# Introduction

Increasing atmospheric ammonia and its presence in precipitation in the Mid-Atlantic region have been well documented (Paerl, 1993; Scudlark, 2005). In the Chesapeake, atmospheric and precipitation ammonia has increased up to 60% in the past 20 years. Coastal waters have been seriously affected by agriculture-dominated nutrient inputs with biotic impacts including toxic algal blooms, decline of submersed aquatic vegetation and fish mortality.

Winter cover crops have been promoted as an agricultural Best Management Practice (BMP) for conserving residual soil nitrate which is highly mobile and can leach into groundwater. The use of cover crops in the Chesapeake Bay watershed is regarded as one of the most important BMPs for the reduction of agriculturally derived non-point nitrogen entering the Bay. For example, nitrate leaching losses were reduced by about 80% with a rye cover crop planted after corn in the Coastal Plain region of the Chesapeake Bay watershed (Staver and Brinsfield, 1998).

Nitrate removal by cover crops is dependent on active uptake and reduction mechanisms that are known to be sensitive to ammonia as low as 1 µM (Haynes and Goh, 1978; Hewitt and Smith, 1975). Scudlark et al. (2005) observed (1) ammonia at 26.3 µM on the Delmarva peninsula and (2) evidence that poultry houses significantly increase ammonia in their vicinity. These observations led us to question whether ammonia in precipitation could interfere with the efficiency of nitrate uptake and reduction in cover crops.

# **Materials and Methods**

#### **Experimental Techniques**

<u>Plant culture (rye and winter wheat):</u>

• Seeded in 7<sup>5/8</sup> x 7<sup>5/8</sup> x 2-in deep flats; distributed 35-40mL of seeds evenly in the cultivating medium (soil or Perlite); topped with 250mL of cultivating medium and watered with dilute (1:10) Hoagland's solution; grown in a Percival® growth chamber with controlled light/dark cycles (12L/12D) at 20C; fed with dilute Hoagland's solution for about 1 week before running experiments

<u>Nitrate Reductase (NRase assay):</u>

• Used whole tissue method developed by Jaworski (1971); determined nitrate produced through NRase colorimetrically by diazotization and expressed as uMoles No<sub>2</sub>-.Hr<sup>-1</sup>.gfw<sup>-1</sup>

#### Experiment #1

<u>Timing of NRase Inhibition (Perlite Experiments):</u>

• Seeded in Perlite (2 treated & 2 untreated flats of each cover crop for a total of 8 flats); all flats were treated with dilute Hoagland's solution

• After 7 days, treated flats received 124mL of 20 µM NH<sup>+</sup> solution every 15 minutes for a period of 1 hour (480mL total) simulating a 0.5-inch rainfall event; untreated flats received the same amount of deionized water; tissue was randomly collected from each flat and analyzed for NRase activity

# Experiment #2

<u>Timing of Nitrate Uptake Inhibition (Hydroponic Experiments):</u>

• PVC rings fitted with double-layer cheese cloth held by a "kerfed" overlapping ring; 2.5g of rye seeds and 10.5g of winter wheat seeds evenly distributed onto cheese cloth; PVC rings placed on top of beakers filled with 1,000mL 100 µM KNO, dilute Hoagland's solution with aeration (see Figure 1)

• After 7 days, 2 rings of each cover crop were treated with 20  $\mu$ M NH<sub>4</sub>+ dilute Hoagland's solution; remaining 2 rings were kept in control with 100 µM KNO<sup>1</sup> dilute Hoagland's solution

• Two (2) mL samples were taken at 0, 2, 4, 6, 8, 10, 15 and 60-minute intervals after NH<sub>4</sub>+ treatment and residual nitrate were determined colorimetrically



Figure 1. Set up of hydroponic experiments

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# **Does Ammonia in Precipitation Inhibit Nitrate Removal in Cover Crops?** Daniel Terlizzi<sup>1</sup>, Patricia Steinhilber<sup>2</sup>, J. Richard Nottingham<sup>3</sup>, Joshua McGrath<sup>2</sup>, Marcia Guedes<sup>2</sup>, and Anastasia Vvedenskaya<sup>2</sup> (1) Center of Marine Biotechnology, 701 E. Pratt St. Baltimore, MD (2) Environmental Science and Technology, University of Maryland, College Park, MD (3) University of Maryland Extension, Princess Anne, MD

# Materials and Methods (cont.)

# **Experiment #3**

<u>Timing of NRase Inhibition (Soils Experiments):</u>

• Fort Mott loamy sand, Hagerstown silt loam and Glenelg loam (see Table 1 for soil taxonomy) were screened, homogenized and distributed into 12 flats, 4 of each soil type; winter wheat was seeded into all 12 flats; design included 3 soil types and 4 different  $NH_{4}^{+}$  concentrations (DI, 5, 10 and 20  $\mu$ M)

