes of mixed layer nitrate and to assess the potential role of N_2 fixation in providing new N, we investigated the depth distribution of nitrate $\delta^{15}N$ and $\delta^{18}O$ at six stations across the Azores Front in the Northeast Atlantic. In addition, we measured the $\delta^{15}N$ of dissolved organic nitrogen (DON) in surface waters and that of sinking particulate nitrogen collected in sediment traps at 2000 m depth (sampled between 2003 and 2005 at the station KIEL276). The nitrate isotope profiles at the majority of the hydrographic stations displayed a decrease in the $\delta^{15}N$ from depth toward low nitrate surface waters, concomitant with an increase in $\delta^{18}O$. Given that nitrate uptake by phytoplankton leads to an equivalent increase in nitrate $\delta^{15}N$ and $\delta^{18}O$, the observed surface water nitrate isotope anomalies (Δ(15;18) up to ~-6‰) indicate that nitrate assimilation is not the sole process controlling the isotopic composition of nitrate in the photic zone. The low $\delta^{15}N$ of ambient nitrate implicates a significant addition of newly fixed nitrogen (N) that is remineralized in surface and subsurface waters. Both the concentration of DON and its $\delta^{15}N$ in surface water were spatially invariant, showing mean values of 4.7±0.5μmol/L and 2.6±0.4‰ (n=35), respectively, supporting the conjecture of a mostly recalcitrant DON pool. The weighted bi-annual mean $\delta^{15}N$ of sinking particulate N (1.8±0.8‰, n=33) was low with respect to thermocline nitrate. The low-$\delta^{15}N$ of export production, together with the anomalous dual nitrate isotope signatures and constantly elevated nitrate-to phosphate ratios strongly suggest that N_2 fixation represents a substantive source of N in the subtropical north-east Atlantic. Simple (N and O) isotope mass balance suggests that N_2 fixation provides between 56-75 mmol N m^{-2} yr^{-1}, accounting for 40% of the estimated export production, on par with the vertical flux of nitrate from below.

Keywords: Nitrogen fixation, Azores Front, subtropical Northeast Atlantic, nitrate $\delta^{15}N$ and $\delta^{18}O$, $\delta^{15}N$ of dissolved organic nitrogen, $\delta^{15}N$ of sinking particulate nitrogen.

1. Introduction

Nitrogen (N) is a central component of marine biomass and one of the principal nutrients required by all phytoplankton. Much of the fixed N in the ocean appears to be generated by marine N_2-fixing cyanobacteria. Estimates on global nitrogen fixation rates have been continually revised upwards over the last two decades, and it is fair to say, that today, no consensus exists on the question of the global N budget being in or out of balance (Codispoti *et al.*, 2001; Gruber, 2004; Codispoti, 2007). Put another way, neither are we certain about the absolute amounts of N added annually to the ocean through biological N_2 fixation, nor do we know whether the global N_2 fixation rate can compensate for the global loss of fixed N by denitrification in suboxic environments.

Direct measurements of N_2 fixation rates and their extrapolation to the ocean-basin scale fluxes are problematic because of their temporal and spatial heterogeneity and artifacts associated with shipboard incubations. Thus, it is desirable to derive N flux estimates from a more integrative scale. Newly fixed N is ultimately subsumed into the oceanic nitrate pool (through the remineralization of algal material and the subsequent nitrification of ammonium). Since oceanic nitrate integrates over relatively large scales of space and time, the problems of spatial and temporal variability in the quantification of N cycling fluxes can partially be overcome by investigating the concentration of water column nitrate relative to phosphate (Gruber and Sarmiento, 1997; Deutsch *et al.*, 2001; Deutsch *et al.*, 2007), and concurrently the isotopic signature of nitrate (e.g. Lehmann *et al.*, 2005; Sigman *et al.*, 2005).

Inputs of N through N_2 fixation are unaccompanied by inputs of Phosphorus (P). Thus, sub-surface excess of N in the tropical and subtropical North Atlantic Ocean, indicated by positive N* values (with N* being a measure of the deviation of the N/P ratio from the Redfield stoechiometry of 16:1), has been interpreted as the consequence

of N_2 fixation, and, together with constraints on ocean circulation and water mass residence times, N_2 fixation rates have been derived from the distribution of N* (Micheals *et al.*, 1996; Gruber and Sarmiento, 1997; Hansell *et al.*, 2004). However, deviations from the Redfield N/P ratio of 16:1 may partially be due to N uptake and/or remineralization at non-redfieldian stoichiometry. For instance, preferential remineralization of P in the tropical North Atlantic and northward advection along isopycnals may be responsible for excess N in the subtropical gyre where residual dissolved organic matter (DOM) with high N/P is ultimately remineralized, resulting in the overestimation of N*-based N_2 fixation (Monteiro and Follows, 2006).

Nitrate N isotope ratios are a valuable tool to trace these N-transformations, helping to partially overcome weaknesses associated with the N* approach. N_2 fixation adds new N with a $\delta^{15}N$ of ~-2-0‰ (Carpenter *et al.*, 1997; Sach and Repeta, 1999), and can thus maintain low nitrate $\delta^{15}N$ in the subsurface where N_2 fixation occurs. Nitrate consumption (i.e., assimilation or denitrification), on the other hand, as a consequence of kinetic N-isotope fractionation, drives increases in the nitrate $\delta^{15}N$ (Cline and Kaplan, 1975; Brandes *et al.*, 1998; Voss *et al.*, 2001; Lehmann *et al.*, 2003; Sigman *et al.*, 2005). N isotope ratios alone do not allow the quantitative separation of nitrate production and consumption. However, the "dual isotope" approach, including the measurement of both nitrate N and O isotopes, has the potential to deconvolve the "opposing" processes in the marine N cycle that have counteracting effects on N* and nitrate N-isotopes alone. The NO_3^- $\delta^{15}N$ and $\delta^{18}O$ has been shown to increase during both assimilatory and dissimilatory nitrate reduction in the ocean or in laboratory experiments, with a constant ratio of N versus O isotope enrichment in heavy isotopes of ~1 for both processes (Casciotti *et al.*, 2002; Sigman *et al.*, 2003; Granger *et al.*, 2004). During nitrate production by nitrification, however, the $\delta^{15}N$ and $\delta^{18}O$ of new nitrate are affected in different ways. In subsurface environments the NO_3^- $\delta^{15}N$ produced is controlled by the $\delta^{15}N$ of the algal organic matter being remineralized (Sigman and Casciotti, 2001), but also by the branching fractionation between nitrification and assimilation of the regenerated, and

rapidly recycled, ammonium in surface waters (Wankel *et al.*, 2007). If all of the regenerated NH_4^+ was either completely reassimilated or nitrified or if the isotope effects (ε) associated with nitrification and NH_4^+ assimilation were equal, this would cause, by mass balance, no net change in the NO_3^- $\delta^{15}N$. Conversely, in the case that both NH_4^+ reassimilation and nitrification are occurring in the euphotic zone, nitrification can add low $\delta^{15}N$ to the nitrate pool if the ε associated with nitrification was higher than the ε associated with NH_4^+ assimilation by phytoplankton. With respect to oxygen, the transformation from ammonium to nitrate (via nitrite) represents an absolute source. It is currently a matter of debate of what exactly controls the $\delta^{18}O$ of nitrate from nitrification. There is conclusive evidence that the O atoms incorporated into nitrate are mainly derived from seawater without significant fractionation (Dispirito and Hooper, 1986; Casciotti, 2002), signifying that newly formed nitrate has a $\delta^{18}O$ close to 0‰. Several studies suggest an invariant $\delta^{18}O$ of deep-water nitrate with a value of ~3‰ (Lehmann *et al.*, 2005; Sigman *et al.*, 2005; Wankel *et al.*, 2007), suggesting that nitrification is returning nitrate with a $\delta^{18}O$ 3‰ higher than seawater (Wankel *et al.*, 2007). However, we suspect that the consistent offset of mean ocean nitrate $\delta^{18}O$ and the $\delta^{18}O$ of seawater is due to the combined processes of marine nitrate production and elimination by denitrification (which results in the elevation in the $\delta^{18}O$ of oceanic nitrate above 0‰). In any case, the $\delta^{18}O$ of newly produced NO_3^- is not sensitive towards the $\delta^{15}N$ of degrading organic matter (be it from N_2 fixing organisms or from nitrate assimilating algae), as it does not depend on the origin of the ammonium being nitrified. As has been elaborated in more detail by Sigman *et al.* (2005), the fundamental difference between nitrate N and O isotopes should allow the separation of N producing and N consuming processes in environments where they occur simultaneously.

Indeed the dual nitrate isotope approach has been successfully applied to water masses along the eastern North Pacific margin, where nitrate isotope data at the upper limit of the denitrification zone show a distinct deviation from the $\varepsilon^{18}O$:$\varepsilon^{15}N$ relationship expected for sole denitrification (i.e., more relative enrichment for ^{18}O than for ^{15}N)

(Sigman *et al.*, 2005). The favoured explanation by the authors for the isotope anomaly was the addition of low-$\delta^{15}N$ nitrate to the shallow thermocline, derived from N_2 fixation in the surface ocean (and subsequent sinking and remineralization of the newly fixed N in the thermocline), yet, other mechanisms for the development of the nitrate N-to-O anomaly were considered and are equally plausible, for example the reoxidation of nitrite at the upper boundary of the oxygen minimum zone (Sigman *et al.*, 2005). In the eastern tropical North Pacific, the dual isotopic signature possibly resulting from N_2 fixation is almost completely masked by the overwhelming isotope effects of water column denitrification. Until today, nitrate N *and* O isotope data for environments where N_2 fixation is occurring without the influence from nearby denitrification (and nitrite redoxidation) zones do not exist. However, they would be very helpful for the calibration of the "pure" N_2 fixation/nitrate assimilation dual isotope signal and, thus, might aid in eliminating part of the ambiguities concerning the nitrate isotope anomaly observed in the subsurface of the eastern tropical North Pacific.

A major motivation for studying the cycling of N in the Azores Front (AF) region is to understand the apparent observational discrepancy between low nitrate input into the euphotic zone from below (Lewis *et al.*, 1986; Dietze *et al.*, 2004) with regard to the comparatively large export production (Jenkins, 1982). In steady state, and in the absence of other sources of nitrogen, like atmospheric deposition of N and N_2 fixation, the export flux of N organic material sinking out of the euphotic zone must be balanced by an upward advection and diffusion of nitrate (Eppley and Peterson, 1979). Despite the fact that direct measurements of N_2 fixation in the AF region in particular, and in the subtropical Northeast (N-E) Atlantic in general, are rare, previous geochemical and isotope geochemical measurements provide arguments for N_2 fixation as an important player over part of the eastern North Atlantic (Mahaffey *et al.*, 2003). Thus, the AF region has potential to be an excellent environment to investigate the combined effects of N_2 fixation and simultaneous nitrate assimilation on the dual isotopic composition of nitrate in the surface and subsurface water column. Moreover, the nitrate-free surface

waters of the subtropical N-E Atlantic provide a nice opportunity to expand the extremely limited data set of oceanic DON-$\delta^{15}N$ and, thus, to investigate the role of dissolved organic N (DON) in the N-isotope mass balance of the AF. No DON N-isotope data exist for the eastern part of the subtropical N-Atlantic, and in general, albeit being an important component of the microbial loop and most often the largest pool of fixed N in oligotrophic surface waters (Doval *et al.*, 2001; Knapp *et al.*, 2005) the exact role of DON fluxes in N isotope budgets is essentially unknown. It is possible that N_2-fixation-derived DON (Karl *et al.*, 1992; Glibert and Bronk, 1994) could represent a significant flux of low-$\delta^{15}N$ N out of surface waters, however, the temporal invariance of DON concentrations and the equally invariant and comparatively high $\delta^{15}N$ of DON at the Bermuda Atlantic Time-series Study site argue for a more or less recalcitrant DON pool that does not actively participate in the shorter-term N cycle dynamics at least in the Sargasso Sea (Knapp *et al.*, 2005).

The goal of this study was to use coupled N and O isotopes of water column nitrate together with other geochemical parameters to trace and quantify N_2 fixation in surface waters in the N-E Atlantic. Moreover, we investigated the spatial distribution of the $\delta^{15}N$ of surface DON to assess its importance to N cycling of the AF region, in particular its behavior with respect to N_2 fixation. Finally, we analyzed the N isotopic composition of export organic matter from sediment traps and established a comprehensive N isotope budget for the AF region, in order to verify the constraints on N_2 fixation gained from the nitrate dual isotope approach, that is, whether the N isotope budget indeed requires additional N inputs from a low-$\delta^{15}N$ source like N_2 fixation.

2. Materials and methods

2.1 Hydrographic setting and sample collection

All samples were collected close to the AF region in the subtropical N-E Atlantic. The Azores Current and the associated AF represent part of the northeastern boundary of the North Atlantic subtropical gyre that separates the warmer and saltier 18°C Mode water originating from the oligotrophic Sargasso Sea from the colder and fresher water masses to the north (Gould, 1985) (Fig. 1). Thus, the AF controls the local nutrient availability in surface waters. The AF was centered at ~34.36°N as inferred from the latitudinal distribution of temperature and salinity and was indicated by an upward displacement of the 15°C isotherm between 200 and 300 m water depth (Fig. 2). During the time of sampling, stations 171, 177, 178, 182 and 187 were all located south of the front and only station 184 was located at, or slightly north, of the AF, were the surface nutrient concentrations can be well above detection limits.

Water column samples were collected by hydrocast using 12-L Niskin bottles from 12 different depths at 6 different stations in the AF region during cruise 321 aboard the R/V *POSEIDON* in May 2005 (Fig. 1). Sub-samples were collected using acid washed and deionized water-rinsed 60-ml HDPE bottles. Vertical temperature, salinity, dissolved oxygen and chlorophyll fluorescence profiles were recorded by a SBE911 CTD. In addition, 35 surface water samples (at 4 m depth) were collected from the underwater supply along the cruise track, for the determination of the concentrations and $\delta^{15}N$ of dissolved organic nitrogen. Water samples were kept frozen at -20°C until analysis.

Sediment trap material was collected for $\delta^{15}N$ analysis of the export production between May 2003 and April 2005 at 2000 m depth at site KIEL276 (33°N, 022°W, water depth of 5300 m), with moorings V276-23 (cruise *POS297*) and V276-24 (cruise

POS321) containing automated 0.5 m^2 sediment traps with acid washed 400 cm^3 polypropylene collection cups (Kremling *et al.*, 1996). Deployment period for each mooring was about 1 year, starting in April. The sampling interval for each cup ranged from 11 to 31 days after an initial 2 week rinse period at depth. In order to prevent bacterial activity, a 4:1 mixture of *in situ* seawater and 5% sodium azide solution with enhanced salt content (38 g l^{-1}) was added in the collection cups (Waniek *et al.*, 2005). Samples were stored in sealed containers and kept refrigerated between 4 and 6°C before further processing and analysis.

2.2 Nitrate

Concentrations of sample nitrate+nitrite were measured by reduction to nitric oxide (NO) in a heated solution of acidic Vanadium (III) and subsequent chemiluminescent detection of the NO (Braman and Hendrix, 1989) using an Antek Model 7020 Nitric Oxide Analyzer, with a precision for replicate analyses of ± 0.1 µmol/L. Since the concentration of nitrite was insignificant in all measured samples, total concentration of nitrate and nitrite will be hereafter referred to as nitrate concentration.

N and O isotope ratios ($\delta^{15}N$ and $\delta^{18}O$) of dissolved nitrate from the water column were measured in duplicate using the "denitrifier method" (Sigman *et al.*, 2001; Casciotti et *al.*, 2002). This method is based on the quantitative conversion of sample nitrate to N_2O by cultured denitrifying bacteria that lack the active N_2O-reductase enzyme. N_2O gas was automatically extracted, purified and analyzed on-line using a Micromass Isoprime™ mass spectrometer coupled to a Micromass Tracegas™ in continuous flow mode. Samples with nitrate concentrations as low as 1 µmol/L were analyzed, with a general target sample size of 20 nmol for samples with a $[NO_3^-]$>4 µmol/L and 10 nmol for samples with a $[NO_3^-]$<4 µmol/L. *Pseudomonas chlororaphis* (ATCC #43928) and

Pseudomonas chlororaphis (ATCC #13985) (formerly *Pseudomonas aureofaciens*) were used to measure nitrate $\delta^{15}N$ and $\delta^{18}O$, respectively. O isotope data were corrected for O isotope exchange with water during the reduction of nitrate to nitrous oxide, following the procedure described in Casciotti *et al.* (2002). O-isotope exchange was always less than 5%. Blank contribution was generally below 0.4 nmol (i.e., 2- 4% of the target sample size). N and O isotope ratios are reported in ‰ relative to air for N [$(^{15}N/^{14}N_{sample}/ ^{15}N/^{14}N_{ref}) - 1] \times 1000$ and V-SMOW for O [$(^{18}O/^{16}O_{sample}/^{18}O/^{16}O_{ref}) - 1] \times 1000$. Isotope values were calibrated using an international KNO_3^- reference material (IAEA-N3) with an assigned $\delta^{15}N$ value of +4.7‰ (air) and a reported value for $\delta^{18}O$ of 25.6‰ (V-SMOW) (Böhlke *et al.*, 2003). Analytical precision of the method was generally better than ±0.2‰ for $\delta^{15}N$ and ±0.4‰ for $\delta^{18}O$.

2.3 Total dissolved N concentration and isotopic composition

The concentration and $\delta^{15}N$ of total dissolved nitrogen (TDN, the sum of NO_3^-, NO_2^-, NH_4^+ and DON) for surface water samples and hydrocast water samples above 200 m water depth were measured using the method developed by Knapp *et al.* (2005), which combines persulfate oxidation of TDN to NO_3^- (Solorzano and Sharp, 1980, Bronk *et al.*, 2000) and the subsequent NO_3^- $\delta^{15}N$ analysis with the denitrifier method. Briefly, for [TDN] determination, 2 ml of persulfate oxidizing reagent (POR) was added to 12 ml of sample in a boro-silicate glass test tube. Samples and three test tubes with 12 ml of POR (blank) were then autoclaved for 45 minutes. After conversion of TDN to NO_3^-, NO_3^- concentrations of the samples and the POR blank were measured by chemiluminescence as described above. Total blank for the POR was generally <3.5μmol/L and [TDN] was corrected for blank contribution, accounting for dilution. Surface water samples were essentially free of particulate N (Doval *et al.*, 2001; Vezzulli *et al.*, 2002) and fixed inorganic N (nitrate and ammonium), so that at water depths <50 m, TDN concentrations and isotopic ratios represent in fact the concentrations/isotopic

composition for DON. For water column samples with significant amounts of NO_3^-, [DON] was derived by subtracting the [NO_3^-] from [TDN]. For hydrocast samples with initial [NO_3^-] higher than the [DON], typically at depth ≥200 m, concentrations and the N isotopic composition of TDN were not measured. Prior to the N-isotope analysis of 10 nmol TDN-derived nitrate with the denitrifier method, the pH of autoclaved samples (and of the POR blank) was lowered to ~2-3 with concentrated HCl. ~30% of the samples were analyzed in duplicate. The $\delta^{15}N$ of DON was determined taking into account the concentration and $\delta^{15}N$ of the POR blank and sample NO_3^-. For each oxidation set, DON oxidation efficiency was tested using two in-house standards (urea and amino-caproic acid (ACA)) at a concentration of 5 μM-N. Total N yields (after correction for the blank) were on average 106.1±5.9%, (n=9) for urea and of 105.8±5.2%, (n=9) for ACA. Urea and ACA also served as our in-house N-isotope standard, for which we determined $\delta^{15}N$ values of -0.43±0.30‰ and 3.48±0.04‰ by direct combustion, respectively. Mutiple N-isotope analyses of DON standards showed that the oxidation/denitrifier method produces accurate and reproducible results, with a $\delta^{15}N$ of -0.30±0.32‰ (n=13) for urea and 3.45±0.28‰ (n=10) for ACA.

