

Bacterial Response from Exposure to selected Aliphatic Nitramines

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Aim

Contribute to environmental risk assessment of amine based Post-combustion CO₂ Capture (PCC) plant by assessing response in bacteria from exposure to nitramines through:

1. Respiration (aerobic)
2. Metabolic community structure

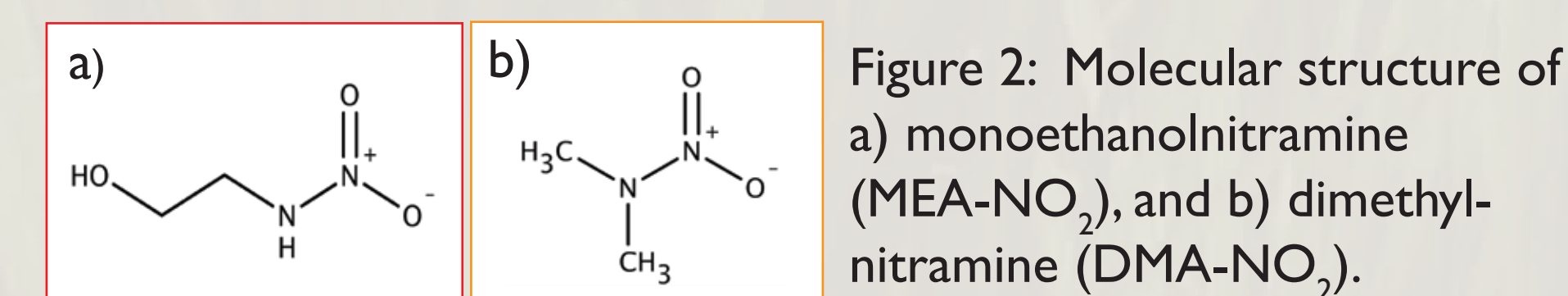
Materials and Methods

Bacterial samples

- i. Lake water containing natural bacterial community was collected from an oligotrophic and a eutrophic lake. Filtrated through 2.0 µm membranefilter.
- ii. Pure bacterial culture of *Bacillus subtilis*. Grown for 24 h in liquid lysogeny broth. Diluted to ~10⁶ cells mL⁻¹ in phosphate buffered saline (PBS).

Nitramine standards

Standards of monoethanolnitramine (MEA-NO₂) and dimethylnitramine (DMA-NO₂) (Fig. 2) of purity > 99%. Dissolved in either PBS or distilled water.



1. Respiration (aerobic)

O₂ levels measured every 15 s for 48 h using PreSens Sensor®Dish reader system (Fig. 3). Concentrations of nitramine standard of ≤ 200 mg L⁻¹. Positive control of CuSO₄ (1×10⁻⁴ M). n = 4.



Figure 3: PreSens Sensor®Dish reader system with multiple 5 mL sample vials. Outline of the sensor spot present in each vial. (www.presens.de)

2. Metabolic community structure

Metabolic community structure of natural bacterial community assessed using BIOLOG Ecoplate™, with 31 different carbon substrates (n=3). Plates incubated in duplicates. Final concentration of the nitramine standards 25 mg L⁻¹. Read periodically over 100 h using a BioTek® Synergy™ Mx plate reader.

Nitramine quantification

Nitramines in samples of pure culture *B. subtilis* was quantified using LC-MS/MS. LC system of UltiMate3000 (Thermo Scientific), and a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific).

Background

Nitramines are formed from atmospheric degradation of amines co-emitted with the cleaned flue gas from an amine based PCC plant^{1,2} (Fig. 1). Nitramines are expected to be long-lived in the environment³. There is currently a lack of knowledge regarding the ecological impact these nitramines may have⁴.

Natural Bacterial Communities are generally the first to encounter newly anthropogenically emitted compounds. These bacterial communities are crucial for a well-functioning ecosystem in that they mediate processes such as in decomposition and in nutrient cycling.

