

Response to Review

The revised version of the ms takes into account the comments made by both reviewers. We thank them for their constructive observations, which were mostly included in the text.

General Comments

The authors poison a subset of samples with mercuric chloride. Mercury is photochemically active (e.g. Ababneh et al., 2006; Pehkonen and Lin, 1998) and can therefore NOT be used to sterilise samples in photochemical studies.

A: The actual effect of HgCl_2 on UV exposure experiments is not clear based on the evidence available in the literature. Reactions of Hg in the presence of Fe or chloroform have been reported but a PH more acid than marine water is required. Also, HgCl_2 as a poison does not show a reversible effect. Hence we expect the time elapsed between addition and the beginning of irradiation to be sufficient to stop biological activity.

Another important point is concentration used. Reactions of free Hg in natural environments rely on ambient concentrations higher than the values used in this study (6 g L^{-1} , dose 1 mL for 500 mL of sample).

It is true that photoreactivity of HgCl_2 has been observed previously (e.g. (Ababneh et al. 2006)). However such reactivity needed specific conditions including low PH (3 to 4) and high concentrations of ferric oxalate ($500\mu\text{M}$). The reaction involves ferrioxalate as a source of radicals. These secondary photoproducts reduce Hg(II) to Hg(0) , which is a dissolved gas mercury product.

Dissolved gas mercury production has also been studied in seawater (Amyot et al. 1997, Costa & Liss 1999) through laboratory experiments as well as in freshwater lakes (Siciliano et al. 2002). It was demonstrated that dissolved gas mercury production was induced by sunlight. However results also showed that chloride promotes Hg(0) oxidation therefore having the opposite effect on mercury concentrations (Amyot et al. 1997).

Others factor may as well influence in mercury photoreactivity, including an increase in artificial humic material in relation to natural DOC concentrations which in our case can also be excluded (Costa & Liss 1999). Additionally many authors suggest that Fe(III) induce photoreduction of mercury (II) in freshwater by the production of free radicals (Zhang & Lindberg 2001, Siciliano et al. 2002, Ababneh et al. 2006) and have also suggested biological reduction and oxidation of mercury in freshwater (Siciliano et al. 2002).

*Our experiments were carried out with diatom exudates (*Chaetoceros muelleri* and *Thalassiosira minuscula* cultures) which are composed of sterilized seawater (Walne 1970), therefore with $\text{pH}\sim 8$. Moreover the mercury photoreduction needs radical production of the photoreduction of Fe complexes at high concentration whereas our culture media contained Fe at a concentration of $\sim 4.8\mu\text{M}$ (Walne, 1970). Considering the conditions*

necessary for the reaction, we conclude that for our ammonium photoproduction experiments, mercury-irradiated-controls are right as biological control, which is possible observed in the higher rates of ammonium production in the not poisoned-irradiated treatments respect to mercury-irradiated treatments (5-16 times). We therefore remain confident in our results. A comment was added to the methodology section.

Secondly, the authors also “sterilized” their samples by filtering through 0.7 μm GF/F filters. This was clearly not effective as microbial cell counts were similar to what one might expect from unfiltered seawater (cell counts were in the order of 10^5 to 10^7 ml⁻¹). It is likely therefore that in irradiated samples abiotic photoammonification and microbial processes (NH₄⁺ regeneration, uptake, nitrification, etc.) co-occurred as the authors point out. However, microbial processes are likely to have been inhibited by light (indeed cell counts in irradiated samples decreased by 50% in Figure 6D). In contrast, microbial processes in the “dark” treatment would not experience light inhibition. This means that the “dark” treatment is not a suitable control for either photochemical or microbial processes. In the future it may be better to use 0.1 μm filters instead. We found that these remove >99% of the microbial community (Kitidis et al., 2011; Kitidis et al., 2006).

A: The objective of this methodology was to study the response of microbial community while simultaneously photoproducing ammonium. By using 0.7 μm we removed the bigger size fraction and left mostly bacterioplankton in our sample. We chose to use that fractionation in order to test our hypothesis (ammonium photoproduced is rapidly used by archaea and bacteria and results in an enhancement of nitrification fluxes). Consequently we did not seek sterilize seawater before performing the experiment.

Nevertheless in future approaches we will use 0.1 μm in order to exclude microbial communities.

