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## Note

## Separation of total nitrogen from sediments into organic and inorganic forms for isotopic analysis

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## ABSTRACT

Nitrogen isotopic ( $\delta^{15}\text{N}$ ) analysis is widely applied in palaeoceanography, palaeolimnology and soil science. Most measurements are performed on untreated bulk samples containing both organic and inorganic nitrogen (ON and IN), which could sometimes confound isotopic signals. Here we propose a sample pretreatment method to physically and quantitatively separate the total nitrogen into ON and IN for subsequent concentration and isotopic measurements. In the method, the sediment is first demineralized with HCl and HCl–HF, adsorbed and bound IN being liberated into the acid solutions during the demineralization. Then, organic matter (OM) dissolved in the acid solutions is recovered by a sorbent of styrene divinyl benzene polymer (Bond Elut PPL, Varian) and is then combined with the acid-resistant OM to obtain the total OM. Finally, IN in the acid solutions passing through the PPL sorbent is recovered by way of steam distillation. Total nitrogen recovery with the method is 92.4% and isotopic mass balance calculations show that the  $\delta^{15}\text{N}$  value of total nitrogen can be quantitatively partitioned into the organic and inorganic forms. The  $\delta^{13}\text{C}$  values of the separated OM show a small positive excursion of 0.2‰ relative to those of bulk OM, ruling out significant loss of ON during the pretreatment. By comparison, the KOBBr method, widely used to remove ON from bulk samples for isotope measurements, gives lower IN concentration and higher  $\delta^{15}\text{N}_{\text{IN}}$  values, attributed to the partial loss of IN and escape of some oxidation-resistant ON.

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## 1. Introduction

Stable nitrogen isotope ratio measurements, or  $\delta^{15}\text{N}$  values, of sedimentary organic matter (OM) can provide valuable information on nitrogen cycling and history. Some important nitrogen processes, e.g. nitrification, denitrification and  $\text{N}_2$  fixation, are microbially mediated and involve quite different kinetic isotope effects discriminating between  $^{14}\text{N}$  and  $^{15}\text{N}$  (Kendall et al., 2007). In marine systems, for example, when the resultant active nitrogen with  $\delta^{15}\text{N}$  value indicative of its processing is completely assimilated by phytoplankton, the processing signals may be finally archived as the  $\delta^{15}\text{N}$  value of organic nitrogen (ON) in sediments via sinking of euphotic particulate OM (Altabet et al., 1995; Thunell and Kepple, 2004). In the case of incomplete assimilation in nitrate-replete environments,  $\delta^{15}\text{N}$  values can be used to track relative nutrient utilization (Altabet and Francois, 1994). Most isotopic studies of sedimentary nitrogen have been based on measurements using bulk sediment, assuming that  $\delta^{15}\text{N}_{\text{bulk}}$  reflects  $\delta^{15}\text{N}$  of total ON (TON) (e.g. Altabet et al., 1995; Thunell and Kepple, 2004; Robinson et al., 2009). However, as demonstrated in earlier studies, inorganic nitrogen (IN),

mostly terrestrial ammonium bound between the lattices of clay minerals, can be a significant fraction of the total nitrogen in marine sediments [20–45% in the central Pacific Ocean according to Müller (1977); up to 34% in the South China Sea according to Kienast et al. (2005); up to 70% in continental margin sediments off Spitsbergen according to Knies et al. (2007)]. Continental climate change could modulate the input and isotopic signals of the terrestrial IN to marginal seas, so may impact greatly on bulk nitrogen content and its  $\delta^{15}\text{N}$  value in sediments, as demonstrated in studies of sediments from the Arctic and the South China Sea (Schubert and Calvert, 2001; Kienast et al., 2005; Knies et al., 2007). Accordingly,  $\delta^{15}\text{N}_{\text{TON}}$  should be measured instead of  $\delta^{15}\text{N}_{\text{bulk}}$  for the accurate interpretation of nitrogen cycle in coastal seas receiving significant terrestrial input.

