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Interactive comment on "Soil Denitrifier Community Size Changes with Land Use Change to Perennial Bioenergy Cropping Systems" *by* K. A. Thompson et al.

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Thank you for your review of our work. Please find responses to your comments below.

First, the adopted DNA soil extraction method do not permit to discriminate between relic DNA pool and the intracellular poll, without considering the PMA approach to discriminate by qPCR between relic and living cells due to contradictory results on its efficiency on soil environment.

Response: Although this is true, at the time this research was conducted (2011-2012), there were no published PMA protocols for environmental matrices, such as soil. Additionally, although some studies have shown an impact of relic DNA on diversity mea-

sures (Carini et al., 2016), others have shown that despite PMA decreasing extracted DNA yields, these decreases did not have a subsequent impact on fingerprinting measures, such as DGGE (Wagner et al., 2015).

In this article, we aren't comparing taxonomic diversity etc. but making comparisons of gene abundances (functional potential) within one soil type between crop treatments. Therefore, the comparisons of gene abundances are still relevant.

Finally, although the use of PMA in environmental matrices is still being improved upon, the efficiency of PMA on different taxa is unknown, and PMA permeability into cells might vary across taxa, indicating that we should interpret PMA-treated data with caution. There has also been some evidence (Taylor et al., 2014) that depending on the environmental matrix assessed and extraction method, at higher concentrations PMA may bind to DNA in viable cells, leaving only dormant state microbes, and therefore not be effective in differentiating viable and non-viable cells.

Paul Carini, Patrick J Marsden, Jonathan W Leff, Emily E Morgan, Michael S Strickland,Noah Fierer. Relic DNA is abundant in soil and obscures estimates of soil microbial diversitybioRxiv 043372; doi: http://dx.doi.org/10.1101/043372

Taylor MJ, Bentham RH, Ross KE. Limitations of Using Propidium Monoazide with qPCR to Discriminate between Live and Dead Legionella in Biofilm Samples. Microbiology Insights. 2014;7:15-24. doi:10.4137/MBI.S17723.

Wagner AO, Praeg N, Reitschuler C, Illmer P. Effect of DNA extraction procedure, repeated extraction and ethidium monoazide (EMA)/propidium monoazide (PMA) treatment on overall DNA yield and impact on microbial fingerprints for bacteria, fungi and archaea in a reference soil. Applied soil ecologyâĂŕ: a section of Agriculture, Ecosystems & Environment. 2015;93:56-64. doi:10.1016/j.apsoil.2015.04.005.

Second, it is no possible to discriminate between the different nitrification/denitrification pathway and the related microbial community.

Response: I think you are inferring that we cannot connect functional potential (gene abundances) with community composition/identification, or process rates. This was not our intent, and we have not attempted to directly link the denitrification pathway with gene abundance data, but have instead assessed the sustainability of these cropping systems based on functional gene abundances involved in the denitrification pathway.

Third, It is also not possible to discriminate which of the detected species is active in the gene function without mRNA detection.

Response: We agree that it is not possible to assess potential activity with DNA-based methods. However, mRNA has a halflife of minutes and was thought to be inappropriate for assessment of denitrifier communities due to the timing of sampling in our study (which was over 2 years). It is more plausible to assess the potential functionality of the soil microbial community to cropping systems when sampling over the long-term than attempting to link differences in mRNA with edaphic factors or agricultural treatments as mRNA may be upregulated in response to short-term (in the order of minutes, hours) changes in soil and climatic properties, while DNA analyses may allow better differentiation of changes due to cropping systems.

Fourth, it is no possible to discriminate between the potential activity and the real activity of the nirS and nosK bacterial species.

Response: We have not attempted to, or claimed to, assess potential activity (mRNA) or real activity (enzymes) of denitrifiers in this study.

Finally result impossible to obtain extremely interesting data by coupling these data with those related to soil N2O/N2 emission.

Response: Although we would have preferred to measure N2O emissions, the field-set up did not permit this. First, the plots were too small and numerous (N=36) to establish eddy covariance/flux towers. Secondly, due to the large root biomass, above ground biomass and overall ground coverage of miscanthus and switchgrass plants,

after consulting with a micrometeorologist, we were advised that it would be impossible to install chambers within our plots without highly disturbing the area, and therefore obtaining biased results. Our focus was in assessing the sustainability of the cropping systems by comparing the functional potential of the soils to produce or consume N2O by quantifying denitrifier gene targets.

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