Nevertheless, I think the authors have done some good work which may be repackaged in a new manuscript with a different emphasis, outlined below. The authors should be upfront about the limitations of their dark control. The authors should remove all of their mercury-treated-light samples and all the relevant discussion. I suggest they keep the mercury-treated-dark samples as these agree well with the filtered-dark for *C.muelleri* exudates. There is a slight NH₄⁺ increase in the filtered-dark compared to the mercury-treated-dark for *T.minuscula* exudates. This would have to be discussed in light of the two main points above. The irradiations of phytoplankton exudates are convincing enough to show photoammonification.

A: Based on the above discussion on HgCl₂ reactivity we are not convinced that our poisoned light control should be excluded from the manuscript. We therefore stand by our

results and modified the discussion and result sections in order to include a discussion on the subject.

The marine DOM irradiations are a lot more difficult to interpret. However, if the authors clearly outline all of the competing and synergistic processes (photochemistry, remineralisation, uptake, nitrification, photo-inhibition...), they may be able to present their results as a net change of NH_4^+ , NO_2^- , NO_3^- and cell abundance under irradiation.

A: The discussion section was modified accordingly.

Please note that NO_2^- , NO_3^- are also photochemically cycled (Kieber et al., 1999; Mack and Bolton, 1999).

A: We agree on the importance of the photochemistry of both compounds. Photoproduction of nitrite has been reported among other by Kieber et al., 1999 and Koopmans and Bronk 2002. Accordingly, ammonium and nitrite can be submitted to photolysis. However, subsequent production of nitrate needs to be mediated (as a general rule) by biological processes such as nitrification. Further insights on the photochemically mediated interactions between NO_2^- and NO_3^- have been made by Mack and Bolton (1999).

Concerning our experiments, we feel that although nitrite undergoes primary photolysis in seawater and can therefore compete with its production, nitrate is unlikely to be produced as a photoproduct from nitrite. Also, nitrite has been proved to remain constant in irradiated sterilized samples. We added a comment on this subject on the discussion sections of the revised text.

Some comparison of the cumulative light dose during irradiation with the respective daily dose in the field would also be required to show that the conclusions are relevant.

A: Such comparison was done in the Table below. Overall our doses are in the range (or even below) of incident radiation levels reported for the study area A comment was also added to the methodology section.

Table 1. Comparison between incident solar radiation values reported off central Chile (36°S) during 2004 (Hernandez et al. 2012) and doses applied to experiments during this study. Daily doses are presented as average $\pm$ standard deviation.

2004	PAR KJm^{-2}		UVA KJm^{-2}		UVB KJm^{-2}	
	Hernandez et al 2012	This study	Hernandez et al 2012	This study	Hernandez et al 2012	This study
Summer	9372 $\pm$ 2681	-	762 $\pm$ 208	-	93 $\pm$ 29	-
Autumn	3892 $\pm$ 2130	-	324 $\pm$ 163		25 $\pm$ 18	-
Winter	3993 $\pm$ 2184	150; 374/--	344 $\pm$ 177	203, 507/ 272; 218 406; 406/ 218	22 $\pm$ 16	4; 11/ 832; 665 9; 9/ 665
Spring	9339 $\pm$ 3034	-	841 $\pm$ 247	-	87 $\pm$ 33	-

The field data are nice, but I would like to see a better attempt at explaining why light attenuation was so low in May and only for PAR. Light attenuation throughout the year seems relatively constant with the exception of this period. Furthermore, the fact that this is only for PAR suggests a completely different spectral distribution for light attenuation. Is this a different water mass, advected or upwelled in early May?

A: The area off central Chile is characterized by intense coastal upwelling that has seasonal patterns, being active during austral summer-spring. During winter however the area is highly influenced by river discharge. A possible explanation for the PAR attenuation may be related to particle concentration in the water column and continental inputs. A comment was added to the text.

Anonymous Referee #2

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This study provides insights about photo- and bio-ammonification in an upwelling region off the coast of Chile. Experiments were conducted under varying conditions to investigate photochemical and microbial ammonification processes and the potential role of photochemical processes in the stimulation microbial ammonification. This is an interesting twist because it suggests photochemical processes could play an important role in making DON more susceptible to microbial ammonification. The nature of the experiments limits their usefulness in providing in situ rates of processes, so the results are indicative of the potential impact on N cycling in the environment. Nonetheless, the study provides data on UV radiation in coastal waters and highlights the role of photochemistry in the N cycle of surface waters.

Comments and Suggestions: Suggested Title: Photochemical and microbial ammonification in the upwelling system off central Chile (36°S)

A: The title of the ms was changed accordingly.

