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Dynamics of nitrous oxide production pathways analysed by $^{15}\text{N}/^{18}\text{O}$ dual isotope labelling – data from a full-scale wastewater treatment plant

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Nitrous oxide production associated with biological nitrogen transformations can contribute substantially to the CO₂ footprint of both man-made and natural systems, but the pathways and regulation of nitrous oxide production are poorly understood. We developed a $^{15}\text{N}/^{18}\text{O}$ dual isotope labelling technique to distinguish and quantify these pathways in mixed communities. The use of $^{18}\text{O}\text{-O}_2$ permits differentiation of hydroxylamine oxidation and nitrifier-denitrification driven nitrous oxide production by ammonium oxidizing bacteria. We analyzed nitrous oxide production pathways during biological nitrogen removal at Lynetten wastewater treatment plant, Denmark. Under anoxia, nitrous oxide accumulated due to denitrification, but nitrous oxide accumulation was ~3 and 1.7 times higher at 30 and 100 $\mu\text{M O}_2$, respectively. Oxic nitrous oxide production was dominated by nitrifier-denitrification, reaching 73% of the total, with the remainder due to hydroxylamine oxidation. Our results demonstrate three active pathways of nitrous oxide production, each with different environmental controls. The dual $^{15}\text{N}/^{18}\text{O}$ isotope labelling approach can contribute to the development of strategies to minimize nitrous oxide emissions from man-made and natural systems.