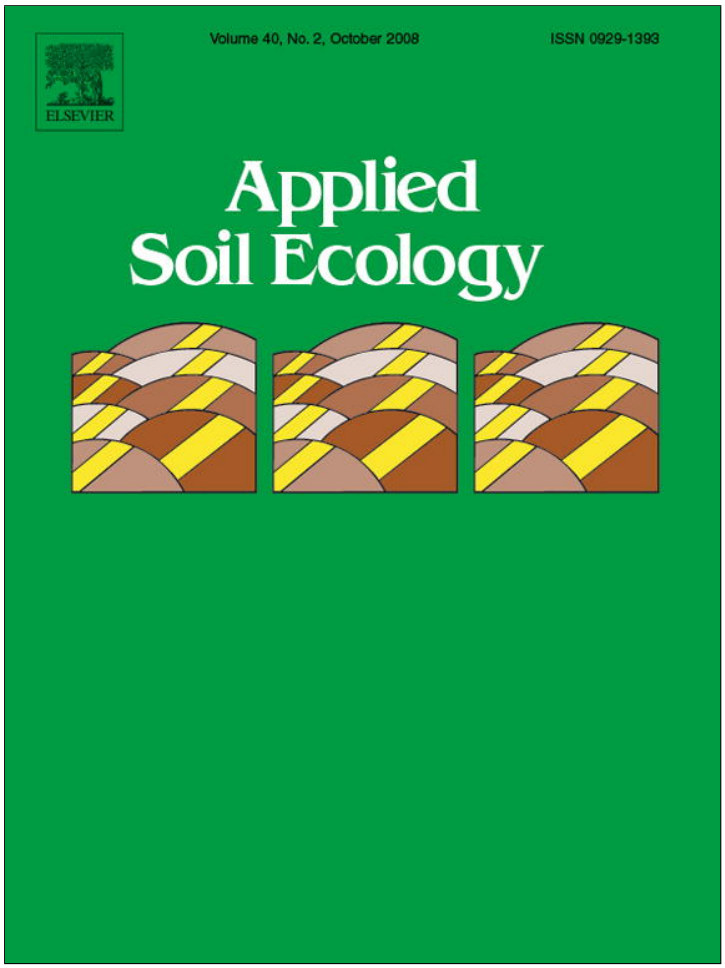

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# Alteration of the soil environment to maximize *Meloidogyne incognita* suppression by an alkaline-stabilized biosolid amendment☆

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ABSTRACT

The ability to identify and manipulate environmental factors conducive for nematode suppression by organic amendments is essential. The impact that soil temperature and moisture, an additional source of nitrogen, and simulated tarping had on the ability of an alkaline-stabilized biosolid amendment (N-Viro Soil; NVS) to suppress *Meloidogyne incognita* was evaluated. A *M. incognita*-infested loamy sand was amended with 2% (dry w $w^{-1}$) NVS plus urea (0.25 g $L^{-1}$ dry soil) and incubated for 5 days at different temperatures (21, 26 and 31 °C), moistures (25, 50 and 75% of water holding capacity (WHC)) in an open or closed incubation environment. Soils were also amended with NVS or urea (additional nitrogen source), alone or in combination. Soil solution pH and ammonia ($NH_3$) concentration (mM) were measured at 0, 3 and 5 days after amendment, and *M. incognita* reproduction on cucumber (eggs $L^{-1}$ dry soil) was assessed. In all experiments soil solution pH increased rapidly after NVS amendment to approximately 10.5 under all factors; by the end of the experiments soil solution pH had decreased to 8.5–9.0 in NVS-amended soil. NVS plus urea amended to soil resulted in greater *M. incognita* suppression than either alone, or compared to an unamended control. This suppression was related to maximum and cumulative $NH_3$ measured in soil over a 5-day period. Nematode suppression was not related to soil solution pH. For the tested factors (temperature, moisture, open vs. closed), *M. incognita* suppression was always greater in NVS plus urea-amended soil compared to the unamended controls, regardless of the tested factor. Differences within the factors were observed for $NH_3$ dynamics in soil solution over time. High temperature (31 °C), low percentage of WHC (25%), and closed incubation resulted in at least twice as much $NH_3$ being accumulated in soil, to levels above 100 mM $NH_3$. Temperature appeared to have increased urea hydrolysis and combined with the high pH resulting from NVS amendment liberated $NH_3 + NH_4^+$ from added urea accumulated as $NH_3$ in soil. Reduced rates of NVS could be applied if combined with a labile source of nitrogen (urea) to promote the rapid production of $NH_3$ under alkaline conditions. The ability of NVS to suppress *M. incognita* could also be improved by manipulation of the soil environment through irrigation and/or tarping.