The occurrence of ammonium bound within clay minerals makes separation of TON from bulk sediments difficult because of the intimate association of OM with the inert clay minerals. As an alternative, a method developed by soil scientists (Silva and Bremner, 1966) to obtain bound IN after removal of TON using KOBBr was applied to marine sediments. Based on the method, the content and isotopic value of TON were indirectly calculated from those of total nitrogen and IN (Kienast et al., 2005; Knies et al., 2007; Shigemitsu et al., 2008). However, a portion of OM has been found to resist KOBBr oxidation (Freudenthal et al.,

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2001) and the error in the method for determining the  $\delta^{15}\text{N}_{\text{TON}}$  values, including processing, measurement and calculation, seems quite large (2.1‰, according to Knies et al., 2007). Moreover, the KOBBr method was found to cause a substantial loss of bound ammonium (6.4 to ca. 32.8%) as a result of partial dissolution of clay edges (Paramasivam and Breitenbeck, 2000). A method avoiding KOBBr pretreatment has been applied to soil samples, whereby the soil is digested with HF–HCl and the ammonium in the acid solution is recovered for isotopic analysis using steam distillation under alkaline conditions (Eudoxie and Gouveia, 2001). However, steam distillation with strong alkali was found to partially recover acid soluble ON (Bremner, 1959; Paramasivam and Breitenbeck, 2000).

Here, we propose that, if the acid soluble OM is recovered effectively before IN collection via steam distillation, then the IN signal will not be confounded by acid soluble ON degradation. Moreover, a sample with the IN removed can be obtained by combining the acid soluble OM with the acid-resistant OM. This composite OM can be subsequently analyzed for  $\delta^{15}\text{N}$ , which may represent the isotopic value of TON. Recently, a styrene divinyl benzene polymer (PPL) sorbent was found to be effective in isolating dissolved OM (DOM), especially nitrogen-rich components, from seawater (Dittmar et al., 2008). Therefore, PPL is likely to be an effective sorbent for the recovery of the acid soluble OM after HF–HCl digestion of soil or sediment samples. Here, we introduce our proposed method, including a procedure for recovering acid soluble OM using PPL.

## 2. Materials and methods

A marine sediment collected from the northern shelf of the South China Sea was used and was air dried before use. After crushing to pass a 2 mm sieve, it was sub-divided into four aliquots (ca. 1 g each) for quadruplicate pretreatment.

The concentration and isotopic values of TOC and TN were first determined. The sample was digested in dilute HCl to remove inorganic carbon and dried to volatilize residual HCl. Decanting of the HCl solution and rinsing of the treated sample, conventional in TOC analysis, were not performed in order to avoid loss of DOM.

The protocol is illustrated in Fig. 1. Each aliquot was first treated with 1 M HCl to remove carbonate and then with 5 M HF/1 M HCl to digest clay minerals. The final dark solid residue was mainly the organic phase. DOM in the HCl and HF–HCl solutions was separately adsorbed onto PPL by way of solid phase extraction (0.5 g PPL cartridge, Varian) and was eluted with MeOH and acetone according to the procedure described by Dittmar et al. (2008). The combined eluate was mixed with the final acid resistant, solid residue to give the extracted TOM (TOM<sub>E</sub>). TOM<sub>E</sub> was thoroughly homogenized and freeze dried before analysis.

The desorbed and liberated IN, predominantly ammonium (perhaps with a trace of nitrate) from minerals, is readily dissolved in the acid solution and passed through the PPL column packed. The HCl and HF–HCl solutions after the solid phase extraction were combined and split into two aliquots for duplicate analysis. In order to recover the IN in each aliquot (ca. 50 ml each) in a 120 ml container, 10 g NaOH was added to raise the pH above 10 and excess Devarda's alloy was added as reducing agent for the reduction of nitrate to ammonia. The container was then sealed quickly with a screw lid. In the container, a Whatman GF-D filter paper (7 mm diameter), which was acidified with 10  $\mu\text{l}$  of 2.5 M KHSO<sub>4</sub> to serve as an ammonium trap, was sealed with PTFE tape and hung above the alkaline solution with a thread adhering to the base of the screw lid. This diffusion unit was gently shaken (2 $\times$ ) every day and was incubated (6 days) at room temperature. These experimental conditions are proper for almost complete recovery of IN. At sampling, the lid was removed, and the ammonium trap was carefully detached from the lid and dried in a desiccator.

