# Hydroxylamine production by *Alcaligenes faecalis* challenges the paradigm of heterotrophic nitrification

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## SUPPLEMENTARY INFORMATION

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- Supplementary Figures S1 - S4

- Supplementary Table S1 (separate Excel file): Log2-fold changes (LFC) of genes that showed significant differential gene expression (LFC > 1.5, FDR < 0.05) in any of pairwise comparison between conditions C/N 20, 8, and 4. Frame, begin, and end columns are relative to AFN_v2_0001. Gene and product columns are a combination of manual and automatic annotations using the MicroScope annotation platform (see methods).

## SUPPLEMENTARY FIGURES

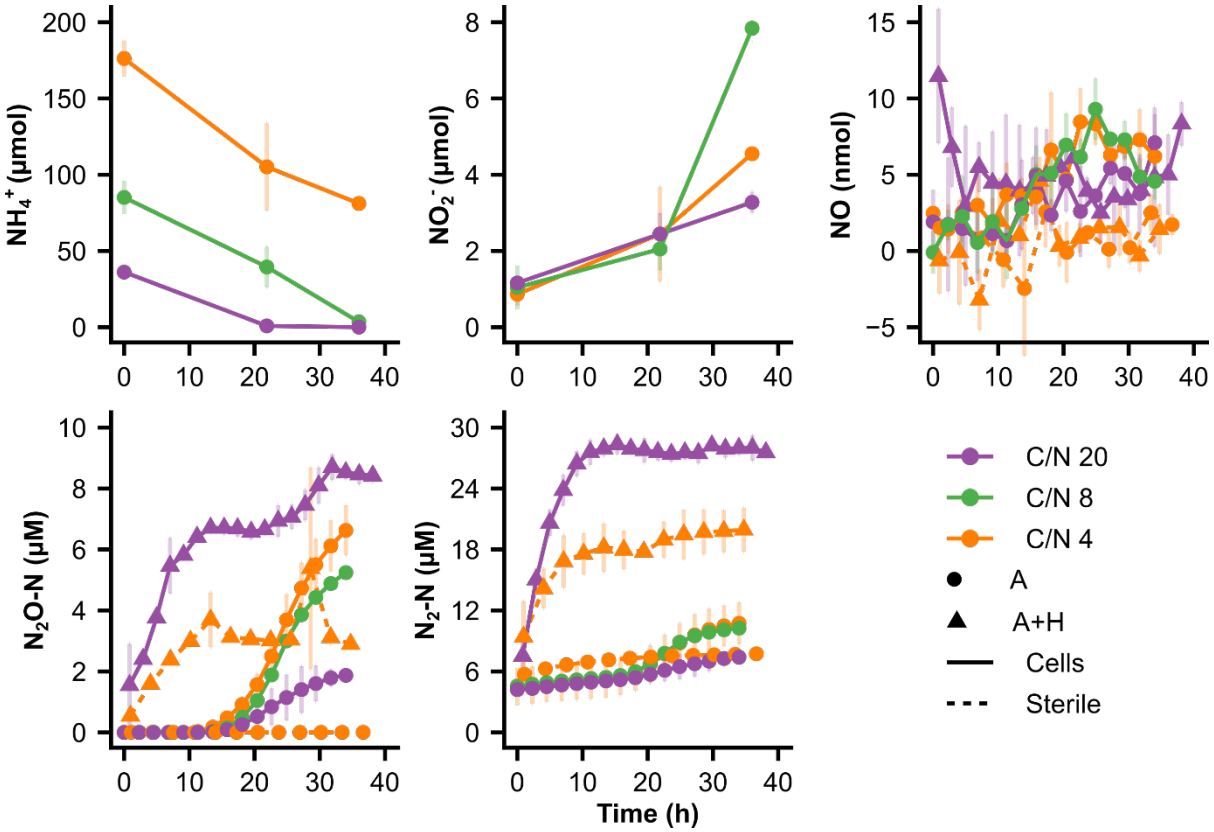


**Figure S1**. $NH_4^+$ consumption and subsequent production of $NO_2^-$, NO, $N_2O$, and $N_2$ by *A. faecalis*. Colors indicate the C/N ratio condition, closed circles represent incubations with $NH_4^+$ as the sole nitrogen source (condition A), closed triangles represent incubations with additionally 1 mM (orange) or 3 mM (purple) $NH_2OH$ added (condition A+H). Solid lines represent incubations with *A. faecalis*, and dotted lines experiments in sterile medium. Data points and error bars represent the mean and standard deviations, respectively, of three biological replicates.

