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Biogeosciences Discussions

Interactive comment on "DON as a source of bioavailable nitrogen for phytoplankton" *by* D. A. Bronk et al.

D. A. Bronk et al.

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Author response to the interactive comment on "DON as a source of bioavailable nitrogen for phytoplankton" by D. A. Bronk et al. Anonymous Referee #3 Received and published: 29 August 2006 Manuscript MS-NR: bgd-2006-0038, Version 1.

Bronk: I have copied Reviewer #3 below in its entirety. I broke it down into the individual comments that required my attention. I've noted how I addressed the comment below it. When I didn't feel the reviewer's comment required me to change the manuscript, I wrote "no response necessary".

Though it is always customary to thank reviewers for their reviews I would like to extend an especially hearty thank you to Reviewer #3. I don't think I have ever received a review with as many good thought-provoking comments! I think the discussion of new

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methods that has been added to the paper as a result will be a very useful addition - particularly for students.

General comments This is a well-written manuscript reviewing the bioavailability of DON as a source of N for phytoplankton. This review does a nice job of summarizing recent field work describing community DON uptake using 15N-labeled substrates and fluorescent probes. It also focuses on the difficulty of separating heterotrophic bacteria from eukaryotic phytoplankton in order to evaluate the importance (proportion) of phytoplankton DON consumption to the total consumption of DON in marine environments.

Bronk: No response necessary.

The review underscores the general trend that diatoms appear largely to be associated with nitrate uptake whereas other eukaryotic phytoplankton appear to be associated with uptake of reduced nitrogen and low molecular weight DON such as urea, amino acids and peptides. This general trend appears contrary to earlier investigations demonstrating that diatoms participated in amino acid uptake, both in culture and in the field, in addition to dinoflagellates (Wheeler et al. 1974, 1977).

Bronk: I added the two Wheeler et al. references to section 3 of the manuscript. I also revised the text to clarify the point that it still seems unlikely to us that a given phytoplankters will evolve a large number of specific uptake mechanisms to use a broad suite of organic compounds.

Recently, it was reported from the genome sequencing project of the diatom Thalassiosira pseudonana that it possesses plasma membrane amino acid transporters (Ambrust et al. 2004). Thus, the long-awaited sequencing of the first diatom genome supported conclusions reached 30 years earlier in field investigations. I think it worth mentioning in the present manuscript how sequencing of phytoplankton genomes may significantly improve on our understanding of phytoplankton nitrogen ecology. For example, how can knowing that diatoms can transport amino acids aid in the design of field experiments? Would or would it not be quantitatively important?

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Bronk: I added a paragraph to section 3.3 that describes how genetic information could be used to study DON uptake.

It may be useful to separate prokaryotic phytoplankton (i.e. cyanobacterial) from eukaryotic phytoplankton DON uptake. It is likely that the enzymes and mechanisms differ significantly. At this time, we know a great deal more about cyanobacterial DON uptake capabilities from genome sequencing efforts (Dufresne et al. 2003, Palenik et al. 2003, Rocap et al. 2003), than we know of eukaryotic phytoplankton capabilities. The scientific community has learned a great deal about nitrogen uptake and assimilation from the cyanobacterial sequencing efforts. As noted by Fuhrman (2003), the cyanobacterium Synechococcus appears much more versatile in the nitrogen substrates (especially DON) it can use compared with the 3 Prochlorococcus species sequenced, who also differ substantially from each other. This may be worth including in the manuscript.

Bronk: I chose not to redraft the manuscript with an eye towards separating prokaryotic from eukaryotic phytoplankton though I think it's a good idea. Both reviewers appeared to like the current format so I didn't want to spend the time changing it too much. There is always the danger of making it worse! There is some very interesting recent work on Prochlorococcus nitrogen nutrition, however, that I did add to section 3.3.

Since the Wheeler et al. (1977) investigation, the use of autoradiography to distinguish DON usage among various eukaryotic phytoplankton has not been employed to a great extent. In contrast, this technique has evolved rapidly for use in distinguishing DON use by heterotrophic bacterial communities. Cottrell and Kirchman (2000) combined microautoradiography and fluorescence in situ hybridization (MICRO FISH) of rRNA-targeted oligonucleotide probes to investigate phylogenetic bacterial groups that dominate uptake of chitin, N-acetyl glucosamine, proteins and amino acids. It seems this technique could also be used in quantifying eukaryotic phytoplankton DON use, and in distinguishing between phytoplankton (prokaryotic and eukaryotic) and heterotrophic bacterial usage. As far as I can tell, this is not being done. Could the present authors speculate on why that is?

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Bronk: I added the Cottrell and Kirchman reference to section 3.3. I really don't have the expertise in the application of these tools to know why they have not been applied to phytoplankton. In talking with colleagues that do work in that area I heard a number of different opinions. Bottom line - I just don't know enough to make an informed opinion and would hate to lead anyone astray.

Also, how can we as researchers use genetic information in combination with "traditional" techniques, i.e. 15N or radiotracers, to improve our understanding of bioavailability of DON to phytoplankton, and to separate rate measurements by class or taxa?

Bronk: I added several sentences about stable isotope probing in section 3.3, which is a really powerful new technique that I was remiss in not mentioning. I thank the reviewer for getting me to think along these lines.