TOM<sub>E</sub> and ammonium traps were weighed and measured for carbon/nitrogen content and isotopes using an elemental analysis-isotope ratio mass spectrometry (EA-IRMS) system (CE EA1112 C/N/S Analyzer interfaced with a DELTAplus XL mass spectrometer). The combustion temperature was 960 °C. Results are reported in per mil (‰) as:

$$\delta = [(R_{\text{sample}} - R_{\text{reference}})/R_{\text{reference}}]/1000$$

where  $\delta$  (‰) is  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  and  $R_{\text{sample}}$  and  $R_{\text{reference}}$  are the isotopic ratios of the sample and reference, respectively. For carbon and nitrogen, the references are Pee Dee Belemnite (PDB) and air, respec-

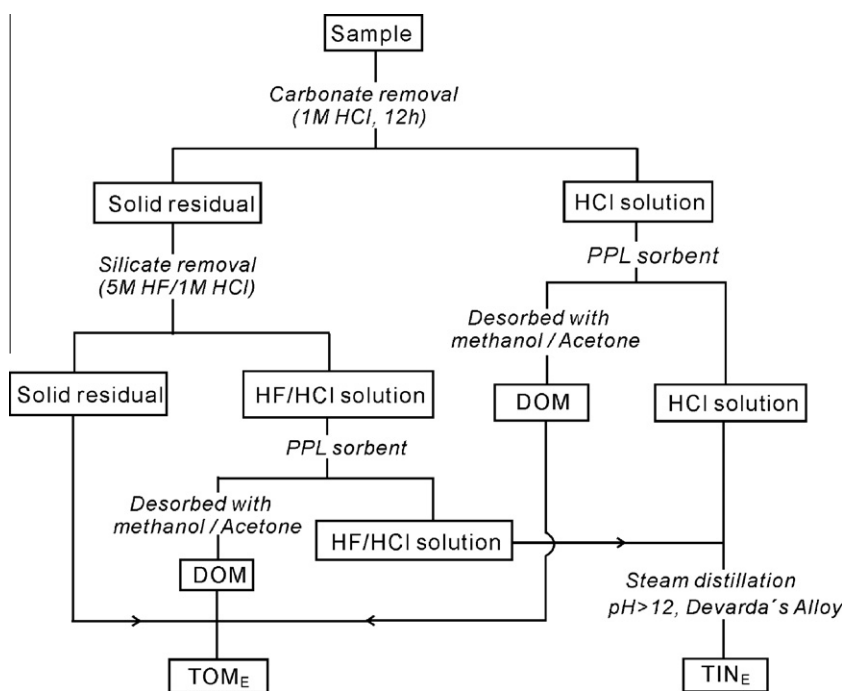


Fig. 1. Procedure for separation of sedimentary total nitrogen into ON and IN.

tively. External standard materials (black carbon with  $\delta^{13}\text{C} - 36.81\text{‰}$  and urea with  $\delta^{15}\text{N} + 0.4\text{‰}$ ) were inserted between every 20 samples to monitor the working conditions of the analyzer. Reproducibility was  $\pm 0.25\text{‰}$  for  $\delta^{13}\text{C}$  and  $\pm 0.5\text{‰}$  for  $\delta^{15}\text{N}$ .

Blank analysis did not show detectable contamination during the whole experimental procedure.

### 3. Results and discussion

#### 3.1. Concentration and isotopic values of TIN and TON

Triplicate analysis of the sample showed that mean values ( $\pm\text{SD}$ ) of TOC and TN were  $8.69 \pm 0.46 \text{ mg g}^{-1}$  and  $1.08 \pm 0.03 \text{ mg g}^{-1}$ , respectively. In our method, TN was physically separated into inorganic and organic forms, i.e.  $\text{TIN}_E$  and  $\text{TON}_E$ . As shown in Table 1, the average  $\text{TIN}_E$  and  $\text{TON}_E$  values were  $0.22 \pm 0.03 \text{ mg g}^{-1}$  and  $0.78 \pm 0.01 \text{ mg g}^{-1}$ , respectively; the recovery of TN was  $92.4 \pm 3.7\%$ . The high recovery is partly supported by the similarly high recovery of TOC ( $97.5 \pm 2.1\%$ ; Table 1).