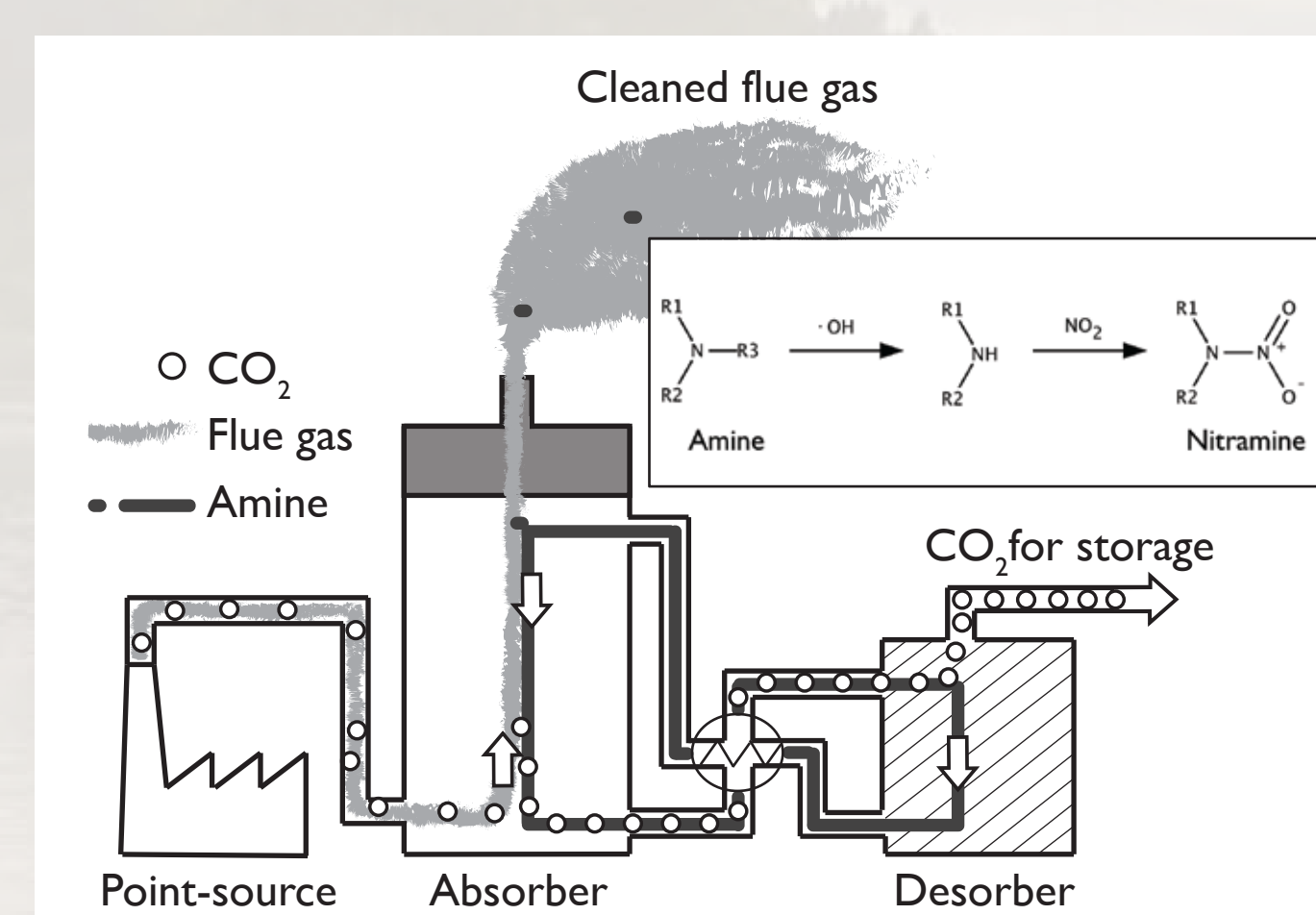


Figure 1: Nitramine formation from atmospheric degradation of amines co-emitted with the cleaned flue gas from a PCC plant.

