Response of nitrification in soil slurry and whole soil assays

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Introduction

Ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) participate in nitrification in soils. Our laboratory has adapted soil slurry nitrification potentials (NP) to discriminate between AOA and AOB contributions in soil by preventing protein synthesis of key enzymes in AOB after inhibition with acetylene. Soil-slurry based potentials are attractive because they are fast, inexpensive, and provide direct evidence that the enzymes responsible for nitrification are present. A short-coming of this assay is that it doesn't necessarily represent real rates of nitrification in the soil because it is optimized to promote maximum activity by maintaining the slurry pH at 7.2, and ensuring that NH_4^+ and O_2 are non-limiting.

We hypothesized that we could inactivate and recover nitrification activity in whole soils. An assay in whole soils (water content ~ 2/3 of water holding capacity, or "field capacity" figure 2) should more accurately represent *in situ* activity.

Table 1. Response of nitrification and recovered nitrification in the presence and absence of antibiotics in soil slurry and whole soil conditions Values in parentheses are standard deviations of three replicates samples.

Treatments	Soil Slurry (µmol/g/day)	Whole Soil (µmol/g/day)
Nitrification	1.00 (0.13)	0.51 (0.09)
Recovery	1.11 (0.08)	0.42 (0.05)
Recovery + Antibiotics	0.09 (0.14)	0.41 (0.05)

►Rates of nitrification in soil slurry were 2-fold greater than rates of nitrification in whole soil.

►Ammonia-oxidizers must synthesize new protein to resume nitrification after acetylene inactivation

►After acetylene inactivation, all nitrification activity recovered in both soil slurry and whole soils, meaning all ammonia oxidizers can synthesize new protein after inactivation.

►800 µg/ml of each kanamycin and gentamycin prevented ~90% of recovery in soil slurry, indicating that AOB were responsible for nitrification in the slurry assay

►Antibiotics show virtually no effect on the whole soil at this concentration. Antibiotics could be bound to soil and ineffective

Rates of nitrification in whole soil may be limited by NH_4^+ availability. Only AOA or AOB that can access NH_4^+ in solution will be able to nitrify.

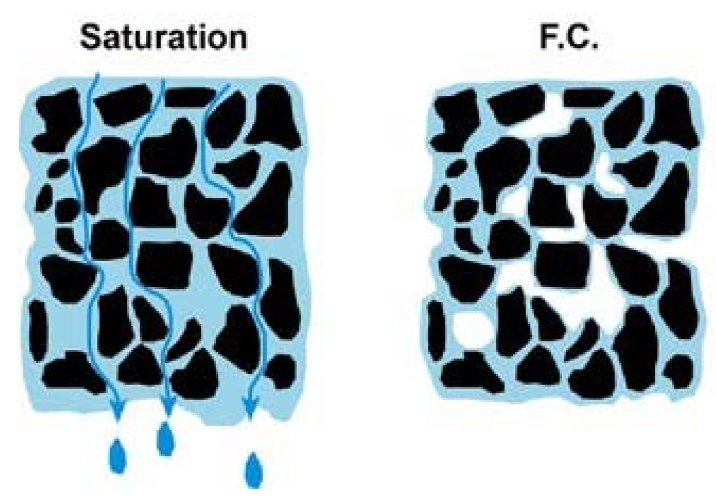

Figure 1. Representation of soils at saturation and field capacity (F.C.)

►When soils are saturated all pore space is water-filled and pores are continuously connected (Figure 1). NH_4+ will be accessible by all ammonia oxidizers, however O_2 may become limiting.

►At field capacity (F.C.) only small pores are water-filled and large pores are filled with air. NH_4+ will only be available to ammonia oxidizers in the small water-filled pores.

