The manuscript by Joshua Haslun et al. provides the application of a Rayleigh approach to determine fractionation factors ε for the multi-step NO_3^- to N_2O reduction by *Pseudomonas aureofaciens* and *Pseudomonas chlororaphis*. They observed a curvilinear relationship for $\delta^{15}N\text{-}N_2O$ and $\delta^{18}O\text{-}N_2O$ versus [–f lnf / (1-f)], which they attributed to the inter-play of fractionation factor of different enzymatic steps and diffusion of substrates and products. They provide a novel approach using non-linear least square regression to calculate net isotope effects (n) for the complete reaction progress (f = 1 to 0), which is of interest for the scientific community. In addition the study provides temporal trends for N_2O SP, showing that SP is a robust indicator for specific production pathways.

The manuscript is very well written and thus easy to read and understand. There are no major weaknesses or shortcomings so I suggest publication after minor revisions.

General comments:

The novel approach to calculate the “net isotope effect” (η) is not very intuitive; therefore it should be rationalized how and why $y = b \times e^{bx}$ (formula 5, Page 5 L14) is equivalent to $\delta^{15}N\text{-}N_2O - \delta^{15}N\text{-}NO_3^-$ the standard approach to calculate the net isotope effect. In addition, results of the standard approach and the novel approach should be compared and discussed in the manuscript.

Specific comments:

Page 1 L17 ff: It should be mentioned here and elsewhere in the text that both bacterial strains lack the enzyme for N_2O reduction as this might not be known by every reader of the manuscript.

Page 4 L5: The isotopic composition of the $NaNO_3$ should be given.

Page 4 L15: Which gas volume or range of volumes was sampled into the serum bottles?

Page 4 L26: The isotopic composition of the standards used for IRMS analysis of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{15}N$ and $\delta^{18}O\text{-}N_2O$ should be given.

Page 4 L31: How was f determined? From the amount of substrate provided minus the cumulative amount of N_2O produced? Please add the respective information here.

Page 6 section 3.1 and 3.2: Please provide results for $\eta^{15}N$ and $\eta^{18}O$ using the standard approach to calculate net isotope effects (e.g. $\delta^{15}N\text{-}N_2O - \delta^{15}N\text{-}NO_3^-$).

Page 7 L5: One experiment with *Ps. aureofaciens* and succinate at 1 mM yields N_2O with low SP similar to *Ps. chlororaphis*. How can this be explained or is it just an outlier?

Page 7 L12 – 21: This section, the application of the Rayleigh model, should be preferably placed in the introductory or method section.

Page 9 L14: The statement that SP depends on $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ is trivial and could be omitted. The differences in SP between *Ps. aureofaciens* and *Ps. chlororaphis* are attributed to differences in the NO pool; which experiment could be used to check this hypothesis?

Page 8 (discussion): Temporal resolved data of N_2O isotopic composition as used in this study could be preferably be collected using an online technique, e.g. laser spectroscopy – Please comment.