The mean  $\delta^{15}\text{N}$  values of  $\text{TIN}_E$  and  $\text{TON}_E$  were  $-1.65 \pm 0.38\text{‰}$  and  $+5.92 \pm 0.22\text{‰}$ , respectively. The  $\delta^{15}\text{N}$  value of total recovered nitrogen ( $\delta^{15}\text{N}_R$ ) was then calculated as follows:

$$\delta^{15}\text{N}_R = \delta^{15}\text{N}_{\text{TIN-E}} \times f + \delta^{15}\text{N}_{\text{TON-E}} \times (1 - f)$$

where  $f$  is the fraction of  $\text{TIN}_E$  in the total recovered nitrogen, i.e.  $f = \text{TIN}_E / (\text{TIN}_E + \text{TON}_E)$ . The calculated  $\delta^{15}\text{N}_R$  averaged  $4.26 \pm 0.43\text{‰}$  (Table 2). This is slightly lower than the measured  $\delta^{15}\text{N}_{\text{Bulk}}$  value of  $4.79 \pm 0.21\text{‰}$ . However, the difference between them is statistically insignificant ( $p > 0.16$ ,  $\alpha > 0.05$ ).

In order to discern whether there was any loss of ON, e.g. transformation of organic nitrogen compounds to inorganic forms, during acid digestion, comparison was made between  $\delta^{13}\text{C}_{\text{Bulk}}$  and  $\delta^{13}\text{C}_{\text{TON-E}}$ . This is related to the idea that nitrogen compounds, such as proteins and amino acids, have more positive  $\delta^{13}\text{C}$  values than other organic fractions, such as lignin and cellulose (Deines, 1980). Therefore, if loss of organic nitrogen compounds occurred during HF–HCl digestion, the OM recovered would be expected to be more negative in  $\delta^{13}\text{C}$  value than the bulk OM. In fact, the results showed a subtle positive excursion of  $\delta^{13}\text{C}_{\text{TON-E}}$  ( $-21.99 \pm 0.04\text{‰}$ ) relative to  $\delta^{13}\text{C}_{\text{Bulk}}$  ( $-22.17 \pm 0.06\text{‰}$ ), thereby ruling out discernable loss of ON. This subtle excursion ( $+0.2\text{‰}$ ) might result from slight loss of a  $^{13}\text{C}$ -depleted OM component, which could not be determined at present work. Nevertheless, we conclude that HCl–HF digestion of sediments does not give rise to significant loss of ON.

The above results demonstrate that our method is robust for quantitatively separating TN into TIN and TON for subsequent isotopic analysis. The PPL procedure is applicable to extraction of acid soluble DOM without major instrumentation and a large number of samples can be processed in parallel. This is important for studies requiring  $\delta^{15}\text{N}$  measurements of ON and involving large numbers of samples.

**Table 1**

Concentration of extracted total IN ( $\text{TIN}_E$ ), total ON ( $\text{TON}_E$ ) and TOC ( $\text{TOC}_E$ ), as well as recovery of TN and TOC.

Aliquot	$\text{TIN}_E$ ( $\text{mg g}^{-1}$ )	$\text{TON}_E$ ( $\text{mg g}^{-1}$ )	Recovery of TN <sup>a</sup> (%)	$\text{TOC}_E$ ( $\text{mg g}^{-1}$ )	Recovery of TOC <sup>b</sup> (%)
1	0.24	0.79	94.7	8.56	98.6
2	0.25	0.78	95.6	8.64	99.4
3	0.22	0.77	92.2	8.21	94.5
4	0.17	0.77	87.3	8.47	97.5
Avg.	0.22	0.78	92.4	8.47	97.5
SD <sup>c</sup>	0.03	0.01	3.7	0.19	2.1

<sup>a</sup>  $1.08 \pm 0.03 \text{ mg g}^{-1}$ .

<sup>b</sup>  $8.69 \pm 0.46 \text{ mg g}^{-1}$ .

<sup>c</sup> Standard deviation.