Methods

Soils were taken from the OSU Hyslop Field Research Laboratory (Woodburn series, Argixerolls, pH 5.9 - 6.5) on the Willamette Valley floor (~85 m elevation), from plots in a two-year crop/fallow rotation.
Nitrification potential (NP): 3.5 g soil + 25 ml 30 mM TES (pH 7.2), shaken at 27ºC with 1mM supplemental NH_4^+. NO_2^- and NO_3^- accumulation followed for 24h.
Recovered nitrification potential (RNP). NP of each field replicate irreversibly inactivated with acetylene. Ammonia monooxygenase (AMO) must be resynthesized for NP to resume. Recovery followed for 48h after acetylene removal.
RNP_{ab} represents RNP treated with 800 µg/ml kanamycin+ 800 µg/ml gentamycin, both bacterial protein synthesis inhibitors.
Whole soil (WS): 3.5 g soil from each field replicate + 860ug/g soil NH_4^+ in 0.8 ml water, and incubated at 27ºC. NO_3^- accumulation followed for 54h. NO_2^- did not accumulate in whole soils.
Whole soil recovery (WSR): WS irreversibly inactivated with acetylene. Ammonia monooxygenase (AMO) must be resynthesized for nitrification to resume. Recovery followed for 48h after acetylene removal.
WS_{ab} represents RNP treated with kanamycin + gentamycin.

► We hypothesized that limitations on NH_4^+ availability could be relieved by increasing the NH_4^+ concentrations added to whole soil, or by increasing the water content.

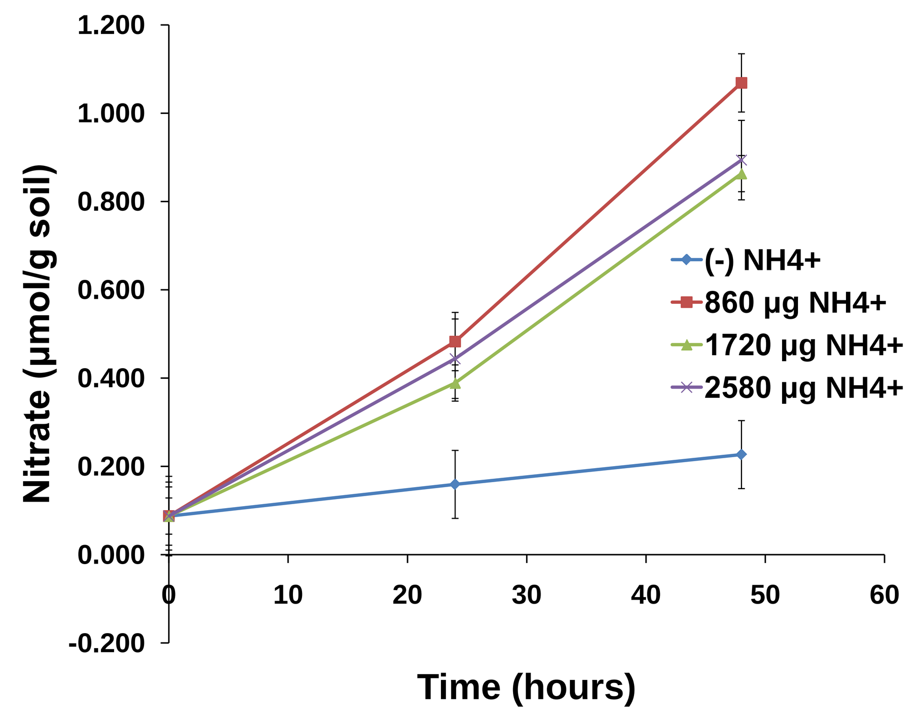

Figure 2. Response of nitrification in whole soils to increased concentrations of NH_4^+ .

►All treatments with added NH_4^+ had greater rates of nitrification than the no added NH_4^+ treatment (Figure 2, Table2).

