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# Dynamics of N<sub>2</sub>O production pathways analysed by <sup>15</sup>N/<sup>18</sup>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 CO<sub>2</sub> footprint of both man-made and natural systems, but the pathways and regulation of N<sub>2</sub>O production are poorly understood. We developed a <sup>15</sup>N/<sup>18</sup>O dual isotope labelling technique to distinguish and quantify these pathways in mixed communities. The use of <sup>18</sup>O-O<sub>2</sub> permits differentiation of hydroxylamine oxidation and nitrifier-denitrification driven N<sub>2</sub>O production by ammonium oxidizing bacteria. We analysed N<sub>2</sub>O production pathways during biological nitrogen removal at Lynetten wastewater treatment plant. Under anoxia, N<sub>2</sub>O accumulated due to denitrification, but N<sub>2</sub>O accumulation was ~3 and 1.7 times higher at 30 and 100 μM O<sub>2</sub>, respectively. Oxic N<sub>2</sub>O 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 N<sub>2</sub>O production, each with different environmental controls. The dual <sup>15</sup>N/<sup>18</sup>O isotope labelling approach can contribute to the development of strategies to minimise N<sub>2</sub>O emissions from man-made and natural systems.