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## 1. Introduction

### 1.1. Alkaline-stabilized biosolid amendments

Biosolids are the nutrient-rich, solid organic matter recovered from the treatment of domestic sewage in wastewater treatment facilities. After processing, these materials are increasingly being marketed as commercial fertilizers, soil conditioners and landfill cover. Although agronomic benefits of biosolids as nutrient sources have been demonstrated (Basta, 2000; Sloan and Basta, 1995), there are concerns about pathogenic microorganisms and the mobility and availability of heavy metals. Technologies have been developed for the treatment of biosolids to yield a pathogen-free and stable product during storage and transportation. One such technology is the N-Viro process™ where digested municipal biosolids are mixed with alkaline reagents such as cement kiln dust, lime kiln dust, coal ash, or flue-gas de-sulfurization byproducts (Logan and Burnham, 1995). Over 100 governmental agencies and private companies utilize the N-Viro process to manufacture over one million tons of product annually (www.nviro.com). The final products, N-Viro Soils (NVS), are solid, granular materials with many desirable agronomic properties.

Alkaline stabilization and low water content differentiate NVS from most other biosolid amendments. To some extent, NVS has similar chemical characteristics to a high organic matter, saline, calcareous soil (Logan and Harrison, 1995). Calcium dominates the chemical characteristics of NVS. Because of its alkalinity, NVS is often marketed as a substitute to liming agents.

### 1.2. Plant-parasitic nematode suppression by NVS

In addition to the soil fertility and organic matter benefits associated with NVS, another benefit is plant-parasitic nematode suppression. In laboratory experiments it was demonstrated that *Heterodera glycines* and *Meloidogyne incognita* were equally sensitive to a NVS amendment (Zasada and Tenuta, 2004). Differences occurred between nematode life stages, with the second-stage juvenile being more susceptible to a NVS amendment than the egg stage. Nematode mortality caused by NVS was positively correlated with sand solution pH levels and to a lesser extent with ammonia ($NH_3$) accumulation following amendment (Zasada and Tenuta, 2004; Zasada, 2005). Results have not been consistent in plant-based experiments. In greenhouse experiments NVS applied at rates equivalent to 2–20 dry t/ha reduced *H. glycines* populations, while in parallel field experiments, a 2.2 dry t $ha^{-1}$ NVS application rate did not reduce *H. glycines* populations (Welacky and Topp, 2001). In another greenhouse experiment, a NVS application equivalent to 60 dry t $ha^{-1}$ suppressed *M. incognita* numbers on cantaloupe while 20 dry t $ha^{-1}$ NVS did not (Meyer et al., 2005). Experimental inconsistencies may have been a result of differences in the NVS used or environments into which NVS was amended.

### 1.3. Environment and amendment efficacy

The environment has a profound influence not only on nematode population dynamics but also on the ability of management practices to suppress nematodes. Previous research has shown that soil properties can have a profound influence on the accumulation of $NH_3$ in soils. A loamy sand of low organic carbon content accumulated more $NH_3$ after amendment with meat and bone meal than a loam of moderate organic carbon (Tenuta and Lazarovits, 2002b). In subsequent experiments, $NH_3$ toxicity occurred in soils with low rates of nitrification, low levels of cation exchange capacity (CEC), moisture levels, and organic carbon contents, but high bulk density and sand contents (Tenuta and Lazarovits, 2004). These results indicated that soil properties and environment conditions were related to the effectiveness of meat and bone meal in reducing the viability of soilborne pathogens. Generally, the quantity of organic amendments generating $NH_3$ required for nematode control in an alkaline soil is less than that in acidic soil (Rodríguez-Kabána et al., 1987). The ability to identify environmental conditions conducive for nematode suppression by NVS is essential. The goal of this research was to evaluate *M. incognita* reproduction on cucumber (*Cucumis sativus*) after the application of NVS under a variety of soil conditions.

