Supplementary information for: The isotopic signatures of nitrous oxide produced by eukaryotic and prokaryotic phototrophs

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Supplementary information 1: Denitrifiers cultures

Denitrifying inocula were cultivated in closed Duran bottles containing simulated domestic wastewater (**Table S1**). These reactors were seeded with a pond sediment suspension (Massey University) and continuously mixed using magnetic agitation at 100 rpm. Once denitrification was confirmed (via nitrate monitoring), the reactors were operated as sequential batch reactors by gradually increasing the feeding rate to reach a hydraulic retention time (HRT) of 10 days. This operation was achieved by i) turning the agitation off and letting biomass to settle for 1 hour before ii) removing 100 ml of culture broth using a syringe (via a sample port) and 3) injecting 100 ml of fresh medium before resuming agitation. The settling period was discontinued once a strong denitrifying ability was evidenced to prevent the excessive accumulation of dead biomass.

Table S1: Denitrification medium

Solution/salt	Composition (g/L)	mL added/L
Organic solution	Starch (2.1), milk powder (2), dried yeast (0.9), soy oil (0.5)/L, peptone (0.3)	100
NO_3	KNO_3 (14.43)	120
Mg	$MgCl_2 \cdot 4H_2O$ (4.19)	100
Phosphate buffer	K_2HPO_4 (2.178) and KH_2PO_4 (0.612)	560
Ca	$CaCl_2 \cdot 2H_2O$ (1.33)	100
Trace element 1	$FeSO_4$ $7H_2O$ (9) and EDTA (6)	10
Trace element 2	EDTA (15), $ZnSO_4 7H_2O$ (0.43), $CoCl_2.6H_2O$ (0.24), $MnCl_2 4H_2O$ (0.99), $CuSO_4.5H_2O$ (0.25), $NaMoO_4 2H_2O$ (0.22), $NiCl_2 6H_2O$ (0.19), H_3BO_3 (0.014)	10

Supplementary information 2: Analytical blind test using isotope analysis to determine fractions of N_2O from two biological sources in a gas mixture

Aliquots of N_2O produced by denitrifiers (60 %) and *Chlorella vulgaris* (40 %) were mixed following the incubation experiments described in the manuscript. N_2O was analysed has described in the manuscript. Using the isotope measurement of N_2O from the pure cultures, the fractions of each N_2O source were calculated in a blind experiment, to test for the robustness of the method developed.

The fractions determined using all measured tracers $\delta^{15}N\alpha$, $\delta^{15}N\beta\alpha$, $\delta^{15}N(bulk)$ and especially SP-N_2O are in good agreement with the fractions used during the preparation (**Figure S2.1**), except for $\delta^{18}O$ (not shown), where the initial values of denitrifiers and *C. vulgaris* are too similar for differentiation with our analysis.

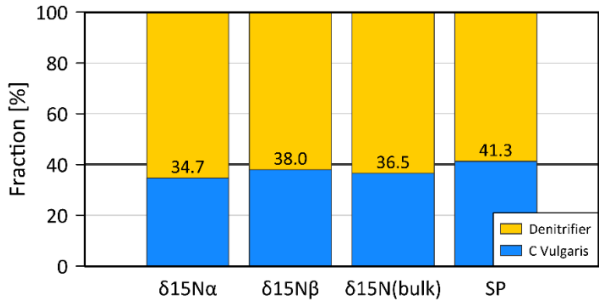

Figure S2.1: Bar plot showing fractions of N_2O from *Chlorella vulgaris* and denitrifiers synthetic gas mixture prepared during incubation experiments. Fractions are calculated based on isotope ratios measured in pure N_2O from the respective culture. The thick black line shows the target in this blind test.