2.4 $\delta^{15}N$ analysis of sinking particulate N

After removal of zooplankton "swimmers", trap samples were centrifuged for 20 min at 7600 rpm and 4°C and washed three times with DI water to remove the sodium azide completely from the sediment trap samples. The supernatant was decanted and particulate samples were frozen freeze-dried and then homogenized using a glass rod. N content was determined using a Carlo Erba Elemental Analyzer (EA) NC2500, with a precision of ±0.3%. Total sinking particulate nitrogen (PN) flux was calculated by multiplication of the total flux of particles and the weight-% N. The $\delta^{15}N$ of particulate N was measured by sample combustion and reduction to N_2, and subsequent gas purification and isotope analysis using a Carlo Erba NC1500™ Elemental Analyser

coupled to a Micromass IsoPrime™ mass spectrometer. Standard reproducibility for replicate analyses was generally better than 0.2‰.

3. Results

3.1 Nitrate concentrations and nitrate N and O isotope ratios

In general, depending on the location of the AF, nitrate is a biolimiting nutrient in the photic zone, with concentrations decreasing from 18-23 μM below 800 m to below detection limits in the upper 50 m of the water column south of the AF and to small but measurable surface water [NO_3^-] (Fig. 3, a) on the northern side of the AF (Station 184). Influence of warmer, saltier and Mediterranean Water masses with lower nutrient levels between ~800-1300 m probably explain lower [NO_3^-] (~13-16 μM) at intermediate depths for some stations.

In agreement with previous reports on generally positive N* (with N* = [NO_3^-] – 16 × [PO_4^-] + 2.9 in μmol/L) for the subtropical N Atlantic (Gruber and Sarmiento, 1997; Hansell *et al.*, 2004), the latitudinal distribution of N* for the sampling area in the AF region indicates values generally above +2.5 $\mu molL^{-1}$ in the upper 1000 m, with maxima of ~+3-3.5 μM confined to depths between ~100 and 750 m (Fig. 4). The positive N* values in the permanent thermocline have traditionally be assumed to reflect the net addition of N by N_2 fixation but other mechanisms such as the elemental discrimination during organic matter remineralization could similarly explain excess nitrate with respect to phosphate (Monteiro and Follows, 2006).

Nitrate $\delta^{15}N$ and $\delta^{18}O$ were generally ~5‰ and ~3‰, respectively, at depths ≥800 m, essentially the mean N isotope values for oceanic nitrate (e.g., Casciotti et *al.*, 2002;

Sigman *et al.*, 2003; Lehmann *et al.*, 2005). NO_3^- $\delta^{15}N$ decreased (except station 184) to a weighted mean value of 3.5±1.2‰ at 100 m for all the stations. At Station 184, NO_3^- $\delta^{15}N$ first decreased to 4.4‰ at 200 m and then increased to a value of 5.1‰ at 100 m (Fig. 3b). Nitrate $\delta^{18}O$, on the other hand, increased continuously upward to a weighted mean value of 6.2±0.5‰ at 100 m for all the stations (Fig. 3c). The $\delta^{18}O$ was strongly anti-correlated with nitrate concentrations, suggesting isotope fractionation, i.e., the preferential incorporation of the lighter isotope ^{16}O, during nitrate uptake by phytoplankton. Similar NO_3^--to-$\delta^{18}O$ relationships have been attributed to fractionating algal nitrate assimilation in the surface waters of the Bering Sea (Lehmann *et al.*, 2005) and the eastern subarctic N-Pacific (Casciotti *et al.*, 2002).

3.2 TDN concentration and $\delta^{15}N$

Both the concentration and $\delta^{15}N$ of surface water TDN at 4 m water depth (which can be assumed to reflect solely DON, since other forms of N are absent in the surface water) were essentially invariable over the whole study area, with mean values of 4.7±0.5 μmol/L and 2.6±0.4‰ (n=35), respectively (Fig. 5). The mean [DON] for the AF found in this study is very similar to mean DON concentrations determined previously in the same region by Doval *et al.*, (2001) (5.2±0.4 μmol/L) and in the surface waters of the western North Atlantic (BATS site) as reported by Knapp *et al.* (2005) (4.2±0.5 μmol/L). To our knowledge, a significant number of oceanic DON $\delta^{15}N$ values does not exists in the literature. The few analyses that have been done on bulk TDN/DON collected from the surface ocean yielded somewhat lower $\delta^{15}N$ values (+1-2‰) for the subtropical N-Pacific Ocean (Abell *et al.*, 1999), and significantly higher values (+3.9‰) for the western subtropical North Atlantic, as reported by Knapp *et al.*, (2005).

An ANOVA test indicates that the mean concentration (4.3±0.7μmol/L) and $\delta^{15}N$ (2.8±0.5‰) of DON measured at the 6 stations for the upper 100 m (Fig. 6) are, at the 99% confidence interval (p=0.014 and 0.35, respectively), not significantly different from those measured in the surface waters along the cruise track, suggesting a homogenous pool of DON. At 200 m depth, [DON] (5.6±2.0 μmol/L) and the $\delta^{15}N$ of DON (2.1±1.8‰) are more variable, which can be partly explained by the greater uncertainty for both [DON] and DON-$\delta^{15}N$, due to the biasing effect of higher NO_3^- concentrations in those samples. However, depth gradients could neither be identified for DON concentrations, nor for the DON N-isotopic composition. The comparatively high [DON] (7.9 μmol/L) and $\delta^{15}N$ of DON (4.8‰) at station 178 at 200 m water depth may either be attributed to the presence of a non-negligible amount of organic particles in that sample (low levels of chlorophyll *a* close to deep-sea water value for this station and depth (data not shown) do not support this hypothesis) or contamination during the experimental protocol.

3.3 Sinking N flux and the $\delta^{15}N$ of export production

Particulate N (PN) fluxes determined at 2000 m depth at the KIEL276 site between May 2003 and April 2005 are shown in Fig. 7. Background N flux was generally below 0.6 mg N m^{-2} d^{-1} throughout most of the two-year period. Elevated PN fluxes were observed during winter and spring months (i.e., between March and May 2004 and December 2004 and March 2005), with a maximum value of 2.2 mg N m^{-2} d^{-1} in March 2005, corresponding to a maximum in total mass flux of 353 mg m^{-2} d^{-1}. Overall variability of our flux data are in general agreement with previously reported fluxes for the years 1993 to 2002 at the same site (Waniek *et al.*, 2005) that showed consistent seasonal trends in flux maxima (occurring between January and April) but significant interannual variability. Flux maxima observed during winter and spring months were previously attributed to periods of enhanced phytoplankton primary production in the

overlying euphotic zone (Waniek *et al.*, 2005) indicating an immediate link between primary production, export production directly below the photic zone, and the particle fluxes determined at 2000 m water depth.

The $\delta^{15}N$ of sinking PN showed relatively subtle variations around a flux-weighted mean of 1.8±0.8‰ (n=33). Lowest $\delta^{15}N$-PN values (0.7‰) were observed in February and March 2005, concomitant with highest PN export production rates (Fig. 7). While the $\delta^{15}N$-to-PN flux relationship during high-flux periods suggests antiproportional behavior, no significant relationship between PN fluxes and the $\delta^{15}N$ of PN could be discerned for the complete data set ($r^2 = 0.18$).

4. Discussion

4.1 Nitrate N-versus-O isotope anomaly:

During nitrate assimilation in the ocean surface waters, the $\delta^{15}N$ and $\delta^{18}O$ of nitrate increase in association with reduced [NO_3^-]. Previous studies observed an N isotope separation factor (ε) for algal nitrate uptake of ~5‰ (e.g., Sigman *et al.*, 1999; Altabet, 2001) and coupled N and O isotope fractionation with a ratio for isotope effects ($^{18}\varepsilon:^{15}\varepsilon$) of ~1 during nitrate assimilation in the surface waters of the Bering Sea (Lehmann *et al.*, 2005), the subarctic North Pacific (Casciotti *et al.*, 2002) and in laboratory culture experiments (Granger *et al.*, 2004). The same $^{18}\varepsilon:^{15}\varepsilon$ ratio was also observed during denitrification in the ocean (Sigman *et al.*, 2003). In contrast, in the AF region, the nitrate $\delta^{15}N$ generally decreases with decreasing [NO_3^-] in oxic surface waters while the nitrate $\delta^{18}O$ increases (Fig. 8). Here, the $^{18}\varepsilon:^{15}\varepsilon$ greatly departs from the 1:1 relationship expected during nitrate assimilation by phytoplankton. The increase in nitrate NO_3^- $\delta^{18}O$ in association with declining [NO_3^-] is, at first, consistent with O-

isotope fractionation during NO_3^- assimilation in the photic zone. However, for all the stations, the Rayleigh Model-based $^{18}\varepsilon$ (1.3‰, r^2=0.9) is significantly lower than 5‰ (Fig. 8, b). The strong nitrate $\delta^{15}N$-to-$\delta^{18}O$ anomaly in the photic zone, together with the lowered $^{18}\varepsilon$ indicate that nitrate assimilation is not the sole process controlling the isotopic composition of nitrate in surface waters.

We can graphically quantify the N-to-O isotope anomaly as deviation relative to a 1:1 fractionation relationship with a slope ($^{18}\varepsilon/^{15}\varepsilon$) of 1 (pure nitrate assimilation) using the Δ(15,18) approach by Sigman *et al.* (2005) :

$$\Delta(15,18) = (\delta^{15}N - \delta^{15}N_m) - (^{15}\varepsilon/^{18}\varepsilon) \times (\delta^{18}O - \delta^{18}O_m) \quad (1)$$

where $\delta^{15}N_m$ and $\delta^{18}O_m$ is the mean $\delta^{15}N$ and $\delta^{18}O$ of deep water and $^{15}\varepsilon$:$^{18}\varepsilon$ is the ratio of N versus O isotope effects during nitrate assimilation, which is 1. We assigned values of 4.8‰ for $\delta^{15}N_m$ and 3.0‰ for $\delta^{18}O_m$, the respective mean $\delta^{15}N$ and $\delta^{18}O$ for depths $\geq$2000 m for all the stations. A negative Δ(15,18) indicates a decrease (or lesser increase) in nitrate $\delta^{15}N$ relative to the $\delta^{18}O$. The Δ(15,18) diminution towards the surface waters, suggesting the interaction of nitrate consumption and production, starts already at about 700 m water depth, well beyond the photic zone, and reaches a mean depth-binned (all six stations) minimum of -4.9‰ at 100 m (Fig. 9.c). The physical/biogeochemical mechanisms which could be responsible for the fact that the N-to-O isotope anomaly starts at depths where no photosynthesis and thus no nitrate assimilation occurs (with associated nitrate O isotope enrichment), is not completely clear. Candidate explanations for the presence of the algal assimilation signal from 200 to ~700 m involve thermocline subduction through Ekman pumping or mixing along isopycnals of surface waters impacted by nitrate assimilation coming from the southward recirculation or the Gulf Stream and the North Atlantic Current. The surface Δ(15,18) minimum for the stations located south of the AF is stronger (-4.5 to -6.4 at 100 m depth) compared to the station 184 north of the front (Δ(15,18)= -3.5, 100 m depth), which suggests that the relative

importance of nitrate assimilation versus potential other N-cycle processes that affect the surface and subsurface nitrate pool changes spatially.

In the sections that follow we will discuss the putative causes for the observed Δ(15,18) distribution, which include internal processes (i.e., the cycle of assimilation-remineralization-nitrification) and external processes such as atmospheric N deposition and the biological fixation of atmospheric N.

4.1.1 The Putative effect of nitrate regeneration in the lower euphotic zone :

Relatively few studies exist that use dual (N and O) nitrate isotopic measurements as a mean to deconvolve the effects of various processes occurring simultaneously in the surface and subsurface waters of the ocean. Sigman *et al.* (2005) previously observed a Δ(15,18) minimum at the upper limit of a denitrification zone (~200 m depth) in the eastern tropical North Pacific and attributed it to remineralization of low $\delta^{15}N$ coming from N_2 fixation and/or the reoxidation of nitrite to nitrate in suboxic zones that would act to increase the NO_3^- $\delta^{18}O$ while having no effect on the NO_3^- $\delta^{15}N$.

Recently, Wankel *et al.* (2007), suggested that the branching between nitrification and NH_4^+ assimilation by phytoplankton could be responsible for the observed un-coupling of nitrate $\delta^{15}N$ versus $\delta^{18}O$ values in the surface waters of the Monterey Bay (California). For a long time, it was common perception that light inhibits nitrification, but it seems now generally accepted that nitrification can occur at the base of the euphotic zone (~5-10% of surface light intensity) contributing to upper-water column nitrate regeneration (Ward *et al.*, 1989; Dore and Karl, 1996; Bianchi *et al.*, 1997; Ward, 2005). Following the ammonification of part of the organic material (OM) (with the remaining part being exported to greater depths), the regenerated NH_4^+ is either rapidly

reassimilated by primary producers (phytoplankton) or oxidized to nitrate. If both processes, NH_4^+ assimilation and nitrification, occurred simultaneously in the euphotic zone, and if the isotope effect associated with nitrification (14-19‰; Casciotti *et al.*, 2002) was larger than the one associated with NH_4^+ uptake by phytoplankton (6.5 to 9‰; Cifuentes *et al.*, 1989; Montoya *et al.*, 1991), the branching of NH_4^+ would add low NO_3^- $\delta^{15}N$ to the NO_3^- pool, while the reassimilation of partially nitrified, and thus ^{15}N enriched NH_4^+, would result in the concomitant ^{15}N-enrichment of algal organic matter. By producing ^{15}N-depleted nitrate, the branching effect could lead to negative $\Delta(15,18)$ values, and several factors, such as the degree of nitrate utilization, the fraction of OM that is remineralized, or the fraction of ammonium oxidized relative to the fraction that is reassimilated would determine the size of the $\Delta(15,18)$ minimum (Wankel *et al.*, 2007). However, the N isotope effects associated with NH_4^+ assimilation and, in particular, with nitrification in the ocean are poorly constrained. If the isotope effect of NH_4^+ assimilation were larger than the one associated with nitrification, nitrification and the branching of ammonium would produce positive $\Delta(15,18)$ values (Wankel *et al.*, 2007).

As mentioned before, from the perspective of the oxygen isotopes, nitrification represents an absolute source that adds nitrate with a NO_3^- $\delta^{18}O$ of ~0 or 3‰ to the existing nitrate pool (Sigman *et al.*, 2005; Wankel *et al.*, 2007). If, during a complete assimilation/remineralization cycle, the $\delta^{18}O$ of new NO_3^- from nitrification is higher than the NO_3^- $\delta^{18}O$ removed during the uptake by phytoplankton, the NO_3^- $\delta^{18}O$ would increase, potentially resulting in a $\Delta(15,18)$ minimum (Sigman *et al.*, 2005). As previously demonstrated by the model of Wankel *et al.* (2007), both negative and positive $\Delta(15,18)$ are possible, depending on the $\delta^{18}O$ added during nitrification.

Nitrification in surface waters, more precisely, the combined processes of ammonium uptake and oxidation and the associated branching fractionation of N isotopes, is a plausible explanation for negative $\Delta(15,18)$ in the Monterey Bay. However,

the fact there are fundamental differences in the degree and quality of the nitrate isotope anomalies observed in this study compared to those reported from the Monterey Bay (Wankel *et al.*, 2007), suggests somewhat different mechanisms behind the negative Δ(15,18) in the two environments. The Δ(15,18) minima observed here (up to -6.4‰ at 100 m depth), while more enhanced than those observed in other open ocean environments (i.e., -3‰ in the eastern North Pacific; Sigman *et al.*, 2005), is generally much smaller than the ones observed in the Monterey Bay (where Wankel *et al.* (2007) observed negative Δ(15,18) of up to ~-34‰). Furthermore, in the AF region, we observed opposite trends for N and O isotope ratios, not only different degrees in enrichment, as is generally the case in other studies; that is, in the AF region the nitrate $\delta^{15}N$ decreases towards the surface concomitant with an increase in $\delta^{18}O$, while in the eastern North Pacific and the Monterey Bay both $\delta^{15}N$ and $\delta^{18}O$ of nitrate increase. Only for one sampling period (September 2003), the surface NO_3^- $\delta^{15}N$ at the investigated three sampling stations in the Monterey Bay was lower than thermocline nitrate. However, the relatively low NO_3^- $\delta^{15}N$ were always associated with very high NO_3^- $\delta^{18}O$ values (up to 34‰), which could not be explained, at this extent, by assimilation-remineralization-nitrification cycle. Alternatively, the authors inferred the input of atmospheric NO_3^-, with low $\delta^{15}N$ and extremely high $\delta^{18}O$, as plausible explanation for the extremely negative Δ(15,18) observed in association with the high $\delta^{18}O$ during the September 2003 sampling campaign (see Section 4.1.2).

While nitrate regeneration and the branching reaction during ammonium consumption represent an elegant explanation for nitrate anomalies observed in the Monterey Bay surface waters, nitrate N and O isotope measurements in other ocean environments (i.e., the Bering Sea and the Gulf of Alaska in the subarctic North Pacific; Casciotti *et al.*, 2002; Lehmann *et al.*, 2005), none of which revealed such nitrate anomalies, beg the question of why isotopic evidence of nitrate regeneration in surface waters is not found there. At this stage clearly more oceanic nitrate N and O measurements are necessary to reveal the utility of coupled N and O isotope

measurements as tracers of nitrate regeneration. We cannot completely exclude the branching reaction during ammonium consumption (assimilation versus nitrification) as an important factor responsible for the observed nitrate N-to-O anomaly in the AF region but there is some indication that this mechanism is at least not solely responsible for the observed depth distributions of nitrate $\delta^{15}N$ and $\delta^{18}O$. As outlined above, the branching between NH_4^+ assimilation and nitrification can lower the $\delta^{15}N$ of nitrate only while increasing that of organic material (giving that the isotope effect for nitrification is generally higher than that for NH_4^+ assimilation). For example, in the Monterey Bay, model simulations by Wankel *et al.* (2007) revealed relatively high values for the $\delta^{15}N$ of PON (7.1-11.4‰) in association with the observed Δ(15,18) minimum in the surface waters. It is reasonable to assume that also in the AF region, a simple nitrification/assimilation cycle would shunt ^{15}N-depleted N into both the PON and the DON pools. However, both the relatively low $\delta^{15}N$-PON of sediment trap material (1.8±0.8‰), as well as the low $\delta^{15}N$ of surface water DON (2.6±0.4‰) argue against such a disproportionation of N isotopes in organic and nitrate N.

The preferential remineralization of ^{14}N during ammonification (nitrate regeneration) of organic particles that sink through the water column, or during transport along isopycnals, could theoretically explain the low NO_3^- $\delta^{15}N$ values observed in the surface waters of the AF region, and, in analogy to the processes described above, could produce opposing nitrate $\delta^{15}N$ versus $\delta^{18}O$ trends even without significant branching of the ammonium consumption (assimilation vs. nitrification). Data from other ocean environments highlight the plausibility of such positive N isotope effects during water column degradation of suspended PON (Altabet, 1988) and early sedimentary diagenesis at the seafloor (Gaze-Haake *et al.*, 2005; Altabet and Francois, 1994). However, the relatively low flux-weighed mean $\delta^{15}N$ of 1.8±0.8‰ for the sinking PN at 2000 m argues against a significant nitrogen isotopic alteration of the primary $\delta^{15}N_{OM}$ during OM degradation towards more ^{15}N enriched values, at least in the AF water column. The liberation of ^{15}N-depleted DIN in the surface and subsurface waters

would necessitate a significant ^{15}N enrichment in the corresponding residual export OM fraction beyond the $\delta^{15}N$ of deep water nitrate (i.e., >5‰), yet this is clearly not observed. Moreover, whether bacterial degradation in the water column and at the sediment water interface really discriminates against the heavier isotope ^{15}N is more than uncertain. For example, incubation experiments show no evidence for N isotope alteration during OM remineralization, at least under oxic conditions (Lehmann *et al.*, 2002), and some sediment trap studies have shown that the $\delta^{15}N$ of sinking PON may in fact be even lower at greater depths compared to the $\delta^{15}N$ of export PN just below the euphotic zone (Altabet *et al.*, 1991; Thunell *et al.*, 2004; Gaye-Haake *et al.*, 2005), suggesting the preferential liberation of ^{15}N-enriched DIN. In conclusion, given the uncertainty regarding the overall direction of potential isotope effects during OM degradation, and given the fact that, although with a generally fairly low isotope effect (<~0.5‰ between shallow (~1000 m) and deeper traps (~2000-3000 m) in the northern Indian Ocean; Gaye-Haake *et al.*, 2005), ^{14}N enrichment was generally observed in the partially degraded sinking OM fraction, it is reasonable to assume that N isotope discrimination during water column remineralization and subsequent nitrification in the lower photic zone could not produce the observed diminution in nitrate $\delta^{15}N$.

