

I.M. McCormick<sup>1</sup>, B. Deen<sup>2</sup>, and K.E. Dunfield<sup>1</sup>. <sup>1</sup> School of Environmental Science, <sup>2</sup> Plant Agriculture, University of Guelph, Guelph, Ontario Canada.

## Introduction

Poor nutrient-use efficiency in agricultural soils remains a major environmental and human health concern. Best Management Practices (BMPs) such as zero-tillage, cover crop use and crop rotation can enhance nutrient-use efficiency, but their long-term ecological effects are not well understood. This study assessed the effects of 30 year-old tillage, crop rotation, and cover crop systems on populations of ammonia oxidizing bacteria (AOB), ammonia oxidizing archaea (AOA), and arbuscular mycorrhizal fungi (AMF).

The objectives of this study were to:

- 1) Quantify ammonia monooxygenase (*amoA*) gene copy numbers in different crop rotation and tillage treatments.
- 2) Determine tillage effects across soil depths on total/active microbial populations and soil properties in a continuous monoculture and a BMP system employing a four year crop rotation with a red clover cover crop.
- 3) Relate microbial population trends and corn yields.

## Experimental Design

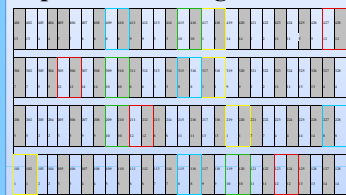


Figure 1a.

Border Colour	Crop Rotation	Symbol	
		Till	No-Till
Yellow	Continuous Corn	CCT	CCNT
Green	Corn-Corn-Soy-Soy	CST	CSNT
Red	Corn-Corn-Soy-Wheat	CSWT	CSWNT
Blue	Corn-Corn-Soy-Wheat (with red clover)	RCT	RCNT

Figure 1c.

**Figure 1b.** **Figure 1abc:** a) Long-term field plots (20'x50') established in 1980 at the University of Guelph's Elora Research Station in Elora, Ontario, Canada. b) Treatments were arranged in a randomized split-plot design with four replications. Till and no-till treatments (sub-plots) were grouped in pairs within different cropping systems (main-plots). c) Colour coded legend for Fig. 1a. and treatment symbols.

## Methodology

- 1) Samples were collected (Fig. 2) on May 3<sup>rd</sup> 2010 (before tillage and corn planting) and on June 30<sup>th</sup> 2010 (after tillage, planting and fertilization events). Surface samples were collected in all study treatments. In CC and RC treatments, samples at 5-15cm and 15-30 cm were also collected (Fig. 3).
- 2) Soils were analyzed for bulk density, % moisture content, and water stable aggregates in the 1-2mm size fraction.
- 3) Molecular analysis (August 2010-Present)
  - 1) DNA/RNA extraction using MoBio Powersoil RNA Extraction kit and accessory DNA elution kit.
  - 2) Reverse-Transcription of RNA to cDNA.
  - 3) Quantitative Polymerase Chain Reaction (qPCR) to quantify *amoA* gene copy numbers and AOA genes.
  - 4) Pyrosequencing of AOB, AOA, and AMF to quantify microbial community diversities.



Figure 2: Four subsamples across a diagonal transect were collected using a 5cm diameter soil corer.



Figure 3: Soil collection across depth. Two grams were placed into MoBio LifeGuard solution and remaining soil was bagged for future analysis.

## Soil Properties

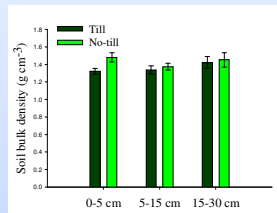


Figure 4: Mean bulk density of CC and RC treatments across sample depths.

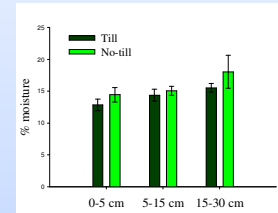


Figure 5: Mean % soil moisture of CC and RC treatments across sample depths.

