

Ammonia oxidizer diversity and N₂O: influence of long-term fertiliser application



Lynne M. Macdonald*, M.J. Mitsch, H.H. Janzen, M.J. Clapperton

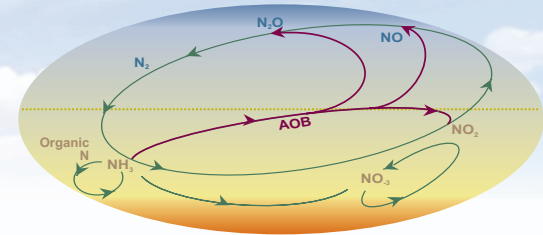
Agriculture and Agri-Food Canada, Research Centre, Lethbridge, Alberta, Canada *macdonaldly@agr.gc.ca

Background

Almost 60% of fertiliser applied to agricultural land is lost from the soil via leaching or gaseous emissions. This loss not only represents economic inefficiency, but is of considerable environmental concern. Nitrate leaching leads to eutrophication of waterways, while N₂O is a potent greenhouse gas and destroys stratospheric ozone. With increasing use of synthetic fertilisers N₂O emissions have greatly increased, accounting for 9.2 Mt CO₂ eq annually from Canadian agriculture.

Understanding the impact of long-term fertiliser application on the nitrogen cycle and micro-organisms involved is key in developing efficient fertiliser use. Ammonia oxidising bacteria (AOB) are responsible for the conversion of ammonia to nitrite, the rate-limiting step of nitrification. In the terrestrial environment the ability to utilise ammonia as a sole energy source, is limited to the subdivision of the class *Proteobacteria*, comprising two genera; *Nitrosomonas* and *Nitrosospira*.

We aimed to determine the influence of long-term fertiliser application on the diversity of AOB, and the N₂O emissions response of the soils to different N sources.



Preliminary studies

- Soil from fertilised (F) and non-fertilised (NF) plots was sampled in spring 2002 (soil details Fig. 1).
- The presence and diversity of AOB was assessed through partial 16S rRNA gene amplification and DGGE.
- Dominant bands underwent sequence analysis and phylogenetic comparison.
- Short-term N₂O incubations were carried out following addition of 2 mg glucose, and 50 g N, g⁻¹ soil (Fig. 2). N was added as either (NH₄)₂SO₄, KNO₃, or NH₄NO₃, and gas sampling occurred 2, 4, 8, 24 and 32 hrs following N addition.

- **Fertiliser application:**
Fertilised plots (F): 45 kg N ha⁻¹ NH₄NO₃ since 1967
- **Non-fertilised (NF): never amendment**
- **Dark brown chernozemic sandy loam**
- **Continuous spring wheat crop**

Figure 1. Site characteristics (Janzen et al., 1995).



Figure 2. Gas sampling 2, 4, 8, 24 and 32 hrs after addition of 50 µg N g⁻¹ soil as KNO₃, (NH₄)₂SO₄, or NH₄NO₃, to non-fertilised and fertilised soils.

Preliminary results

- The AOB 16S rRNA DGGE profiles had 2 dominant bands and did not differ between the two soils (Fig. 3).
- Sequence analysis revealed that the bands corresponded to *Nitrosospira* genus, and phylogenetic comparison grouped them into the previously described cluster 3 (Fig. 4).
- N₂O production from the two soils did not differ when un-amended, and (NH₄)₂SO₄ addition had no effect in either soil (Fig. 5).
- Addition of KNO₃ or NH₄NO₃ significantly (P<0.001) increased cumulative N₂O production in both soils, but to a significantly (P<0.001) greater level in F compared to NF soils.
- The time course of N₂O production reveals differences between KNO₃ and NH₄NO₃ treatments that follow a similar pattern in both soil types (Fig. 5). Soil amended with the dual N source emitted significantly (P<0.001) more N₂O for a period around the 4-8 hour period, before dropping to levels below that of soil amended with KNO₃-only.

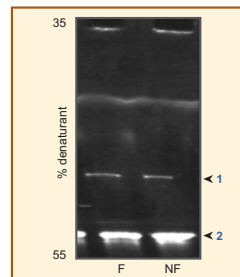


Figure 3. DGGE polyacrylamide gel of 465 bp PCR product of 16S rRNA gene fragments isolated from long-term fertilised (F) and non-fertilised (NF) soils. Arrows indicate bands isolated and sequenced.

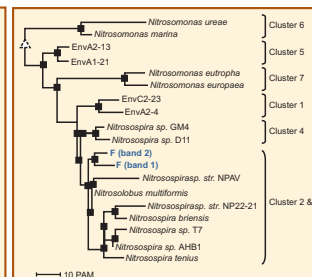


Figure 4. Phylogenetic tree constructed with partial 16S rRNA gene sequences. Sequences obtained from long-term rotations are high-lighted in blue. Groupings indicated are as suggested by Stephen et al., 1996.

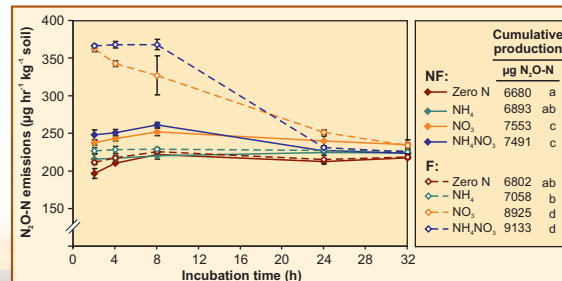


Figure 5. Time course of N₂O-N production from long-term non-fertilised (NF) and fertilised (F) soil incubated following amendment with 2 mg⁻¹ soil glucose, and either zero N, or 50 mg N g⁻¹ soil of (NH₄)₂SO₄, KNO₃, or NH₄NO₃. Cumulative total N₂O-N is presented alongside legend, with significant (P<0.001) differences indicated with different letters.

Discussion

- Domination of agricultural soil by *Nitrosospira* cluster 3 has been previously reported (Stephen et al., 1998; Mendum et al., 1999; Ibekwe et al., 2002). Where *Nitrosospira* cluster 3 dominates non-fertilised soils, long-term fertiliser application has been shown to have no effect on AOB diversity (Phillips et al., 2000). However, the size and/or activity of the AOB community may differ, as demonstrated by cPCR by Phillips et al. (2000).
- The large difference in N₂O production between NF and F soils in the presence of a large nitrate pool suggests that long-term fertiliser application has altered the denitrifying community size, activity and/or structure.
- Efforts to characterize the denitrifying community using *nirS* and *nirK* have proved frustrating. While amplification with *nirS* yielded no PCR product, *nirK* products were detected, however yield was low and DGGE separation was poor (data not shown).

Future work

Further investigations employing acetylene inhibition assays to determine the N₂:N₂O ratio of gaseous N loss, potential denitrification rates and denitrification enzyme activity are required. There is also a need to investigate the stability of the microbial community throughout the growing season.

Acknowledgements

This work is funded by Natural Sciences and Engineering Research Council of Canada (NSERC) and Agriculture and Agri-Food Canada (AAFC).

References

Ibekwe et al., 2002. FEMS Micro. Ecol. 39; Janzen, 1995. Can. J. Soil. Soil Sci. 75; Mendum and Hirst 2002. SBB 34; Mendum et al., 1999. AEM 65; Phillips et al., 2000. AEM 66; Stephen et al., 1996. AEM 62; Stephen et al., 1998. AEM 64