**Table 2**

Measured  $\delta^{15}\text{N}$  values of TN ( $\delta^{15}\text{N}_{\text{TN}}$ ), extracted TIN ( $\delta^{15}\text{N}_{\text{TIN-E}}$ ) and TON ( $\delta^{15}\text{N}_{\text{TON-E}}$ ), as well as calculated  $\delta^{15}\text{N}$  values of total extracted nitrogen ( $\delta^{15}\text{N}_{\text{TN-E}}$ ).

Aliquot	$\delta^{15}\text{N}_{\text{TIN-E}}$ (‰)	$\delta^{15}\text{N}_{\text{TON-E}}$ (‰)	$\delta^{15}\text{N}_{\text{TN-E}}$ (‰)	$\delta^{15}\text{N}_{\text{TN}}$ (‰)
1	-1.87	5.88	4.09	4.97
2	-1.72	5.82	4.00	4.63
3	-1.92	5.75	4.04	4.59
4	-1.09	6.25	4.91	4.98
Avg.	-1.65	5.92	4.26	4.79
SD <sup>a</sup>	0.38	0.22	0.43	0.21

<sup>a</sup> Standard deviation.

#### 3.2. Comparison with KOBBr method

The KOBBr method has been widely used to remove ON from bulk samples, after which  $\delta^{15}\text{N}$  measurement is performed to obtain the  $\delta^{15}\text{N}$  values of bound IN ( $\delta^{15}\text{N}_{\text{KBrO}}$ ). This method was also applied to the test sample for comparison with our proposed method.

The IN concentration in the test sample pretreated using KOBBr method ( $\text{TIN}_{\text{KOBBr}}$ ) was  $0.16 \pm 0.01 \text{ mg g}^{-1}$  and the  $\delta^{15}\text{N}_{\text{KOBBr}}$   $2.77 \pm 0.49\text{‰}$ , based on triplicate analyses. The values are apparently lower than  $\text{TIN}_E$  and higher than  $\delta^{15}\text{N}_{\text{TIN-E}}$ , respectively, obtained from our method. The lower  $\text{TIN}_{\text{KOBBr}}$  value may be caused by loss of bound ammonium as a result of partial dissolution of clay edges (which can be as high as 33% according to Paramasivam and Breitenbeck, 2000) and by loss of adsorbed inorganic ammonium and nitrate during sample pretreatment. However, the loss of IN is hard to explain the obviously higher  $\delta^{15}\text{N}_{\text{KOBBr}}$  value. We speculated that some OM might escape KOBBr oxidation. Consequently, the KOBBr-treated sample was further treated to extract the possible OM with our proposed method. As we had speculated, there was some ON present in the KOBBr-treated sample, which accounted for 26.8% of TN in the KOBBr-treated sample and 5.6% of the TON in the raw sample. Therefore, although ca. 95% ON was removed from the raw sample, escaped ON could still be a significant fraction of TN in the KOBBr-treated sample, which would confound determination of the  $\delta^{15}\text{N}$  value of IN.

### 4. Conclusions

Sedimentary total nitrogen was quantitatively separated into organic and inorganic forms with a recovery of 92.4% using the proposed method. Isotopic mass balance calculation showed that the  $\delta^{15}\text{N}$  value of total nitrogen could be quantitatively partitioned into the organic and inorganic forms. The nearly unchanged  $\delta^{13}\text{C}$  value of the separated OM suggested no loss of organic N during the pretreatment. Therefore, using the proposed method, both organic and inorganic nitrogen can be reliably obtained for concentration and isotopic measurement. Although the method is still a kind of bulk isotopic analysis and may be less refined than the compound specific measurements proposed

recently (e.g. Chikaraishi et al., 2008; Higgins et al., 2009), its simplicity and low cost are valuable for  $\delta^{15}\text{N}$  studies requiring large numbers of samples to be analysed, such as in palaeoceanography, palaeolimnology and soil science.