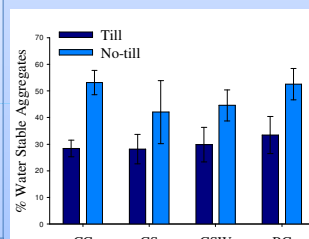


Figure 6: Mean % water stable aggregates (1-2mm).

Bulk density increased with depth in till plots and was higher in no-till plots (Fig. 4). Moisture increased with depth (Fig. 5,  $p < 0.05$ ) and was higher in no-till plots. CCNT at 15-30cm had the highest soil moisture at 22.7%.

There were 18.2% more water stable aggregates (WSA, 1-2mm fraction) in no-till soils compared to tilled (Fig. 6,  $p < 0.05$ ). Red clover cover crop incorporation enhanced WSA in both tilled and no-till soils by 3.6% and 8.0% respectively as compared to an identical crop rotation with no red clover (RC vs. CSW).

## Plot Yields

There was a trend with higher yields in tilled soils, and in the cover cropped treatment (RC). Interestingly, RCT had the highest yields, but RCNT had the second lowest yields amongst all treatments. However, averaged 30 year corn yields were not significantly different between treatments.

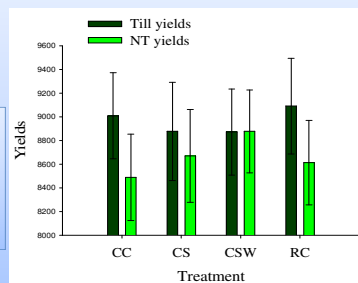


Figure 7: Mean corn yields over 30 years.

## *amoA* Quantification

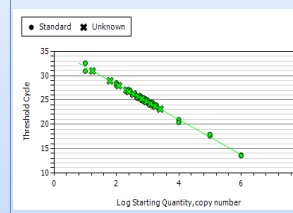


Figure 8: Sample standard curve of *amoA* gene copy numbers from dilution series of DNA plasmid. PCR efficiency averaged 91.5%,  $R^2 = 0.99$ , and slope = -3.543.

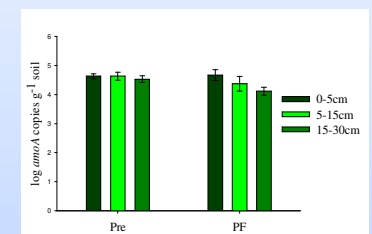


Figure 9: Mean *amoA* copy numbers in surface samples (0-5 cm) before tillage, planting and fertilization (Pre) and after (PF).

Quantitative analysis of *amoA* (Fig. 8) in extracted DNA revealed gene copy numbers were affected by depth of sampling in the CC and RC plots (Fig. 9,  $p < 0.05$ ). Copy numbers also changed significantly after tillage and fertilization (Pre vs. PF) within the 5-15 cm and 15-30 cm depth fractions (Fig. 9,  $p < 0.05$ ). Interestingly surface samples (0-5 cm) contained the highest copies of *amoA* and were maintained throughout the experiment. Crop rotation and tillage did not affect *amoA* copy numbers (Fig. 10).

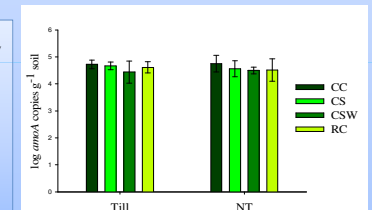


Figure 10: Mean *amoA* copy numbers in different crop rotations with till and no-till (NT) treatments.

## Summary

- 1) Lowest corn yields were found in NT plots and corresponded with soils exhibiting the greatest water stable aggregation and soil moisture.
- 2) *amoA* copy number was dependent on soil depth and sample time. Interestingly, long term crop rotation and tillage did not significantly alter the quantity of *amoA* genes in soil.

## Future work

- 1) Quantify AOB and AOA community size in relation to total soil bacteria and archaea.
- 2) Quantify diversities of AOB, AOA, and AMF.
- 3) Relate microbial population data with corn yields and total microbial biomass carbon and nitrogen.

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