

# Amino acid d13C values of diatoms, bacteria, and core M772-003-2 samples from the R/V Meteor (M77) in the Peruvian ocean margin from November to December 2008.

Website: <https://www.bco-dmo.org/dataset/715160>

Data Type: Cruise Results

Version: 1

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## Project

» [The Use of Nitrogen Isotopes of Amino Acids To Understand Marine Sedimentary 15N Records](#) (Amino Acid Sediment 15N)

Contributors	Affiliation	Role
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## Abstract

Amino acid d13C values of diatoms, bacteria, and core M772-003-2 samples from the R/V Meteor (M77) in the Peruvian ocean margin from November to December 2008.

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## Coverage

**Temporal Extent:** 2008-11 - 2008-12

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## Dataset Description

Amino acid d13C values of diatoms, bacteria and core M772-003-2 samples

**These data were published in: Larsen et al., 2015**

## Acquisition Description

### Culturing

The marine diatom *Thalassiosira weissflogii* Grunow (strain CCMP 1010) was cultured in sterile filtered natural North Sea water (Schleswig-Holstein, Germany) or Baltic Sea water (Schleswig-Holstein, Germany). The medium was enriched with f/4 concentrations of macro- and micronutrients (nitrate, phosphate, silicic acid, trace metal mixture, vitamin mixture (Guillard and Ryther, 1962). All experiments were performed in sterile 2.1 L Schott Duran glass bottles. These bottles were made of borosilicate glass (filters UV radiation <310 nm) except for the quartz glass bottles (pure silica without UV radiation filter) used in the UV experiment. The cultures were either incubated in climate chambers with 400 –700 nm radiation or 10 cm below water level at low tide in Kiel Fjord in May 2011. Water temperature and light irradiance data were obtained from the weather station maintained by the GEOMAR institute in Kiel, Germany. Growth conditions for the various treatments, i.e. salinity, pH, temperature and irradiance are given in Table 1. pH values (reported on free scale) were measured with separate glass and reference electrodes (Metrohm) and calculated with equation 3 from DOE 2007 chapter 6b (Dickson et al., 2007) as described in (Bach et al., 2012). Cultures were inoculated with densities of 20 cells ml<sup>-1</sup>. Cell densities and equivalent spherical diameters were determined with a Coulter Counter (Beckman Coulter) at the beginning and the end of the experiment, respectively. When incubations ended, cells were filtered on 47 mm diameter, 5 µm mesh size Nucleopore Track-Etch Membrane filters (Whatman) and frozen at -18 deg C immediately after filtration.

### Sediment sampling

Sediment samples were retrieved from a 14.97 m core, station M772-003-2, collected November 26, 2008 by the Meteor cruise M77 at 271 m water depth within the main

upwelling area off Peru (15 deg 06.21 'S, 75 deg 41.28 'W). The Peruvian ocean margin is characterized by a high particle flux and a well-defined oxygen minimum zone. At the time of sampling, the O<sub>2</sub> concentration at the seafloor was measured to 1.1  $\mu$ M, the salinity to 34.9 psu and the temperature to 12.2 deg C.

Prior to analysis, sediment samples were pre-treated with an acid-alkali-acid cleaning with HCl and NaOH (Grootes et al., 2004.)

## **Analyses**

Both diatom and sediment samples were freeze dried prior to isotopic analysis. To prepare aliquots for derivatization of amino acids, we used 3-4 mg of diatoms and 100-150 mg of sediments. The samples were transferred to Pyrex culture tubes (13 x 100 mm), flushed with N<sub>2</sub> gas, sealed, and hydrolysed in 1 ml 6N HCl at 110 deg C for 20 h. After hydrolysis, lipophilic compounds were removed by vortexing with 2 ml n-hexane/DCM (6:5, v/v) for 30 sec. The aqueous phase was subsequently transferred through disposable glass pipettes lined with glass wool into 4 ml dram vials. Samples were evaporated to dryness under a stream of N<sub>2</sub> gas for 30 min at 110 deg C before being stored at 18 deg C until required for analysis. The derivatisation procedure was modified from Corr et al. (2007) as described by Larsen et al. (2013). In short, the dried samples were methylated with acidified methanol and subsequently acetylated with a mixture of acetic anhydride, triethylamine, and acetone, forming N-acetyl methyl ester derivatives. As a precautionary measure to reduce the oxidation of amino acids, we flushed and sealed reaction vials with N<sub>2</sub> gas prior to methylation and acetylation. Another modification from Corr et al. (2007) was that icebaths were substituted with solid aluminum blocks at room temperature. We used known d<sup>13</sup>C values of pure amino acids prepared and analyzed under the same conditions as the samples to calculate correction factors specific to each amino acid to account for carbon addition and fractionation during derivatization. The derivatised AAs were dissolved in 250  $\mu$ l ethyl acetate and stored at 18 deg C until required for analysis.