## 2. Materials and methods

### 2.1. General experimental parameters

All experiments were conducted using a Norfolk A loamy sand (sand:silt:clay 87:6:7; pH 7.3; OM 0.4), air dried, passed through a 2-mm sieve and stored at 4 °C until use. Dry soil, 2 kg, was added to a 4-L, sealable plastic bag prior to treatment establishment. In all experiments NVS was added at a rate of 2% dry $g^{-1}$ dry (w $w^{-1}$) soil with or without urea (0.25 g $L^{-1}$ dry soil). No additional source of nutrients was applied. The NVS used was highly alkaline with a pH reaction in water of approximately 12.0, a total solids content of 75%, 160 kg $Mg^{-1}$ calcium, 53 kg $Mg^{-1}$ sulfur, 17 kg $Mg^{-1}$ magnesium, 10 kg $Mg^{-1}$ potassium, 4 kg $Mg^{-1}$ total nitrogen, 2.8 kg $Mg^{-1}$ phosphorus, and 0.3 kg $Mg^{-1}$ $NH_4^+$-N (Biocheck Laboratories, Toledo, OH). *M. incognita*, 5000 eggs $L^{-1}$ soil, were added to soil (amended or unamended). The *M. incognita* used was originally isolated from a field near Salisbury, MD and cultured on greenhouse-grown pepper (*Capsicum annuum*) 'PA-136'. Eggs were obtained from roots with 0.5% sodium hypochlorite (Hussey and Barker, 1973), and used immediately. After the addition of amendments and nematodes, the soil was adjusted to a desired moisture level, depending upon the experiment, and gently mixed in the bags before being placed in incubators (specific parameters stated below) for 5 days. During the incubation period, 5 g soil samples were removed from each bag for pH and $NH_3$ determination at 0, 3 and 5 days. Soil solution pH was also determined upon completion of the experiments. At the end of the incubation period the soil was transferred to 1475 $cm^3$ pots and nematode survival was assayed with a cucumber plant.

Cucumber 'Sweet Slice' seeds were planted in flats of Premier Pro-Mix 'PGX' plug and germination medium (Premier Horticulture Inc., Quakertown, PA), and the seeds were germinated in a greenhouse maintained between 24 and 29 °C. Natural and supplemental lighting were combined to achieve a 16-h day length. Fourteen-day-old seedlings were

transplanted into treated soils. Plants were allowed to mature for 40–45 days. At harvest, shoot dry weight (all plant parts above the soil line) was determined after removal of the shoot from the pots and oven-drying for 7 days at 60 °C. Soil and roots from each pot were collected and placed in a cold room until processing for root weight and *M. incognita* egg densities. Wet root weight was determined after removal of adhering soil and then roots were processed to collect *M. incognita* eggs. Roots were macerated with scissors and then placed in a Warring blender with 10% sodium hypochlorite for 2 min. The contents of the blender were pored over 60- and 500-mesh sieves and the eggs were rinsed with water and collected from the 500-mesh sieve. All aqueous egg suspensions were refrigerated until counted. Aliquots were counted at a magnification of 10× to estimate numbers of eggs $L^{-1}$ dry soil.

In all experiments soil pH was determined by weighing 5 g of soil into a 50 polyethylene tube and adding 25 ml deionized water. The slurry was agitated using a reciprocal shaker at 350 RPM for 15 min, the supernatant was allowed to clear for 15 min, and the pH of the supernatant was then determined. For ammonium ($NH_4^+$) plus $NH_3$ analysis, 1 ml of the supernatant was removed and filtered through a PVDF 0.22-μm-pore filter (Pall Corporation, East Hills, NY) into a 1.5 ml Eppendorf tube. The $NH_4^+$ plus $NH_3$ concentration of the filtered soil solution was measured using a colorimetric method based upon the Berthelot reaction (Rhine et al., 1998). Ammonia concentration in sand solution was estimated using the Henderson-Hasselbalch equation describing the pH-dependent equilibrium between $NH_4^+$ and $NH_3$ (Tenuta and Lazarovits, 2002b).

