How Do Soil Properties Affect the Efficacy of Nitrification Inhibitors On Nitrous Oxide Emission and Distribution of Ammonia Oxidizers?

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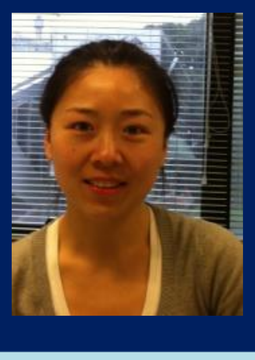

Introduction

Nitrous oxide (N_2O) is a major GHG produced by agricultural practices. Denitrification and nitrification are the two main N_2O producing processes in soils.
The application of nitrification inhibitors is one strategy to reduce N_2O losses as they inhibit the bacterial ammonia monooxygenase (AMO) enzyme that is involved in the oxidation of NH_3 to NH_2OH, the first step of nitrification (Fig.1). However, previous studies have indicated that the efficacy of nitrification inhibitors is variable both spatially and temporally, and this depends greatly on the environmental conditions and soil properties. It has been observed that application of fertilizers treated with nitrification inhibitors had impact on soil ammonia-oxidizing bacterial (AOB) populations but not on ammonia oxidizing-archaeal (AOA) populations (Di et al., 2010; O'Callaghan et al., 2010). However, recent results have indicated that nitrification inhibitors can stop AOA growth (Zhang et al., 2012). Differences in soil properties seems to be a key parameter responsible for the variation in AOA & AOB distributions.

Objective

To investigate how soil physical, chemical and microbial (AOB and AOA abundance) properties influence the efficacy of the nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) and acetylene (C_2H_2) in reducing nitrification and N_2O production.

Materials and Methods

- Laboratory Incubation Experiment
- Soils: See Table 1
- Treatment: NH_4Cl (100 µg NH_4-N/g soil (for all treatments)) (Control)
  NH_4Cl + DMPP (0.1% active ingredient) (DMPP)
  NH_4Cl + C_2H_2 (1% v/v) (C_2H_2)

4 replications at 25 ℃ and 60% water-filled pore space (WFPS)

- Measurements:

➢ N_2O – flux measurements taken over 72 hrs on days 1, 4, 8 and 12, analysed using gas chromatograph.
➢ Soil mineral N – extracted weekly with 2M KCl, analysed by segmented-flow analyser (Skalar SAN++)
➢ Gene Abundance - DNA extraction (DNA Isolation Kit (MOBIO Laboratories, Inc., US)) ⟶ cloning (TOPO cloning kit) ⟶ sequencing ⟶ quantifying (qPCR)

Gas collection vial

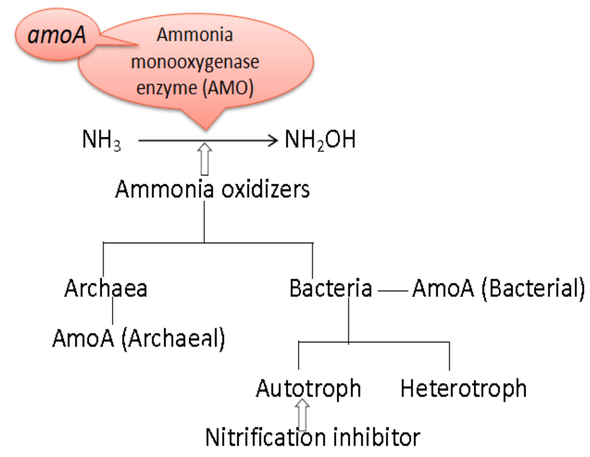

Fig. 1 Using functional *amo*A gene to separate archaea and bacteria

Table 1. Selected soils properties

Location	Clare	Tamworth	Hamilton
Soil Type	Neutral Clay loam	Alkaline Clay loam	Acid loam
pH (H_2O)	7.0	8.0	4.6
Organic C %	4.7	1.5	6.2
Nitrate-N mg/kg	7.6	65.3	93.0
Ammonium-N mg/kg	4.6	6.2	13.0

Results

Nitrification and N_2O

- Nitrification rates followed the order neutral clay loam< acid loam < alkaline clay loam (Table. 2).
- DMPP and C_2H_2 were most effective in the neutral clay loam (Table 2).
- C_2H_2 is more effective than DMPP in all three soils.
- DMPP and C_2H_2 can reduce N_2O emission flux from all three soils (Fig. 2).
- DMPP was less effective than C_2H_2 on N_2O emissions in the acid loam (Fig. 2, Table. 3).

