Research Project Report.

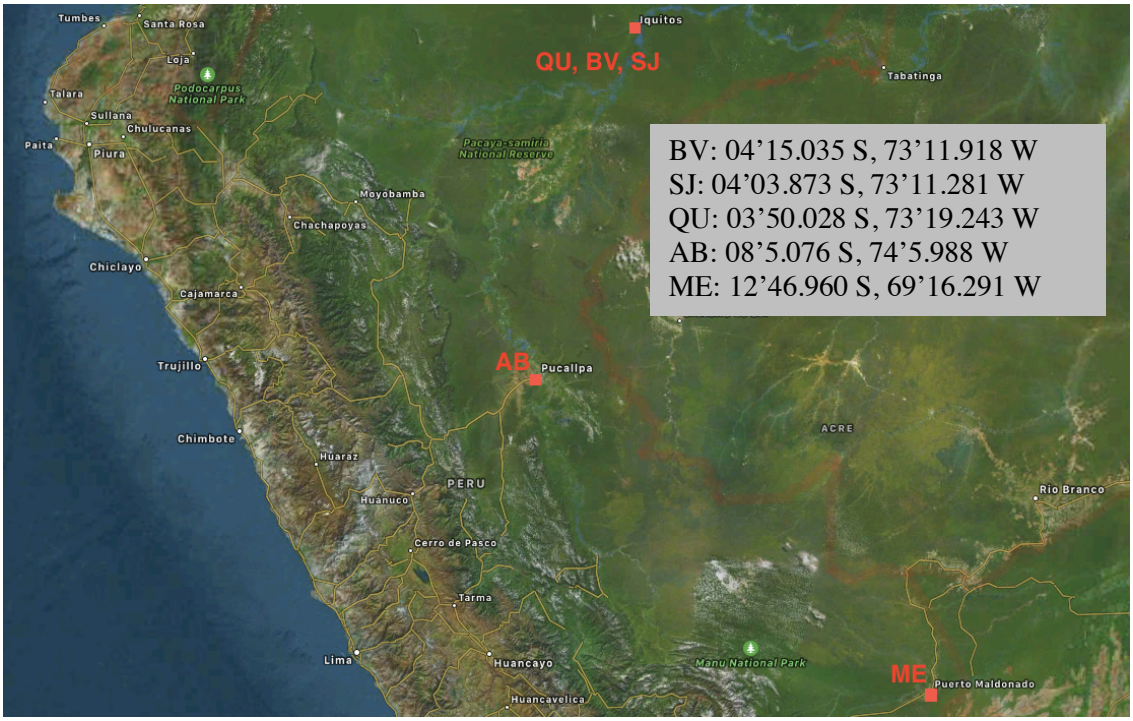

Fig. 1. Map of Peru showing the sampling sites with coordinates (box) in tropical peatlands.

The overarching goal of this study was to decipher abiotic and biotic components of nitrous oxide (N_2O) production from tropical peat soil with diverse iron (Fe) contents. We chose a suite of peatlands from the lowlands of Iquitos in the Amazon basin to more elevated sites in Pucallpa and Puerto Maldonado (Fig. 1). N_2O is of high interest, because it is a strong greenhouse gas (global warming potential 300 times that of CO_2) and an important oxidant for microbial respiratory processes in anoxic soil. Reduced Fe has been found to play a crucial role in the production of N_2O without enzymatic mediation [chemodenitrification, (*1*)]. The uncertainty in N_2O emissions is extremely high for South America and the Amazon region in general (*2*). A better understanding of the controlling factors on the production of this potent greenhouse gas will diminish the uncertainty and make predictions more accurate. Organic-rich soils such as peat are typically waterlogged bogs or fens with high contents (>85%) of organic matter and varying mineral fraction. Microbial communities inhabit this system that is deprived of oxygen and dominated by anaerobic metabolisms, such as methanogenesis, dissimilatory Fe reduction, or denitrification. N_2O has a high redox

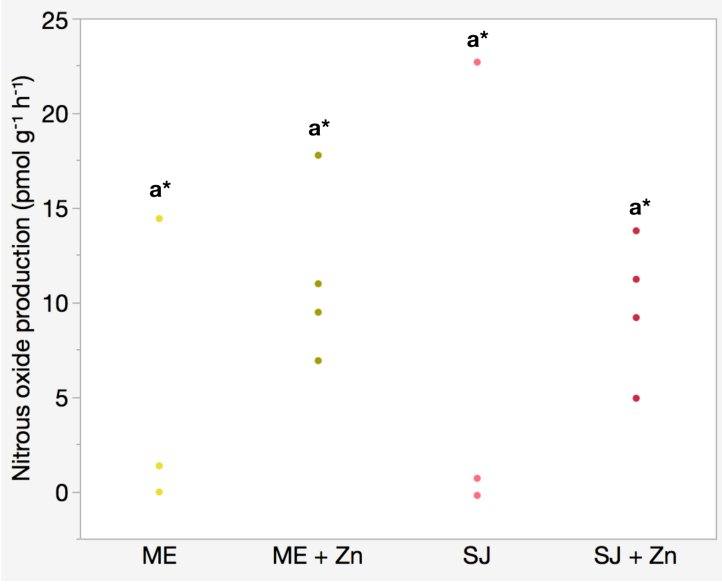

Fig. 2. Nitrous oxide production based on $^{15}N_2O$ measurements in soils with and without zinc treatment.

