# Assessing Bacterial and Fungal Contribution to $N_2O$ Production in Soils under Different Tillage Practices

**Shiva Ladan and Pierre-Andre Jacinthe**

Department of Earth Sciences, Indiana University Purdue University Indianapolis (IUPUI)

Contact: shladan@iupui.edu

## Background

- Nitrous oxide ($N_2O$) is an atmospheric constituent implicated in the accelerated greenhouse effect and stratospheric ozone depletion.
- Among anthropogenic activities, agriculture is the largest contributor (about 69 %) to $N_2O$ emission.
- The biological processes of nitrification and denitrification are the primary pathways of $N_2O$ production in soil ecosystems.
- During the last several decades, no-till (NT) farming has been widely adopted as an alternative tillage practice to conventional plow till (PT).
- There are mixed results about the net effect of NT practice on $N_2O$ emission, and the contributing soil factors are not well elucidated.
- Through its impact on soil biophysical conditions, NT farming could change the fungal :bacterial ratio of soils.
- Standard methods are needed to quantify the contribution of fungi and bacteria to $N_2O$ production in agricultural soils.

## Objectives

- Identify the types and concentration of biocides (fungicide and bactericide) that provide optimum inhibition of denitrification in agricultural soils.
- Determine the relative contribution of fungi and bacteria to denitrification in soils under various forms of land-use and tillage practices.

## Materials and Methods

Soil Samples (0-10 cm)

S. Charleston (OH): Plow till (PT1), No-till (NT), Woodlot (WL)

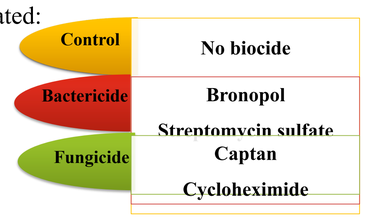
Danville Farm (IN): Plow till (PT2)

Starkey Farm (IN): Plow till (PT3)

Selective Inhibition (SI) of Denitrification

Field-moist sieved soil samples were left overnight at room temperature (22 °C) for acclimation. Soil samples (10 g of PT1 soil) were placed in serum bottles (250 mL) and amended as follows:

- Denitrification enzyme activity (DEA) media: 100 mg $NO_3$-N $kg^{-1}$, and 40 mg dextrose-C $kg^{-1}$.
- Biocides addition either concurrently with DEA media (Exp 1) or 1 hour before DEA media (Exp 2).
- The following biocides were evaluated:




Biocides were applied at concentrations ranging between 1 and 16 mg $g^{-1}$ soil. Each treatment was tested in triplicate.

Serum bottles were evacuated and flushed with UHP $N_2$ at least 3 times, injected with $C_2H_2$ (10 kPa), and incubated (25 °C). Gas samples were taken from bottles headspace after 3, 8, 24 and 48 hours. Concentration of $N_2O$ was measured by gas chromatography (electron capture detector, ECD).

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## Results

**$N_2O$ and $CO_2$ production**

To identify the type, concentration and mode of application of biocides that result in the strongest inhibition of denitrification activity.

Fig 1. Experiment 1 (Media and biocides added simultaneously)