Results and Discussion

1. Respiration (aerobic)

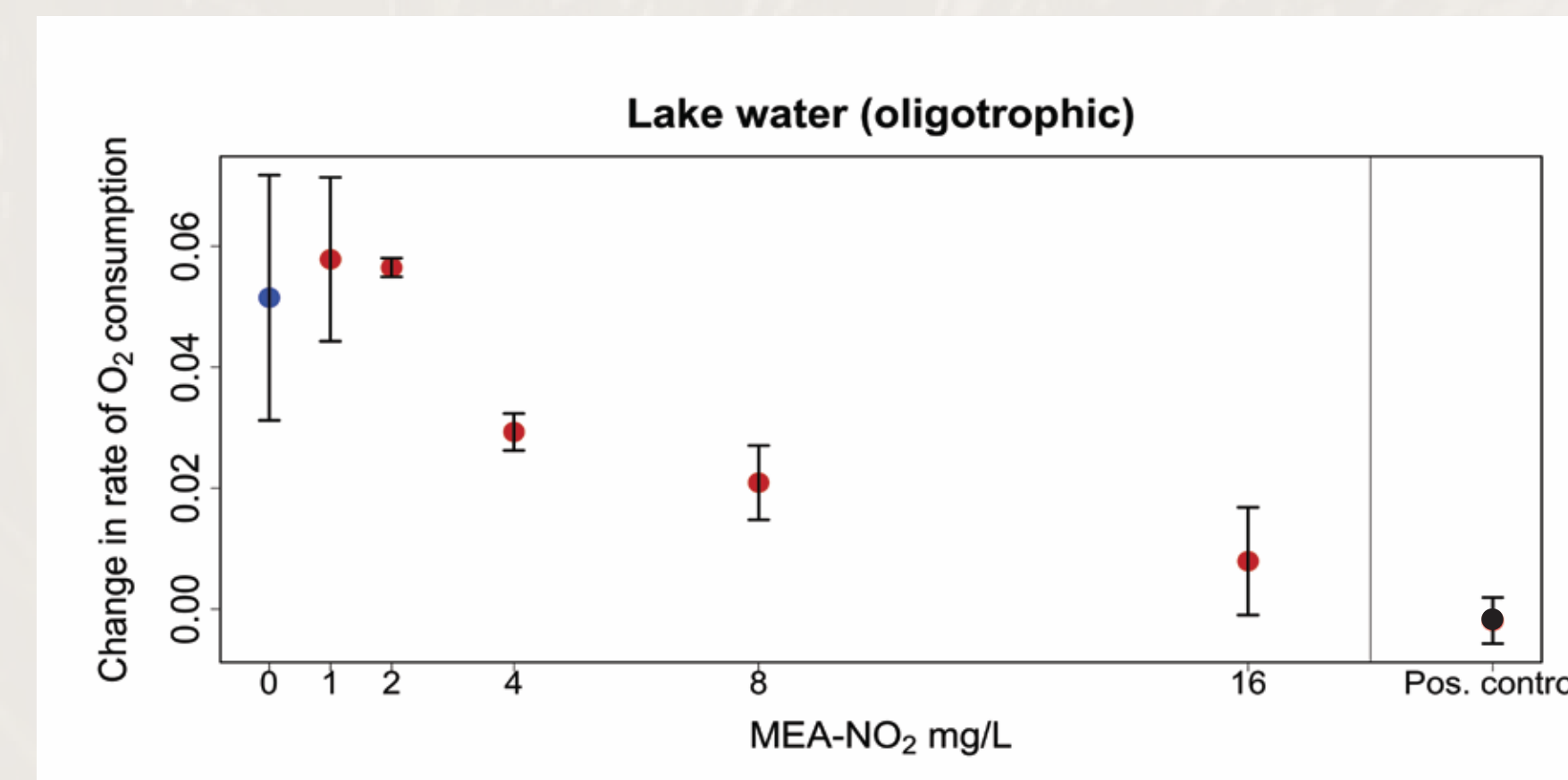


Figure 4: Change in rate of O₂ consumption versus exposure level of MEA-NO₂ (red) for natural bacterial community from the oligotrophic lake. Blank (blue) and positive control (black) included for comparison.