### 2.2. Experiments

#### 2.2.1. Additional nitrogen source

Four treatments were applied to soil in bags: 2% NVS only, urea only, 2% NVS plus urea and an unamended control. Bags were adjusted to 70% of water holding capacity (WHC) (determined by the methodology described by Cassel and Nielsen (1986)), *M. incognita* added, and then the bags were closed and incubated at 26 °C for 5 days. All other experimental parameters were as described above.

#### 2.2.2. Open vs. closed experiments

Bags containing 1-L Norfolk A sandy loam were treated in a 2 × 2 factorial design. Soils received or did not receive 2% NVS plus urea and were then left open or closed during incubation. *M. incognita* was added to the amended soils, adjusted to 70% of WHC and incubated at 21 °C for 5 days. All other experimental parameters were as described above.

#### 2.2.3. Moisture experiment

A Norfolk loamy sand amended with or without 2% NVS plus urea and adjusted to 25, 50 and 70% of WHC (Cassel and Nielsen, 1986) in a 2 × 3 factorial design. Treated soils were inoculated with *M. incognita*, the bags were closed, and incubated at 25 °C for 5 days. All other experimental parameters were as described above.

#### 2.2.4. Temperature experiment

A 2 × 3 factorial experiment was employed for this experiment: with or without 2% NVS plus urea and incubated at different temperatures (21, 26, and 31 °C). Soils were adjusted to 70% of WHC, *M. incognita* was added and the treated soils were incubated at the appropriate temperature for 5 days. All other experimental parameters were as described above.

### 2.3. Statistical analysis

All experiments were randomized designs, conducted at least two times and treatments were replicated 5 times within experiments. Statistics were performed using SAS version 9.1 (SAS Institute, Cary, NC). Soil solution pH data was not transformed, while nematode, $NH_3$ and plant data was log transformed when necessary to achieve homogeneity of variance. Trials for each experiment were combined when possible ($P > 0.05$), and combined data is presented unless otherwise noted. Differences in nematode survival among trials and treatments were determined by analysis of variance using PROC MIXED, and means were compared using Tukey's adjusted for multiple comparisons ($P < 0.05$). Differences in $NH_3$ concentrations over time were determined by analysis of variance using a REPEATED statement and means compared similar to that described for nematode survival ($P < 0.05$). Cumulative $NH_3$ across 5 days was calculated as the area under the curve. The relationships between maximum pH and maximum and cumulative $NH_3$ exposure values and harvest *M. incognita* eggs $L^{-1}$ dry soil were determined using linear least squares regression models. Untransformed means are presented and errors are presented as ±standard errors.

## 3. Results

### 3.1. Additional nitrogen source

At 26 °C, it was necessary to combine NVS with an additional nitrogen source to maximize reduction in nematode survival. Nematode reproduction was greater ($P < 0.001$) in Trial 1 than Trial 2, and while the same trend in nematode survival was observed only data from Trial 1 is presented (Fig. 1A). Urea alone did not result in a significant reduction in eggs $L^{-1}$ soil compared to the unamended control. NVS alone was intermediate in its affect on nematode survival between urea and NVS plus urea. There was a significant interaction between NVS and urea ($P < 0.001$). Plant dry shoot weights increased with the addition of NVS or urea, alone or in combination. In Trial 1, there was an interaction between NVS and urea ($P < 0.001$) with larger plants being produced, 19.7 (±0.3) g, than either NVS (17.0 g (±0.4)) or urea (15.7 g (±0.5)) alone. Plant weight in the unamended control was 12.0 (±0.5) g. In Trial 2 all nitrogen additions resulted in an increase in shoot dry weight (18.6–19.6 g) compared to the unamended control (15.9 g (±0.7)). There was no difference in root wet weights between the nitrogen amendments and the unamended control (data not shown) in either Trial.