►Concentrations of NH_4^+ greater than 860 µg NH_4^+/g soil do not stimulate higher rates of nitrification

Table 2. Comparison of rates of nitrification in response to increasing concentrations of NH_4^+

Treatments	Nitrification Rates (µmol/g/day)
- NH_4^+	0.07 (0.08)
860 µg NH_4^+/g soil	0.49 (0.07)
1720 µg NH_4^+/g soil	0.39 (0.04)
2580 µg NH_4^+/g soil	0.40 (0.09)

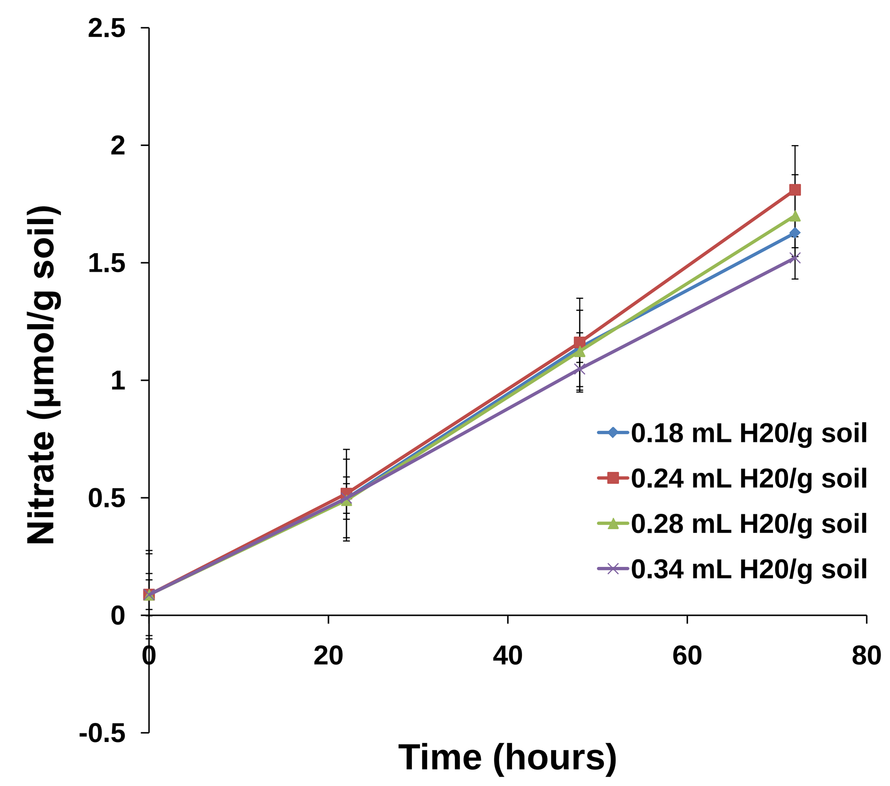

Figure 3. Effects of varying amounts of water – whole soil experiment.

►Varying the volume of water used in the bottles showed no significant effect on rates of nitrification (Figure 3, Table 3)

Table 3. Comparison of rates of nitrification in response to increased moisture contents of soil

Treatments	Nitrification Rates (µmol/g/day)
0.18 mL H_2O/g soil	0.59 (0.06)
0.24 mL H_2O/g soil	0.67 (0.19)
0.28 mL H_2O/g soil	0.63 (0.17)
0.34 mL H_2O/g soil	0.53 (0.09)

Conclusions

►Rates of nitrification in slurry assays are ~2-fold greater than in whole soil

►Rates of nitrification with added NH_4^+ are seven times greater than rates in the absence of NH_4^+

►Rates of nitrification in whole soil assays was not limited by NH_4^+ concentrations or moisture content of the soil

►**Future work:**
►Whole soil recovery with higher concentrations of antibiotics

►Attempt to control the pH in whole soil experiments

►Test the assay for whole soil recovery on other soils

References:
[1]A.E. Taylor, L.H. Zeglin, D.D. Myrold, P.J. Bottomley. 2010. Evidence for different contributions of archaea and bacteria to the ammonia-oxidizing potential of diverse Oregon soils. Applied and Environmental Microbiolgy, vol. 76, p 7691-7698.