4.1.2 Effects of N atmospheric depositions

Both dry and wet atmospheric depositions display relatively high N:P ratios (>100:1) (Duce, 1986). Hastings *et al.* (2003) measured the $\delta^{15}N$ and $\delta^{18}O$ of nitrate in atmospheric depositions over Bermuda in the western subtropical Atlantic from January 2000 to January 2001 and found nitrate $\delta^{15}N$ and $\delta^{18}O$ values of -2.1‰ to -5.9‰ and 68.6‰ to 76‰, respectively. As a consequence, one may assume that atmospheric depositions of nitrogen over the North Atlantic may significantly contribute to the observed surface water nitrate isotope anomalies (by decreasing the NO_3^- $\delta^{15}N$ and

strongly increasing the NO_3^- $\delta^{18}O$ of surface waters with respect to deep water nitrate) concomitant with the increase in surface water N*.

In fact, low $\delta^{15}N$ (2.5‰) together with very high $\delta^{18}O$ of nitrate (up to ~34‰) observed in the Monterey Bay in September 2003 have been attributed to atmospherically derived nitrate producing an extremely negative $\Delta(15,18)$ of ~-34‰ (Wankel *et al.*, 2007). In this study, the $\Delta(15,18)$ decreases (up to 6.4‰ at 100 m depth) is much smaller, and, more important, it is associated with relatively low $\delta^{18}O$ values (up to 6.8‰, 100 m depth). In fact, not even a minimal addition of atmospheric nitrate needs to be inferred to explain the increase in nitrate $\delta^{18}O$ towards the surface waters in the AF, because even a relatively small O-isotope effect (~1.3‰) for nitrate assimilation could already account for the observed raising of the nitrate $\delta^{18}O$ beyond its deep water value. On the other hand, we cannot rule out the remineralization of atmospheric DON that could, upon remineralization, add a low nitrate $\delta^{15}N$ and $\delta^{18}O$ (~0 or 3‰) to the euphotic zone, yet, the estimated total atmospheric depositions of nitrogen in the open subtropical N-E Atlantic ocean is fairly low, contributing only ~10 mmol N m^{-2} yr^{-1} to the total external N flux in this region, with DON, NO_x, and NH_4^+ accounting for ~50%, 30%, and 20% of it, respectively (Prospero *et al.*, 1996). While the low $\delta^{18}O$ of surface water nitrate already indicates that (at least at the time of sampling) atmospheric N deposition is not significant, future work should include the measurement of $\delta^{17}O$-NO_3^- as unequivocal tracer of atmospheric NO_x which will yield more conclusive evidence as to the relative importance of atmospheric N in the AF N isotope dynamics.

4.1.3 Addition of newly fixed N

Deep oceanic (>2 km) nitrate has a mean NO_3^- $\delta^{15}N$ of ~5‰, (Liu and Kaplan, 1989; Sigman *et al.*, 2000). Lower NO_3^- $\delta^{15}N$ values in marine environments have

previously been observed only in the euphotic zone of the Pacific and North Atlantic subtropical oceans (Liu et *al.*, 1996; Karl *et al.*, 2002; Knapp *et al.*, 2005; Sigman *et al.*, 2005). There, the low $\delta^{15}N$ has been attributed to complete ammonification and nitrification of newly fixed N that adds nitrate with a $\delta^{15}N$ of ~-2-0‰ to surface waters (Carpenter *et al.*, 1997; Sach and Repeta, 1999). Indeed, N_2 fixation arises as the only plausible explanation that can cause both a decrease in nitrate $\delta^{15}N$ in the surface zone concomitant with a modest $\Delta(15,18)$ minimum. We suggest that the observed nitrate anomalies in the AF photic zone (upper 100 m) are due to the combined effects of diapycnal and isopycnal mixing with waters at 200 m depth (the maximum mixing depth in winter), injecting nitrate with a $\delta^{15}N$ of 3.7‰ and a $\delta^{18}O$ of 5.1‰ into the surface waters from below, nitrate assimilation removing nitrate with similar isotope effects for N and O isotopes of 5‰, respectively, and the remineralization of organic material that is partially derived from newly fixed nitrogen ($\delta^{15}N$ ~-1‰), which ultimately adds nitrate with a $\delta^{15}N$ of -1‰ and a $\delta^{18}O$ of 0‰ (or 3‰) (Fig. 10). These processes all act together to maintain a $\Delta(15,18)$ minimum in the euphotic zone, the extend of which provides some indication as to the relative importance of algal production by assimilation of regenerated and new nitrate versus N_2 fixation. Based on the steady-state box model presented in Figure 10, six highly simplified scenarios have been calculated using simple mass balance equations (see Auxiliary materials) and will exemplify the potential to use nitrate $\Delta(15,18)$ for the assessment of the relative contribution of N_2 fixation to the total primary productivity and with respect to new production fuelled from thermocline nitrate (Table 1). In the simulated scenarios we consider ratios of N_2 fixation/export production between 10 and 90%, and set ratios of N_2 fixation/gross primary production by nitrate uptake (assuming complete nitrification of regenerated ammonium) to 5 or 10%. Results for the respective cases are shown in Table 1. An increase in N_2 fixation/export production from 10 to 90% causes a decreases in the $\delta^{15}N$ (~2‰) which is significantly more prominent than that for $\delta^{18}O$ (~0.3‰), generating increasingly negative $\Delta(15,18)$ values in surface waters. The decrease in $\Delta(15,18)$ is even more pronounced (i.e., greater nitrate isotope anomaly) if we assume that the $\delta^{18}O$ added

during OM nitrification is in fact of 3% (instead of 0%), as the nitrate $\delta^{18}O$ now increases with N_2 fixation (along with decreasing $\delta^{15}N$). Indeed, we observe variations in $\Delta(15,18)$ of similar magnitude in the AF, pointing to the fact that changes in the relative importance of N_2 fixation are plausible.

These examples and our data indicate that the nitrate N versus O isotope anomaly can potentially be used to quantify the relative importance of the respective processes (mixing, N_2 fixation and nitrate regeneration, and nitrate assimilation). Yet at this point, it is not possible to extract more quantitative estimates on N_2 fixation rates without knowledge of one or several of the other fluxes. Moreover, in our simplified scenarios, we have ignored the possible impact by the branching effect during ammonium consumption (i.e., we have ignored ammonium assimilation). However, our nitrate isotope data suggest several important points: (1) that the $\Delta(15,18)$ of photic zone nitrate is a good indicator of N_2 fixation, however, the quality and degree of the nitrate anomalies is dependent on the rate of co-occurring reactions and fluxes (i.e., the balance of nitrate assimilation, N_2 fixation, and the re-supply of nitrate from below), (2) that N_2 fixation must play a quantitative role in the AF N budget, probably of similar importance for the total primary production as the nitrate flux from below and (3) that there are clear spatial variations in the relative importance of N_2 fixation versus other fluxes across the AF, as highlighted by changes in the quality and the degree of observed nitrate N-to-O isotope anomalies.

N_2 fixation is a well documented phenomenon in the subtropical Atlantic, with several biological studies focussing on the filamentous cyanobacteria of the genus *Trichodesmium* (e.g. Capone *et al.*, 1997; 2005; Karl *et al.*, 2002, Carpenter *et al.*, 2004). Support for N_2 fixation as our favourite explanation for the observed $\Delta(15,18)$ minima in the AF region comes not only from the observed N* and the low $\delta^{15}N$ for both DON and PON, but also from a previous geochemical studies in nearby regions. For example,

chemotaxonomic data by Mahaffey *et al.* (2003) revealed a dominance of cyanobacteria and prochlorophytes, and related the low $\delta^{15}N$ of suspended PON (2.25±0.36‰) between 26°N to 32°N along 20°W to N_2 fixation. Indeed, N_2 fixers flourish in N-limited well-lit tropical and subtropical waters with large eolian Fe supply (Niemi, 1979; Berman-Frank *et al.*, 2001), and it has been previously suggested that the relatively high dust input from the Sahara could deliver enough iron, usually the limiting nutrient during N_2 fixation, to meet the N_2 fixer's requirements in the eastern North Atlantic (Mahaffey *et al.* 2003).

Moreover, our interpretation is supported by the previous observation of a negative nitrate $\Delta(15,18)$ anomaly at the upper boundary of the denitrification zone in the eastern tropical North Pacific, which has also been attributed to the addition of N_2-fixation derived nitrate (Sigman *et al.*, 2005). From the study of both, the tropical Pacific and the AF $\Delta(15,18)$ profiles it becomes clear how denitrification and the advection of water masses that carry the nitrate isotope signal of N_2 fixation can combine to produce distinct $\Delta(15,18)$ minima in the shallow thermocline, such as observed in the eastern tropical North Pacific (Sigman *et al.*, 2005).

4.2 Surface water DON in the AF region

The fact that DON concentrations as well as its $\delta^{15}N$ were essentially invariant over the entire AF region is consistent with previous reports of the bulk marine surface DON pool being little dynamic and mostly recalcitrant (Mahaffey *et al.*, 2004; Knapp *et al.*, 2005). Mahaffey *et al.* (2004) estimated that the labile and semi-labile DON fractions represent less than 0.6% and 10%, respectively, of the total DON pool in surface waters (7 m depth) along a transect in the Atlantic Ocean (35°S, 49°W to 48°N, 20°W). DON in surface open ocean waters is primarily released during phytoplankton primary

production. Not much is known about the N isotope partitioning (DON versus PON) of organic N in N_2 fixing environments, but assuming that both the possible excretion of DON from N_2 fixing organisms and/or the breakdown of PON into DON during the biosynthesis of N_2-fixation-derived OM are not associated with significant N-isotope fractionation, it is reasonable to assume that newly fixed DON will have a $\delta^{15}N$ close to that of atmospheric nitrogen (~0‰). This way, N_2 fixation, by adding newly fixed organic N to surface waters, may significantly increase the [DON] (Karl *et al.*, 1992; Glibert and Bronk, 1994) while lowering the $\delta^{15}N$ of the DON pool. One putative explanation for the spatially invariant DON concentrations and its isotopic stability observed throughout the study area is that, if there were indeed marked inputs of labile DON produced by diazotrophs and non-diazotrophs, this labile DON pool must be completely and rapidly recycled, analogous to the cycling and complete consumption of NH_4^+ in the euphotic zone. In this case, the isotopic composition of the recalcitrant DON pool would not be significantly affected but would be set to a more or less constant value, given its relatively large size (Mahaffey *et al.*, 2004). However, from Figures 3 and 5 it becomes evident that, for all the stations, the $\delta^{15}N$ of surface DON is lower than that of deep water nitrate and even lower than the mean $\delta^{15}N$ for photic zone nitrate (mean $\delta^{15}N_{DON}$=2.6‰ at 4 m versus mean $\delta^{15}N_{NO3^-}$=3.5‰ at 100 m), suggesting an input of ^{15}N-depleted N that likely shift the whole DON pool, including the recalcitrant fraction, towards lowered bulk DON-$\delta^{15}N$ values. This low $\delta^{15}N$ most probably derives from the exudation of low DON-$\delta^{15}N$ by N_2 fixing organisms or the simple initial degradation of larger N_2 fixing cells into DON. Clearly more work is needed that will address the controls that set the absolute values of [DON] and DON-$\delta^{15}N$; that is, the N isotope effects associated with DON consumption and production. At this point, we can only speculate about causes for the significant difference in surface DON-$\delta^{15}N$ in the western (4.1‰) (Knapp *et al.*, 2005) versus the eastern subtropical North Atlantic (2.6‰), at comparable DON concentrations and nitrate $\delta^{15}N$ values. The most plausible explanation could be that N_2 fixation plays an even more important role in the N budget of the eastern subtropical North Atlantic than in that of the western

side, shunting relatively large amounts of ^{15}N-depleted N into the surface N pool. A more quantitative treatment of the AF nitrogen budget follows in the next section; however, given the apparently recalcitrant character of the surface water DON pool, we neglected DON in our N isotope budget below.

4.3 Quantitative estimates of N_2 fixation in the Azores Front region from N isotope mass balance considerations

Adopting the approach by Altabet (1988) and Knapp *et al.* (2005) in the western subtropical N-Atlantic, we used a simplified N isotope mass balance in order to derive more quantitative estimates of N_2 fixation in the AF area. In steady state, the PN export from the surface waters (both ^{15}N and ^{14}N, respectively) should be balanced by the sum of the nitrate flux (diffusion and advection) from below the thermocline and the supply of new N by N_2 fixation.

In our conceptual model, surface water is represented by the 0-100 m depth interval, with 100 m being the lower limit of the euphotic zone. The surface water pool exchanges nitrate with the waters below at a depth of 200 m which is the maximum mixing depth during winter. The mass balance equation for N is as follows:

$$(N_B - N_U) \times K_U + N_{fix} = F_{PN} \qquad (2)$$

where N_U and N_B, represent the mean integrated nitrate concentration in the upper 100 m and at 200 m, respectively, K_U is the vertical exchange between surface and subsurface waters, N_{fix} is the N_2 fixation rate and F_{PN} is the flux of particulate nitrogen leaving the euphotic zone at 100 m (Table 2). Regarding the isotope budget, the following equation applies:

$$((\delta^{15}N_B \times N_B) - (\delta^{15}N_U \times N_U)) \times K_U + \delta^{15}N\text{-}N_{fix} \times N_{fix} = \delta^{15}N\text{-}PN \times F_{PN} \quad (3)$$

where $\delta^{15}N_U$, $\delta^{15}N_B$, $\delta^{15}N\text{-}N_{fix}$ and $\delta^{15}N\text{-}PN$ represent the mean weighted $\delta^{15}N$ values for surface and subsurface waters, the $\delta^{15}N$ of newly fixed N (assumed to be -1‰) and the flux-weighted mean $\delta^{15}N$ of sinking PN measured at station KIEL276 (33°N, 22°W) from May 2003 to April 2005 (Table 2), respectively. Unfortunately, we only have data on the sinking PN at 2000 m, with mean PN fluxes observed in the deep sediment traps between May 2003 and April 2005 of 0.56±0.48 mg N m^{-2} d^{-1} (n=33). To estimate the export flux at 100 m depth, we assumed an exponential decrease of PN flux with depth according to Pace *et al.* (1987):

$$F_{PN} = 0.432Z^{-0.843} \times PP^{1.123} \quad (4)$$

where z is the depth in m and PP is the primary production in the photic zone in mg m^{-2} d^{-1}. Using the PN flux at 2000m, we can calculate PP, which we subsequently used to assess a $F_{PN(100m)}$, the export flux at 100 m, of 183 mmol N m^{-2} yr^{-1}. This approach is validated by the fact that the calculated value for PP is 379 mg C m^{-2} d^{-1}, in close agreement to estimates for primary productivity in the same region ranging from 300-500 mg C m^{-2} d^{-1} (Fernandez and Pingree, 1996; Joint *et al.*, 2002).

If we resolve for K_u (equation 2) and substitute into equation 3, we obtain an N_{fix} estimate of 75±43 mmol N m^{-2} yr^{-1}. We assume that the $\delta^{15}N$ of PN measured at 2000 m was the same than the $\delta^{15}N$ of PN at 100 m. The possible effects of N isotope alteration during bacterial OM degradation have been discussed in detail in section 4.1.1. Given the fact that both the DON and DIN pools have a low $\delta^{15}N$ compared to deep water nitrate, it is reasonable to assume that if at all, N isotope alteration would have lead to an enrichment in the sinking POM during sinking, thus rendering the above-given N_2 fixation rate a conservative estimate. Yet, even if we assume 0.5‰ higher value for the $\delta^{15}N$ of exported PN, we still yield a significant N_{fix} estimate of 56±43 mmol N m^{-2} yr^{-1}.

Our N_2 fixation rate estimates for the AF region fall within the upper range of reported values derived from most recent direct measurements (15-87 mmol N m^{-2} yr^{-1}) in the western subtropical N-Atlantic and are in good agreement with geochemical estimates for the North Atlantic Ocean (Table 3).

We are aware of the fact that the mean PN flux and the δ^{15}N-PN measured near the AF front (station KIEL276) are probably not representative for all the stations. Primary productivity is likely to display large variations, depending on the position relative to the AF and this imposes a great uncertainty on our N_{fix} estimate, because we derive the export flux relating the sinking flux of N at 2000 m to PP in the surface. Also, since *Trichodesmium* blooms generally occur in marine habitat with temperature >25°C and because N_2 fixers are more competitive in nitrate depleted waters (Karl *et al.*, 2002), we expect even higher N_2 fixation rates in the oligotrophic waters further south of the AF. Indeed, as outlined above, the variation in the geometry of nitrate N and O isotope profiles between stations provides some indication for spatial changes in the contribution of N_2 fixation to the new production. For example, at station 184, we observe a positive curvature in the nitrate δ^{15}N in surface waters (NO_3^- δ^{15}N=5.1‰, 100 m depth) associated with a fairly low Δ(15,18) minimum (3.5‰). Considering that the station 184 is located north of the AF, where surface waters are colder and more productive, this likely indicates more nitrate flux from below with respect to N_2 fixation (for example, see scenario 1, Table 1) in comparison to the stations located further south.

Moreover, it is reasonable to assume that N_2 fixation and/or export production undergo significant temporal variability, as indicated by the seasonal sinking flux and $\delta^{15}N_{PON}$ variations observed at Station KIEL 276 (Fig. 7). It is important to understand that the here-derived N_2 fixation rate represents an integrated mean value (based on two

years of sinking PON data), that does not provide any information on possibly much higher peak rates in spring.

4.4 Reconciling the discrepancy between N export and N input in the subtropical Northeast Atlantic

The few available observational data from the AF and nearby regions suggest a large apparent imbalance in the N cycle of the subtropical Northeast Atlantic, with a relatively low flux of nitrate into the euphotic zone by vertical turbulent diffusion, mesoscale eddies, advection of PON and labile/semilabile DON from outside of the gyre and atmospheric depositions (<~125-220 mmol N m^{-2} yr^{-1}, Table 4) and a much higher export production (based on oxygen utilization rates in a nearby environment) (630±150 mmol N m^{-2} yr^{-1}) (Jenkins, 1982, Oschlies, 2002b). While alternative explanations, primarily invoking the export of nitrogen-poor DOM, interannual variability and artifacts due to non-correspondance of respective measurements (Oschlies, 2002b), are plausible, our combined nitrate and PON isotope data suggest that N_2 fixation is an important element of the AF/subtropical N-E Atlantic N cycle, that could, if not close the N budget, at least help to decrease the apparent imbalance.

Dependent on whether we use an export production estimate in the oligotrophic N-E Atlantic of 183 mmol N m^{-2} yr^{-1} (derived in this study (Table 2), and consistent with a modeled export production of 200 mmol N m^{-2} yr^{-1} for the subtropical North Atlantic (Roussenov *et al.*, 2006), or that of Jenkins (1982) (630±150 mmol N m^{-2} yr^{-1}), our mean N_2 fixation rate estimate of 56-75 mmol N m^{-2} yr^{-1} implies that between 9 and 41% of the export production can be attributed to the supply of new N by N_2 fixation, and, that N_2 fixation is of the same order of magnitude as new production fueled by nitrate from below and thus, constitutes an important source of nitrogen to the euphotic zone (Table 4). In close agreement, Deutsch *et al.* (2007) estimated that ~50% of the exported PN in

the subtropical gyres comes from N_2 fixation, making it the principal N supply for primary production in low-latitude environments. Moreover, N_2 fixation would represents ~5 % of the N required for total primary production (1.65 mol N m^{-2} yr^{-1} if we consider a PP of 400 mg C m^{-2} d^{-1} and a C:N ratio of 7.2 (Körtzinger *et al.*, 2001)).