Amino acid d<sup>13</sup>C values were obtained from Leibniz-Laboratory for Radiometric Dating and Stable Isotope Research in Kiel. We injected the AA derivatives into a PTV injector held at 250 deg C for 4 min. before GC separation on an Agilent 6890N GC. Diatom samples were separated on an Rtx-200 column (60m x 0.32mm x 0.25 $\mu$ m, Fig. S1) and sediment samples on a Thermo Trace GOLD TG-200MS GC column (60m x 0.32mm x 0.25 $\mu$ m). For both GC columns, the oven temperature of the GC was started at 50 deg C and heated at 15 deg C min<sup>-1</sup> to 140 deg C, followed by 3 deg C min<sup>-1</sup> to 152 deg C and held for 4 min, then 10 deg C min<sup>-1</sup> to 245 deg C and held for 10 min, and finally 5 deg C min<sup>-1</sup> to 290 deg C and held for 5 min. The GC was interfaced with a MAT 253 isotope ratio mass spectrometer (IRMS) via a GC-III combustion (C) interface (Thermo-Finnigan Corporation). We obtained consistently good chromatography for alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), asparagine/aspartate (Asx), threonine (Thr), methionine (Met), glutamine/glutamate

(Glx), phenylalanine (Phe), tyrosine (Tyr), lysine (Lys), and arginine (Arg) with the exception that Asx and Thr partially coeluted with the Rtx-200 column. Serine (Ser) and proline (Pro) coeluted on both columns. The average reproducibility for the norleucine internal standard was  $\pm 0.4\%$  (n = 3 for each sample), and the reproducibility of amino acid standards ranged from  $\pm 0.1\%$  for Phe to  $\pm 0.6\%$  for Thr (n = 3).

Amino acid composition of the diatom samples was determined with the derivative samples used for d13CAA analysis. The amino acids were separated on an Rxi-35SIL MS column (30m x 0.32mm x 0.25um) with an Agilent 6890 N GC with a flame ionization detector. With this column we obtained good chromatography for Ala, Asx, Glx, Gly, Ser, Tyr, Arg, Ile, Leu, Lys, Met, Phe, Thr, and Val. For quantification, we used internal references consisting of pure amino acids (Alfa Aesar, Karlsruhe, Germany). The composition of the amino acids are shown in Table 3 according to the following biosynthetic families: Pyruvate (Ala, Leu, Val), Oxaloacetate (Asx, Ile, Lys, Met, Thr),  $\alpha$ -ketoglutarate (Arg, Glx), 3-phosphoglycerate (Gly, Ser), and Shikimate (Phe, Tyr).

Bulk  $^{13}\text{C}$ ,  $\delta^{13}\text{C}$ ,  $^{15}\text{N}$  and  $\delta^{15}\text{N}$  values of the diatom samples were determined at the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The dry weight of the samples ranged between 1.5 and 2.5 mg. During analysis, samples were interspersed with several replicates of at least three different laboratory standards. These laboratory standards, which were selected to be compositionally similar to the samples being analyzed, had previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). A sample's preliminary isotope ratio was measured relative to reference gases analyzed with each sample. These preliminary values were finalized by correcting the values for the entire batch based on the known values of the included laboratory standards. The long term standard deviation is  $0.2\%$  for  $^{13}\text{C}$  and  $0.3\%$  for  $^{15}\text{N}$ .

## Processing Description

Thermo-Finnigan Isodat software and Microsoft Excel 2013.