There was a dramatic difference in $NH_3$ concentration dynamics in soil solution over time among the treatments. Urea and NVS, both applied alone to soil, reached maximum $NH_3$ concentrations after 0 days, with little subsequent increases over time (Fig. 1B). This was in contrast to NVS plus urea-amended soil where the concentration of $NH_3$ in soil

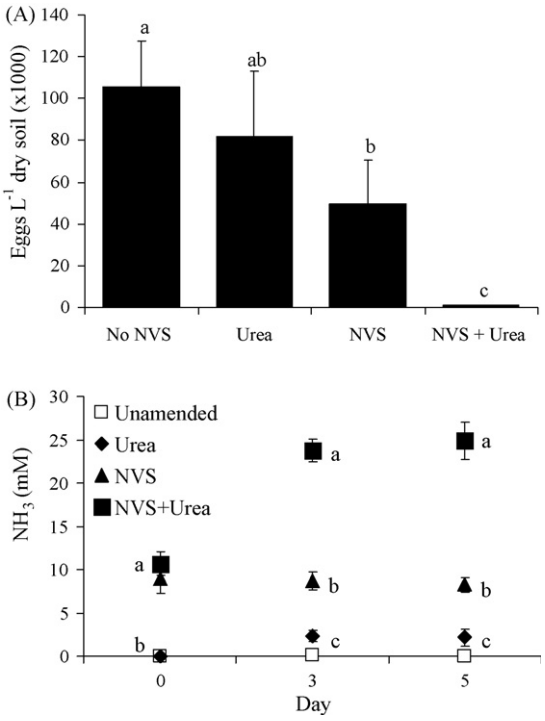


**Fig. 1 – *Meloidogyne incognita* suppression (A) and production of $NH_3$ over time (B) after the addition of 2% N-Viro Soil (dry w $w^{-1}$) or urea (0.25 g $L^{-1}$ dry soil), alone or in combination. Points or bars with the same letter are not statistically different according to Tukey's adjustment for multiple comparison test ($P < 0.05$). Standard errors (±1) are presented and $N = 10$ for each mean.**

solution almost doubled between 0 and 3 days to a level three times or higher than concentrations measured in the other amended soils. This trend was also reflected in maximum and cumulative $NH_3$ (Table 1), with nematodes being exposed to twice as much cumulative $NH_3$ in NVS plus urea-amended soils than soils receiving either alone. The addition of NVS, alone or in combination with urea, resulted in maximum pH levels higher than that measured in the urea-amended soil (Table 1). By the end of the experiment urea-amended soil had a pH of 6.2 (±0.1), while soil receiving NVS had a pH of 8.5 (±0.1), compared to 6.6 (±0.1) in the unamended control. A reduction in *M. incognita* survival was most closely related to cumulative and maximum $NH_3$ (Fig. 2A and B). There was only a weak relationship between the maximum pH to which nematodes were exposed and nematode survival (Fig. 2C).

### 3.2. *Open vs. closed experiments*

There was not a significant ($P = 0.19$)) reduction in *M. incognita* eggs $L^{-1}$ dry soil when the bag was closed after NVS plus urea amendment rather than left open (Table 2). There were always fewer eggs retrieved from soil amended with NVS plus urea compared to the unamended soil, regardless of incubation condition. The addition of nitrogen in the form of NVS plus urea significantly ($P < 0.001$) increased dry shoot and wet root weights. Shoot dry weights ranged from 19.2–19.3 g in NVS plus urea-amended soils compared to 15.4–16.7 g in unamended soils. Wet root weights were twice as big in soils receiving NVS plus urea compared to those not receiving additional nitrogen (data not shown).

The addition of NVS and urea to soil immediately increased soil solution pH to an average maximum of 10.6 (Table 1), compared to a pH range of 6.8–7.3 in the unamended soils. There was no influence of having the bags open or closed on