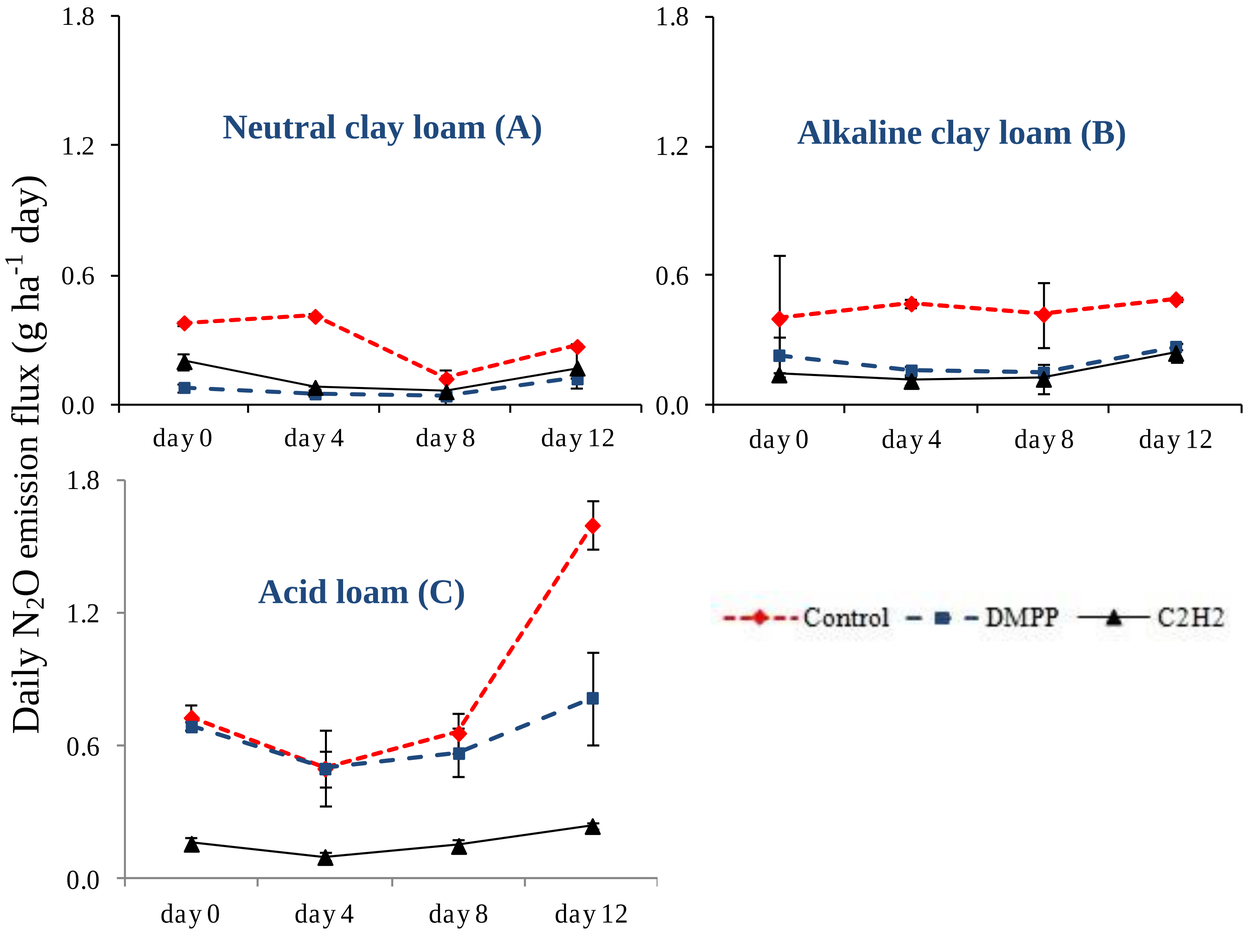

Fig. 2 Daily N_2O flux at 25◦C. Error bars indicate standard deviations of four replicates.