Our upper PON-based value for the N_2 fixation/export ratio of 40% does at least not stand in contradiction with the observed nitrate anomalies in the AF. In the AF, the observed weighted mean (for all the stations) nitrate $\delta^{15}N$, $\delta^{18}O$, and mean $\Delta(15,18)$ values for the upper 100 m are 3.5‰, 6.3‰ and -4.9‰, respectively. Given nitrate isotope values derived from our different scenarios outlined in section 4.1.3, the observational nitrate isotope data from the AF study area suggest that N_2 fixation could account for up to 40% of the new production (Table 1). However, more reliable estimates of this ratio based on nitrate N and O isotopes are only possible with a better understanding of 1) the branching fractionation during ammonium consumption (which has not been considered here) and 2) the exact mechanisms that determine the $\delta^{18}O$ of newly nitrified nitrate (0 versus 3‰).

5. Summary and concluding remarks

Here we report combined nitrate N and O isotope measurements from the water column in the Azores Front region. We observed, for the first time in an open marine study, distinct nitrate isotope anomalies in the $\delta^{15}N/\delta^{18}O$ relationship (as indicated by pronounced $\Delta(15,18)$ minima of up to ~-6‰) in the surface waters of the AF, which result from the decreases of NO_3^- $\delta^{15}N$ concomitant with an increase of the NO_3^- $\delta^{18}O$. The nitrate anomalies can most likely be ascribed to the combined processes of DIN uptake and nitrate regeneration, advection and entrainment of new nitrate from below the euphotic zone, as well as N_2 fixation, adding nitrate with a distinct N and O isotopic composition. While previous work has outlined the plausibility of surface nitrate

Δ(15,18) minima being due to the branching fractionation during ammonium consumption by assimilation and nitrification, atmospheric precipitation, and/or N-isotope discrimination during remineralization, we provide arguments that these mechanisms and processes, if at all important in the AF region, cannot explain by themselves the geometry of the observed $\delta^{15}N$ versus $\delta^{18}O$ profiles. We propose that the addition of low-$\delta^{15}N$ nitrate by N_2 fixation arises as the most plausible explanation that can cause both a decrease in nitrate $\delta^{15}N$ concomitant with a modest Δ(15,18) minimum in surface waters. This assumption is further supported by the low $\delta^{15}N$ values observed for sinking PN at the station KIEL276 (1.8‰), as well as the positive N* (~3-3.5 μM) in the subsurface waters.

We also expand here the highly limited data set on concentrations and the N-isotope composition of marine DON. The invariability of both [DON] and $\delta^{15}N$ of DON in the surface waters of the AF region suggests that DON is mostly recalcitrant and that a more labile fraction is rapidly and completely recycled in the surface waters. This is consistent with observations in the Sargasso Sea (Knapp *et al.*, 2005), suggesting an essentially static character of DON throughout the subtropical N-Atlantic. In agreement with both nitrate N and O isotope data and the ^{15}N-depleted sinking PON, the generally low $\delta^{15}N$ of DON (lower than the $\delta^{15}N$ of thermocline nitrate) probably results from the exudation of low-$\delta^{15}N$-DON by N_2 fixers, which ultimately acts to shift the $\delta^{15}N$ of the total fixed N inventory in surface waters of the AF region (including the recalcitrant DON fraction) towards relatively low $\delta^{15}N$ values.

Based on simple mass balance considerations, we estimate an average N_2 fixation rate of 56-75 mmol N m^{-2} yr^{-1} for the AF region in the Eastern Subtropical North Atlantic. Consequently, our data suggest that N_2 fixation is an important component of the N cycle in the AF area. More precisely, our estimate would indicate that the new production by N_2 fixation could explain ~9-41% of the export production, and thus

would be almost equally important as the nitrate supply from below the photic zone. Indeed, this estimate is not violated by the observed distribution of nitrate $\delta^{15}N$ versus $\delta^{18}O$. Assuming a N_2 fixation/export production of 40% in our nitrate N and O mass balance calculations would yield a $\Delta(15,18)$ minimum relatively close to the observational mean $\Delta(15,18)$ for the AF region (especially for a $\delta^{18}O_{ntr}$ of 3‰). Our mass balance-derived estimate for N_2 fixation represents an integrative mean value for the study region. However, variations in the nitrate $\Delta(15,18)$ between stations suggest there is significant spatial variability regarding the dynamics of the AF nitrogen cycle. We interpret the variations in the magnitude of the nitrate anomaly as indicative for variations in the relative importance of N_2 fixation for new production, with more pronounced N-to-O isotope anomalies for the stations located south of the AF where the oligotrophic conditions favor high N_2 fixation rates, and a lower $\Delta(15,18)$ minimum at station 184 located in the more productive and nutrient replete waters slightly north of the Front, where high N_2 fixation rates are less likely to occur.

This study represents one of the initial efforts to use dual nitrate isotope measurements to disentangle various N processes in the marine environment. However, a number of uncertainties remain that preclude, at least at this point, a more quantitative use of the $\Delta(15,18)$ to assess integrated N_2 fixation rates in the study region. First, estimates on the addition of newly fixed nitrate depends on the quantitative understanding of vertical water movement and the re-supply of nitrate from below the photic zone, the rates of which are not well constrained in the AF region. Second, we are not able to explain the exact mechanisms that are responsible for the fact that the decrease in nitrate $\Delta(15,18)$ already starts at a depth of 700 m. Obviously, some sort of downwelling is required to transfer nitrate isotope signatures intrinsic to photic zone processes down to these depths. Third, neither do we know the exact N isotope effects for nitrification and ammonium assimilation, nor can we assume that these isotope effects are invariant in the ocean environment. Fourth, it is still unclear what the exact mechanisms are that determine the $\delta^{18}O$ of newly nitrified nitrate. As suggested by

previous work (Wankel *et al.*, 2007), the nitrate Δ(15,18) is highly sensitive to both the importance of the branching during ammonium consumption (and in particular the associated N isotope effects) and the $\delta^{18}O$ of regenerated nitrate (be it from the remineralization of N_2 fixing or fixed-N assimilating phytoplankton). Thus, this study should be motivation for more work on the nitrate N and O isotope effects during nitrate regeneration.

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Figures captions

Figure 1. Map of the Azores Front region showing hydrocast (HC, white circles), sediment trap (KIEL276, white diamond) and DON (black dots) sampling locations during cruise *POS321* (*POSEIDON*). The approximate location of the Azores Current is also indicated.

Figure 2. Temperature distribution along the transect during *POS321*. The vertical resolution is 5 m. The approximate location of the AF is indicated by an upward displacement of the 15°C isotherm (starting at ~34.36°N, shaded area).

Figure 3. Depth profiles for a) $[NO_3^-]$, b) NO_3^- $\delta^{15}N$ and c) NO_3^- $\delta^{18}O$. Plotted values represent mean values from replicate analyses at any given depth.

Figure 4. Latitudinal N* distribution in the Eastern North Atlantic (15 to 24°W and 28 to 36°N). Data are taken from the Global Ocean Data Analysis Project (GLODAP) (http://cdiac.ornl.gov/oceans/glodap/GlopDV.htm) using objectively analyzed 1° nutrient fields (Key *et al.*, 2004). Black dots represent individual analysis.

Figure 5. Latitudinal distribution of [DON] and DON $\delta^{15}N$ (at 4 m) along the cruise transect (Fig. 1) in the Azores Front region in May 2005. Lines indicate average values.

Figure 6. Integrated values of a) [DON] and b) DON $\delta^{15}N$ in the upper water column of the Azores Front region in May 2005.

Figure 7. Particulate nitrogen (PN) sinking flux and $\delta^{15}N$ of PN at 2000 m depth at station KIEL276 (33°N, 22°W) between May 2003 and April 2005. Values shown for May and June 2003, January, March, April, May and June 2004 and February and March 2005 represent the mean value of two consecutive measurements made during the respective month.

Figure 8. Relation between ln $[NO_3^-]$ (μM) and nitrate $\delta^{15}N$ (a) and $\delta^{18}O$ (b).

Figure 9. Depth profiles of a) NO_3^- $\delta^{15}N$, b) NO_3^- $\delta^{18}O$ and c) nitrate $\delta^{15}N$:$\delta^{18}O$ anomaly from a 1:1 relationship expected during algal assimilation (Δ(15,18)). Plotted values are mean values of replicate analyses at a given depth for each individual station. Bold symbols show the depth-binned average for all stations.

Figure 10. Conceptual model used to evaluate the nitrate dual (N and O) isotopic composition ($\delta^{15}N_{Box}$ and $\delta^{18}O_{Box}$) and the resulting Δ(15,18) in the euphotic zone (upper 100 m) of the subtropical North Atlantic Ocean from isotope balance considerations as a function of relative changes in N_2 fixation (see Auxiliary materials, results in Table 1)). Four processes are considered: A: addition of newly fixed nitrate (N_{fix}) with a $\delta^{15}N$ and a $\delta^{18}O$ of -1‰ and 0 or 3‰, respectively (see text), B: net input of nitrate by diffusive and advective mixing (using mean values for the AF region of 3.7‰ and 5.1‰, for nitrate $\delta^{15}N$ and $\delta^{18}O$ respectively), C: export of N removing fixed N with an N isotope effect of 5‰ (the internal N cycling (nitrate assimilation and remineralization) is not assumed to affect the $\delta^{15}N$), D: gross nitrate assimilation removing nitrate with an O-isotope effect of 5‰, E) remineralization of OM and nitrification (recycled production) in the euphotic zone, which is assumed to return nitrate with a $\delta^{18}O$ ($\delta^{18}O_{ntr}$) of 0 or 3‰. Flux D-E in the right panel is equal to the export production C in the left panel.

Table 1. Expected nitrate $\delta^{15}N$, $\delta^{18}O$ and $\Delta(15, 18)$ in the euphotic zone (upper 100 m) of the AF region for various ratios of N_2 fixation versus export production and gross nitrate assimilation, based on the box model scheme and assumed isotope values presented in Figure 10 (see text and Fig. 10 for details)

Scenarios	1	2	3	4	5	6
N_2 fix/export production (%)	10	40	40	70	70	90
N_2 fix/gross NO_3^- assimilation (%)	5	10	5	10	5	10
NO_3^- $\delta^{15}N$	4.11	3.40	3.40	2.70	2.70	2.23
NO_3^- $\delta^{18}O$[a]	4.86 (5.96)	4.61 (6.65)	4.78 (7.25)	4.57 (7.08)	4.77 (7.51)	4.55 (7.22)
$\Delta(15,18)$[a]	-2.56 (-3.66)	-3.01 (-5.05)	-3.18 (-5.65)	-3.66 (-6.18)	-3.87 (-6,61)	-4.12 (-6.79)

[a] Values in parentheses represent results obtained using a value of 3‰ (instead of 0%) for the $\delta^{18}O$ of nitrate added to the nitrate pool following OM remineralization and subsequent nitrification (see also Auxiliary materials)

Table 2. Water column parameters used in the N isotope budget (Section 4.3)

Mean [NO_3^-] (μmol/L), upper 100 m	0.92±0.36
Mean NO_3^- (μmol/L), 200 m	4.51±1.46
Weighted mean NO_3^- $\delta^{15}N$ (‰ versus air), upper 100 m	3.47±1.25
Weighted mean NO_3^- $\delta^{15}N$ (‰ versus air), 200 m	3.68±0.65
Export production at 100 m (mmol N m^{-2} yr^{-1})	183
$\delta^{15}N$ (‰ versus air) of export PN[a]	1.79±0.75
$\delta^{15}N$ of new N added by N_2 fixation (‰)	-1

[a] Mean value for sinking PN at station KIEL276 (2000 m depth) between May 2003 and April 2005

Table 3. N_2 fixation rates in the North Atlantic based on geochemical approaches and recent direct biological measurements

Geochemical approaches	N_2 fixation rate (mmol N m^{-2} yr^{-1})	Reference
North Atlantic	133-230[a]	Michaels *et al.* (1996)
North Atlantic (10°-50°N, 10°-90°W)	72[a]	Gruber and Sarmiento (1997)
Atlantic 40°N-0°	66-99[b]	Lee *et al.* (2002)
North Atlantic (15°-25°N, 25°-75°W)	45[a]	Hansell *et al.* (2004)
North Atlantic	310[c]	Capone *et al.* (2005)
Atlantic	23[d]	Deutsch *et al.* (2007)
Direct biological measurements		
BATS site	15[e]	Orcutt *et al.* (2001)
North Atlantic	87 ± 14[f]	Capone *et al.* (2005)

[a] N* or excess DIN approach

[b] C_t inventory

[c] ^{15}N isotope mass balance

[d] Ocean circulation model using the reduction in P* and residence time of water masses

[e] Direct measurements of the amount of ^{15}N-labelled N_2 gas incorporated by individual colonies of *Trichodesmium* spp. and extrapolation to the North Atlantic

[f] Acetylene reduction method using a conversion ratio of 3:1

Table 4. N supply in the euphotic zone of the Subtropical Northeast Atlantic Ocean

N sources	Location	Flux (mmol N m^{-2} yr^{-1})	% of the export production[a]	Reference
N_2 fixation	29.17°-35.50°N, 15.50°-23.00°W	56-75±43	9-41	This study
NO_3^- from vertical turbulent diffusion	28.50°N, 23.00°W	51 (1-325)[b]	8-28	Lewis *et al.* (1986)
	24.00°-32.00°N, 34.50°-25.50°W	100±51[c]	16-55	Dietze *et al.* (2004)
Eddy induced nitrate supply	Subtropical N Atlantic	<50	8-27	Oschlies (2002a)
DON advection	10.00°-39.00°N, ~20.00°W	6[d]	1-3	Mahaffey *et al.* (2004)
	Subtropical N Atlantic	50[e]	8-27	Roussenov *et al.* (2006)
PON advection	Subtropical N-E Atlantic	10	2-5	Oschlies (2002b)
Atmopheric dry and wet N depositions	20.00°-40.00°N, 10.00°-30.00°W	10	2-5	Prospero *et al.* (1996)

[a] Based on estimates for export production of 630±150 mmol N m^{-2} yr^{-1} from oxygen utilization rate measurements below 100 m depth (Jenkins, 1982) (lower value) and of 183 mmol N m^{-2} yr^{-1} (at 100 m depth) estimated in this study (Section 4.3, upper value)

[b] 95% confidence interval

[c] Total of nitrate supplied by turbulent diffusion due to internal-waves (15±7 mmol m^{-2} yr^{-1}) and to salt-fingering (85±44 mmol m^{-2} yr^{-1})

[d] This amount accounts only for the semilabile fraction of DON that represents about 10% of the total DON pool (Mahaffey *et al.*, 2004)

[e] Based on a simplified cycling and transport model for inorganic nutrients, DON and DOP and assuming that 50% of the DON produced in the tropics and transported northward into the North Atlantic subtropical gyres is semilabile. Alternatively, if DON was considered to be entirely refractory, it would not contribute to export production

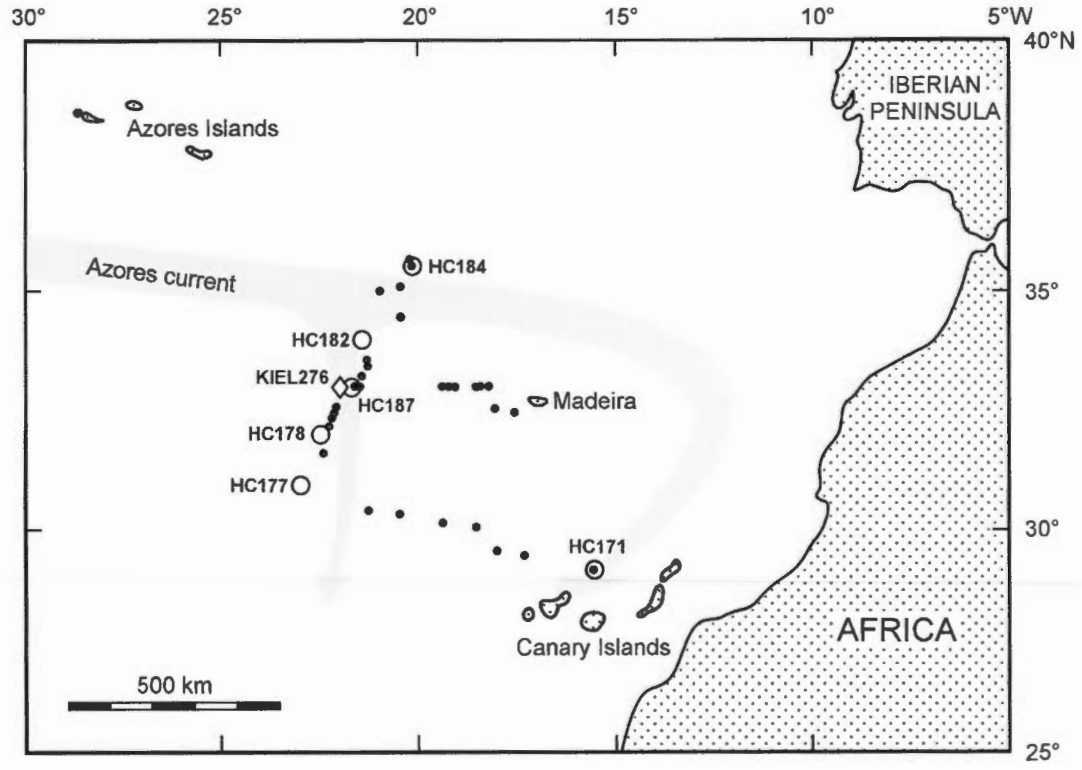
30°
25°
20°
15°
10°
5°W
40°N
35°
30°
25°
IBERIAN
PENINSULA
Azores Islands
Azores current
HC184
HC182
KIEL276
HC187
Madeira
HC178
HC177
HC171
Canary Islands
AFRICA
500 km

Figure 1.

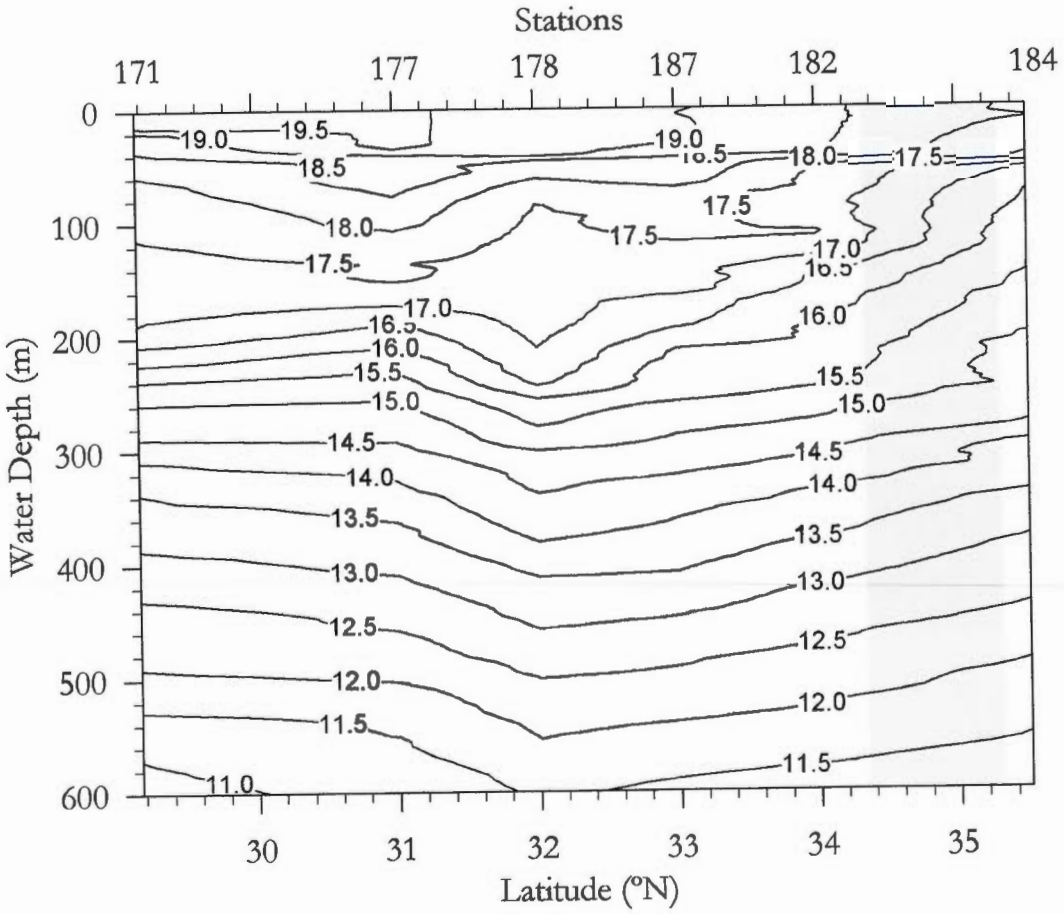
Stations
171
177
178
187
182
184
0
100
200
300
400
500
600
Water Depth (m)
19.0
19.5
18.5
18.0
17.5
17.0
16.5
16.0
15.5
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
11.0
30
31
32
33
34
35
Latitude (°N)

Figure 2.