References Ambrust et al. 2004. Science 306: 79-86 Cottrell and Kirchman. 2000. Appl Environ Microbiol. 66:1692-1697 Dufresne et al. 2003. Proc Natl Acad Sci 100:10020-10025 Fuhrman. 2003. Nature 424: 1001-1003 Palenik et al. 2003. Nature 424: 1037-1042 Rocap et al. 2003. Nature 424: 1042-1046 Wheeler et al. 1974. Limnol Oceanogr 19: 249-259. Wheeler et al. 1977. Limnol Oceanogr 22: 900-910

Bronk: All of the references have been added to the revised manuscript, as well as many additional ones that I thought appropriate given the additional text.

Specific comments p. 1249. Line 4, other references on DON concentrations: Hansell et al. 1993, Libby and Wheeler 1997, Church et al. 2002.

Bronk: I added these references.

Line 7: Please include proportions of the various constituents of high molecular weight DON (or recalcitrant DON as described in the text) in Table 1 and in the text starting on line 7: Recent investigations show that amide-linked nitrogen comprises the largest fraction of high molecular weight DON; Amides constitute 92% of marine HMW DON while amines constitute 8% (Aluwihare et al. Nature 2005).

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Bronk: I added the percentages to the text but did not add them to Table 1 because it is not clear how the HMW versus LMW characterization merges with the labile, semi-labile, and refractory scheme. I revised the text separating out these two ways of characterizing DON (section 2.1) to make the point that these are two different ways of looking at the issue.

Humic and fulvic acids are generally not detectable in NMR spectroscopy and therefore represent a very small proportion of HMW DON, even in fresh water systems (Repeta et al. Geochim. Cosmochim. Acta 2002). Line 21: There is a substantial discrepancy in the proportion of DON that humic substances comprise between Aluwihare and Repeta (above) versus Alberts and Takacs (1999). Can the authors 1) include all 3 citations and 2) comment on this discrepancy?

Bronk: I added the references. I suspect the difference has to do with coastal versus open ocean systems. Terrestrial-derived humics are much more prevalent along the coast and within rivers and estuaries. I also added some of my own data from coastal Georgia that supports the view that humics can be a large fraction of the DON pool in some systems.

p. 1253 Line 2: Nitrate and ammonium transporters have been described for diatoms, these citations can be added to Syrett 1988 reference (Hildebrand 2005, Hildebrand and Dahlin 2000).

Bronk: All of these references have been added.

From the T. pseudonana sequencing project, it appears that diatoms did evolve amino acid transporters (Ambrust et al. 2004) despite their low concentrations. Perhaps sentence starting “It is unlikely that they would evolve” could be modified. Moreover, earlier data demonstrated diatom uptake of amino acids using autoradiography (Wheeler et al. 1977).

Bronk: I modified the text accordingly.

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Line 5: In the same vein, early uptake studies of 14C labeled substrates indicated that carbon was excreted following uptake of amino acids by marine phytoplankton (Stephens and North 1971 L&O 16:752), prompting researchers to speculate that the amino nitrogen was retained in the cell and the carbon skeleton excreted, consistent with extracellular deamination, proposed as early as in 1948 by Algeus (Algeus S. Physiol. Plant. 1: 382-386).

Bronk: The references have been added and the text has been revised accordingly. I'm especially grateful for the Algeus reference. I had not seen that one before.

p. 1256 Line 1: Urea is generally not considered important to bacterial N nutrition (see Cho et al. 1996, Tamminen and Irmisch (1996). Maybe the importance of urea and amino acid uptake to phytoplankton and bacteria could be separated by adding "respectively" into the sentence as follows: Urea and amino acids are the most frequently studies DON forms, not only because of their importance to phytoplankton and bacterial N nutrition, respectively, but because they are readily available in labeled form.

Bronk: I added the references. I also modified the text to reflect that urea was used primarily because it was available commercially. I was hesitant to include the phytoplankton versus bacterial N nutrition point, however. I don't think it is that clear cut. I also added a recent reference for bacterial use of urea by Jorgensen (2006).

p. 1257 Line 5: Add reference Kristiansen S. 1983 Mar Biol 74: 17-24. This citation contains the highest urea uptake rates ever recorded.

Bronk: The reference was added.

p. 1258 Line 9: The reference Berg et al. (1997) does not refer to dinoflagellates. Please substitute other references for dinoflagellate DON affinity (i.e. Palenik and Morel 1990, 1991, Glibert and Terlizzi 1999, Dyhrman and Anderson 2003, Fan et al. 2003)

Bronk: The reviewer is correct - my apologies! I added the references, as well as some

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new 2007 references that speak to this topic (Herndon and Cochlan 2007 and Howard et al. 2007).

p. 1262 Line 10: At the time of the study by Mulholland et al. (2002), axenic cultures of A. anophagefferens were not commercially available and the culture experiments described in this paper were non-axenic.

Bronk: This text has been corrected.

Technical comments p. 1260 Line 15: Sentence star ting “The f-ratio, which is” appears to have a typo in it. It can be combined with the following sentence to read: For example, the f-ratio, which is the ratio of new to total (new plus regenerated) production (Eppley and Peterson 1979), has traditionally been calculated by dividing 15NO3- uptake (i.e. new production) by the summed uptake of 15NH4+ (i.e. regenerated production) and 15NO3- (e.g. Harrison et al. 1987).

Bronk: The sentence as been corrected and the paragraph modified for clarity.

Interactive comment on Biogeosciences Discuss., 3, 1247, 2006.