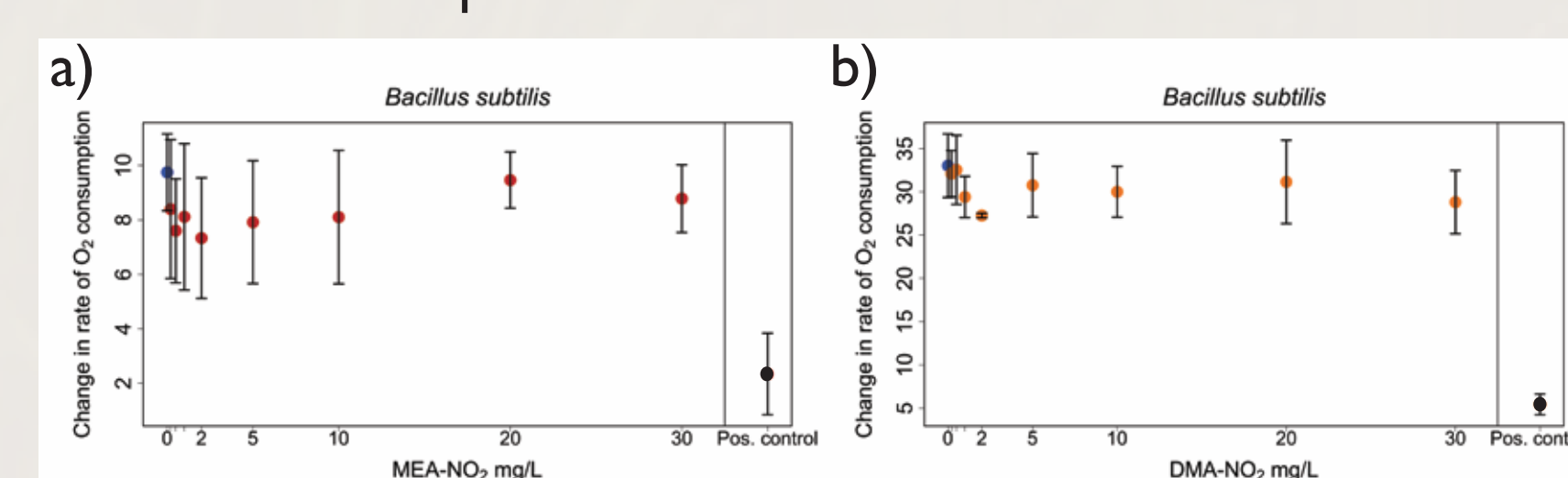


Figure 5: Change in rate of O₂ consumption versus exposure level of a) MEA-NO₂ (red) and b) DMA-NO₂ (orange) for pure culture of *B. subtilis*. Blank (blue) and positive control (black) included for comparison.

2. Metabolic community structure

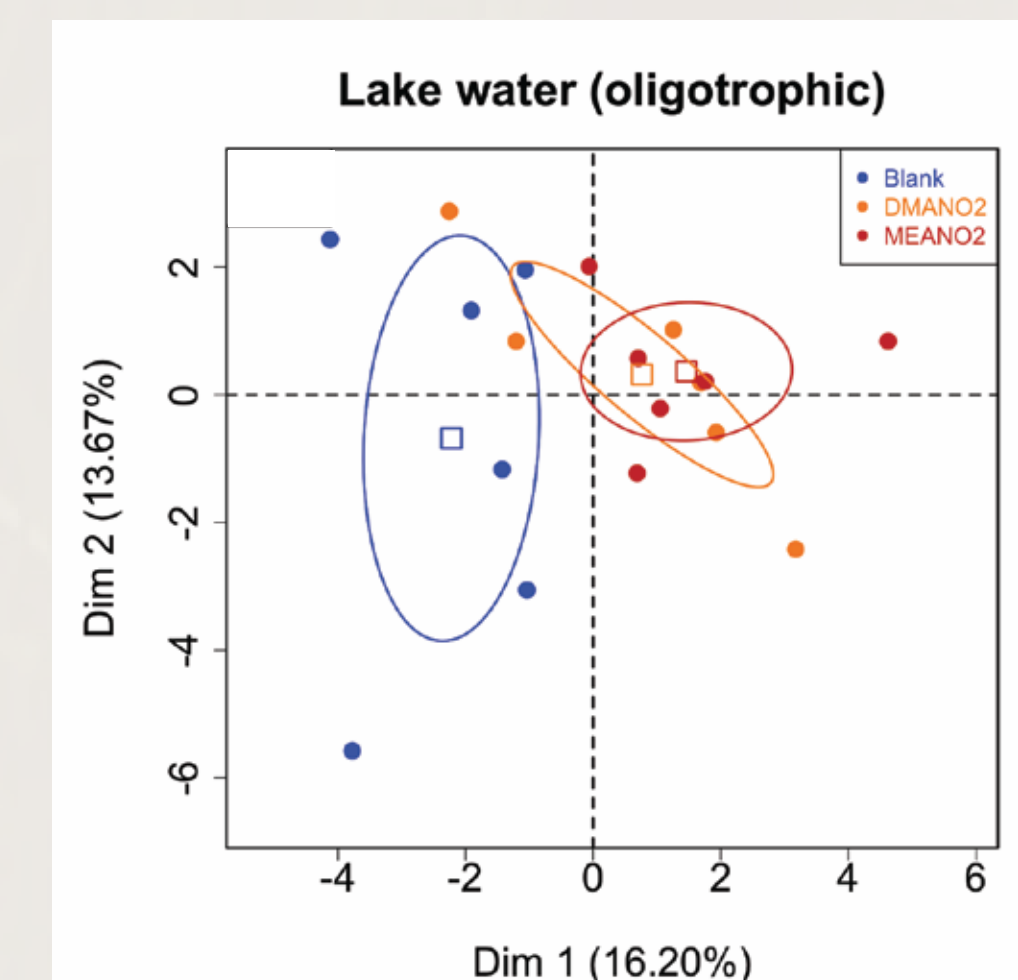


Figure 6: Principle component analysis of carbon substrate utilization data for the oligotrophic bacterial community.

