

ARTEFACTS AND PITFALLS OF STERILIZATION TECHNIQUES TO ASSESS ABIOTIC N₂O PRODUCTION FROM SOIL S. Buessecker¹, K. Tylor¹, J. Nye², K. E. Holbert³, H. E. Hartnett², J. B. Glass⁴ and H. Cadillo-Quiroz¹, ¹Arizona State University, School of Life Sciences, ²Arizona State University, School of Earth and Space Exploration, ³Arizona State University, School of Electrical, Computer and Energy Engineering, ⁴Georgia Institute of Technology, School of Earth and Atmospheric Sciences

On Earth, natural and agricultural soils represent the major sources of nitrous oxide (N₂O) with atmospheric emission of over 6.6 Tg N yr⁻¹ (IPCC). N₂O is of outstanding significance, because first, it has a global warming potential ~300 times that of CO₂ and second, its abundance and proportion to other nitrogen species in the atmosphere has great potential to serve as biosignature. Therefore, its role in the regulation of planetary climate and its importance for life processes are intimately linked. However, in recent years, a multitude of studies revealed previously unknown production pathways with several lines of evidence that N₂O can be formed by abiotic reactions under diverse conditions. For instance, Heil et al. demonstrated N₂O production from the oxidation of hydroxylamine in a forest soil (1). N₂O is also the product of the reduction of nitrite, but the responsible reductants remain enigmatic. Reduced iron (Fe²⁺) has been identified as viable reactant (2, 3), but natural organic matter revealed enormous potential to retain introduced N and to emit N₂O as well (4). All these studies share one experimental obstacle, that is the sterilization of samples in order to solely allow non-enzymatic chemical reactions. As common ground, sterilants are meant to cause the least analytical bias possible, but often this is a true challenge in light of the typically high heterogeneity of soils. Here, we present experimental results of (i) a comparison of 6 different chemical and physical sterilization methods with respect to artifacts interfering in the production pathways of N₂O, and (ii) the first abiotic N₂O production described for a tropical soil.

In a series of batch incubations we treated samples from a tropical peatland with Zn, Hg, azide, chloroform, exposed them to γ -irradiation, or autoclaved them. Then, the soil was amended with nitrite, nitrate, or hydroxylamine to stimulate distinct N₂O production pathways. In the course of the incubation, N₂O and CO₂ was monitored, and Fe speciation along with dissolved organic matter (DOM) based on fluorescence was analyzed.

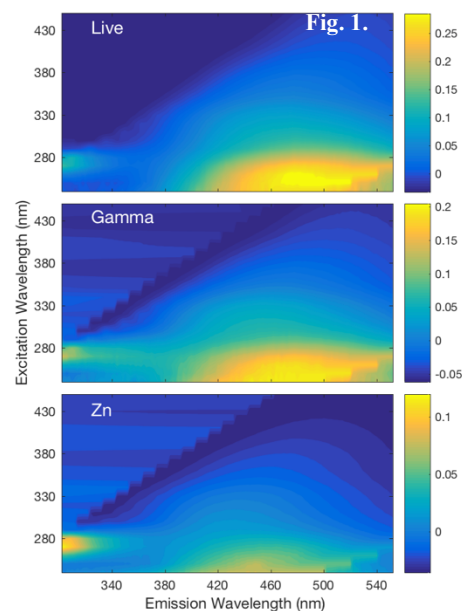
Our results show significant differences in N₂O production rates. For nitrite additions, linear N₂O production apparent in all incubations except for live controls were enveloped by high rates of up to 6.3 nmoles N₂O h⁻¹ in azide treatments and low rates of 1.3 nmoles N₂O h⁻¹ in γ -irradiated samples. This difference of about a

factor of 5 is consistent with variations in the abundance of extractable Fe species and the signal of fluorescing DOM. Fig. 1 provides an insight in our data gained from the DOM fluorescence analysis.

Noteworthy,

a pool characterized as “tyrosine-like” compounds (ex. 270 nm, em. 300 nm) is heavily reduced when nitrite is added. Consistent with the reaction scheme outlined by a study based on NMR spectroscopy (4), phenolic groups are very receptive for soil nitrite derived from the reduction of nitrate or nitrification.

Tropical peatsoils have to be considered as sources of abiotically produced N₂O (ii). The chemical artifacts induced by the different sterilants influence the speciation of soil Fe and the availability of reactive organic groups. Since the reaction route and abiotic production capacity is dependent on the sterilization technique used, we recommend to carefully evaluate the choice of the latter based on the focus question. Our work provides a fundament for studies elucidating N₂O emissions from different soils and gives insight in the variety of inorganic and organic reactants with nitrogenous compounds leading to the abiotic formation of N₂O. These possible reactants are of special relevance when assessing N₂O as a biosignature gas.



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