The manuscript of Malte Winther et al. describes the real-time analysis of site-specific  $N_2O$  isotopic composition from two denitrifying bacterial strains with a novel Picarro CRDS analyser. A setup for a closed-loop experiment was designed and applied in a number of prototype experiments. A correction function was developed for the spectrometer raw data and a modified Rayleigh model applied to derive fractionation factors.

The manuscript is an important contribution to research on N<sub>2</sub>O isotopes and therefore of interest for a number of readers of Biogeosciences. The presented interpretation of singular incubation experiments might be questionable; at least given the "surprising" results, e.g. for  $\epsilon_{SP}$  of N<sub>2</sub>O reduction. But the manuscript should still be accepted after a number of minor revisions as detailed below:

Page 1 Line 8: The main application of the instrument might be for biogeochemical applications, e.g. soil sciences, at enhanced concentrations and not for atmospheric chemistry.

Page 1 Line 10 – 11: The expression "... reveal the transient pattern" is incomplete.

Page 1 Line 15 – 17: The explanation for the SP isotopic fractionation for  $N_2O$  reduction above zero, "diffusive isotopic fractionation and a difference in active enzymes during production of  $N_2O$ ", is not convincing.

Page 2 Line 18 – 19: Please rephrase the sentence "The position in the N<sub>2</sub>O molecule are named ... " to "N<sub>2</sub>O molecules with <sup>15</sup>N substitution in the central or terminal position are named <sup>15</sup>N<sup> $\alpha$ </sup> for <sup>14</sup>N<sup>15</sup>N<sup>16</sup>O or <sup>15</sup>N<sup> $\beta$ </sup> for <sup>15</sup>N<sup>14</sup>N<sup>16</sup>O, respectively."

Page 2 Line 25: Please rephrase the expression to "... to enable continuous and selective measurements of the isotopomer abundances."

Page 3 Line 4 – 6: The sentences "The primary anthropogenic sources of  $N_2O$  are organic and inorganic N fertilizers used for agriculture. The natural sources are primarily nitrification and denitrification in terrestrial and aquatic ecosystems." are misleading as the biotic (and abiotic) source processes for anthropogenic and natural  $N_2O$  emissions are similar, but anthropogenic emissions are enhanced due to fertilizer application. Please rephrase the sentences.

Page 3 Line 17 – 18: The expression "..., the cleavage of N<sub>2</sub>O is expected to have an increased fractionation effect on <sup>15</sup>N<sup> $\alpha$ </sup>, due to ..." might be rephrased to "the cleavage of N<sub>2</sub>O is expected to fractionate in favour of the <sup>15</sup>N<sup> $\alpha$ </sup> molecule, due to ...".

Page 3 Line 17 – 19: The statement that "diffusion into the cell (Tilsner et al., 2003) and enzymatic reduction (Wrage et al., 2004)" might be deleted.

Page 4 Line 8: The phrase "by placing the sample delivery system ... in a closed loop" might be rephrased to "by a closed-loop gas flow through the ...".

Page 4 Line 13:  $Mg(CIO_4)_2$  is the chemical formula for Magnesiumperchlorate used for drying the measuring gas, but not for Ascarite used for removing  $CO_2$ . The scheme in Figure 1 shows the correct setup of the trap.

Page 4 Line 22 – 29: Please re-write this section, as the same information, that a concentration dependent correction for delta values is needed is given several times.

Page 5 Line 5: The section on the O2 correction (now Page 6 Line 18 - 23) could better be placed here.

Page 5 Section calibration gases (Table 1): Please check whether there is a mistake in the mean values in Table 1, e.g. the mean of 1.34, 1.08, 2.62 is not 1.32.

Page 6 Line 6: The statement to give  $\delta^{15}N^{\alpha}$  and  $\delta^{15}N^{\beta}$  values for KNO<sub>3</sub> is wrong or at least a misunderstanding.

Page 12 Line 3: The statement that differences in net production rates affect  $\epsilon_{SP}$  seems questionable.

Page 12 Line 26: The statement that higher  $\epsilon_{SP}$  values as reported in literature could be rationalized by diffusive isotope fractionation seems questionable, as diffusion is generally assumed to not affect the N<sub>2</sub>O SP.

Page 13 Line 2: The term "isotope depletion" is incomplete, it should be mentioned which isotopic species is depleted.