• After 7 days, NH<sub>4</sub><sup>+</sup> solution was applied every 15 minutes for a period of 1 hour simulating a 0.5-inch rainfall event; untreated flats received the same amount of deionized water; tissue was randomly collected from each flat and analyzed for NRase activity

# Table 1. Relevant features of geographically representative soils used in study

Soil series	Physiographic province	Taxonomy	Textural class	Exchangeable ammonium (mg/kg)	Exchangeable nitrate (mg/kg)
Fort Mott	Coastal Plain	Arenic Hapludult	loamy sand	1.2	18.8
Hagerstown	Valley & Ridge	Typic Hapludalf	silt loam	7.7	26.4
Glenelg	Piedmont	Typic Hapludult	loam	4.4	25.6

# **Results and Discussion**

# **Experiment #1**

<u>Timing of NRase Inhibition (Perlite Experiments):</u>

• Significant inhibition (P< .05) of NRase was observed in rye seedlings in Perlite exposed to 20  $\mu$ M NH<sub>4</sub><sup>+</sup> and the DI control at 2-hour post treatment (see Figure 2); NRase activity was significantly lower (P< .05) in the 20  $\mu$ M NH<sub>4</sub><sup>+</sup> treatment vs. the DI control; winter wheat seedlings in Perlite significant reduction in NRase activity was only observed in the 20  $\mu$ M NH<sub>4</sub><sup>+</sup> treatment; DI control was not significantly reduced at 2 hours post treatment • Magnitude of inhibition was greater in winter wheat (44%) than in rye (21%); winter wheat was more sensitive to NH<sub>4</sub>+ inhibition than rye; suggests that rye may be a better choice in sensitive watershed areas or regions with higher NH<sub>4</sub><sup>+</sup> concentrations in



Figure 2. NRase inhibition of ammonia in rye and winter wheat in Perlite

# **Results and Discussion (cont.)**

# **Experiment #2**

Timing of NRase Inhibition (Hydroponic Experiments):

• Previous research on barley shows that  $NH_{A}^+$  treatment inhibits nitrate uptake within minutes of exposure (Kronzucker et al., 1999); our results support this finding (see Figure 3)

• Nitrate removal from controls was higher (80%) in rye than in winter wheat (60%);  $NH_4^+$  exposure at 20  $\mu$ M inhibited  $NO_3^+$  uptake in both rye and winter wheat; inhibition of net NO,<sup>-</sup> uptake in plants attributed to direct inhibition of NO,<sup>-</sup> influx or simulation of NO<sup>3</sup> efflux (Kronzucker et al., 1999; Aslam et al., 1997) • These data show the ability of winter wheat and rye to absorb NO<sub>3</sub><sup>-</sup> rapidly is inhibited by exposure to NH<sub>4</sub><sup>+</sup> at concentrations known to occur in precipitation







# **Results and Discussion (cont.)**

#### **Experiment #3**

Timing of NRase Inhibition (Soils Experiments): • NRase inhibition in winter wheat was examined at 0, 5, 10, and 20  $\mu$ M NH<sub>4</sub><sup>+</sup> in 3 geographically representative soils from the Chesapeake region (see Figure 4)

# Figure 4. Sample sites representing the Valley & Ridge, Piedmont, and Coastal Plain regions of Maryland

• NH<sub>4</sub><sup>+</sup> inhibition of NRase not significant in Glenelg loam or Hagerstown silt loam; significant inhibition observed in Fort Mott loamy sand from the Coastal Plain (see Figure 5) • Three (3) days after exposure to  $NH_{4}^{+}$  50% and 62% reduction in NRase was observed at 10 and 20  $\mu$ M  $NH_{4}^{+}$ ; inhibition of NRase observed at 5 µM NH<sub>4</sub><sup>+</sup> at days 6 and 11 post treatment, suggesting efficiency of winter wheat may be reduced at modest  $NH_{A}^{+}$  concentration in precipitation; the reduced efficiency of nitrate assimilation in winter wheat persists for 13 days after  $NH_{A}^{+}$ exposure in the 20  $\mu$ M NH<sub>4</sub><sup>+</sup> treatment



Figure 5. Ammonia concentration effects in winter wheat in the geographically represented soils: a) Fort Mott loamy sand, b) Hagerstown silt loam, c) Glenelg loam

# Conclusions

• In winter wheat and rye, exposure to  $NH_{4}^{+}$  at concentrations observed in precipitation in the Mid-Atlantic region inhibits  $NO_{3}^{-}$  uptake and assimilation

• Winter wheat appears more sensitive than rye to  $NH_4^+$  inhibition; NRase inhibition by  $NH_4^+$  (in winter wheat in geographically representative soils) was significant in Fort Mott loamy sand from Coastal Plain but not in silt loam and loam soils from Valley & Ridge and Piedmont regions, respectively • If  $NH_{4}^{+}$  in precipitation is shown to inhibit cover crop performance in the field and inhibition is dependent on cover crop and soil type, this knowledge can guide cost share and management decisions for increased protection of water quality



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