### **BCO-DMO Processing:**

- first column name added as "sample\_description"
- changed column names to comply with BCO-DMO standards
- changed the name of the standard deviation columns to include the amino acid they were referencing. Example Thr\_st\_dev.
- filled in all blank cells with nd

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### **Related Publications**

Bach, L. T., Bauke, C., Meier, K. J. S., Riebesell, U., & Schulz, K. G. (2012). Influence of changing carbonate chemistry on morphology and weight of coccoliths formed by *Emiliana huxleyi* Biogeosciences, 9(8), 3449–3463. doi:[10.5194/bg-9-3449-2012](https://doi.org/10.5194/bg-9-3449-2012)

Corr, L. T., Berstan, R., & Evershed, R. P. (2007). Development of N-Acetyl Methyl Ester Derivatives for the Determination of  $\delta^{13}\text{C}$  Values of Amino Acids Using Gas Chromatography-Combustion- Isotope Ratio Mass Spectrometry. Analytical Chemistry, 79(23), 9082–9090. doi:[10.1021/ac071223b](https://doi.org/10.1021/ac071223b)

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO<sub>2</sub> measurements. PICES Special Publication 3, 191 pp. ISBN: 1-897176-07-4. Handle: <http://hdl.handle.net/11329/249>. URL: [https://www.nodc.noaa.gov/ocads/oceans/Handbook\\_2007.html](https://www.nodc.noaa.gov/ocads/oceans/Handbook_2007.html)

Grootes, P. M., Nadeau, M.-J., & Rieck, A. (2004).  $^{14}\text{C}$ -AMS at the Leibniz-Labor: radiometric dating and isotope research. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 223-224, 55–61. doi:[10.1016/j.nimb.2004.04.015](https://doi.org/10.1016/j.nimb.2004.04.015)

Guillard, R. R. L., & Ryther, J. H. (1962). STUDIES OF MARINE PLANKTONIC DIATOMS: I. CYCLOTELLA NANA HUSTEDT, AND DETONULA CONFERVACEA (CLEVE) GRAN. Canadian Journal of Microbiology, 8(2), 229–239. doi:[10.1139/m62-029](https://doi.org/10.1139/m62-029)

Larsen, T., Bach, L. T., Salvatteci, R., Wang, Y. V., Andersen, N., Ventura, M., & McCarthy, M. D. (2015). Assessing the potential of amino acid  $^{13}\text{C}$  patterns as a carbon source tracer in marine sediments: effects of algal growth conditions and sedimentary diagenesis. Biogeosciences, 12(16), 4979–4992. doi:[10.5194/bg-12-4979-2015](https://doi.org/10.5194/bg-12-4979-2015)

Larsen, T., Ventura, M., Andersen, N., O'Brien, D. M., Piatkowski, U., & McCarthy, M. D. (2013). Tracing Carbon Sources through Aquatic and Terrestrial Food Webs Using Amino Acid Stable Isotope Fingerprinting. PLoS ONE, 8(9), e73441. doi:[10.1371/journal.pone.0073441](https://doi.org/10.1371/journal.pone.0073441)

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## Parameters

<b>Parameter</b>	<b>Description</b>	<b>Units</b>
sample_description	Type of sample	unitless
ID	Sample ID	unitless
Ala	Alanine d13C value	per mil
Ala_st_dev	Standard deviation	per mil
Asx	Aspartic Acid + Asparagine d13C value	per mil
Asx_st_dev	Standard deviation	per mil
Glx_st_dev	Standard deviation	per mil
Gly	Glycine d13C value	per mil
Gly_st_dev	Standard deviation	per mil
His	Histodine d13C value	per mil
His_st_dev	Standard deviation	per mil
Ile	Isoleucine d13C value	per mil
Ile_st_dev	Standard deviation	per mil
Leu	Leucine d13C value	per mil
Leu_st_dev	Standard deviation	per mil
Lys	Lysine d13C value	per mil
Lys_st_dev	Standard deviation	per mil
Met	Methoinine d13C value	per mil
Met_st_dev	Standard deviation	per mil
Phe	Phenylalanine d13C value	per mil
Phe_st_dev	Standard deviation	per mil
Thr	Threonine d13C value	per mil
Thr_st_dev	Standard deviation	per mil
Tyr_st_dev	Standard deviation	per mil
Val	Valine d13C value	per mil
Val_st_dev	Standard deviation	per mil
Glx	Glutamic Acid + Glutamine d13C value	per mil
Tyr	Tyrosine d13C value	per mil

