

Proposal for a SCOR Working Group on The reassessment of marine dinitrogen fixation methodology and measurements

Abstract

Biological dinitrogen (N₂) fixation rate measurements are critical for determining the input of new fixed nitrogen to the oceanic nitrogen inventory. Recent development in N₂ fixation research has identified that the method widely applied in the last 15 years has led to a systematic, significant, and variable underestimation of the real N₂ fixation rates. Experimentally, the most widely applied method to measure N₂ fixation in the ocean involves adding ¹⁵N₂ gas to a water sample through a gas tight septum in a bottle, which is incubated in situ or under simulated in situ conditions, terminating the experiment by filtering the water onto a filter, and measuring the ¹⁴N and ¹⁵N isotopic abundances (or relative abundances) using a mass spectrometer. It has now been demonstrated that the rate of dissolution of the added ¹⁵N₂ gas bubble is slow, taking between 6 to 12 hours to reach equilibrium (saturation) in the dissolved pool. Thus, the resulting rates are inherently in error, since the ¹⁵N percent in the aqueous phase is changing throughout the incubation rather than being constant, as assumed in the rate calculation. Both laboratory and field measurements predict on average a ~2 fold underestimation of the rates measured up to now. The research community needs to develop an acceptable alternative method for accurate measurements of N₂ fixation. An initial workshop on this topic has already taken place on February 6-8 2012 in Kiel, Germany where a group of experts has come together to begin the initiation of a community attempt to rectify the problem. The outcome of the workshop indicates that there will be an additional requirement to meet periodically over the next 3-4 years to develop a widely accepted new method. The proposed SCOR group would continue this initiative of the SOLAS-sponsored workshop by coordinating the work of a group of international scientists that will develop a consensus methodology for N₂ fixation rate measurements. The outcome of the working group would be 1) a short position paper explaining the problem and the possible solutions, 2) the planning of experiments and inter-calibration exercises to validate the new approach to N₂ fixation measurements, 3) production of accepted and tested protocols for N₂ fixation rate measurements, 4) publishing of a 'Guide to Best Practice to Marine N₂ Fixation Research', and 5) evaluation of the historical data on N₂ fixation to provide a best estimate of the magnitude of the underestimation and provide guidelines for the utilization of historical N₂ fixation rate measurements in biogeochemical and global change research and models.

Rationale

The nitrogen cycle is intricately linked to the productivity of the ocean (Duce et al. 2008). The oceanic fixed nitrogen inventory is balanced by loss and gain processes, which are controlled by marine microbes (Gruber and Sarmiento 1997). Loss processes are diverse and include autotrophic and heterotrophic denitrification and anammox reactions, while N₂ fixation is the only direct biological input of new fixed nitrogen to the ocean. Thus, biological dinitrogen fixation rate measurements are critical for determining the magnitude of new fixed N input to the oceanic N inventory (Karl et al. 2002, Luo et al. 2012, Duce et al. 2008). Since dinitrogen (N₂) is a gas, these measurements require incubation of seawater samples with a gas, either isotopically labeled N₂ molecules, or a N₂ analogue (such as acetylene) (Capone 1993). In the last 15 years, the most widely accepted method to measure N₂ fixation has been to incubate water samples containing natural microbial communities, including N₂ fixing microorganisms, with tracer amount of the ¹⁵N (a stable isotope form of N) labeled N₂ gas. The rate of N₂ fixation is then determined by measuring the isotopic enrichment of the particulate nitrogen, which becomes enriched in ¹⁵N through the enzymatic conversion of N₂ into ammonium, and subsequently into amino acids and other organic cellular material (Montoya et al. 1996). The calculations of the rates are based on measurements of the ¹⁵N in the particulate material and on the natural abundance of ¹⁵N in the particulate material, both measured with a mass spectrometer. A third critical parameter is the ¹⁵N isotope ratio of the seawater phase after the addition of the tracer, which is calculated assuming complete and instantaneous equilibration of the

added $^{15}\text{N}_2$ gas with the seawater, according to Weiss (1970), and Hamme and Emmerson (2004). It has recently been demonstrated experimentally that the latter assumption is not valid and therefore results in an underestimation of the real N_2 fixation rates (Mohr et al. 2010a).