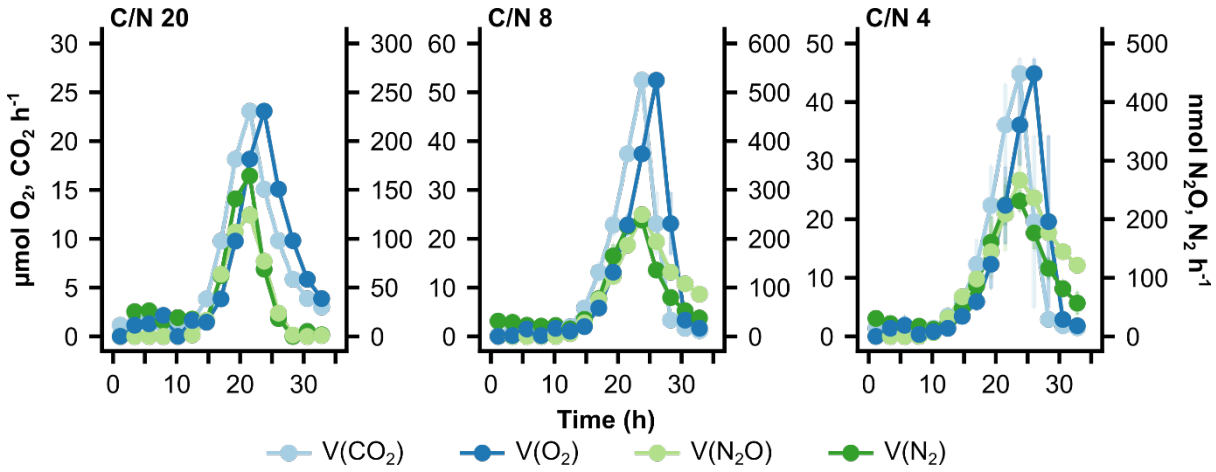


**Figure S2**. Rate kinetics of $CO_2$, $N_2O$, and $N_2$ production and $O_2$ consumption by *A. faecalis* in batch culture at C/N ratios 20, 8, and 4. Data points and error bars represent the mean and standard deviations, respectively, of three biological replicates.