## Instruments

<b>Dataset-specific Instrument Name</b>	PDZ Europa 20-20 isotope ratio mass spectrometer
<b>Generic Instrument Name</b>	Isotope-ratio Mass Spectrometer
<b>Dataset-specific Description</b>	Used with PDZ Europa ANCA-GSL elemental analyzer
<b>Generic Instrument Description</b>	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

<b>Dataset-specific Instrument Name</b>	MAT 253 isotope ratio mass spectrometer (IRMS)
<b>Generic Instrument Name</b>	Isotope-ratio Mass Spectrometer
<b>Dataset-specific Description</b>	Used with Thermo Trace GOLD GC
<b>Generic Instrument Description</b>	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

<b>Dataset-specific Instrument Name</b>	Thermo Trace GOLD GC
<b>Generic Instrument Name</b>	Gas Chromatograph
<b>Dataset-specific Description</b>	Used with MAT 253 isotope ratio mass spectrometer (IRMS) via a GC-III combustion (C) interface (Thermo-Finnigan Corporation)
<b>Generic Instrument Description</b>	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

<b>Dataset-specific Instrument Name</b>	PDZ Europa ANCA-GSL elemental analyzer
<b>Generic Instrument Name</b>	Elemental Analyzer
<b>Dataset-specific Description</b>	Interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK)
<b>Generic Instrument Description</b>	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

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## Deployments

## M77

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/715334">https://www.bco-dmo.org/deployment/715334</a>
<b>Platform</b>	R/V Meteor
<b>Report</b>	<a href="https://www.lfd.uni-hamburg.de/meteor/wochenberichte/wochenberichte-meteor/m77/m77-2-scr.pdf">https://www.lfd.uni-hamburg.de/meteor/wochenberichte/wochenberichte-meteor/m77/m77-2-scr.pdf</a>
<b>Start Date</b>	2008-11-24
<b>End Date</b>	2008-12-21
<b>Description</b>	Main research topic of cruise M77-2 was the investigation of the oxygen minimum zone (OMZ) in the coastal upwelling areas off Peru and off Ecuador.

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## Project Information

## The Use of Nitrogen Isotopes of Amino Acids To Understand Marine Sedimentary $^{15}\text{N}$ Records (Amino Acid Sediment $^{15}\text{N}$ )

**Coverage:** California Margin , Santa Barbara Basin , CA current system, Eastern Tropical Pacific

The bioavailability of nutrients plays a crucial role in oceanic biological productivity, the carbon cycle, and climate change. The global ocean inventory of nitrogen (N) is determined by the balance of N-fixation (sources) and denitrification (sinks). In this three-year project, a researcher from the University of California, Santa Cruz, will focus on developing compound-specific N isotope ( $\delta^{15}\text{N}$ ) analysis of amino acids as a new tool for understanding N source and transformation of organic matter in paleo-reservoirs. The offsets in the isotopic ratios of individual amino acid groups may yield information about trophic transfer, heterotrophic microbial reworking, and autotrophic versus heterotrophic sources. By measuring and comparing the bulk and amino acid  $\delta^{15}\text{N}$  in size-fractionated samples from plankton tows, sediments traps, and multi-cores in oxic and suboxic depositional environments, the researcher will: (1) Provide a proxy of the  $\delta^{15}\text{N}$  of average exported photoautotrophic organic matter; and (2) Provide a new level of detail into sedimentary organic N degradation and preservation. Broader impacts: This project will improve understanding of the fundamental underpinnings and behaviors of  $\delta^{15}\text{N}$  amino acid patterns and how they behave in contrasting sedimentary environments, while also developing a potential paleoceanographic proxy. Funding will support a graduate student and undergraduate research at the institution. The researcher will also conduct community outreach in the form of a workshop/tutorial on the proxy development.

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### Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1131816</a>

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