potential which makes it a suitable terminal electron acceptor for anaerobic microorganisms. Nitrous oxide reductase (Nos) is the enzyme that catalyzes the reduction of N_2O to dinitrogen gas (N_2). Its active site contains copper (Cu) atoms that are essential for the redox capability of the enzyme. This conversion represents the final step of the denitrification pathway, but it may also occur separately from previous denitrification steps. Soil sink capacities are important factors to ecosystem N_2O fluxes and tied to the activity of microbial respiration. Thus, the production of N_2O can occur over microbially mediated or purely chemical (abiotic) reactions, whereas substantial N_2O consumption can only be of biotic nature under the conditions met in anoxic peat soils. Since Fe plays a central role in the abiotic formation of N_2O (*3*, *4*) and Cu in the consumption of N_2O, we specifically addressed dynamics of soil metals as possible underlying regulators for soil N_2O emissions.

To what extent does abiotic N_2O production occur (i) and how does soil Fe and Cu content correlate with production rates (ii)? We approached question (i) using ^{15}N-labelled nitrite that we added to Argon-flushed soil microcosms in the field. Control incubations (water added) were accompanied by incubations amended with zinc chloride. Zinc has been used as inhibitor of microbial activity (*5*) and we added it to halt microbial interference, at least for the time of the experiment. All five sites received also a ^{15}N-N_2O tracer in separate incubations that served the purpose to track N_2O consumption. Unfortunately, we ran into analytical problems with detecting ^{15}N-N_2 (product pool) and we are currently optimizing the