**Table 1 – Maximum pH, and cumulative and maximum $NH_3$ (mM) measured in a sandy loam over a 5 day period under different conditions after amendment with 2% N-Viro Soil (dry w $w^{-1}$) and/or urea (0.25 g $L^{-1}$ dry soil)**

| | Maximum pH[a] | Maximum $NH_3$[a,b] | Cumulative $NH_3$[a,b] |
|---|---|---|---|
| Additional nitrogen source | | | |
| Urea | 8.3 (±0.3) a | 2.8 (±2.9) a | 8.1 (±8.2) a |
| NVS | 10.7 (±0.6) b | 11.4 (±4.5) b | 43.4 (±14.1) b |
| NVS + urea | 10.7 (±0.8) b | 27.2 (±5.0) c | 100.3 (±18.1) c |
| Open vs. closed incubation | | | |
| Open | 10.6 (±0.3) a | 17.7 (±1.4) a | 56.6 (±10.4) a |
| Closed | 10.8 (±0.3) a | 28.7 (±4.0) b | 95.1 (±11.2) b |
| Percentage of WHC[c] | | | |
| 25% | 10.6 (±0.2) a[d] | 44.2 (±5.2) a | 152.3 (±20.6) a |
| 50% | 10.2 (±0.3) a | 24.9 (±3.1) b | 84.8 (±12.1) b |
| 75% | 10.5 (±0.3) a | 18.7 (±1.1) c | 70.6 (±5.4) b |
| Temperature | | | |
| 21 °C | 10.7 (±0.3) a | 18.0 (±1.9) a | 62.9 (±4.0) a |
| 26 °C | 10.9 (±0.3) a | 28.7 (±2.1) b | 108.6 (±5.3) b |
| 31 °C | 11.0 (±0.2) a | 39.0 (±4.5) c | 134.1 (±12.6) c |

[a] Soil solution pH was determined on a 1:5 dilution shaken for 15 min and allowed to settle for 15 min.
[b] Concentration of $NH_3$ in soil solution was determined by the Henderson-Hasselbach equation (Tenuta and Lazarovits, 2002b). Data was log transformed ($\log_{10}(x + 1)$) prior to analysis; non-transformed data shown.
[c] Water holding capacity (WHC) was determined by the methodology described by Cassel and Nielsen (1986).
[d] Means within a column followed by the same letter are not statistical different according to Tukey's adjustment for multiple comparison test ($P < 0.001$). Means are followed by the ±standard error and $N = 10$.

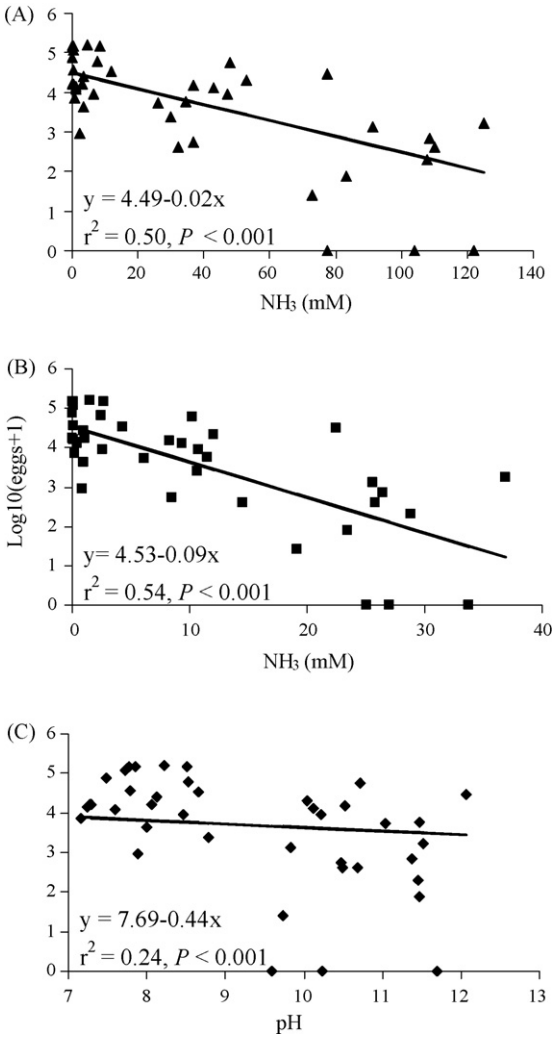


**Fig. 2 – Relationships between cumulative (A) and maximum (B) $NH_3$ (mM) and maximum pH (C) measured over a 5 day period and *Meloidogyne incognita* suppression. Nematode data (eggs $L^{-1}$ dry soil) was transformed ($log_{10}(x + 1)$) prior to analysis and log transformed data is presented.**