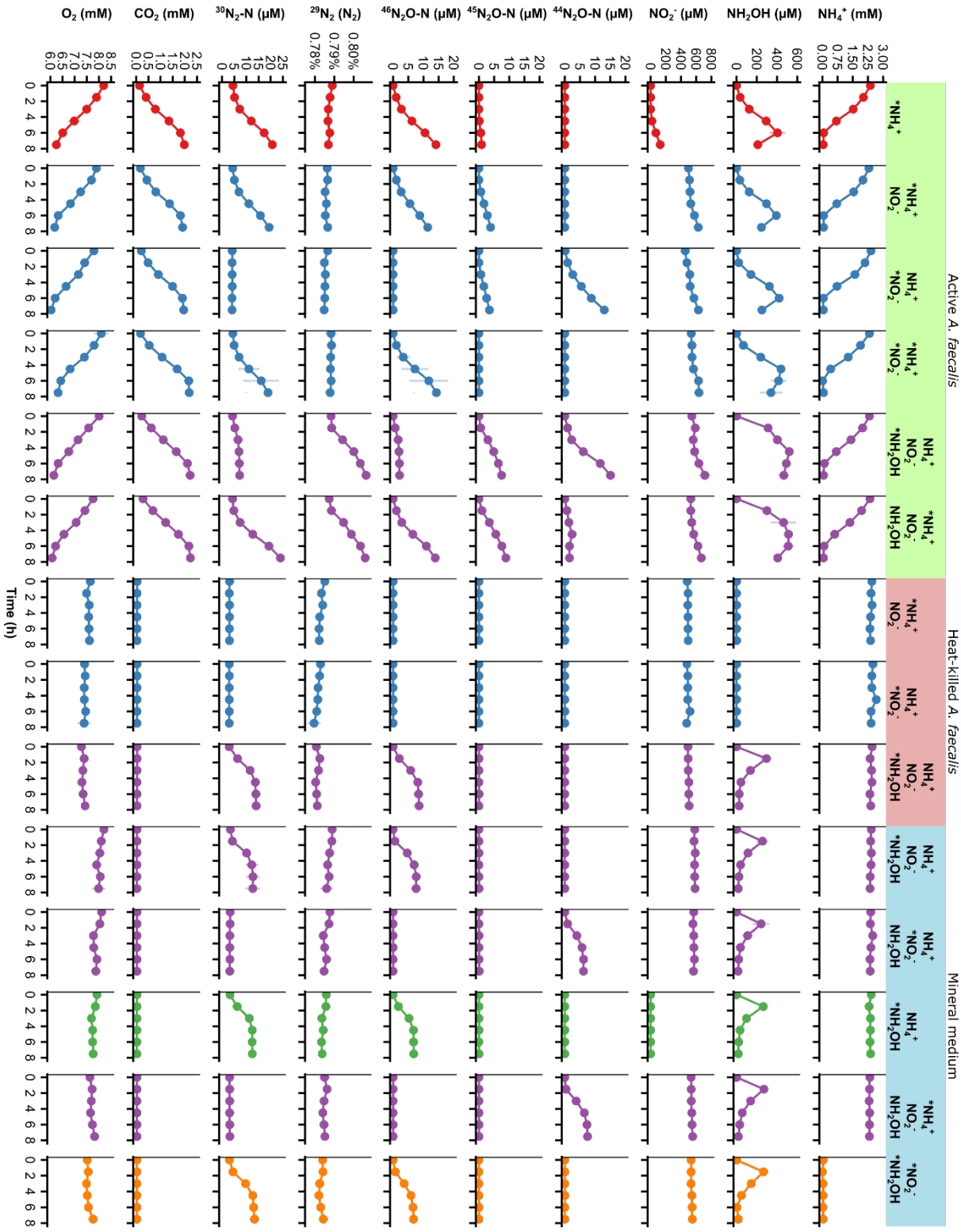


**Figure S3**. Measurements of $CO_2$, $O_2$, and nitrogen compounds in batch incubations with *A. faecalis*, heat-killed *A. faecalis*, or mineral medium. Line colors indicate which nitrogen compounds were added (column headers, vertical). Asterisks in the column headers indicate the nitrogen compounds added as $^{15}N$ stable isotope. Data points and error bars represent the mean and standard deviations, respectively, of three biological replicates.

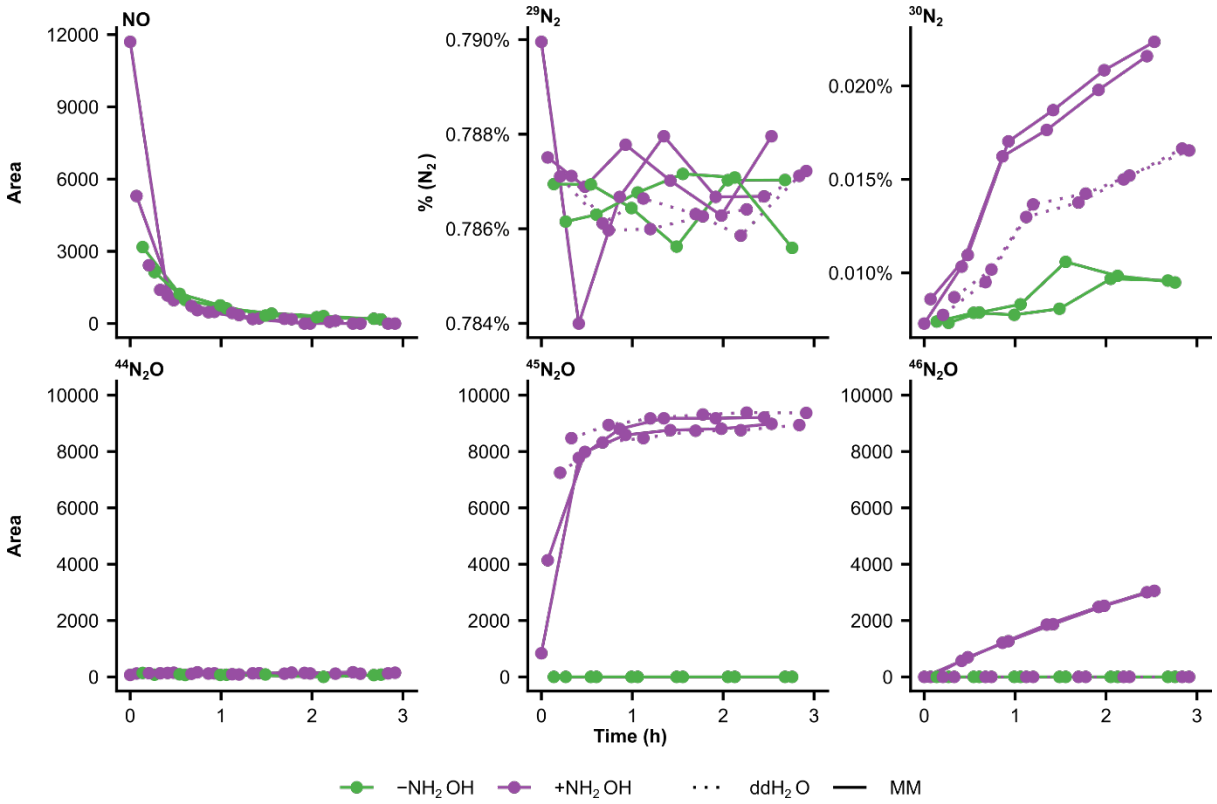


**Figure S4.** Abiotic reactions of NO with and without $NH_2OH$ to $N_2O$ and $N_2$. Shown are incubations with NO only (green lines) and with NO and $^{15}$N-labelled $NH_2OH$ (purple lines), either in demineralized water (dotted lines) or in mineral medium (solid lines). Note that $^{28}N_2$ formation was not determined. Duplicate experiments are shown as separate curves.