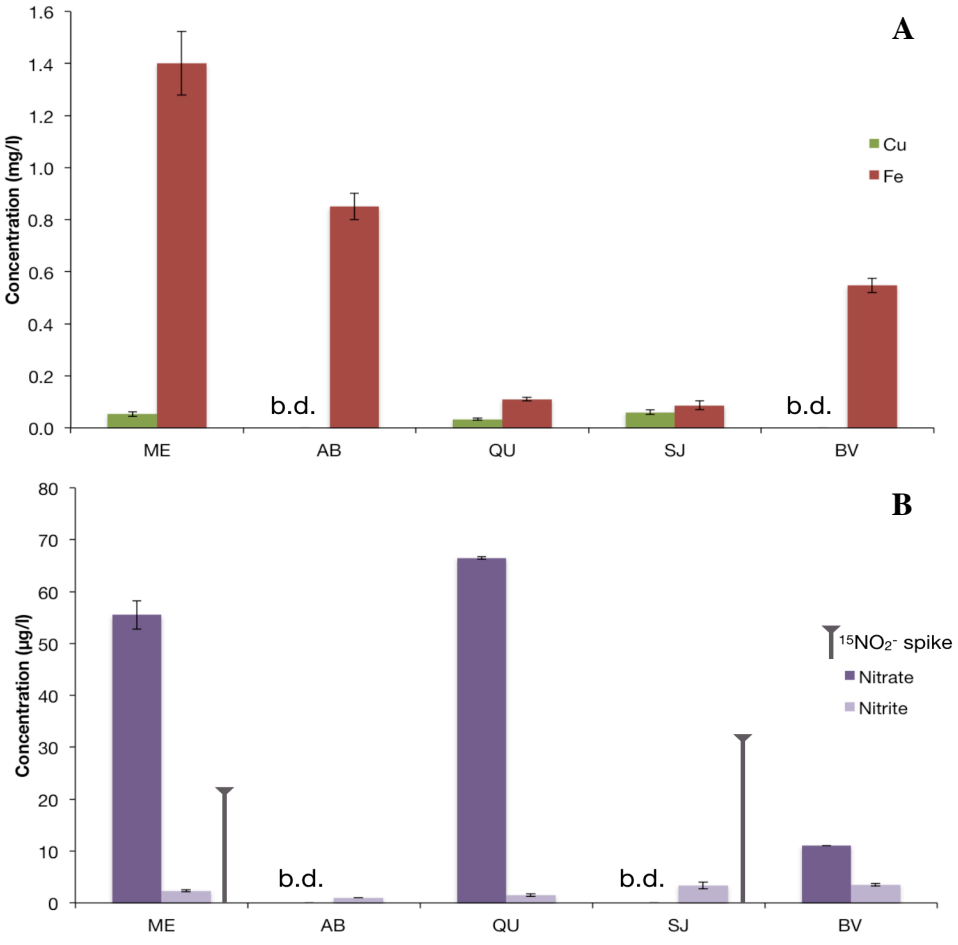

Fig. 3. Soil pore water concentrations of metals (**A**) and inorganic nitrogen (**B**) from various tropical peat soils. Metal concentrations comprise the total amount of Fe and Cu species in dissolved form. Panel B contains a mark for the height of the ^{15}N-nitrite spike caused by injection into the anoxic microcosms. Missing columns mean the measured result was below the detection limit (b.d.) of the photometer.

experimental set up to retrieve data on N_2O consumption. Two sites were chosen for the ^{15}N-nitrite tracer experiment, that were very different in terms of Fe abundance and pH. Soil from ME and SJ was prepared in an anaerobic glove bag in the field and incubated for 24 hours with initially 1.5 μM ^{15}N-nitrite. The label fraction was 10%, meaning $X = {}^{15}N / ({}^{15}N + {}^{14}N) = 0.1$ (*4*, *6*). The microcosms were kept in thermo-cylinders to lower the fluctuations of temperature which may influence the reaction rates. After the incubation, headspace of the closed microcosms was sampled with a gas-tight syringe and stored with high care taken to minimize any possible N_2 contamination from the air. Bulk ^{15}N-N_2O was measured on an isotope ratio-mass spectrometer (IR-MS) interfaced with a trace gas chromatographic system. Rates of N_2O production were calculated as described previously (*4*). Figure 2 shows insignificant differences (student's t-test on non-transformed and normalized data, $P > 0.05$) between a) the soils and b) the treatments. The rates in zinc-treated incubations (abiotic signal) are comparable to previous observations (*3*). To assess a) we looked at metal abundances to test for possible correlations in the apparent production rates with Fe and Cu concentrations (next paragraph). To explain b), we concluded the abiotic component of N_2O production to be competitive to the biotic one. This would mean that nitrite, which can either be produced from the reduction of nitrate, or via nitrification (typically an aerobic process), can react spontaneously with soil constituents or can be respired by denitrifying bacteria to similar extents. It should be noted that in order to fully elucidate the proportions we have to determine the rate of concomitant N_2O consumption in the non-treated controls. Consumption may explain why some microcosms showed lower rates (< 5 pmol g^{-1} h^{-1}) when zinc was absent (Fig. 2). Nevertheless, the apparent potential of abiotic N_2O production is remarkable, especially in light of N_2O production that is in models commonly accounted for as part of microbial denitrification only.

We tackled question (ii) by photometrically measuring Fe and Cu levels in soil pore water of all sites. In addition we also quantified nitrate and nitrite to reveal the native abundance and to determine the relative amount of substrate (^{15}N-nitrite) to be added. For this, pore water of the top 5 cm soil was withdrawn with a hand-made sampler and filtered using a 0.2 μm syringe filter. A portable photometer was then used to measure compound concentrations based on reaction with respective reagents that were added as tablets. The results of the *in-situ* photometric analysis are illustrated in Figure 3. A succession of the Fe abundance in pore water becomes apparent, with declining concentrations from ME (highest), over AB, to the lowland sites BV, QU, SJ. This pattern makes sense in that the higher altitude sites are more affected by Fe mineral sources from weathering bedrock. The ombrotrophic site SJ is a dome-type bog with high organic matter deposition and hence low levels of Fe. Cu levels were generally low across all sites. Nitrate concentrations peaked in ME and QU. Nitrite showed a low but consistent background measured in all sites. We infer dynamic production and consumption processes to shape the nitrite pool. Nitrite is a very transient inorganic nitrogen species and is reactive towards inorganic and organic soil constituents. Any nitrite produced may rapidly be consumed.

Conclusively, there is no apparent correlation between N_2O production rates and Fe or Cu content with the rates of abiotic or biotic N_2O production. This does not mean that the parameters investigated do not interact with each other, but that the methodology used may pose limits in determining evidence of interaction. For example, Fe^{2+} is the tentative reductant of nitrite in the chemodenitrification pathway. However, based on the photometry applied we were only able to monitor total Fe content and not the ratio of reduced versus oxidized Fe. Moreover, humic substances likely play a role in the re-reduction of Fe, which we did not account for in our experimental design. Nevertheless, the preliminary results on the abiotic N_2O production rates are intriguing evidence of the importance of non-enzymatic reactions in these ecosystems. N_2O emissions may be based on spontaneous chemical transformations of nitrite to yet underestimated proportions. In the future, we will attempt to tease out the drivers of this coupled abiotic-biotic process in order to better understand the molecular mechanism behind our observations.

References

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