Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-3-RC2, 2018

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Programmed cell death in diazotrophs and the fate of organic matter in the Western Tropical South Pacific Ocean during the OUTPACE cruise" *by* Dina Spungin et al.

Anonymous Referee #2

Received and published: 15 March 2018

INTERACTIVE COMMENT on "Programmed cell death in diazotrophs and the fate of organic matter in the Western Tropical South Pacific Ocean during the OUTPACE cruise" By Spungin et al.

GENERAL COMMENTS: The manuscript by Spungin et al. reports on the induction of cell death by nutrient limitation during blooms of the diazotroph Trichodesmium (and also diazotrophs associated to diatoms) during a cruise in the South Pacific Ocean. The study analyses whether cell death is a relevant mechanism driving Trichodesmium mortality , and whether this contributes to vertical export of organic matter.

The aim of this work is to provide evidence of correlation between bloom termina-

Printer-friendly version

Discussion paper

tion, cell death and vertical export, with the mediation of specific proteases such as caspase-like and metacaspases. Trichodesmium is responsible for roughly half of the nitrogen fixed in the ocean. The study hereby presented focuses on a relevant topic for marine biologists which helps elucidate the impact of cell death of a globally relevant species on the ecosystem, warranting important consequences for the C and N cycles. The paper clearly disserves publication since most of the conclussion are strong.

However, I have several concerns that (in my opinion) need to be addressed by the authors before it can be published.

SPECIFIC COMMENTS:

A) I have two major concerns. The first, relates to gene expression and activity of metacaspases and caspase like-proteins and their role in a death cascade (initiation and execution of PCD).

1. The mechanisms by which cell death (CD) (programmed or not programmed) occurs, considering that cell death in phytoplankton leads to the complete demise of the organism/colonies, are always intriguing and there still are many unanswered questions. Among them, which is the proteolytic machinery involved and how it works. Metacaspases, belong to the CD clan of cysteine proteases, were thought to perform similar functions than caspases. It has been shown by multiple labs working with several organisms from yeast, plants and protists that metacaspases are quite distinct in terms of target site specificity from caspases . They target substrate sites are either arginine (R) or lysine (V) at the P1 position. The authors consider this approach right and use a substrate typically hydrolysed by MCs. I was wondering why this specific (VRPR) substrate was used and no other? and, why in the concentration described, 50mM?

Did not the authors test for the optimal substrate concentrations for Trichodesmium before the analyses? The reference they give is based on Arabidopsis thaliana assays and that certainly is very different to cyanobacteria. Clarification is needed.

Printer-friendly version

Discussion paper

2.-Same applies with the caspase like substrate IETD, but in this case, I assume that this has been previously tested according to Berman-Frank and Baar-Zev former studies. Caspase -like activities have been reported in vascular plants, phytoplankton, yeast and protozoa. However, their nature is controversial . Up to date, is still not clear, who is the responsible for the observed caspase-like activity in phytoplankton. In vascular plants some authors have pointed to the serine protease family proteins to perform this hydrolysis (see Bonneau et al., 2008) and/or the vacuolar processing enzyme (Hara-Nishimura and Hatsugai, 2011). It has also been reported that some caspase-like activities are attributable to the plant subtilisin-like proteases-saspases and phytaspases (see Vartapetian et al 2011). Hence, clarify this in the text please.

To me the question is:

Since we are measuring these enzymatic activities in phytoplankton's cell free extracts and not in purified proteins result of gene overexpression, we shall be very careful when ascribing the activity to a species. What I mean is : in a cell free extract there are many proteins potentially users of the mentioned substrates. For this reason, I find the use the term "caspase" is not correct, but instead use the term "caspase-like" throughout the whole MS. It is appropriate that the activity must be referred to as "IETDase, etc Therefore, substitute "caspase activity" by "Caspase-like" (or CL). The same applies to metacaspases, and so VRPRase must be used. Otherwise it can lead to confusion. By the way, revise the nomenclature of the substrate: "Av-VRPR", what group linked to the peptide is Av? Could possible be that Av is in reality Ac?

3.-Along the same thought, the gene expression measurement is very important, but I must say, that does not mean that the enzymatic activity you are measuring corresponds to the expressed gene , if , as said before, that specific activity has not been measured in a purified protein. Hence, caution is needed on this respect when interpreting your data.

4.-Aditionally, I think we all must accept that we do not really know if there are ini-

Printer-friendly version

Discussion paper

tiator or executor CLs or MCs in phytoplankton. Two types of metacaspases (types I and II) are defined based on the presence of a prodomain analogous to the classification of caspases into initiator or executioner caspases. The molecular role of a prodomain in initiator caspases is the recruitment of caspases to multicomponent signalling complexes for caspase activation. However, phytoplankton metacaspases often lack prodomains (Choi and Berges 2013). As I see it, to use this homology can lead into mistake, so I would not describe the enzymes involved as executors of the cell, or initiators of the cascade (although for vascular plants is widely used, it is different, they know exactly which protease which is, and what they do).

5.-Last but not least, just would like to know your opinion on this actual heated-debate: Do you think that at the time being caspase-like proteins, in phytoplankton, could hydrolyse R or V ?

B) The second major concern relates to the fact of bloom/ cell dismissal in the water column.

1.-When working in the field, dead cells are rarely seen at later stages (Berges and Choi 2014) or not seen (Segovia et al., 2018), only because they have been cleared away from the system. Any source of energy that cellular debris may provide to the neighbourhood will be immediately used by other species within the food web. So, it is very unlikely to see cellular rests consequence of CD on the water column. Yet, POC downward flux is the way to have some estimates. In my opinion and experience, this can be applied to cultures in the lab under controlled conditions, but I find it truly complicated in natural communities / ecosystem level. Please, clarify how this fits within your sampling/sample analyses time framework . Has that to do with the blooming condition excluding other components of the trophic web of the niche?

2.- Nothing is said about viruses affecting C losses, which is important for C cycling and definitively affect C export. Viruses were not measured the text says. But in my opinion, this shall at least be discussed and do not directly exclude this possibility as a

Printer-friendly version

Discussion paper

possible cause for bloom demise. Is there any long-term study done on Trichodesmium blooms termination affected by viruses that at least allows you to compare with other situations?

BIBLIOGRAPHY: Bonneau L, Ge Y, Drury GE, Gallois P. 2008. What happened to plant caspases? Journal of Experimental Botany 59, 491-499).

Vartapetian AB, Tuzhikov AI, Chichkova NV, Taliansky M, Wolpert TJ (2011) A plant alternative to animal caspases: subtilisin-like proteases. Cell Death Differ 18:1289-1297

Choi CJ, Berges JA. New types of metacaspases in phytoplankton reveal diverse origins of cell death proteases . Cell Death and Disease (2013) 4, e490; doi:10.1038/cddis.2013.21

Berges JA Choi CJ. Cell death in algae: physiological processes and relationships with stress (2014).Perspectives in Phycology .1:103-112âĂĺ

Segovia, M., Lorenzo, M.R., Iñiguez, C., García-Gómez C., (2018). Physiological stress response associated with elevated CO2 and dissolved iron in a phytoplankton community dominated by the coccolithophore Emiliania huxleyi. Mar. Ecol. Prog. Ser. 586-73-89

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-3, 2018.

Printer-friendly version

Discussion paper