During a recent workshop where a group of 24 experts in marine N_2 fixation research came together to discuss these problems, it was established that an improved method should be developed by the scientific community rather than by individual scientists. This will ensure thorough testing of the method, circumventing new problems such that the best possible alternative method can rapidly gain acceptance in the scientific community. Although several researchers have begun to adapt the method to circumvent the equilibration problem, working as a group rather than in isolation will ensure that the new method will be widely tested and benefit from the ideas and expertise of the scientific community involved in N_2 fixation research. Coordination and discussion of the various individual efforts during the initial workshop, primarily sponsored by SOLAS, has already proven beneficial in terms of standardizing protocols and sharing information. During the initial workshop, a work plan has been proposed for the next 3-4 years. It was also discussed that a SCOR working group would be ideal to fulfill the goals of establishing the new method and assessing the consequences of the underestimation in the last 15 years of N_2 fixation rate measurements, which are widely used in biogeochemical models and marine N cycle and global change research. The development and implementation of a new method which corrects for the underestimation will most likely lead to higher estimates of marine N_2 fixation over the next few years and starting in 2012. It is very important that this time point (2012) is clearly documented in the peer-reviewed literature as the time when the method was readjusted to prevent misperception decades later that marine N_2 fixation has increased due to natural or anthropogenic changes in environmental conditions that would favor N_2 fixing organisms (diazotrophs).

In addition to designing a new method, it is important to recommend a strategy to regain a basic data set of N_2 fixation rate measurements in the ocean, and assess the level of underestimation that may have taken place in the last 15 years of measurements. Additionally, the discovery of new diazotrophs in unsuspected regions calls for a systematic re-evaluation of N_2 fixation in the ocean, requiring a strategic planning for oceanographic campaigns, where N_2 fixation rate measurements will be carried out. A SCOR working group is the best mechanism to ensure a coordinated international scientific effort to develop, test and apply a N_2 fixation method that circumvent the problems of the widely applied method, which currently underestimates N_2 fixation rates. The scientific rationale for the working group is to distribute the tasks of designing and testing an improved method among the interested groups that cover different scientific backgrounds, ranging from chemists to ecologists. It will also provide a broad range of expertise and instrumentation that would otherwise not be readily available to individual groups (e.g., MIMS (membrane inlet mass spectrometry) measurements), which allows us to speed up the task of designing a new method and distributing it broadly.

Scientific Background

The importance of N_2 fixation in the oceanic N cycle is well established (Duce et al 2008) and will not be reviewed in detail here. Marine N_2 fixation has been the subject of recent reviews (e.g., Luo et al. 2012) and has been implemented in several coupled ocean-atmosphere biogeochemical models (Monteiro et al 2011, Monteiro and Follows 2009). Indirect estimates of N_2 fixation based on geochemical approaches have so far been higher than direct field-based rate measurements (Gruber and Sarmiento, 1997, Gruber, 2008). Recent experimental work (Mohr et al. 2010a) indicates that the discrepancy may have arisen to a large extent because of a misconception in the assumptions involved in the direct measurements of marine N_2 fixation as described above.

The N_2 fixation measurements have typically been performed by injecting a gas bubble (typically a few milliliters) into a bottle filled with seawater, inverting the bottle to mix the bubble, and assuming