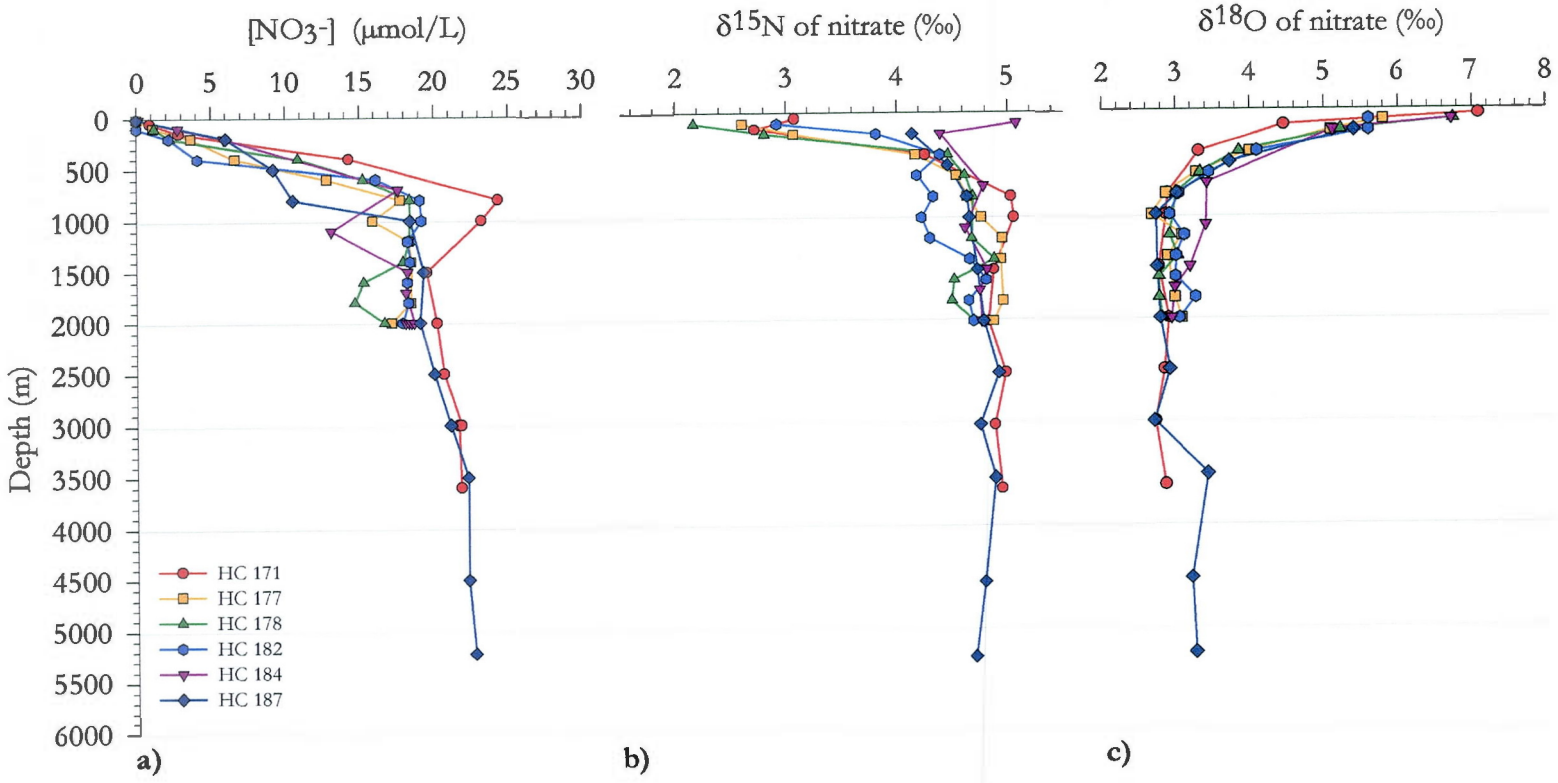
[NO3-] (µmol/L)
δ15N of nitrate (‰)
δ18O of nitrate (‰)
Depth (m)
HC 171
HC 177
HC 178
HC 182
HC 184
HC 187
a)
b)
c)

Figure 3.

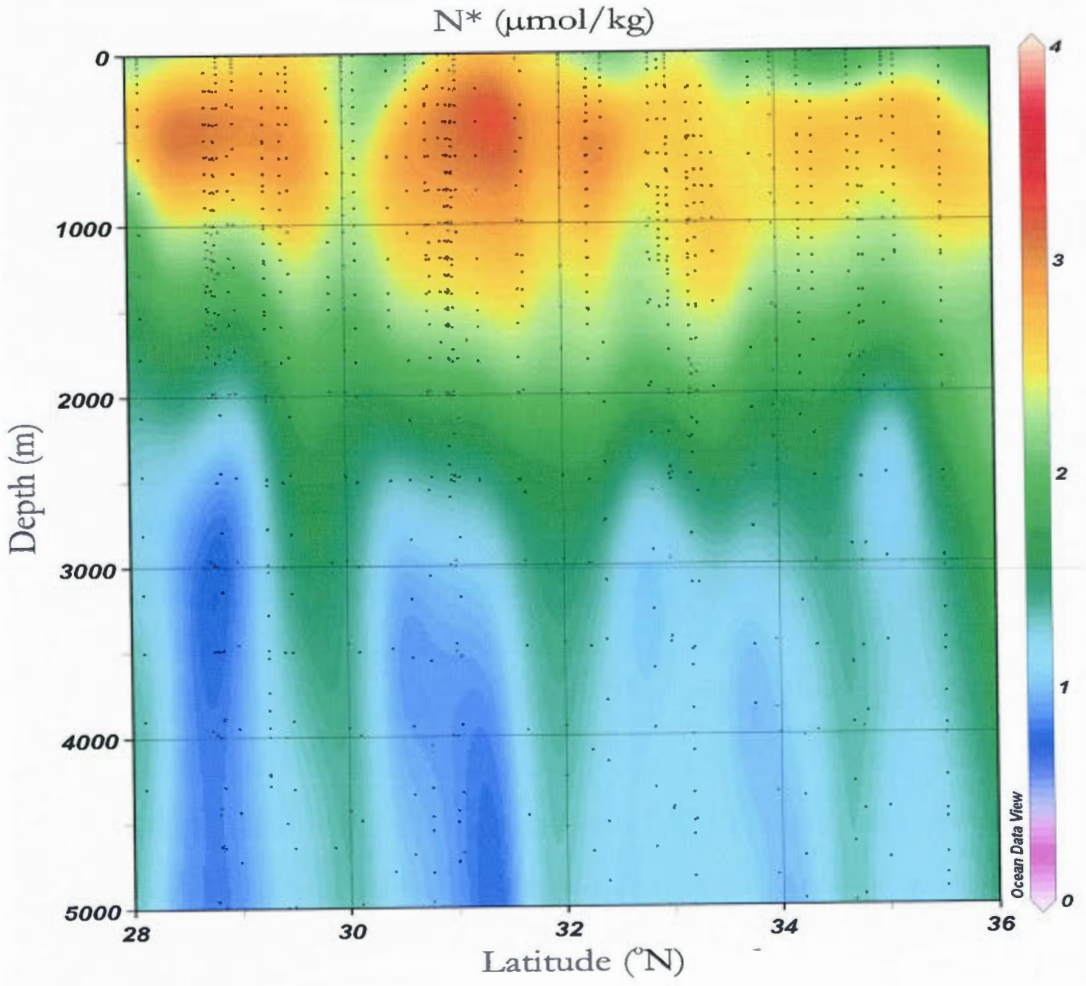
N* (µmol/kg)
Depth (m)
0
1000
2000
3000
4000
5000
28
30
32
34
36
Latitude (°N)
4
3
2
1
0
Ocean Data View

Figure 4.

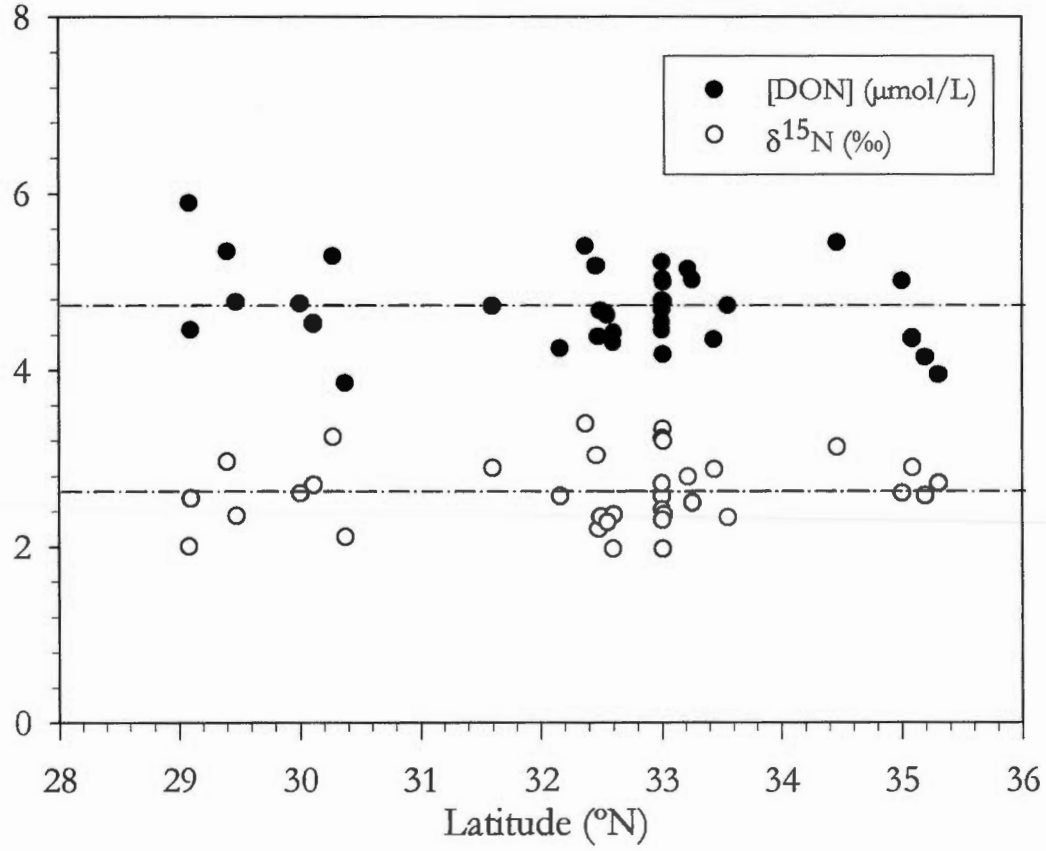

Figure 5.

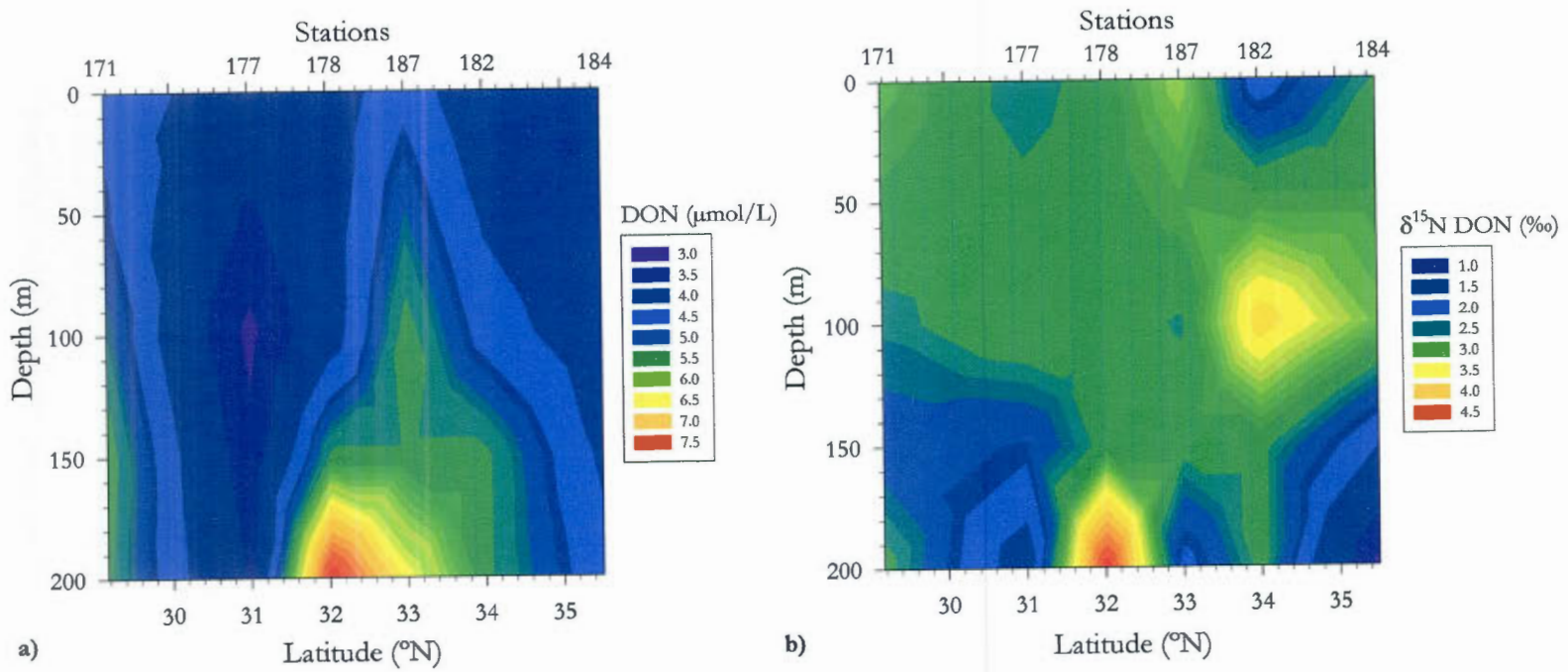
Stations
171
177
178
187
182
184
0
50
100
150
200
Depth (m)
30
31
32
33
34
35
Latitude (°N)
a)
DON (µmol/L)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Stations
171
177
178
187
182
184
0
50
100
150
200
Depth (m)
30
31
32
33
34
35
Latitude (°N)
b)
δ15N DON (‰)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

Figure 6.

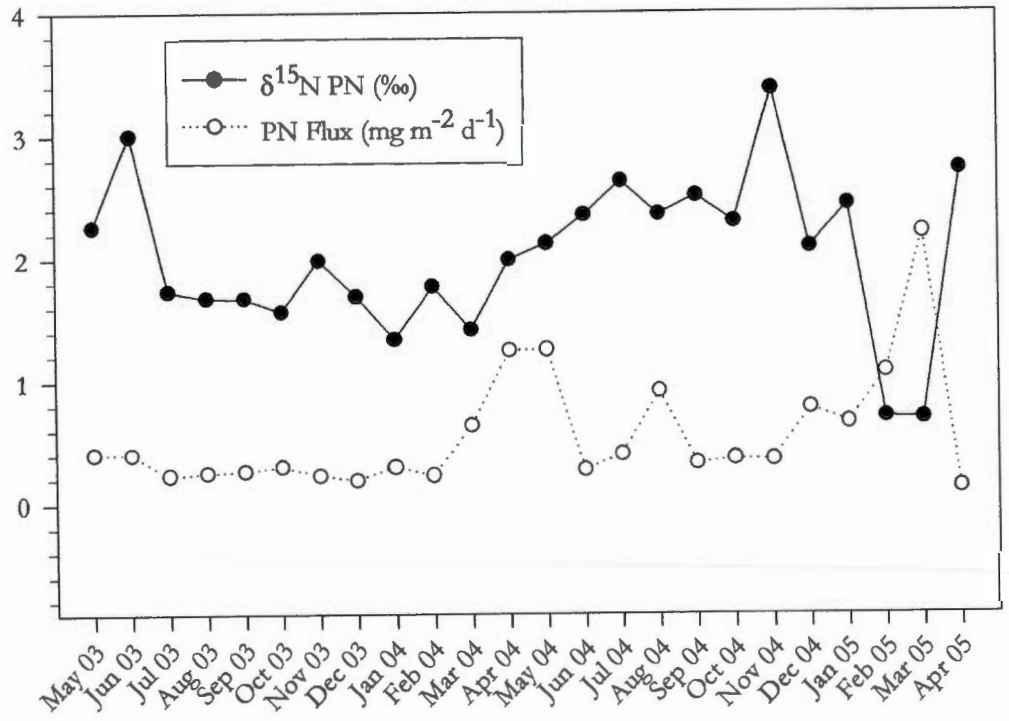
δ15N PN (‰)
PN Flux (mg m-2 d-1)
4
3
2
1
0
May 03
Jun 03
Jul 03
Aug 03
Sep 03
Oct 03
Nov 03
Dec 03
Jan 04
Feb 04
Mar 04
Apr 04
May 04
Jun 04
Jul 04
Aug 04
Sep 04
Oct 04
Nov 04
Dec 04
Jan 05
Feb 05
Mar 05
Apr 05

Figure 7.

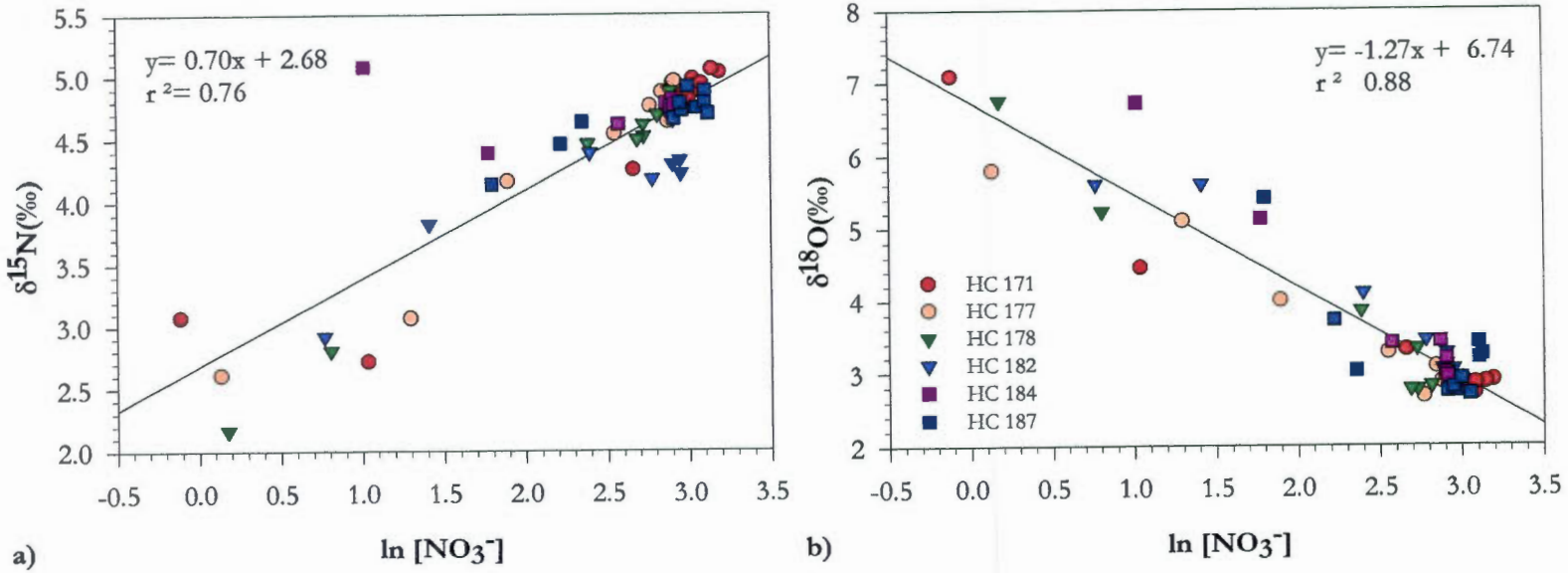
y= 0.70x + 2.68
r ²= 0.76
δ15N(‰)
ln [NO3-]
a)
y= -1.27x + 6.74
r ² 0.88
δ18O(‰)
HC 171
HC 177
HC 178
HC 182
HC 184
HC 187
ln [NO3-]
b)

Figure 8.

