Review of Ibraim et al: “Attribution of N2O sources in a grassland soil…”

By Nathaniel E. Ostrom, Michigan State University

The authors have carefully addressed the issues raised in review and overall this is an excellent manuscript with a compelling data set. I remain concerned with three points that were raised in my initial review.

1. Reporting of isotope standards outside the range of the standards. There is no doubt that the authors have an extensive history in the development of N2O standards and international collaboration to attain that goal. And I agree that the authors are conducting the “current best practice(s)” in the field. But this does not avoid the issue that, particularly with regard to d18O, the sample values are well outside the range of the standards and, further, the range in d18O values for standards is quite small. The statement that “… the linearity of the delta scale for QCLAS measurements was demonstrated already in 2008 (Waechter et al., 2008)” is not very satisfactory as spectroscopic instruments are very susceptible to drift and require “frequent calibration” (Waechter et al., 2008). Further, linearity does not equate to accuracy. In that study, the d18O values for the standards ranged about 100 per mil vs only a few per mil in this study. As in archery, accuracy becomes much more difficult the further from the target you are. I agree that international standards are not available in this range, but Waechter et al. clearly demonstrated that working standards can be generated (although I expect that there is some uncertainty in the accuracy of those standard values). Thus, the authors are following best practices, but the standard should not be best practices, but do we have sufficient confidence in the accuracy of the sample values to publish? Calibration is far behind the applications and this is largely because of the difficulty in developing standards, lack of support from funding agencies and insufficient attention by the scientific community. I, for one, have chosen not to publish d18O values generated spectroscopically because of the limited range of values in our standards. I believe the isotope values I obtain are accurate, but the problem is that I don’t know for certain. Further, the casual reader is very unlikely to be able to make this distinction. I honestly don’t know the solution for this problem and have no desire to hold up publication. My leaning, as indicated, is not to publish the d18O values but, at the least, the authors should indicate that many isotope values are outside the range of the standards which can be problematic to precise calibration.

2. The “benchmark value of 10 per mil for the SP standard deviation” seems rather large given that the difference between microbial sources of N2O is about 30-40 per mil. As long as the authors are clear about this and, perhaps, report the standard deviations the reader will have the ability to evaluate accuracy.

3. I agree that given full exchange between N2O and water during bacterial denitrification the d18O values for N2O should be constant. I find the word “stable” is this context to be less accurate than “constant”. But, what can we expect the d18O values to be when exchange is not 100% and how often does this happen?