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Interactive comment on "Short-and long-term temperature responses of soil denitrifier net N_2O efflux rates, inter-profile N_2O dynamics, and microbial genetic potentials" *by* Kate M. Buckeridge et al.

Anonymous Referee #3

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In this manuscript the authors present a laboratory-based study to address three uncertainties in climate projections of net N2O fluxes from soil: (1) short v long-term responses to warming, (2) interactions among soil horizons, and (3) temperatures responses of different steps in the denitrification pathway. While the study itself is sound (although see my comment below about clarification of how net N2O fluxes were estimated), the authors treat denitrifiers superficially and interpret their results without deep consideration of the mechanisms driving the observed patternsâĂŤthat is, they never mention any of the known controls on nitrate reduction and nitrous oxide reduc-

tion by denitrifiers and how warming or soil property differences among soil horizons would affect those controls. Below I detail some of my concerns and hope that my suggestions will help the authors improve the manuscript such that their findings can clearly advance our understanding of how warming affects soil denitrification.

The physiological rationale for enhanced rates of complete denitrification under long-term temperature regimes should be explained in order to justify this hypothesis. The following language is currently used to justify and describe this hypothesis: "less effective processing" by denitrifiers (Line 75) leading to more incomplete denitrification; denitrifying communities as "efficient transformers of NO3- to N2" (Line 137); "a soil denitrifying community well-adapted to its temperature regime is adept at complete denitrification"; and "denitrifier performance" (Line 415). But what is meant by "effective, "efficient," "adept," and "performance" as it relates to microbial physiology? Denitrifiers are mostly facultative anaerobes that can utilize various metabolisms other than nitrate reduction or nitrous oxide reduction depending on environmental conditions. There was no mention of controls on the actual processes of nitrate reduction and nitrous oxide reduction (e.g., nitrate availability, soil redox) anywhere in the manuscript, which severely undercuts the hypothesis and the interpretation of the results.

Lines 138-146: These three hypotheses are actually predictions (i.e., expected results). In the introduction, there is little justification presented for why these results would be expected other than similar patterns have been observed for heterotrophic respiration. As the authors acknowledge, denitrification is a more complicated process because it includes multiple enzymatic steps. But physiologically, the controls on denitrification are also different from heterotrophic respiration, and that needs to be considered.

The calculation of net N2O fluxes is core to the validity of the results of this study, so this vague statement on lines 202-203 needs to be clarified. Please explain what is meant by "the robustness of the final 60 h time point measure," how the multiple times points were used to verify this, and what is meant by "the net results of these samples." In addition, I recommend that the authors add a supplementary figure that shows the

net N2O fluxes calculated for each of the time points so that the readers can see what patterns got washed out by averaging the fluxes observed over the 60 hour incubation (Lines 219-222).

Line 227-242: Based on the equation presented in line 238, it is not the change in 15N enrichment of the N2O that is used to estimate N2O reduction rates as stated on line 227 but rather 15N2O abundance. Also, on lines 231, 234, 440, and elsewhere in the manuscript, only "15N2O" is referred to but it would be clearer to the reader if "15N2O abundance" was specified.

Lines 255-258: Please clarify what is meant by nirS and nosZ clade I as being "the most tractable indicators of N2O production and reduction." What does "tractable" mean, and how was this assessed? Please also specify which other functional gene primers were tested (including citations for the primers used) so that readers can interpret why these genes may have failed to amplify. Primers vary in their coverage of the diversity of microbes harboring a given functional gene, so the selected primers may not have been able to detect the relevant organisms present (see Ma et al. 2019, Environmental Microbiology). For example, only recently was a suite of primers developed that provides better coverage for the many subclades of nosZ clade II (see Chee-Sanford et al. 2020, Journal of Microbiological Methods).

Lines 411-413: This discussion of differences among the sites representing different long-term climate regimes needs to be expanded on. How would differences in soil organic matter input and nitrogen availability among the sites confound the interpretation of warming effects on N2O dynamics? The authors should consider how these potential confounding factors influence nitrate reduction and nitrous oxide reduction rates based on our understanding of controls on denitrification.

Lines 498-509: I would delete this paragraph about "contrasting efficiencies of N2O scavenging" which is speculative and not founded in an understanding of microbial physiology. What is meant by "efficiency"? The rationale presented does not consider

why microbes would reduce N2O nor differing conditions in mineral versus organic soils that would cause differences in N2O reduction rates in these two horizons.

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