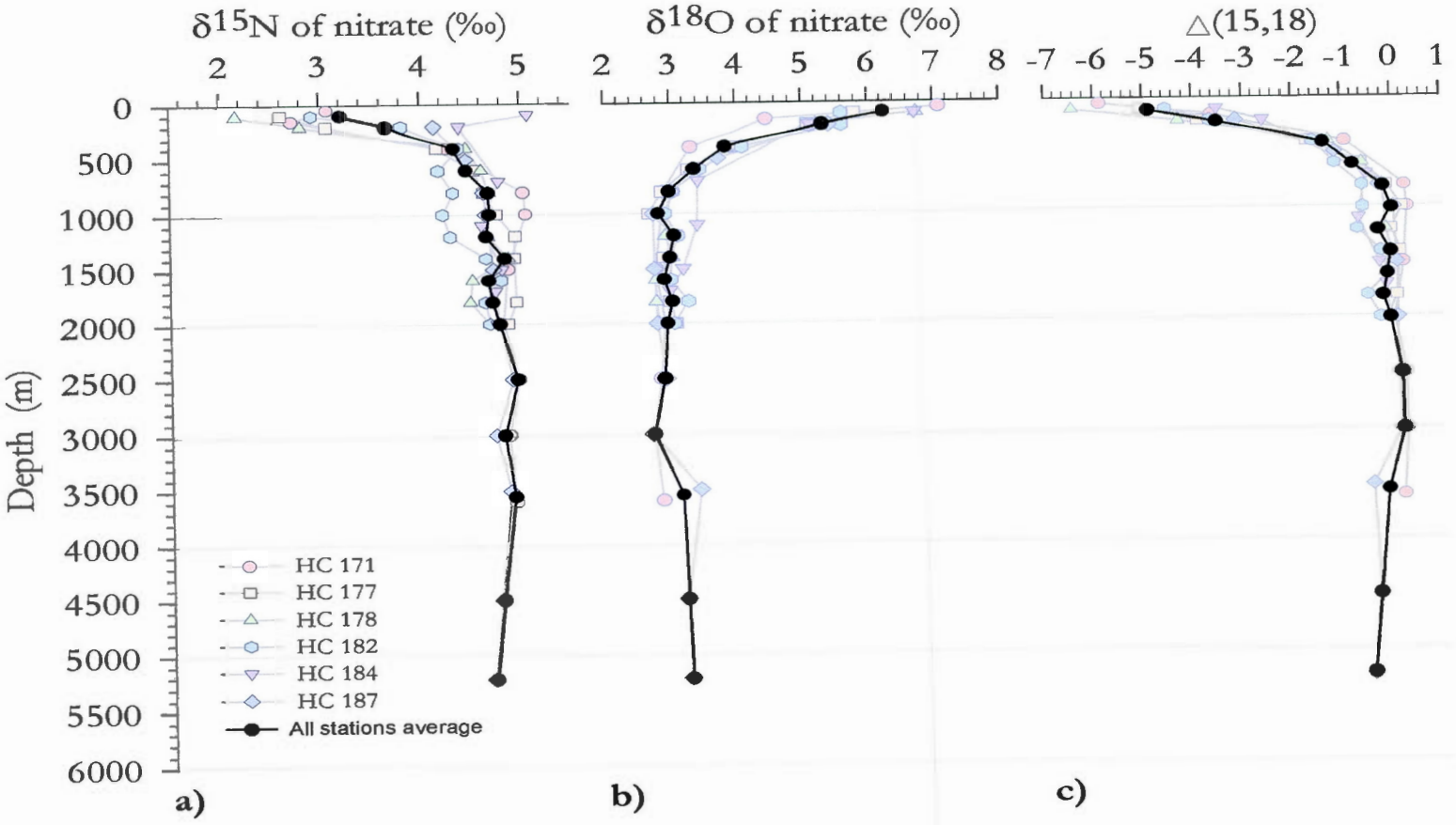
δ15N of nitrate (‰)
δ18O of nitrate (‰)
△(15,18)
Depth (m)
HC 171
HC 177
HC 178
HC 182
HC 184
HC 187
All stations average
a)
b)
c)

Figure 9.

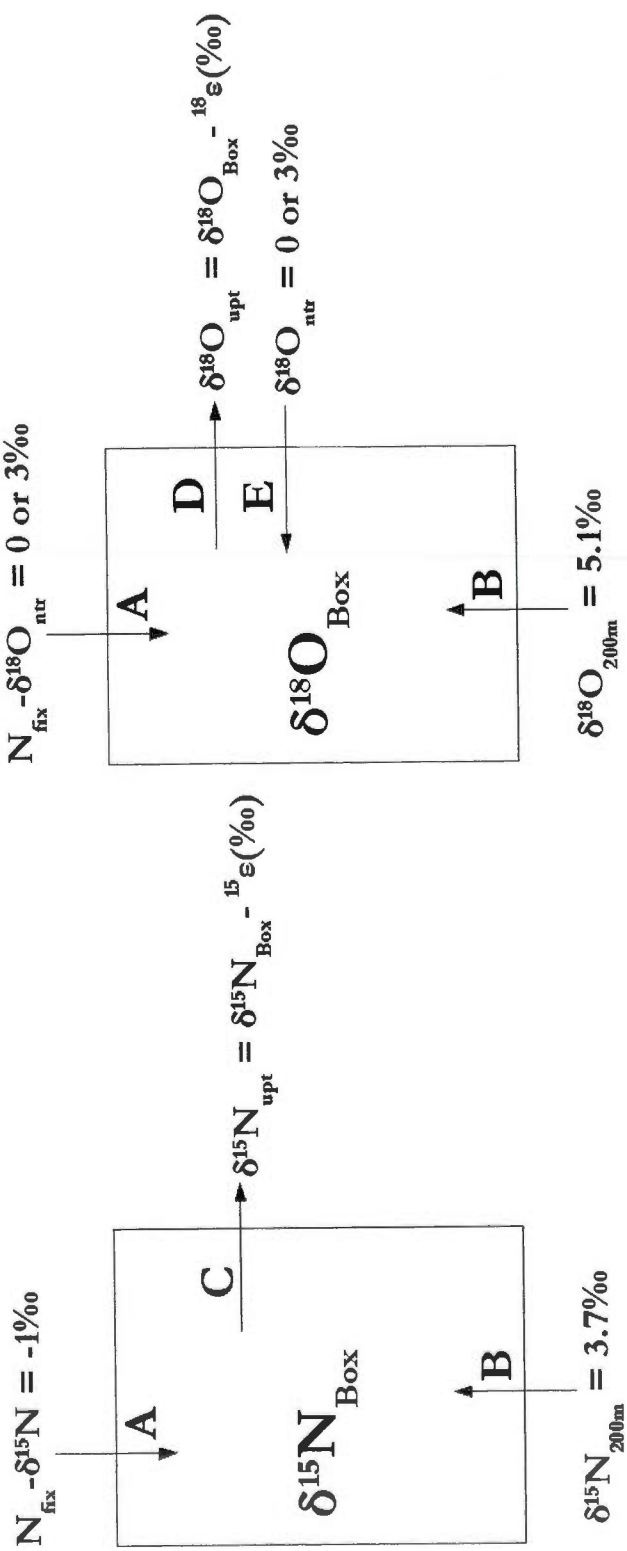
N_{fix}-$\delta^{15}N$ = -1‰
A
C
$\delta^{15}N_{upt} = \delta^{15}N_{Box} - {}^{15}\varepsilon$(‰)
$\delta^{15}N_{Box}$
B
$\delta^{15}N_{200m}$ = 3.7‰
N_{fix}-$\delta^{18}O_{ntr}$ = 0 or 3‰
A
D
E
$\delta^{18}O_{upt} = \delta^{18}O_{Box} - {}^{18}\varepsilon$(‰)
$\delta^{18}O_{ntr}$ = 0 or 3‰
$\delta^{18}O_{Box}$
B
$\delta^{18}O_{200m}$ = 5.1‰

Figure 10.

Auxiliary materials

Isotope balance calculations (section 4.1.3):

Under steady state, nitrate $\delta^{15}N$ and $\delta^{18}O$ values (and $\Delta(15,18)$) for a surface water parcel (upper 100 m depth) in the AF region can be derived from the isotope mass balance equations below for different values of the parameters A, B, C, D and E :

$$\delta^{15}N_{Box} = (A \times N_{fix}\text{-}\delta^{15}N) + (B \times \delta^{15}N_{200m}) - [C \times (\delta^{15}N_{Box} - {}^{15}\varepsilon\text{-}NO_3^-{}_{upt})]$$

$$\delta^{18}O_{Box} = (A \times \delta^{18}O_{ntr}) + (B \times \delta^{18}O_{200m}) - [(D \times (\delta^{18}O_{Box} - {}^{18}\varepsilon\text{-}NO_3^-{}_{upt})) - (E \times \delta^{18}O_{ntr})]$$

where:

A: Flux of nitrate coming from N_2 fixation

B: Flux of nitrate from below (200 m depth)

C: Export of N

D : Total nitrate assimilation

E : Nitrate regeneration

and

$\delta^{15}N_{Box}$ and $\delta^{18}O_{Box}$: Nitrate $\delta^{15}N$ and $\delta^{18}O$ values in the euphotic zone (upper 100 m).

N_{fix}-$\delta^{15}N$: $\delta^{15}N$ (of -1‰) added from newly fixed N ($\delta^{18}O$ of 0 or 3‰).

$\delta^{15}N_{200m}$ and $\delta^{18}O_{200m}$: mean weighted NO_3^- $\delta^{15}N$ and $\delta^{18}O$ at 200 m depth (maximum mixing depth in winter) for all the stations (NO_3^- $\delta^{15}N$ = 3.7‰ and NO_3^- $\delta^{18}O$ = 5.1‰).

${}^{15}\varepsilon\text{-}NO_3^-{}_{upt}$: Isotope effect during nitrate assimilation (${}^{15}\varepsilon$ = 5‰ and ${}^{18}\varepsilon$:${}^{15}\varepsilon$ = 1).

$\delta^{18}O_{ntr}$: $\delta^{18}O$ added to the nitrate pool following the remineralization (ammonification and nitrification) of organic material (0‰ or 3‰).

In steady-state, A+B (new production) is equal to the export production, which its self equals the difference between D and E. Accordingly, Flux E can be considered as recycled production.

CONCLUSIONS

Dans le cadre de cette étude, nous avons mesuré le $\delta^{15}N$ et le $\delta^{18}O$ du nitrate dans la colonne d'eau de la région du front des Açores dans l'Atlantique nord-est subtropical. Nous avons observé une diminution du $\delta^{15}N$ associée à une augmentation du $\delta^{18}O$ du nitrate dans les eaux de surface, causant des anomalies isotopiques comparativement au fractionnement observé durant l'assimilation du nitrate seulement. Ces anomalies isotopiques, générant un minimum du $\Delta(15,18)$ jusqu'à ~-6‰, peuvent être expliquées par la combinaison de différents processus, c'est-à-dire l'assimilation de l'azote inorganique dissous, la reminéralisation de la matière organique, l'advection et la diffusion du nitrate provenant des eaux profondes à travers la thermocline et la fixation de l'azote. Quoique des études aient suggéré d'autres processus pouvant générer de telles anomalies isotopiques du nitrate dans les eaux de surface, tels que le fractionnement relatif entre l'assimilation de l'ammonium et la nitrification, les précipitations atmosphériques et le fractionnement isotopique durant la reminéralisation de la matière organique, plusieurs évidences suggèrent que ces processus ne peuvent expliquer, à eux seuls, la géométrie des profils du $\delta^{15}N$ versus $\delta^{18}O$ du nitrate observés. Nous suggérons que l'addition d'un faible $\delta^{15}N$ par la fixation de l'azote est l'explication la plus probable pouvant causer une diminution du $\delta^{15}N$ du nitrate associée à un relativement faible minimum du $\Delta(15,18)$ dans les eaux de surface. Cette hypothèse est supportée par le faible $\delta^{15}N$ de l'AP exporté à la station KIEL276 (1,8‰) et les valeurs positives de N* observées dans les eaux de sous surface (~3-3,5 µM).

Nous avons également mesuré le $\delta^{15}N$ de l'AOD dans les eaux de surface de la région du front des Açores. L'invariabilité de la concentration et du $\delta^{15}N$ de l'AOD des eaux de surface suggère que l'AOD est récalcitrant et qu'une fraction labile est rapidement et complètement recyclée. Ceci est en accord avec une étude réalisée dans la mer des Sargasses (Knapp *et al.*, 2005), ce qui implique que l'AOD serait essentiellement

(N et O) du nitrate et le faible $\delta^{15}N$ de l'AP exporté à la station KIEL276, le faible $\delta^{15}N$ de l'AOD, plus faible que le $\delta^{15}N$ du nitrate de la thermocline, provient possiblement de l'exudation d'AOD ayant un faible $\delta^{15}N$ par les organismes fixateurs d'azote. La fixation de l'azote ferait donc ultimement diminuer le $\delta^{15}N$ du réservoir total d'azote fixé dans les eaux de surface de la région du front des Açores, incluant la fraction d'AOD récalcitrante.

Selon un simple bilan massique, la fixation de l'azote serait une composante importante du cycle de l'azote dans la région du front des Açores, avec un taux moyen de 56-75 mmol N m^{-2} an^{-1}. Plus précisément, notre estimé indique que la fixation de l'azote pourrait expliquer ~9-41% de la production exportée et être aussi importante que la diffusion du nitrate à travers la thermocline pour soutenir la production nouvelle. Cet estimé est également en accord avec les profils du $\delta^{15}N$ et $\delta^{18}O$ du nitrate. En effet, un ratio de fixation de N_2/production exportée de 40% dans les bilans isotopiques élaborés ci-dessus pour le N et O du nitrate donne un minimum du $\Delta(15,18)$ relativement près de la moyenne du $\Delta(15,18)$ observée dans la région du front des Açores, spécialement pour un $\delta^{18}O$ ajouté lors de la nitrification de 3‰. Notre taux de fixation d'azote estimé par bilan massique représente une valeur moyenne pour la région étudiée. Cependant, des variations du $\Delta(15,18)$ du nitrate entre les stations indiquent qu'il y a une grande variabilité spatiale en ce qui a trait à l'importance relative des processus contrôlant le cycle de l'azote dans cette région. En effet, les conditions oligotrophes pour les stations situées au sud du front des Açores favorisent des taux de fixation d'azote plus élevés (et un minimum du $\Delta(15,18)$ plus prononcé) que pour la station 184 située légèrement au nord du front des Açores, ou les eaux sont plus riches en nutriments et productives.

Cette étude représente un effort initial dans l'utilisation de la composition isotopique couplée ($\delta^{15}N$ et $\delta^{18}O$) du nitrate afin de différencier différents processus reliés au cycle de l'azote dans un environnement marin. Par contre, beaucoup

d'incertitudes demeurent avant d'obtenir un estimé quantitatif plus précis du taux de fixation d'azote pour la région étudiée. Premièrement, le taux de diffusion du nitrate à travers la thermocline est mal connu pour la région du front des Açores. Deuxièment, nous ne connaissons pas le mécanisme responsable de l'augmentation du $\delta^{18}O\text{-}NO_3^-$ (et la diminution du $\Delta(15,18)$) à partir d'une profondeur de 700 m, où il n'y a pas de processus biologiques, ce qui implique probablement une descente des eaux de surface. Troisièmement, nous ne connaissons pas précisément les effets isotopiques de la nitrification et de l'assimilation de l'ammonium et nous ne pouvons pas nous plus assumer que ces effets sont invariables dans les environnements marins. En quatrième lieu, nous ne connaissons pas les mécanismes exacts déterminant le $\delta^{18}O$ du nitrate lors de la nitrification. Or, selon Wankel *et al.* (2007), le $\Delta(15,18)$ du nitrate dépendrait de l'importance du fractionnement relatif entre l'assimilation de l'ammonium et la nitrification, particulièrement les effets isotopiques associés au N, et du $\delta^{18}O$ du nitrate ajouté lors de la nitrification. De ce fait, de futures études devraient étudier davantage les effets isotopiques du O et N du nitrate durant la régénération de celui-ci.

