

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

Anonymous Referee #3

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Manuscript MS-NR: bgd-2006-0038, Version 1. DON as a source of bioavailable nitrogen for phytoplankton by DA Bronk et al.

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 ^{15}N -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. The review underscores the general trend that diatoms appear largely to be associated with nitrate

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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). 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?

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.

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)

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

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

Specific comments

p. 1249. Line 4, other references on DON concentrations: Hansell et al. 1993, Libby and Wheeler 1997, Church et al. 2002. 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). Humic and fulvic acids are generally not detectable in NMR spectroscopy and therefore represent a very small proportion of 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? p. 1253 Line 2: Nitrate and ammonium transporters have been described for diatoms, these citations

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can be added to Syrett 1988 reference (Hildebrand 2005, Hildebrand and Dahlin 2000). 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). 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). 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. p. 1257 Line 5: Add reference Kristiansen S. 1983 Mar Biol 74: 17-24. This citation contains the highest urea uptake rates ever recorded. 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) 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.

Technical comments

p. 1260 Line 15: Sentence starting “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),

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has traditionally been calculated by dividing $^{15}\text{NO}_3^-$ uptake (i.e. new production) by the summed uptake of $^{15}\text{NH}_4^+$ (i.e. regenerated production) and $^{15}\text{NO}_3^-$ (e.g. Harrison et al. 1987).

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