Terminology: photoammonification should be used instead of abiotic ammonification, which is vague and does not describe the process. The term, “gross ammonium photoproduction”, is confusing because most of the ammonium being produced is not directly from photoammonification.

A: Abiotic ammonification was replaced by photoammonification in the text. The term gross ammonium photoproduction was replaced by gross ammonium production.

Were any chlorophyll measurements made in the GFF filtered seawater samples used for experiments? It is unclear whether phytoplankton were present in the experiments and influenced ammonium dynamics.

A: We did not follow Chla concentrations in our experiments. However we believe phytoplankton interference was absent because:

-Exudates of diatom cultures were filtered through 0.2 μ m.

-Irradiated and control natural samples of filtered water did not show dramatic increases in cell abundance, nor a depletion of ammonium or nitrate which would have been indicative of phytoplankton consumption.

Mercuric chloride was used in killed controls to estimate photoammonification, and it is unclear whether mercuric chloride influences the photoammonification process.

A: The actual effect of HgCl₂ on UV exposure experiments is not clear based on the evidence available in the literature. Reactions of Hg in seawater in the presence of Fe or chloroform have been reported but a PH more acid than marine water is required. Also, HgCl₂ as a poison does not show a reversible effect. Hence we expect the time elapsed between addition and the beginning or irradiation to be sufficient to stop biological activity.

Another important point is concentration used. Reactions of free Hg in natural environments rely on ambient concentrations higher than the values used in this study (6%).

It is true that photoreactivity of HgCl₂ has been observed previously (Ababneh et al. 2006). However such reactivity needed specific conditions including low PH (3 to 4) and high concentrations of ferric oxalate (500μM). The reaction involves ferrioxalate as a source of radicals. These secondary photoproducts reduce Hg(II) to Hg(0,) which is a dissolved gas mercury product.

Dissolved gas mercury production has also been studied in seawater (Amyot et al. 1997, Costa & Liss 1999) through laboratory experiments as well as in freshwater lakes (Siciliano et al. 2002). It was demonstrated that dissolved gas mercury production was induced by sunlight. However results also showed that chloride promotes Hg(0) oxidation (Amyot et al. 1997).

Others factor may as well influence in mercury photoreactivity, including an increase in artificial humic material in relation to natural DOC concentrations which In our case can also be excluded (Costa & Liss 1999). Additionally many authors suggest that Fe(III) induce photoreduction of mercury (II) in freshwater by the production of free radicals (Zhang & Lindberg 2001, Siciliano et al. 2002, Ababneh et al. 2006) and have also suggested biological reduction and oxidation of mercury in freshwater (Siciliano et al. 2002).

Our experiments were carried out with diatom exudates (Chaetoceros muelleri and Thalassiosira minuscula cultures) which are composed of sterilized seawater (Walne 1970), therefore with pH~8. Moreover the mercury photoreduction needs radical production of the photoreduction of Fe complexes at high concentration whereas our culture media contained Fe at a concentration of ~4.8 μM (Walne, 1970). Considering the conditions necessary for the reaction, we conclude that for our ammonium photoproduction experiments, mercury-irradiated-controls are right as biological control, which is possible observed in the higher rates of ammonium production in the not poisoned-irradiated treatments respect to mercury-irradiated treatments (5-16 times).

Were any filtered seawater (<0.2 μm) controls used for comparison to mercuric chloride controls?

A: We did not use filtered seawater for comparison purposes because the fraction below 0.2 μm corresponds to the DOM pool which we were trying to photodegrade in order to obtain ammonium. We felt such a comparison would bias our results.

The data in Table 1 indicates different light exposures were used for samples and that simulated UV radiation was relatively high.

A: We used different light exposures for testing the occurrence of ammonium photoproduction from diatom exudates and to evaluate a response in bacterial community.

Although the doses might seem high, they are within the range of values reported for this geographical area. In fact, atmospheric data Collected in December 2012 using a GUV-511C (Biospherical Instruments) showed that our doses are in the range or below incident UVA ($1062 \pm 240 \text{ KJm}^{-2}$) and UVB ($102 \pm 35 \text{ KJm}^{-2}$) values (average of three full days of measurements).

For our “Full sun light” treatment (PAR + UVA + UVB), the doses for both UVA and UVB were in the range of natural variability reported for the winter season by a previous study (Hernandez et al. 2011). For the experiments using marine samples, PAR doses were lower than natural levels while UVA and UVB doses were in the range of levels reported for winter time(Hernandez et al. 2011). Experiments carried out in spring showed only an excess of UVB radiation with respect to previous doses reported. UVA levels used were below or equivalent to average winter doses reported by Hernandez et al., (2011).