Table 2. Nitrification rate (ug/g d^{-1}) and inhibition (%)[a] by DMPP and C_2H_2 at day 28

Soil	Nitrification rate			Nitrification inhibition	
	Control	DMPP	C_2H_2	DMPP	C_2H_2
Neutral clay loam	1.7	1.1	0.5	81.2	84.1
Alkaline clay loam	5.7	3.8	0	52.8	62.4
Acid loam	3.2	1.9	0	42.3	62.6

[a] %inhibition of nitrification = ((NO_3-N produced in control treatment)-(NO_3-N produced in inhibitor-treated soil))/(NO_3-N produced in control soil) × 100

Table 3. Mineral N concentration and cumulative N_2O emission at day 14[b]

Soils	[NH_4^+-N] (µg /g soil)			[NO_3^--N] (µg /g soil)			N_2O-N cumulative emission (g/ha)			N_2O/Nitrification [c] (%)	
	Control	DMPP	C_2H_2	Control	DMPP	C_2H_2	Control	DMPP	C_2H_2	DMPP	C_2H_2
Neutral Clay loam	49.0	73.0	82.2	37.8	11.1	9.2	3.4	0.6	0.8	25.2	28.3
Alkaline Clay loam	13.8	74.7	97.4	158.9	80.8	66.8	4.4	1.5	1.2	3.6	4.8
Acid loam	81.6	82.2	119.0	71.5	55.2	34.9	12.7	5.1	1.2	13.8	33.0

[b] 14 days was the final collection time after 72 hrs closure (day 12 sample)
[c] %N_2O/Nitrification = ((N_2O-N produced by nitrification over incubation period/(NO_3-N produced over incubation period) × 100

Gene Abundance

- All three soils had detectable functional *amo*A gene from bacteria and archaea (Fig. 3).

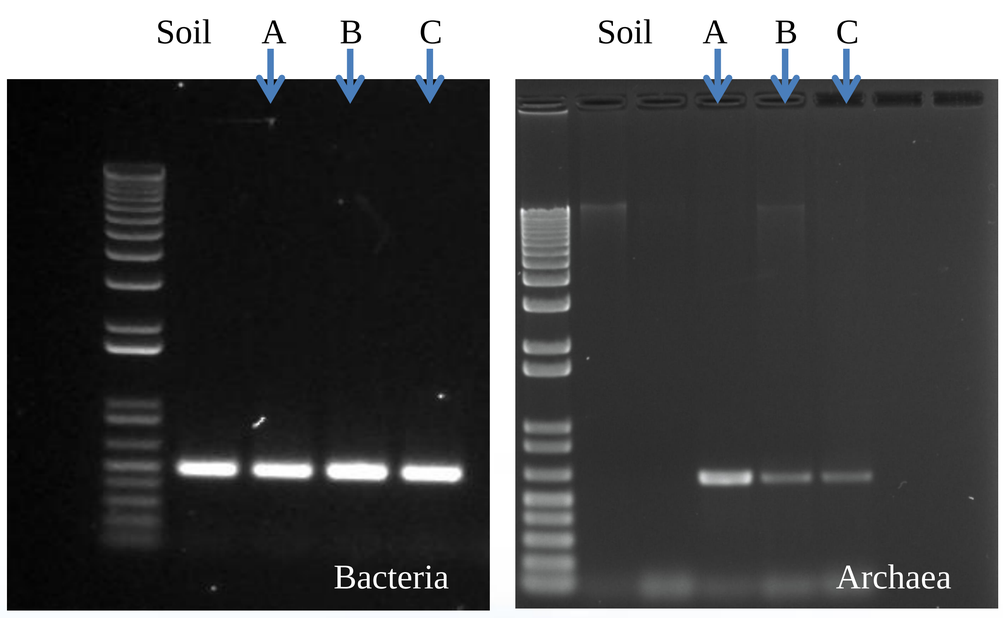

Fig. 3 Gel images showing detection of *amo*A gene from bacteria and archaea from PCR product

Future work

- Quantification of the specific functional gene (*amo*A) to test for relationship between these nitrifiers and soil properties.

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