that the injected gas rapidly equilibrates with the dissolved N₂ pool (Montoya et al. 1996). Recent research has demonstrated that the dissolution of the gas bubble is slow, taking between 6-12 hours to reach close to equilibrium (saturation) in the dissolved pool, depending on the incubation conditions. Thus, the resulting rates are inherently in error, since the ¹⁵N % labeling is changing throughout the incubation time while a constant value is applied in the calculation of the N₂ fixation rate leading to the underestimation. One additional consequence of the slow equilibration is that the time of initiation of the experiment is critical, as is the nature of the N₂ fixing microorganisms. For example, some N₂ fixing microorganisms fix only in the light period (e.g. *Trichodesmium*) and if the incubation is begun at the beginning of the light period, there will be a large degree of error, since the isotope will not be in equilibrium during the period that N₂ fixation occurs. In contrast, if the incubation is begun the previous evening, the isotope has time to equilibrate prior to the daytime N₂ fixation of *Trichodesmium*. Additionally, some organisms fix only during the dark (Mohr et al. 2010b), and some fix in both the light and dark (e.g. the UCYN-A cyanobacteria, Goebel et al. 2007). As a result, the bubble method is inconsistent in how it underestimates N₂ fixation. The currently utilized method is therefore in violation of the principles of tracer experiments and a new method in which isotopic equilibrium is reached completely and rapidly at the beginning of the incubation is needed. Until we rectify the method and obtain a series of new measurements, we need to consider the current direct estimates of marine N₂ fixation as lower estimates for this process. It is likely that with the development of the new methods the oceanic N cycle will need to be revised significantly to include higher estimates of N₂ fixation. Given that N cycle is affected by anthropogenic activities, it is important to have an accurate method to directly measure N₂ fixation at the local, regional and global scale in order to assess, for example, the relative contribution of atmospheric N deposition and N₂ fixation to the N oceanic inventory as the contribution of atmospheric deposition is predicted to increase significantly in the future (Duce et al. 2008). Additionally, the recent discovery of new diazotrophs (Foster et al., 2006, Moisaner et al., 2010, Rieman et al., 2010) and the long-term commitments of N₂ fixation rate measurements at time-series sites such as HOT and BATS, where diazotrophs are known to be important members of the microbial community, all point to the urgent need to develop and apply accurate and standardized methods for measuring N₂ fixation. During the initial workshop sponsored by SOLAS, we have already begun the task of designing a method without the drawbacks of the technique currently in use. At the initial workshop, it became evident that the process of establishing a more accurate method will require extensive testing and coordination of these activities through regular meetings would be the best way to reach our goals. As the work on N₂ fixation is ongoing, a set of initial recommendations were made at the SOLAS workshop but the upgraded method will require more time to become established. Guidelines are needed for N₂ fixation work and for interpreting previous measurements and for recommendations on the future plans for strategic measurements.

Terms of Reference

The working group will pursue the following terms of reference:

1. Write a short paper after the first meeting, to be submitted to *Frontiers in Aquatic Microbiology-Perspective*, explaining the inherent problems of the current N₂ fixation rate measurement method, the associated consequences and the possible solutions.
2. Develop and test a more accurate method for the direct measurement of oceanic N₂ fixation rates
 - Test method for producing ¹⁵N₂ enriched water
 - Storage of standard enriched seawater
 - Test the effect of adding ¹⁵N₂ enriched sea water to natural communities of microorganisms
3. Coordinate and execute an intercalibration exercise and a workshop to train people on the application of the new method
4. Review and assess the consequences of the underestimation in the historical measurements and provide a list of recommendation for their utilization.

5. Produce a series of protocols to measure marine N₂ fixation, accepted by the group and distribute them to the wider scientific community. Eventually, also publish these in a peer-reviewed methods journal.
6. Produce and publish a document entitled 'Best practice guide to N₂ fixation research' with chapters contributed by various members of the working group and complemented (if necessary) from solicited contribution from additional members of the scientific community at large.

Working Group Membership

The work proposed here would be carried out by a group of 10 Full Members and 18 Associate Members, including the scientists that were involved in the initial workshop. The list of proposed experts includes all of the participants who attended the first workshop but has been extended to include scientists from other geographical areas. The full members all have ongoing programs to measure N₂ fixation in the ocean or interest in the N cycle and are therefore highly motivated to carry out this task.

