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Dynamics of N₂O production pathways analysed by ¹⁵N/¹⁸O 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 N₂O production are poorly understood. We developed a ¹⁵N/¹⁸O dual isotope labelling technique to distinguish and quantify these pathways in mixed communities. The use of ¹⁸O-O₂ permits differentiation of hydroxylamine oxidation and nitrifier-denitrification driven N₂O production by ammonium oxidizing bacteria. We analysed N₂O production pathways during biological nitrogen removal at Lynetten wastewater treatment plant. Under anoxia, N₂O accumulated due to denitrification, but N₂O accumulation was ~3 and 1.7 times higher at 30 and 100 μM O₂, respectively. Oxic N₂O 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 N₂O production, each with different environmental controls. The dual ¹⁵N/¹⁸O isotope labelling approach can contribute to the development of strategies to minimise N₂O emissions from man-made and natural systems.