Accordingly, the doses used during this study are representative of the season and geographical area that was sampled. We therefore conclude that our experiments are representative of the natural levels of incident solar radiation in central Chile (36°S) observed during winter season. A possible consequence of the above is that our rates of photoammonification may be underestimated for spring and summer seasons. A comment was added to the text.

Table 1. Comparison between incident solar radiation values reported off central Chile (36°S) during 2004 (Hernandez et al. 2012) and doses applied to experiments during this study. Daily doses are presented as average $\pm$ standard deviation.

2004	PAR KJm^{-2}		UVA KJm^{-2}		UVB KJm^{-2}	
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Fig. 2 – it would be useful to add the mixed layer and chlorophyll maximum depths to the time series data in panel B.

A: Unfortunately, during our sampling campaign we did not have access to Chlorophyll or mixed layer depth data. This is because samplings at Coliumo Bay and Concepcion (from which our time series data was generated) were done in the very surface layer without the use of a CTD.

Pg 18481, Line 24 – should be “Orinoco River plume”

A: The sentence was modified

Pg 18502, Line 5 – “glycine” should be “glycine”

A: The sentence was modified

The authors should see the articles by Xie et al. 2012 in Biogeosciences on photoammonification in the Beaufort Sea and Smith 2005 AquatMicroEcol on the rapid heterotrophic utilization of ammonium released during photoammonification.

A: Both papers were analyzed and some comments were added to our discussion section.

References

- Ababneh FA, Scott SL, Al-Reasi HA, Lean DRS (2006) Photochemical reduction and reoxidation of aqueous mercuric chloride in the presence of ferrioxalate and air. *Science of the Total Environment* 367:831-839
- Amyot M, Gill G, Morel M (1997) Production and Loss of Dissolved Gaseous Mercury in Coastal Seawater. *Environ Sci Technol* 31:3606-3611
- Costa M, Liss P (1999) Photoreduction of mercury in sea water and its possible implications for Hg⁰ air-sea fluxes. *Marine Chemistry* 68:87-95
- Hernandez K, Yanicelli B, Montecinos A, Ramos M, Gonzalez H, Daneri G (2012) Temporal variability of incidental solar radiation and modulating factors in a coastal upwelling area (36°S). *Progress in Oceanography*. doi:10.1016/j.pocean.2011.07.011
- Kieber, R.J., Li, A., Seaton, P.J., 1999. Production of Nitrite from the Photodegradation of Dissolved Organic Matter in Natural Waters. *Environmental Science and Technology* 33, 993-998.
- Kitidis, V., Uher, G., Upstill-Goddard, R.C., Mantoura, R.F.C., Spyres, G., Woodward, E.M.S., (2006). Photochemical production of ammonium in the oligotrophic Cyprus Gyre (Eastern Mediterranean). *Biogeosciences* 3, 439-449.
- Kitidis, V., Tilstone, G., Smyth, T., Torres, R., Law, C.S., 2011. Carbon Monoxide Emission from a Mauritanian Upwelling Filament *Marine Chemistry* 127, 123-133.
- Koopmans DJ, Bronk D (2002) Photochemical production of dissolved inorganic nitrogen and primary amines from dissolved organic nitrogen in waters of two estuaries and adjacent surficial groundwaters. *Aquatic Microbial Ecology* 26:295-304
- Mack, J., Bolton, J.R., 1999. Photochemistry of nitrite and nitrate in aqueous solution: a review. *Journal of Photochemistry and Photobiology A: Chemistry* 128, 1-13.
- Pehkonen, S.O., Lin, C.J., 1998. Aqueous photochemistry of mercury with organic acids. *Journal of the Air & Waste Management Association* 48, 144-150.

Razuvaev, G.A., Vasileiskava, N.S. 1965 Photoreaction of mercuric chloride with chloroform in the presence of pyridine. Bulletin of the Academy of Sciences of the USSR, Division of chemicalscience. 14 (7): 1254-1255.

Siciliano S, O'Driscoll NS, Lean DRS (2002) Microbial Reduction and Oxidation of Mercury in Freshwater Lakes. Environ Sci Technol 36:3064–3068

Walne PR (1970) Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria, and Mytilus. Fish Invest 26:1-62

Zhang H, Lindberg SE (2001) Sunlight and Iron(III)-Induced Photochemical Production of Dissolved Gaseous Mercury in Freshwater Environ Sci Technol 35:928-935