APPENDICE A

TABLEAUX DES RÉSULTATS

A.1 Concentrations et compositions isotopiques du nitrate, de l'ATD et de l'AOD pour les stations échantillonnées

Tableau A.1.1 Station 171 (Lat.: 29°10,14'N; Long.: 15°30,15'O)

Profondeur (m)	$[NO_3^-]$ (µmol/L)	$\delta^{15}N\text{-}NO_3^-$ (‰, air)	$\delta^{18}O\text{-}NO_3^-$ (‰, VSMOW)	Δ(15,18) (‰)	[ATD] (µmol/L)	[AOD] (µmol/L)	$\delta^{15}N$-ATD (‰, air)	$\delta^{15}N$-AOD (‰, air)
10	0,00	–	–	–	4,70	4,70	2,96	2,96
50	0,89	3,08	7,09	-5,86	–	–	–	–
150	2,82	2,72	4,46	-3,60	8,39	5,56	2,43	2,28
400	14,30	4,25	3,31	-0,91	–	–	–	–
800	24,33	5,03	2,89	0,28	–	–	–	–
1000	23,18	5,05	2,87	0,33	–	–	–	–
1500	19,53	4,87	2,77	0,25	–	–	–	–
2000	20,20	4,82	2,91	0,07	–	–	–	–
2500	20,67	4,98	2,84	0,28	–	–	–	–
3000	21,71	4,87	2,72	0,31	–	–	–	–
3600	21,78	4,94	2,85	0,23	–	–	–	–

Tableau A.1.2 Station 177 (Lat.: 30°59,88'N; Long.: 23°00,08'O)

Profondeur (m)	$[NO_3^-]$ (µmol/L)	$\delta^{15}N\text{-}NO_3^-$ (‰, air)	$\delta^{18}O\text{-}NO_3^-$ (‰, VSMOW)	Δ(15,18) (‰)	[ATD] (µmol/L)	[AOD] (µmol/L)	$\delta^{15}N$-ATD (‰, air)	$\delta^{15}N$-AOD (‰, air)
10	0,00	–	–	–	4,08	4,08	2,51	2,51
100	1,14	2,61	5,80	-5,04	4,10	2,96	2,75	2,80
200	3,65	3,07	5,10	-3,88	7,21	3,56	2,33	1,56
400	6,64	4,17	4,00	-1,68	9,13	2,49	3,27	0,86
600	12,79	4,54	3,28	-0,59	–	–	–	–
800	17,76	4,64	2,87	-0,08	–	–	–	–
1000	15,91	4,76	2,68	0,23	–	–	–	–
1200	18,32	4,95	3,09	0,01	–	–	–	–
1400	18,49	4,94	2,90	0,19	–	–	–	–
1800	18,47	4,96	2,99	0,12	–	–	–	–
2000	17,17	4,87	3,08	-0,06	–	–	–	–

Tableau A.1.3 Station 178 (Lat.: 32°00,92'N; Long.: 22°29,75'O)

Profondeur (m)	$[NO_3^-]$ (µmol/L)	$\delta^{15}N\text{-}NO_3^-$ (‰, air)	$\delta^{18}O\text{-}NO_3^-$ (‰, VSMOW)	Δ(15,18) (‰)	[ATD] (µmol/L)	[AOD] (µmol/L)	$\delta^{15}N$-ATD (‰, air)	$\delta^{15}N$-AOD (‰, air)
100	1,20	2,17	6,75	-6,43	5,30	4,10	2,55	2,67
200	2,24	2,80	5,23	-4,27	10,18	7,95	4,37	4,81
400	10,87	4,46	3,86	-1,24	–	–	–	–
600	15,27	4,61	3,33	-0,57	–	–	–	–
800	18,38	4,69	3,01	-0,17	–	–	–	–
1200	18,37	4,67	2,92	-0,10	–	–	–	–
1400	17,96	4,88	3,03	0,00	–	–	–	–
1600	15,31	4,52	2,77	-0,11	–	–	–	–
1800	14,70	4,49	2,78	-0,13	–	–	–	–
2000	16,69	4,69	2,82	0,02	–	–	–	–

Tableau A.1.4 Station 182 (Lat.: 34°00,00'N; Long.: 21°29,91'O)

Profondeur (m)	$[NO_3^-]$ (µmol/L)	$\delta^{15}N\text{-}NO_3^-$ (‰, air)	$\delta^{18}O\text{-}NO_3^-$ (‰, VSMOW)	Δ(15,18) (‰)	[ATD] (µmol/L)	[AOD] (µmol/L)	$\delta^{15}N$-ATD (‰, air)	$\delta^{15}N$-AOD (‰, air)
10	0,00	–	–	–	4,01	4,01	1,92	1,92
100	2,15	2,92	5,61	-4,54	6,79	4,64	3,49	3,75
200	4,10	3,82	5,61	-3,64	–	–	–	–
400	11,01	4,39	4,10	-1,56	–	–	–	–
600	16,14	4,18	3,45	-1,12	–	–	–	–
800	19,08	4,33	3,04	-0,57	–	–	–	–
1000	19,18	4,22	2,92	-0,55	–	–	–	–
1200	18,25	4,30	3,11	-0,67	–	–	–	–
1400	18,41	4,66	3,00	-0,20	–	–	–	–
1600	18,22	4,80	3,00	-0,04	–	–	–	–
1800	18,31	4,65	3,26	-0,47	–	–	–	–
2000	17,91	4,69	3,05	-0,21	–	–	–	–

Tableau A.1.5 Station 184 (Lat.: 35°29,96'N; Long.: 20°14,79'O)

Profondeur (m)	$[NO_3^-]$ (µmol/L)	$\delta^{15}N\text{-}NO_3^-$ (‰, air)	$\delta^{18}O\text{-}NO_3^-$ (‰, VSMOW)	Δ(15,18) (‰)	[ATD] (µmol/L)	[AOD] (µmol/L)	$\delta^{15}N$-ATD (‰, air)	$\delta^{15}N$-AOD (‰, air)
10	0,11	–	–	–	3,94	3,83	2,55	2,62
100	2,77	5,08	6,72	-3,49	6,84	4,07	3,87	3,05
200	5,90	4,39	5,12	-2,58	10,45	4,55	2,73	0,58
700	17,60	4,78	3,42	-0,49	–	–	–	–
1100	13,10	4,62	3,41	-0,64	–	–	–	–
1500	18,25	4,81	3,19	-0,23	–	–	–	–
1700	18,13	4,75	2,99	-0,09	–	–	–	–
2000	18,41	4,76	2,94	-0,03	–	–	–	–

Tableau A.1.6 Station 187 (Lat.: 33°00,04'N; Long.: 21°58,87'O)

Profondeur (m)	$[NO_3^-]$ (µmol/L)	$\delta^{15}N\text{-}NO_3^-$ (‰, air)	$\delta^{18}O\text{-}NO_3^-$ (‰, VSMOW)	Δ(15,18) (‰)	[ATD] (µmol/L)	[AOD] (µmol/L)	$\delta^{15}N$-ATD (‰, air)	$\delta^{15}N$-AOD (‰, air)
10	0,00	–	–	–	4,68	4,68	3,19	3,19
200	6,04	4,14	5,41	-3,12	12,42	6,38	3,03	1,98
500	9,20	4,46	3,73	-1,12	–	–	–	–
800	10,52	4,63	3,02	-0,24	–	–	–	–
1000	18,41	4,65	2,74	0,06	–	–	–	–
1500	19,32	4,73	2,75	0,12	–	–	–	–
2000	19,08	4,78	2,79	0,14	–	–	–	–
2500	20,03	4,91	2,92	0,15	–	–	–	–
3000	21,10	4,74	2,71	0,19	–	–	–	–
3500	22,25	4,88	3,42	-0,39	–	–	–	–
4500	22,26	4,78	3,20	-0,27	–	–	–	–
5221	22,70	4,70	3,25	-0,40	–	–	–	–

Tableau A.2 Concentrations et $\delta^{15}N$ de l'AOD de l'eau de surface (4 m de profondeur) le long du transect

Location	[AOD] (µmol/L)	$\delta^{15}N$-AOD (‰, air)
29°09'N;15°30'O	5,89	2,01
29°10'N;15°30'O	4,46	2,55
29°40'N;17°32'O	5,35	2,96
29°48'N;18°03'O	4,77	2,35
30°00'N;18°55'O	4,75	2,61
30°11'N;19°40'O	4,53	2,70
30°28'N;20°46'O	5,29	3,24
30°38'N;21°27'O	3,86	2,11
31°60'N;22°29'O	4,73	2,89
32°16'N;22°20'O	4,25	2,58
32°40'N;22°08'O	5,41	3,39
32°48'N;22°01'O	4,38	2,20
32°49'N;22°00'O	4,67	2,34
33°00'N;22°00'O	4,31	1,98
33°25'N;21°47'O	5,02	2,50
33°55'N;21°33'O	4,73	2,33
35°00'N;21°00'O	5,01	2,61
35°09'N;20°47'O	4,36	2,89
35°30'N;20°15'O	3,95	2,72
35°19'N;20°23'O	4,15	2,58
34°47'N;20°46'O	5,44	3,13
33°44'N;21°30'O	4,35	2,87
33°22'N;21°45'O	5,15	2,79
33°01'N;21°57'O	4,18	1,98
32°60'N;22°00'O	4,42	2,36
33°01'N;21°59'O	5,03	3,33
33°00'N;21°48'O	4,69	2,57
33°00'N;19°43'O	4,54	2,71
33°00'N;19°22'O	4,79	2,42
33°00'N;19°06'O	4,45	3,23
33°01'N;18°53'O	5,00	3,19
33°02'N;18°43'O	4,77	2,37
33°00'N;18°23'O	5,23	2,30
32°55'N;18°08'O	4,62	2,28
32°46'N;17°58'O	5,18	3,03

Tableau A.3 $\delta^{15}N$ de l'AP exporté, flux de particules, % d'azote et flux d'AP à la station KIEL276 (Lat.: 33°00'N; Long.: 22°00'O, 2000 m de profondeur)

Mois d'échantillonnage	$\delta^{15}N$ de l'AP exporté (‰, air)	Flux de particules total (mg m^{-2} j^{-1})	% d'azote (%)	Flux d'azote particulaire (mg m^{-2} j^{-1})
Mai 2003*	2,26	65,80	0,67	0,41
Juin 2003*	3,00	62,99	0,64	0,41
Juillet 2003	1,74	37,80	0,61	0,23
Août 2003	1,68	36,78	0,69	0,25
Septembre 2003	1,68	41,02	0,65	0,27
Octobre 2003	1,57	45,13	0,67	0,30
Novembre 2003	1,99	35,87	0,66	0,23
Décembre 2003	1,70	37,22	0,52	0,19
Janvier 2004*	1,34	49,41	0,61	0,30
Février 2004	1,77	46,08	0,32	0,23
Mars 2004*	1,42	115,56	0,63	0,64
Avril 2004*	1,99	247,12	0,47	1,25
Mai 2004*	2,12	253,32	0,50	1,26
Juin 2004*	2,35	53,28	0,49	0,28
Juillet 2004	2,63	59,35	0,67	0,40
Août 2004	2,36	144,13	0,64	0,92
Septembre 2004	2,51	49,30	0,67	0,33
Octobre 2004	2,30	72,87	0,50	0,37
Novembre 2004	3,38	64,09	0,56	0,36
Décembre 2004	2,09	172,55	0,45	0,78
Janvier 2005	2,43	91,26	0,72	0,66
Février 2005*	0,70	176,09	0,59	1,07
Mars 2005*	0,69	352,99	0,62	2,21
Avril 2005	2,72	15,66	0,80	0,13

*Mois pour lesquels les moyennes du $\delta^{15}N$ de l'AP exporté, du flux de particules total, du % d'azote et du flux d'AP de deux échantillons pris pendant des intervalles de temps consécutifs de 11 à 15 jours pendant le mois sont indiquées.

APPENDICE B

ANALYSE DU $\delta^{15}N$ ET DU $\delta^{18}O$ DU NITRATE AVEC LA MÉTHODE DES BACTÉRIES DÉNITRIFIANTES

La méthode des bactéries dénitrifiantes (Casciotti *et al.*, 2002; Sigman *et al.*, 2001) requiert des concentrations de nitrate beaucoup plus faibles (jusqu'à 10 nmol) que les autres méthodes conventionnelles et permet d'analyser de façon simultanée le $\delta^{15}N$ et le $\delta^{18}O$ du nitrate. En résumé, les échantillons sont inoculés avec une culture pure des bactéries dénitrifiantes *Pseudomonas chlororaphis* (*P. Chlororaphis*) ou *Pseudomonas aureofaciens* (*P. aureofaciens*) qui convertissent complètement le nitrate en N_2O gazeux (équation B.1). Le N_2O ainsi produit est ensuite analysé pour la composition isotopique (N et O) avec un chromatographe en phase gazeuse couplé à un spectromètre de masse.

$$2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow N_2O \qquad \text{(B.1)}$$

Deux bactéries différentes sont utilisées : *P. chlororaphis* ($\delta^{15}N$ du nitrate) et *P. aureofaciens*. La bactérie *P. aureofaciens* est utilisée pour l'analyse du $\delta^{18}O\text{-}NO_3^-$ car, contrairement à la bactérie *P. chlororaphis*, l'incorporation d'atomes d'oxygène de l'eau lors de la réduction des nitrates en oxyde nitreux (équation B.1) est faible (généralement $\leq$5%) et peut être corrigée (voir étape 12). La bactérie *P. chlororaphis* est utilisée préférentiellement à la bactérie *P. aureofaciens* pour l'analyse du $\delta^{15}N$ du nitrate car elle donne généralement des résultats plus exacts et reproductibles, surtout pour les échantillons ayant de faibles concentrations de nitrate ($\leq$5 µM).

1. Réhydratation de la bactérie lyophilisée

La bactérie *P. chlororaphis* (#43928) n'est plus disponible à l'American Type and Culture Collection (ATCC). La bactérie *P. aureofaciens* (*renommée P. chlororaphis*) peut être achetée à l'ATCC (#13985). Afin d'éviter toute confusion, l'ancienne appellation de la bactérie *P. chlororaphis* (c'est-à-dire *P. aureofaciens*) sera utilisée tout au long de ce texte.

Afin de préparer le médium de croissance pour la bactérie *P. aureofaciens*, dissoudre 1.6 g de bouillon de nutriments « nutrient broth » dans 200 ml d'eau ultrapure dans une bouteille de médium de 250 ml. Stériliser au cycle pour les liquides à 121°C pendant 20 minutes. Pipetter ensuite 5 ml de la solution stérile de bouillon de nutriments dans des éprouvettes stériles. Ranger la bouteille de bouillon de nutriments au frigidaire jusqu'au prochain usage. La bouteille peut ainsi se conserver plusieurs mois.

Ouvrir le contenant de bactéries séchées à froid (tube provenant de l'ATCC) en chauffant le bout à la flamme d'un brûleur Bunsen, mettre quelques gouttes d'eau, casser à l'aide d'une lime et enlever le coton à l'intérieur avec une pince stérile. Il faut ensuite pipetter ~0,5-1 ml de bouillon de nutriments provenant de l'éprouvette stérile, réhydrater la bactérie solide et verser rapidement la bactérie réhydratée dans une éprouvette stérile en prenant bien soin de chauffer les extrémités des contenants à la flamme avant l'opération et brasser. Utiliser plusieurs gouttes de cette suspension pour inoculer d'autres éprouvettes de 5 ml de bouillon de nutriments afin de créer une réserve de bactéries. Mettre toutes les éprouvettes dans un brasseur pendant 12-24 heures. Il est important que la densité des bactéries soit modérée (il ne faut pas atteindre la phase stationnaire de croissance) afin de pouvoir les congeler. La solution de bouillon de nutriments dans les éprouvettes devrait être brouillée avec l'apparition d'un précipité jaunâtre (fig. B.1).

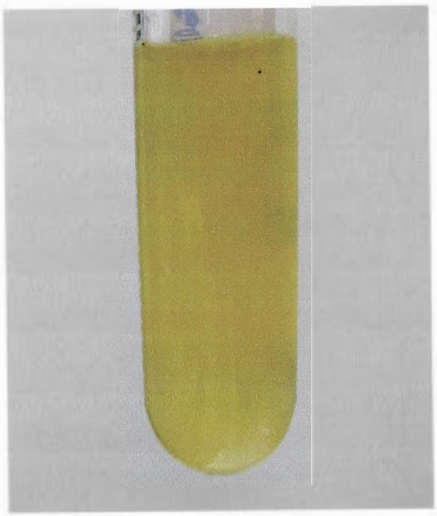

Figure B.1 Éprouvette de 5 ml de bouillon de nutriments inoculé avec la bactérie *P. Aureofaciens.*

2. Création de la réserve de bactéries

À partir d'une éprouvette de bouillon de nutriments inoculé avec la bactérie (étape 1), ajouter 1.275 ml de solution bactérienne dans 5 petits cryotubes stériles de 2 ml. Ajouter ~0.225 ml de glycérol stérile (121°C pendant 20 minutes au cycle liquide), fermer et bien brasser les contenants à l'aide d'un vortex. De cette façon on obtient une concentration d'environ 15-20% de glycérol qui est optimale pour la préservation des bactéries. Répéter cette procédure plusieurs fois pour chaque culture afin d'obtenir une réserve de bactéries. Mettre les contenants dans une boîte bien identifiée avec le nom de la bactérie, la date, le nom du responsable et le numéro de téléphone du laboratoire et mettre au congélateur à -80°C. De cette façon, les bactéries devraient se conserver plusieurs années.

Il est également possible de créer une réserve de bactéries à partir d'une culture sur une plaque agar (étape 4). Pour ce faire, utiliser les plaques #2 ou 3 seulement, inoculer

dans des éprouvettes de 5 ml de bouillon de nutriments, mettre dans un brasseur pendant 12-24 heures et suivre la procédure ci-dessus afin de congeler les bactéries.

3. Préparation des plaques agar:

Peser 20 g de TSA (Tryptic Soy Agar) et 0.5 g de KNO_3 et mettre dans un Erlenmeyer de 1 L. Ajouter 500 ml d'eau ultra pure et un agitateur magnétique. Mettre sur une plaque qui chauffe et agite la solution et attendre que le solide se dissolve complètement. Couvrir avec un papier d'aluminium et stériliser au cycle des liquides à 121°C pendant 20 minutes.

Désinfecter le comptoir de travail avec du germicide, allumer un brûleur Bunsen et prendre environ 40 plaques de culture stériles. Ouvrir une plaque de culture stérile à un angle de 45° et verser doucement la solution d'agar liquide stérile de façon à recouvrir entièrement le fond, puis refermer le couvercle. Répéter l'opération pour plusieurs plaques. Si la solution d'agar se solidifie partiellement, chauffer celle-ci afin de liquéfier de nouveau l'agar. Empiler les plaques à proximité du brûleur jusqu'à ce que la solution d'agar se solidifie. Mettre un papier parafilm tout autour des plaques et incuber celles-ci en position inverse (agar sur le dessus) à 37°C pendant 12 heures pour enlever l'humidité. Ranger dans le frigidaire jusqu'au prochain usage.

4. Faire revivre les bactéries congelées

Identifier 2 plaques agar avec le nom de la bactérie et la date. À partir de la réserve de bactéries congelées, prendre un cure-dent stérile, prélever quelques bactéries et mettre dans un coin de la plaque agar en évitant les contaminations le plus possible. Laisser

dégeler un peu et utiliser une loupe de platinium-iridium pour étendre sur la plaque #1. Avant de toucher les bactéries avec la loupe, refroidir celle-ci des deux côtés en touchant une portion d'agar. Il est toujours préférable d'inoculer deux plaques ou plus au cas ou une plaque serait contaminée ou ne montrerait pas de signe de croissance bactérienne. Incuber la plaque #1 à 26±5°C pendant 3-4 jours (*P. aureofaciens*) ou à la température de la pièce pendant 2-3 jours (*P. chlororaphis*). Les colonies sur les plaques devraient être de couleur jaune-orange (*P. aureofaciens*) ou jaune foncé (*P.chlororaphis*), entières, circulaires, lisses et plates (fig. B.2). À l'aide de la loupe, transférer une colonie de bactéries de la plaque #1 à la plaque #2 et incuber à 26±5°C pendant 3-4 jours (*P. aureofaciens*) ou à la température de la pièce pendant 2-3 jours (*P. chlororaphis*). Si nécessaire, préparer une plaque #3 à partir de la plaque #2. Utiliser seulement les plaques #2 ou #3 pour l'inoculation dans les bouteilles de médium (étape 6).

Figure B.2 Plaque #2 des *bactéries P. aureofaciens* (gauche) et *P. chlororaphis* (droite).

5. Préparation du médium TSB

Peser 60 g de TSB (Tryptic Soy Broth), 10 g de K_2HPO_4, 2 g de KNO_3 et 2 g de $(NH_4)_2SO_4$ et mettre dans une bouteille en verre de 5 L. Ajouter 2 L d'eau ultra pure et brasser jusqu'à ce que le solide se dissolve complètement.

Pour la bactérie *P. aureofaciens*, verser 130 ml de médium à l'aide d'un cylindre gradué dans des bouteilles de verre de 160 ml. Mettre un bouchon de butyl et fermer avec un scellant d'aluminium. Pour la bactérie *P. chlororaphis*, verser 400 ml de médium dans des bouteilles de médium de 500 ml. Stériliser les bouteilles au cycle des liquides pendant 85 minutes en tout (55 minutes de stérilisation) à 121°C. Une fois la solution stérilisée, la couleur du médium devrait être brun-rougeâtre (fig. B.3). Identifier les bouteilles de médium avec les ingrédients et la date de préparation et ranger à la température de la pièce. De cette façon, les solutions se conservent pendant quelques mois. Si il y a apparition d'un précipité, cela signifie que le médium n'est plus utilisable.

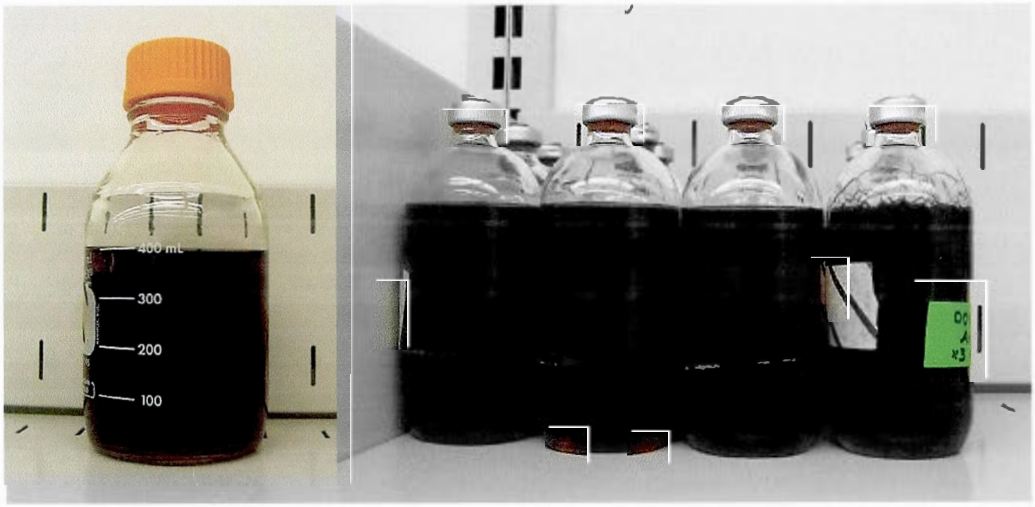

Figure B.3 Bouteilles de médium TSB de 500 ml (gauche) et 160 ml (droite).

