The Nitrogen Cycle & Denitrification
- The nitrogen cycle (Fig. 1) is a complex biogeochemical cycle of great economic and environmental significance, owing to its involvement in agricultural productivity and its production of useful products and greenhouse gases.
- Nitrous oxide (N₂O) has 298 times global warming potential of the molar equivalent of CO₂ and is produced by insensible denitrification.
- Denitrification is the microbially mediated soil water reduction of NO₃⁻ to gases including N₂ and N₂O. This is a modular process, with soil organisms having the full suite of denitrification enzymes.
- Denitrification is thought to account for 48% of global N₂O emissions and is the sole biological sink for N₂O in agricultural soils.
- Little is currently understood about the effects of microbial community structure on nitrogen use efficiency in agriculture.
- The project will investigate whether soil management practices and diversity of denitrification genes are correlated in soils with different land management histories, including different fertilizer and tillage strategies.

Effects of fertilizer use and tillage strategies
- Land management strategies are known to affect N₂O emissions.
- Fertilizer use increases denitrification by an average of 3% which increases N₂O emissions.
- Tillage increases soil moisture, reduces water conservation and increases N₂O emissions when applied long term. Tillage also reduces denitrification, altering nitrogen and carbon cycling.
- Little is currently understood about the effects of microbial community structure on nitrogen use efficiency in agriculture.

Materials and methods

The Site
- Soil samples were taken in the East Hemmel experiment (Fig. 2). The site comprises mainly of magentic sandy-loam soil (loamy sand).
- The trial was established in 2011 and follows a split plot design with combinations of tillage and fertility management (Fig. 3), with four Site x 16 replicate fields for each treatment.

Sample preparation & analysis
- Soil samples were taken from the soil surface to 10 cm depths were bulked and homogenised to create one composite sample per subplot.
- These samples were oven dried and stored at -20°C.
- Known amounts of DNA from Thermus thermophilus were added to the soil as an internal standard in order to quantify gene abundance.
- DNA was extracted using DNeasy PowerSoil kit and amplified using PCR with universal primers for the 16S rRNA gene or universal primers for the 16S rRNA gene.
- Amplions were sequenced using the MiSeq platform, Illumina® generating 2x300bp paired-end reads.
- Amplions were analysed using DADA2 & quantified relativem to the internal standard DNA. Functional genes were verified using blasto.

Soil sampling from Nafferton Factorial Systems Comparison (NFSC) field trial

Physicochemical analysis of soil properties

DNA extraction from soil

PCR amplification of 16S rRNA and denitrification genes

DNA sequencing using Illumina® MiSeq

Bioinformatics analysis using QIME & blastx

Soil physicochemical properties

The 16S rDNA rRNA gene sequence was used to investigate bacterial diversity. This gene is often used for phylogenetic studies of bacteria & archaea, because it contains highly conserved regions which universal primers can bind to, as well as non-competitive regions for species specific signatures which can be used to identify bacteria & archaea.

By adding a known quantity of DNA from an organisms that isn’t found in soil, Thermus thermophilus, it is possible to quantitatively evaluate the absolute 16S rRNA gene abundance per gram of soil (Fig. 4).

Although absolute abundance of 16S genes was not significantly different between treatments, due to high variance, there appears to be a trend for greater microbial abundance in soils treated with minimum tillage compared to conventional tillage (Fig. 5). This suggests that reduced disturbance has a positive impact on microbial abundance in the soil.

16S rDNA gene abundance

16S rDNA diversity

like rib ‘gene signatures, organisms were not found to cluster exclusively by treatment (Fig. 4), although fertilizer treatment was found to have a significant effect at the genus level (PERMANOVA: F = 1.6; d.f. = 1,8; p< 0.05).

Microbial biodiversity in the soil was found to cluster predominantly by field block (Fig. 6), with block having a highly significant effect on species distribution (PERMANOVA: F = 4.4; d.f. = 18; p < 0.001).

It appears as though soil physical properties and/or soil physical properties have a greater effect on species distribution than tillage or fertilization regime. This suggests that using conventional tillage systems and synthetic fertilizers have little effect on microbial diversity.

Acknowledgements

Thank you to everyone who has contributed to this project. Samples were collected from Nafferton Ecological Farming Group. By students from the University of Newcastle, under the supervision of Dr Julia Cooper. Sequencing was undertaken within the Genomics and Bioinformatics Lab within the Bioscience Technology Facility. Project funding was provided by BBSRC through its NFP Leadership with Precision Decisions. as the industrial partner would also like to thank my supervisors James MaI, Thoren Helgason and Calvin Dytham, and ITP members Keith Redder and Sylvia Tait.

Conclusions

- Microbial communities in the soil appear to be largely unaffected by land management history, and are likely to be more affected by physical factors within the soil as evidenced by clustering by field block rather than treatment. A significant factor for this is likely to be water and oxygen availability within the soil. Elevation and organic matter content within the soil were found to have an effect on the microbial community.

- Fertilizer was shown to have an effect on the microbial community as the effect was not on the nPK genes. This may suggest that the variation in microbial diversity observed to fertilizer application may be predominantly affecting non-nPK containing organisms.

- Using an internal standard to quantify microbial abundance whilst simultaneously measuring community composition is an extremely useful way to quantify how the total amounts of 16S rRNA genes may vary across samples, rather than just the relative abundance of species. This technique should continue to be used in microbial biodiversity studies.

- Minimum tillage has a positive impact on below-ground biomass, regardless of fertility management strategy and use of organic fertilizer may have a positive impact on available carbon in Nafferton Factorial Systems Comparison (NFSC) field trials, even within 6 months after application. This may signify leaching of fertilizer into water courses.

References


The authors declare no competing financial interests. This study was funded by BBSRC through NewSEER project, with precision Decisions as the industrial partner, and ITP members Keith Redder and Sylvia Tait.

Fig. 1. The nitrogen cycle

Fig. 2. The Field site

Fig. 3. Soil sampling

Fig. 4. DNA extraction

Fig. 5. PCR amplification

Fig. 6. DNA sequencing

Fig. 7. Bioinformatics analysis

Fig. 8. Soil physicochemical properties

Fig. 9. nPK diversity

Fig. 10. 16S rRNA gene abundance

Fig. 11. 16S rRNA diversity

Fig. 12. Conclusions

Fig. 13. Acknowledgements