There is a slight shift in the utilization of the 31 different ecological relevant carbon substrates. The three different treatments significantly accounts for variation in data along Dim1 for both lake samples (Fig. 6 and 7). Blank treatment (blue) is significantly different from the nitramine exposures. For eutrophic lake, MEA-NO₂ (red) is also significantly distinct (Fig. 7).

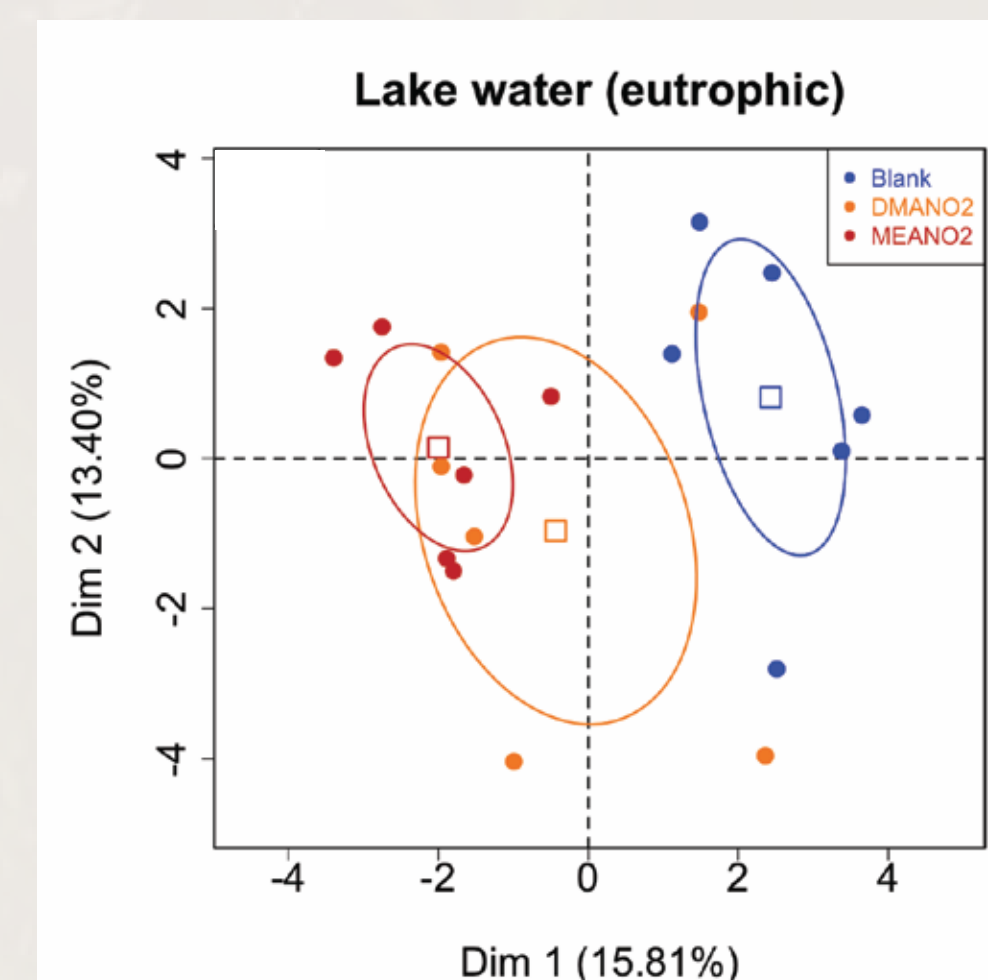


Figure 7: Principle component analysis of carbon substrate utilization data for the eutrophic bacterial community.

Conclusions and Future work

- Results show that the nitramines tested affect natural bacterial communities from lake waters, both by reducing rate of aerobic respiration (MEA-NO₂) and by inducing shifts in the utilization of different carbon substrates (MEA-NO₂ and DMA-NO₂).
- No effect is found in aerobic respiration of pure culture *Bacillus subtilis* from exposure to neither MEA-NO₂ nor DMA-NO₂.
- More experiments are needed to fully reveal what effect these aliphatic nitramines may have on bacteria.
- It is recommended for future studies to also focus on anaerobic processes, and especially those involved in the nitrogen cycle.