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### References

- Altabet, M.A., Francois, R., 1994. Sedimentary nitrogen isotopic ratio records surface ocean  $\text{NO}_3^-$  utilization. *Global Biogeochemical Cycles* 8, 103–116.
- Altabet, M.A., Francois, R., Murray, D.W., Prell, W.L., 1995. Climate-related variations in denitrification in the Arabian Sea from sediment  $^{15}\text{N}/^{14}\text{N}$  ratios. *Nature* 373, 506–509.
- Bremner, J.M., 1959. Determination of fixed ammonium in soil. *The Journal of Agricultural Science* 52, 147–160.
- Chikaraishi, Y., Kashiyama, Y., Ogawa, N.O., Kitazato, H., Satoh, M., Nomoto, S., Ohkouchi, N., 2008. A compound-specific isotope method for measuring the stable nitrogen isotopic composition of tetrapyrroles. *Organic Geochemistry* 39, 510–520.
- Deines, P., 1980. The isotopic composition of reduced organic carbon. In: Fritz, P., Fontes, J.C. (Eds.), *Handbook of Environmental Isotope Geochemistry*, the Terrestrial Environment. Elsevier, New York, pp. 329–406.
- Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography: Methods* 6, 230–235.
- Eudoxie, G.D., Gouveia, G.A., 2001. Microdiffusion of strongly fixed ammonium in soil-acid digests prior to automated  $^{15}\text{N}$  analysis. *Soil Science Society of America Journal* 65, 1846–1852.
- Freudenthal, T., Wagner, T., Wenzhöfer, F., Zabel, M., Wefer, G., 2001. Early diagenesis of organic matter from sediments of the eastern subtropical Atlantic: evidence from stable nitrogen and carbon isotopes. *Geochimica et Cosmochimica Acta* 65, 1795–1808.
- Higgins, M.B., Robinson, R.S., Casciotti, K.L., McIlvin, M.R., Pearson, A., 2009. A method for determining the nitrogen isotopic composition of porphyrins. *Analytical Chemistry* 81, 184–192.
- Kendall, C., Elliott, E.M., Wankel, S.D., 2007. Tracing anthropogenic inputs of nitrogen to ecosystems. In: Lajtha, K., Michener, R.H. (Eds.), *Stable Isotopes in Ecology and Environmental Science*, second ed. Blackwell, Oxford, pp. 375–449.
- Kienast, M., Higginson, M.J., Mollenhauer, G., Eglinton, T.I., Chen, M.T., Calvert, S.E., 2005. On the sedimentological origin of down-core variations of bulk sedimentary nitrogen isotope ratios. *Paleoceanography* 20, PA2009.
- Knies, J., Brookes, S., Schubert, C.J., 2007. Re-assessing the nitrogen signal in continental margin sediments: new insights from the high northern latitudes. *Earth and Planetary Science Letters* 253, 471–484.
- Müller, P.J., 1977. C/N ratio in Pacific deep-sea sediments: effect of inorganic ammonium and organic nitrogen compounds sorbed by clays. *Geochimica et Cosmochimica Acta* 41, 765–776.
- Paramasivam, S., Breitenbeck, G.A., 2000. Quantitative determination of recently fixed nonexchangeable ammonium in soils. *Soil Science Society of America Journal* 64, 1859–1866.
- Robinson, R.S., Martinez, P., Pena, L.D., Cacho, I., 2009. Nitrogen isotopic evidence for deglacial changes in nutrient supply in the eastern equatorial Pacific. *Paleoceanography* 24, PA4213.
- Schubert, C.J., Calvert, S.E., 2001. Nitrogen and carbon isotopic composition of marine and terrestrial organic matter in Arctic Ocean sediments: implications for nutrient utilization and organic matter composition. *Deep-Sea Research I* 48, 789–810.
- Shigemitsu, M., Watanabe, Y.W., Narita, H., 2008. Time variations of  $\delta^{15}\text{N}$  of organic nitrogen in deep western subarctic Pacific sediment over the last 145 ka. *Geochemistry Geophysics Geosystems* 9, Q10012.
- Silva, J.A., Bremner, J.M., 1966. Determination and isotope ratio analysis of different forms of nitrogen in soils. 5. Fixed ammonium. *Soil Science Society of America Proceedings* 30, 587–594.
- Thunell, R.C., Kepple, A.B., 2004. Glacial–Holocene  $\delta^{15}\text{N}$  record from the Gulf of Tehuantepec, Mexico: implications for denitrification in the eastern equatorial Pacific and changes in atmospheric  $\text{N}_2\text{O}$ . *Global Biogeochemical Cycles* 18, GB1001.