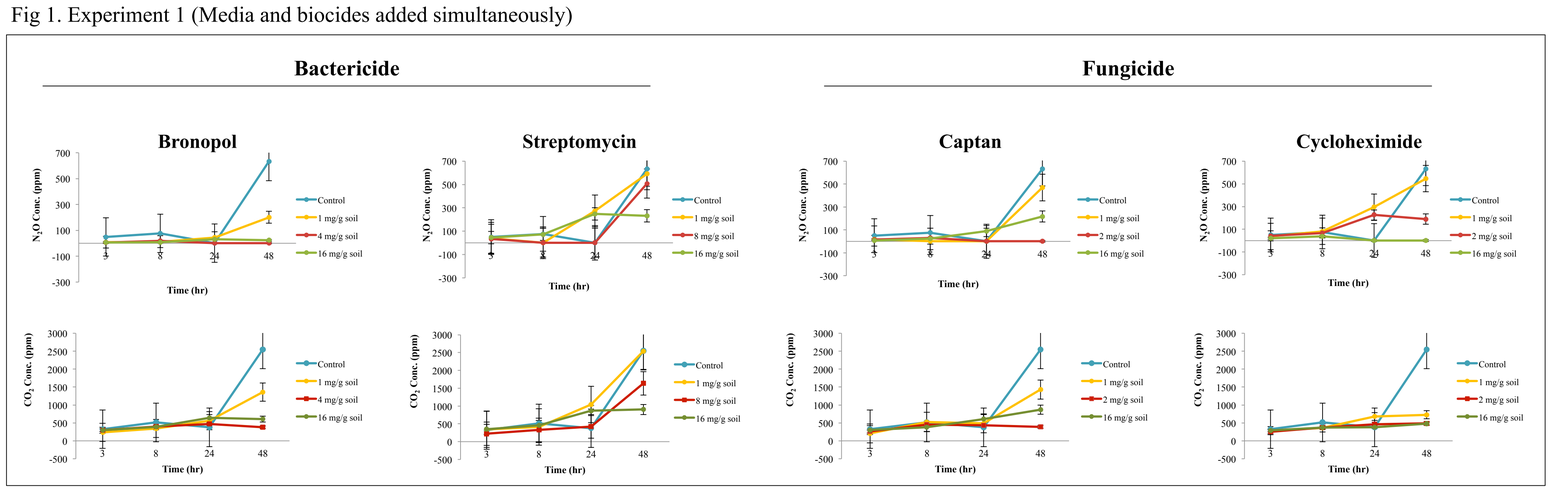
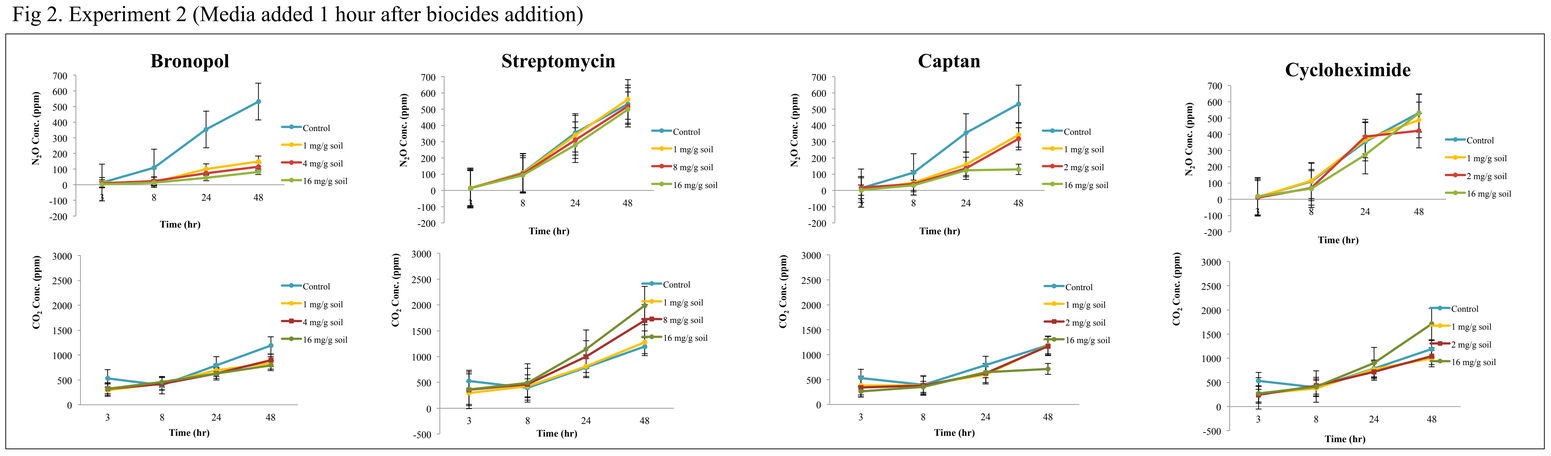



Fig 2. Experiment 2 (Media added 1 hour after biocides addition)




**Results showed that: (i) time of media addition had no significant effect on $N_2O$ and $CO_2$ production, (ii) streptomycin was the least effective biocide, often resulting in stimulation of $N_2O$ and $CO_2$ production, (iii) bronopol and captan (both at 16 mg $g^{-1}$ soil) had the strongest inhibitory effect in the production of both gases. Therefore, these biocides were selected for subsequent assays conducted with the other soils (Tables 1 and 2).**

Table 1. Percent inhibition of $N_2O$ and $CO_2$ production in different soils amended with **bronopol** (16 mg $g^{-1}$ soil)

| Soil | $N_2O$ Production Inhibition % | $CO_2$ Production Inhibition % |
|---|---|---|
| **PT1** | 88.8 | 75.9 |
| **PT2** | 84.4 | 57.0 |
| **PT3** | 84.7 | 34.5 |
| **NT** | 65.8 | 76.4 |
| **WL** | 40.6 | 5.1 |

Table 2. Percent inhibition of $N_2O$ and $CO_2$ production in different soils amended with **captan** (16 mg $g^{-1}$ soil)

| Soil | $N_2O$ Production Inhibition % | $CO_2$ Production Inhibition % |
|---|---|---|
| **PT1** | 71.5 | 76.0 |
| **PT2** | 67.4 | 62.0 |
| **PT3** | 56.6 | 53.3 |
| **NT** | 57.2 | 80.7 |
| **WL** | 30.0 | 53.8 |

**In the soils tested, bronopol caused greater inhibition in $N_2O$ production compared to captan. These results suggest a greater contribution of bacteria than fungi to $N_2O$ production. $CO_2$ production, however was more strongly inhibited by captan than by bronopol.**

## Summary

- The amount of $N_2O$ and $CO_2$ production was not affected by the time of media addition.
- Among the biocides evaluated in this study, streptomycin was the least effective, whereas bronopol and captan were the most effective inhibitors of both $N_2O$ and $CO_2$ production.
- Since greater inhibition in $N_2O$ production was achieved with bronopol than with captan, results suggest greater contribution of bacteria than fungi to $N_2O$ production in soils investigated

## Future Directions

- Additional experiments, using soils from different regions, management practices, and incubated at different moisture levels will be conducted to expand the range of application of the method.
- Additional experiments will also be conducted with $^{15}N$-labeled $NO_3$ to improve the internal validity of the method, and address limitations of the acetylene ($C_2H_2$) block technique.