6. Inoculation des bactéries dans les bouteilles de médium

Pour la bactérie *P. aureofaciens*, préparer une éprouvette de 5 ml de bouillon de nutriments (étape 1) en utilisant une pipette stérile en verre de 10 ml ou une seringue stérile. Inoculer celle-ci avec une seule colonie de la bactérie *P. aureofaciens* et mettre dans un agitateur automatique pendant ~12 hrs. Injecter ensuite 0.8 ml de la solution bactérienne dans les bouteilles de médium de 160 ml en utilisant une seringue stérile de 1 ml et une aiguille 23G1. Identifier les bouteilles avec le nom de la bactérie ainsi que la date d'inoculation. Mettre dans un agitateur automatique en position horizontale pendant 3 à 5 jours afin de permettre la croissance bactérienne. Il est important de déterminer par la suite si les solutions de bactéries sont utilisables pour l'analyse isotopique à l'aide d'un test pour détecter la présence de nitrites (étape 7).

Pour la bactérie *P. chlororaphis*, inoculer directement la bouteille de médium de 500 ml à l'aide d'une seule colonie de la bactérie. Identifier les bouteilles avec le nom de la bactérie et la date d'inoculation et mettre dans un agitateur automatique en position horizontale pendant 2 à 3 jours. Le test pour détecter la présence des nitrites n'est pas nécessaire pour cette bactérie.

7. Tests des nitrites (P. aureofaciens seulement)

Ouvrir chacune des bouteilles de 160 ml et pipetter 5 ml dans une éprouvette. Pipetter 100 µl d'une solution de sulfanilamide 1% m/v et 100 µl d'une solution de N-(1-Naphthyl)-éthylènediamine dihydrochloridrique HCl, 0.1% et ajouter à l'éprouvette. Si la solution devient rouge ou rose, il y a présence de nitrites et la solution bactérienne n'est plus utilisable. Une odeur de chlore est souvent associée avec la présence de nitrites.

8. Concentration des bactéries

Une fois les bouteilles de 160 ml (*P. aureofaciens*) ou de 500 ml (*P. chlororaphis*) ouvertes, ajouter environ 10-30 gouttes d'antimousse et brasser doucement. Diviser la culture bactérienne en aliquots de 40 ml dans des tubes de plastique à fond conique de 50 ml. Centrifuger pour 10 minutes à 7500 rpm dans une centrifuge à angle fixe réfrigérée à 18°C. Verser le surnageant dans la bouteille de 160 ml ou 500 ml (selon la bactérie) (fig. B.4) et pipetter 4 ml (*P. aureofaciens*) ou 6 ml (*P. chlororaphis*) de ce liquide dans le tube de 50 ml. On obtient ainsi des bactéries 10 et ~6.5 fois plus concentrées pour *P. aureofaciens* et *P. chlororaphis* respectivement. Utiliser un vortex pour mélanger le concentré de bactéries au fond du tube avec le 4 ou 6 ml de surnageant. Mettre 40 ml de bactéries concentrées dans un seul tube de 50 ml. Ajouter 0.1 ml de $(NH_4)_2SO_4$ 6.06 M au 40 ml de bactéries concentrées en utilisant une seringue stérile de 1 ml. Pour préparer le sulfate d'ammonium 6.06 M, dissoudre 36 g de $(NH_4)_2SO_4$ dans 90 ml d'eau ultra pure en prenant soin de bien mélanger.

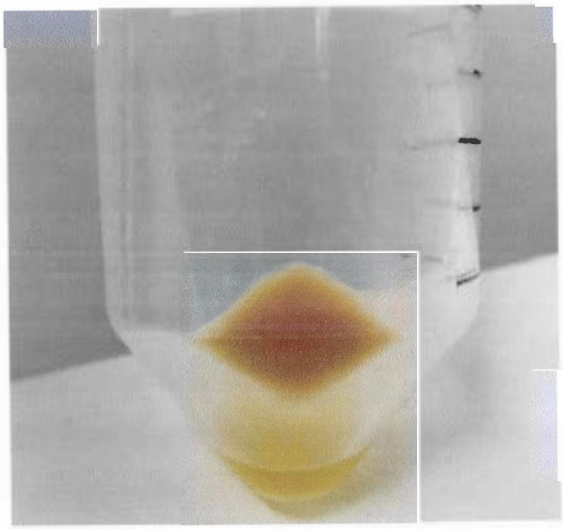

Figure B.4 Bactéries (*P. chlororaphis*) concentrées après la centrifugation.

Pipetter ensuite 2 ml de la solution de bactéries concentrées dans des contenants en verre de 20 ml et fermer avec un bouchon de téflon et un scellant d'aluminium (Casciotti *et al.*, 2002; Sigman *et al.*, 2001).

9. Purge des solutions de bactéries concentrées

Purger les solutions de bactéries concentrées avec de l'hélium pendant au moins 4 heures pour *P. chlororaphis* et 6 heures pour *P. aureofaciens*. Cette opération élimine la majorité du N_2O dans la solution ce qui a pour effet de réduire considérablement le bruit de fond (blanc) et enlève l'oxygène. Le gaz est introduit par une aiguille 26G3/8" à travers le septum, doit faire des bulles dans le médium et sort par une aiguille 25G1/5" qui doit être au-dessus du niveau du liquide (fig. B.5) (Casciotti *et al.*, 2002; Sigman *et al.*, 2001).

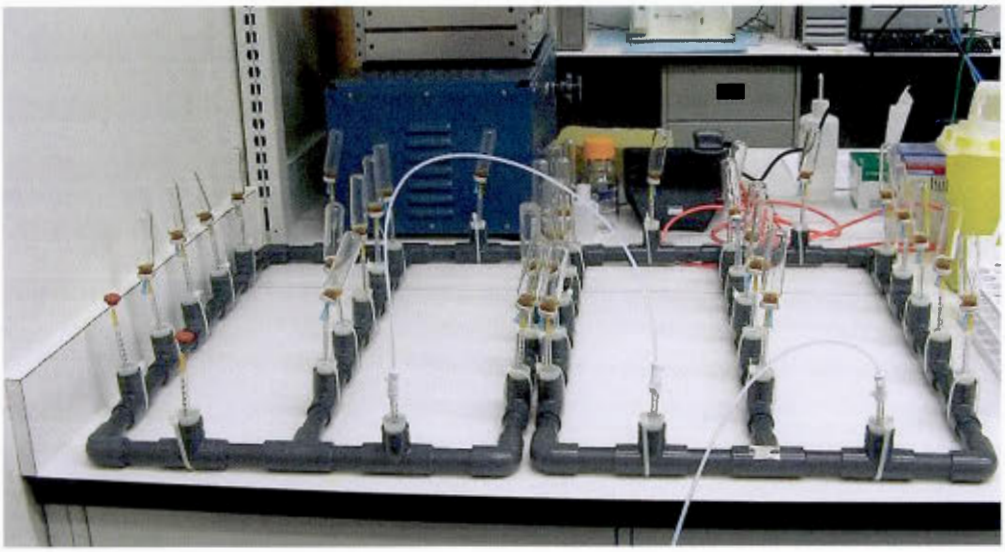

Figure B.5 Purge des solutions de bactéries concentrées.

10. Injection des échantillons et standards et préparation pour l'analyse isotopique

Préparer des solutions (selon les concentrations des échantillons à analyser) du standard international de nitrate IAEA-NO3 (No 150) et conserver celles-ci pour usages ultérieurs au congélateur. Injecter 10 ou 20 nmoles de NO_3^- (5 ou 10 nmoles de N_2O) dans chaque contenant pour les échantillons et les standards en utilisant une seringue étanche en verre. Le volume d'échantillon injecté ne doit pas être supérieur à 10 ml. De plus, il est préférable d'avoir la même quantité finale de N_2O pour les échantillons et les standards. Incuber les contenants en position inversée pendant environ 12 heures (une nuit) pour permettre la conversion du nitrate en N_2O par l'action des bactéries. Ensuite, injecter 0.1 ml d'hydroxyde de sodium 10 N dans les contenants afin de tuer les bactéries et immobiliser le CO_2 dissous de l'échantillon. Il faut noter que la masse du CO_2 est de 44 g/mol, soit la même que N_2O, ce qui pourrait causer une interférence lors de l'analyse isotopique. Injecter environ 3 gouttes d'antimousse dans chaque contenant à l'aide d'une seringue avant les analyses isotopiques (Casciotti et *al.*, 2002; Sigman *et al.*, 2001).

11. Extraction et analyse isotopique avec le spectromètre de masse

Les analyses isotopiques, en mode automatique, sont réalisées à l'aide d'un chromatographe (Micromass TraceGas) couplé à un spectromètre de masse (Micromass Isoprime) en flux continu. Brièvement, le spectromètre de masse comporte une source d'ionisation et un ou plusieurs analyseurs qui séparent les ions produits selon leur rapport masse/charge, d'un détecteur qui compte les ions et amplifie le signal, et enfin d'un système informatique pour le traitement des données. Les contenants de 20 ml sont d'abord placés dans un échantillonneur automatique (Gilson). Lors de l'analyse, le N_2O est extrait des contenants de 20 ml, entraîné par le gaz porteur (He) et congelé dans des trappes plongées dans l'azote liquide (-180°C) pendant ~10-15 minutes. Le N_2O ainsi

extrait et concentré est entraîné à travers des trappes chimiques pour enlever l'eau et le CO_2 et passe ensuite à travers un chromatographe (TraceGas), afin de séparer le N_2O du CO_2 restant. Le N_2O passe finalement dans un spectromètre de masse (Isoprime) en flux continu afin d'obtenir le $\delta^{18}O$ et $\delta^{15}N$ du N_2O/nitrate (fig. B.6) (mesure des masses 44, 45 et 46) par rapport à un gaz de référence (N_2O). Les ratios isotopiques ($^{15}N/^{14}N$ et $^{18}O/^{16}O$) du nitrate sont ensuite calibrés par rapport à l'air et V-SMOW à l'aide du standard international de nitrate IAEA N3. Pour plus de détails sur les différents paramètres utilisés lors des analyses, consulter les articles de Sigman *et al.* (2001) et Casciotti *et al.* (2002).

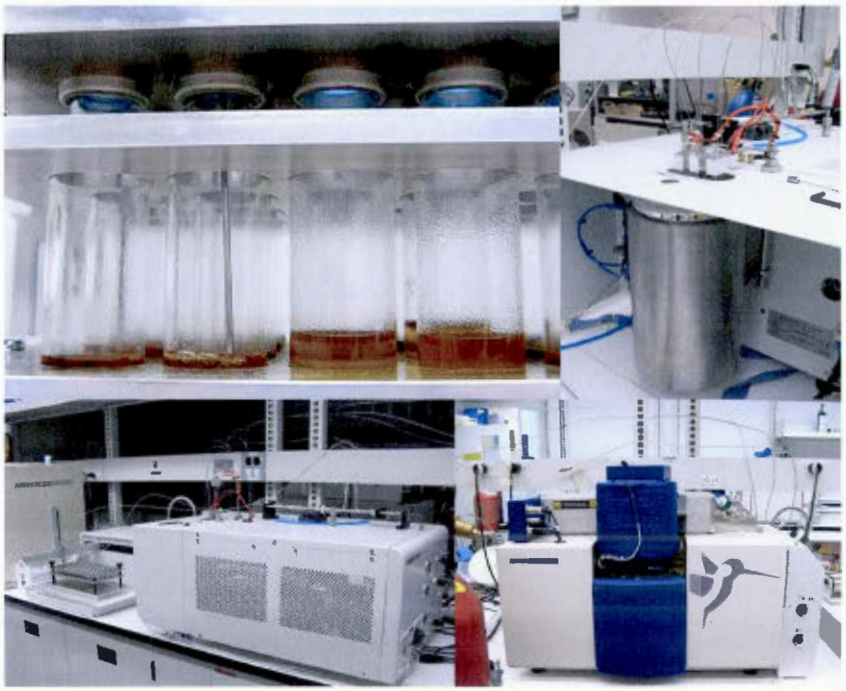

Figure B.6 Photos montrant (de gauche à droite et de haut en bas) 1) l'extraction du gaz N_2O des contenants de 20 ml, 2) la concentration du N_2O dans les trappes plongées dans l'azote liquide, 3) le système de préparation « TraceGas » et 4) le spectromètre de masse (Isoprime) utilisé pour l'analyse isotopique du N et O des nitrates.

12. Corrections

12.1 Corrections pour les valeurs de $\delta^{15}N\text{-}NO_3^-$:

12.1.1 Corriger pour la contribution de ^{17}O à la masse 45 (i.e. $^{14}N^{14}N^{17}O$) :

$$\text{«}\delta^{15}N_d\text{»} = ((1/(2\times0.0036765)) \times [(1+[\delta^{15}N]/1000) \times (0.0003859+2\times0.0036765)-0.0003859\times(1+0.5\times[\delta^{18}O]/1000)]-1) \times 1000$$

où $\delta^{15}N = \delta45/44$ et $\delta^{18}O = \delta46/44$

12.1.2 Corriger pour la contribution du blanc en multipliant $\delta^{15}N_d$ par (s+b)/s, s étant la quantité d'échantillon (en nmoles) et b la quantité du blanc (en nmoles) :

$$\delta^{15}N_b = \delta^{15}N_d \times ((s+b)/s)$$

quantité du blanc : [aire du pic du blanc/moyenne de l'aire des pics des standards IAEA N3]*nombre de moles d'échantillon ajouté (10 ou 20 nmoles)

12.1.3 Convertir $\delta^{15}N_{\text{IAEA vs gaz de référence}}$ en $\delta^{15}N_{\text{gaz de référence vs IAEA}}$ pour les standards IAEA N3 :

$$\delta^{15}N_{\text{gaz de réf. vs IAEA}} = -\delta^{15}N_{\text{IEAE vs gaz de réf.}} \times 1000/(\delta^{15}N_{\text{IAEA vs gaz de réf.}} + 1000)$$

Calculer la moyenne de $\delta^{15}N_{\text{gaz de réf. vs IAEA}}$.

1.2.1.4 Convertir $\delta^{15}N_{\text{échantillon vs gaz de référence}}$ en $\delta^{15}N_{\text{échantillon vs IAEA}}$ pour tous les échantillons :

$$\delta^{15}N_{\text{éch. vs IAEA}} = \delta^{15}N_{\text{éch. vs gaz de réf.}} \times ((\delta^{15}N_{\text{gaz de réf. vs IAEA}}/1000) + 1) + \delta^{15}N_{\text{gaz de réf. vs IAEA}}$$

1.2.1.5 Convertir $\delta^{15}N_{\text{échantillon vs IAEA}}$ en $\delta^{15}N_{\text{échantillon vs air}}$ pour tous les échantillons :

$$\delta^{15}N_{\text{éch. vs air}} = \delta^{15}N_{\text{éch. vs IAEA}} \times ((\delta^{15}N_{\text{IAEA vs air}}/1000) + 1) + \delta^{15}N_{\text{IAEA vs air}}$$

où $\delta^{15}N_{\text{IAEA vs air}} = 4.7‰$

1.2.1.6 Faire la moyenne des réplicats.

12.2 Corrections pour les valeurs de $\delta^{18}O\text{-}NO_3^-$:

12.2.1 Convertir $\delta^{18}O_{\text{IAEA vs gaz de référence}}$ en $\delta^{18}O_{\text{gaz de référence vs IAEA}}$ pour les standards IAEA N3 :

$$\delta^{18}O_{\text{gaz réf. vs IAEA}} = -\delta^{18}O_{\text{IEAE vs gaz de réf.}} \times 1000/(\delta^{18}O_{\text{IAEA vs gaz de réf.}}+1000)$$

Calculer la moyenne de $\delta^{18}O_{\text{gaz de référence vs IAEA}}$.

1.2.2.2 Convertir $\delta^{18}O_{\text{échantillon vs gaz de référence}}$ en $\delta^{18}O_{\text{échantillon vs IAEA}}$ pour tous les échantillons :

$$\delta^{18}O_{\text{éch. vs IAEA}} = \delta^{18}O_{\text{éch. vs gaz de réf.}} \times ((\delta^{18}O_{\text{gaz de réf. vs IAEA}}/1000) + 1) + \delta^{18}O_{\text{gaz de réf. vs IAEA}}$$

1.2.2.3 Convertir $\delta^{18}O_{\text{échantillon vs IAEA}}$ en $\delta^{18}O_{\text{échantillon vs V-SMOW}}$ pour tous les échantillons :

$$\delta^{18}O_{\text{éch. vs V-SMOW}} = \delta^{18}O_{\text{éch. vs IAEA}} \times ((\delta^{18}O_{\text{IAEA vs V-SMOW}}/1000) + 1) + \delta^{18}O_{\text{IAEA vs V-SMOW}}$$

où $\delta^{18}O_{\text{IAEA vs V-SMOW}} = 25.6‰$

1.2.2.4 Calculer l'échange avec l'eau :

Lors de la réaction avec la bactérie *P. Aureofaciens*, l'atome d'oxygène incorporé dans le produit (N_2O) provient essentiellement du nitrate. Il y a néanmoins un faible échange (généralement 5% ou moins) avec les atomes d'oxygène provenant de l'eau. Afin de calculer cet échange on fait un graphique du $\delta^{18}O\text{-}NO_3^-$ (V-SMOW) versus $\delta^{18}O\text{-}H_2O$ (V-SMOW) pour la moyenne des standards IAEA N3 préparés avec de l'eau ultra-pure ($\delta^{18}O\text{-}H_2O$ de ~-7‰ et $\delta^{18}O\text{-}NO_3^-{}_{\text{IAEA vs V-SMOW}} = 25.6‰$) et un standard IAEA N3 préparé avec de l'eau enrichie ($\delta^{18}O\text{-}H_2O_{\text{initial}}$ de ~850‰). Ne pas oublier de tenir compte de la dilution lors de l'ajout du 10 ou 20 nmol de IAEA N3 préparé avec de l'eau enrichie ($\delta^{18}O\text{-}H_2O$ de ~850‰) dans le 2 ml de bactéries concentrées ($\delta^{18}O\text{-}H_2O$ de ~-7‰). La pente de la droite indique la quantité d'échange (par exemple, si m = 0.02, échange = 2%).

1.2.2.5 Corriger la quantité d'échange pour le blanc, s étant la quantité d'échantillon (en nmoles) et b la quantité du blanc (en nmoles) :

quantité d'échange × (s+b)/s

quantité du blanc : [aire du pic du blanc/moyenne de l'aire des pics des standards IAEA N3]*nombre de moles d'échantillon ajouté (ex : 10 ou 20 nmoles)

1.2.2.6 Calculer le facteur de correction, s étant la quantité d'échantillon (en nmoles), b la quantité du blanc (en nmoles) et x, la quantité d'échange:

facteur de correction : $(s+b)/s\times(1-x)$

1.2.2.7 Calculer le $\delta^{18}O_{\text{échantillon vs V-SMOW}}$ et faire la moyenne pour les réplicats :

$$\delta^{18}O_{\text{éch. vs V-SMOW}} = ((\delta^{18}O_{\text{éch. vs V-SMOW}} - \delta^{18}O_{\text{IAEA vs V-SMOW}}) \times ((s+b)/s\times(1-x)) + \delta^{18}O_{\text{IAEA vs V-SMOW}}$$

où $\delta^{18}O_{\text{IAEA vs V-SMOW}} = 25.6‰$

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