Full Members

1. Julie LaRoche (Canada) - Co-chair – Marine Biogeochemistry
2. Lucas Stal (Netherlands) - Co-chair – Marine Microbiology
3. Jonathan Zehr (USA)-Biological Oceanography
4. Eric Achterberg (UK)– Chemical Oceanography
5. Hongbin Liu (China) Biological Oceanography
6. Cliff Law (New Zealand) - Biogeochemistry
7. Anya Waite (Australia) – Biological Oceanography
8. Wajih Naqvi (India)- Biological Oceanography
9. Helle Plough (Sweden) - Biology
10. Wiebke Mohr (USA)-Marine Biogeochemistry

Associate Members

1. Sophie Bonnet (France) - Biology
2. Mark Altabet (USA)-Chemical oceanography
3. Margaret Mulholland (USA) - Biology
4. Lasse Riemann (Denmark) - Ecology
5. Ricardo Letelier (USA) Biological Oceanography
6. Ilana Berman-Frank (Israel) Biological Oceanography
7. Emilio Maranon (Spain) Ecology
8. Pia Moisander (USA) Biological Oceanography
9. Angelique White (USA) Biological Oceanography
10. Mark Moore (UK) Marine Biogeochemistry
11. Matt Church (USA) Biological Oceanography
12. David Karl (USA) Marine Biogeochemistry
13. Gaute Lavik (Germany) Marine Biogeochemistry
14. Maren Voß (Germany) – Biological Oceanography
15. Claire Mahaffey (UK) Biological Oceanography
16. Rachel Foster (Germany) Biological Oceanography
17. Daniella Böttjer (USA) Biological Oceanography
18. Sam Wilson (USA) Biological Oceanography

Working Group Activities

If approved, the working group would organize its first meeting in early 2013, potentially in conjunction with the ASLO meeting in New Orleans (February) or the annual EGU meeting (late April). At the first meeting, the members will present results from the activities that were carried out since the first meeting held in February 2012. The initial results will determine how to follow and

fulfill the terms of references. As in the initial SOLAS meeting, tasks will be assigned to groups of individual scientists to insure that all of the terms of references will be covered during the 3-year period. The implemented task groups will discuss their requirements and work plans for the next year (e.g., how they will coordinate their activities and whether they will require an additional meeting or workshop to best achieve their goals). They will also plan a joint cruise that will serve both as a training and an intercalibration exercise, and additionally explore options to acquire additional funds for the field work.

The second meeting will be held at Dalhousie University in Halifax Canada in late 2013 or early 2014. A detailed outline of a publication entitled 'Guide to best practices to marine N₂ fixation research' will be produced at the meeting with the various chapters of the book assigned to specific authors. This publication will be in a format similar to the successfully completed 'Guide to best practice to Ocean acidification research' (<http://www.epoca-project.eu/index.php/guide-to-best-practices-for-ocean-acidification-research-and-data-reporting.html>) which is widely accepted and used by the scientific community. In addition, the working group members will plan to write a method's paper (if possible to a journal such as *Nature Methods*, that encourages the synthesis of large experimental trials). We will also discuss at that time how to obtain the financial means to produce and distribute widely the publications either electronically or as printed material.

The final meeting in late 2014 or early 2015 will be to complete the best practice guide document and to produce a set of protocols for the methods paper, also collecting and reviewing the rate measurements made with the new method. In addition, the final meeting will be used to finalize the recommendations on 1) the utilization of the historical data and 2) the determination of a new series of N₂ fixation rate measurements that can be used as a baseline for global change research on the oceanic N cycle from 2012 and beyond.

The topic and activities of the proposed group have already been judged important for SOLAS, as demonstrated from their support of an initial workshop. In addition, they will be useful to IMBER and GEOTRACES activities. The working group will ensure that links are established with these other programs.

Capacity Building

Nitrogen fixation measurements are made worldwide, in developed and developing countries. It will be important to ensure that the new methods are transmitted effectively to developing countries. This goal will be achieved through participation of developing country scientists on the working group and in the group's intercalibration and training activities. In addition, the publications of the outcomes of the SCOR group results will be made open access, including the best guide to N₂ measurements and the potential 'Nature Methods' publication. The group will seek additional funding from SCOR and other sources for participation of developing country scientists in group activities.

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