soil solution pH. In both trials pH dropped rapidly with time; after 5 days pH ranged from 8.5–9.5 and upon completion of the experiment pH ranged from 8.1–8.8 in NVS and urea-amended soils. A greater concentration of $NH_3$ was present ($P < 0.001$) in soil at days 3 and 5 when bags were kept closed compared to open. There was a difference between maximum and cumulative $NH_3$ in soil solution between open and closed (Table 1), with more $NH_3$ being present in closed vs. open amended-bags. $NH_3$ accumulation was more strongly related to a reduction in nematode survival, ($r^2 = 0.53$, $P < 0.001$) compared to maximum pH ($r^2 = 0.42$, $P < 0.001$) and maximum $NH_3$ ($r^2 = 0.43$, $P < 0.001$).

### 3.3. *Moisture experiment*

Despite significant difference in $NH_3$ dynamics between percentages of WHC (Table 1; Fig. 3B), there was no difference

**Table 2 – Influence of different factors on the ability of N-Viro Soil (2% dry w $w^{-1}$; +NVS) plus urea (0.25 g $L^{-1}$ dry soil) or no NVS (0% dry w $w^{-1}$; −NVS) to suppress *Meloidogyne incognita* (eggs $L^{-1}$ dry soil)**

| | Open vs. closed incubation[a] | |
|---|---|---|
| | Open | Closed |
| +NVS | 2545 (±780) b | 908 (±458) b |
| −NVS | 22156 (±4379) a | 26050 (±7855) a |
| | Percentage of water holding capacity[a] | |
| | 25 | 50 | 75 |
| +NVS | 545 (+301) b[b] | 535 (+101) b | 289 (+96) b |
| −NVS | 48500 (+10766) a | 42275 (+9592) a | 58625 (+11289) a |
| | Temperature (°C)[c] | | |
| | 20 | 25 | 30 |
| +NVS | 900 (+477) b | 800 (+280) b | 1675 (+592) b |
| −NVS | 120800 (+14413) a | 105600 (+21851) a | 79840 (+25479) a |

[a] $N = 10$.
[b] Different letters within a factor indicate significant differences according to Tukey's adjustment for multiple comparison test ($P < 0.05$). Data was transformed ($log_{10}(x + 1)$) prior to analysis; non-transformed data shown.
[c] $N = 5$.

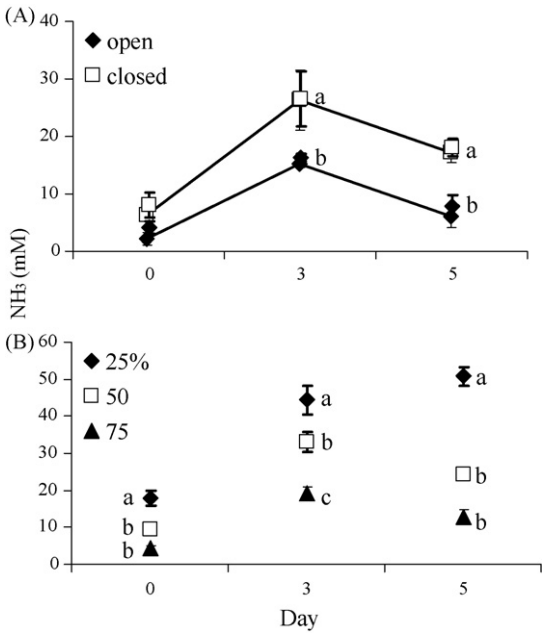


**Fig. 3 – Ammonia ($NH_3$) accumulation in soil solution over time in open vs. closed bags (A), and different percentages of water holding capacity (B) after the application of 2% (dry w $w^{-1}$) N-Viro Soil or urea (0.25 g $L^{-1}$ dry soil) to soil. Points within an environmental factor and sampling time with different letters are significantly different according to Tukey's adjustment for multiple comparison test ($P < 0.05$). Standard errors (±1) are presented and N = 5 or 10 for each mean.**

**Table 3 – Influence of incubation temperature on soil solution pH, $NH_3$ and $NH_3 + NH_4^+$ concentrations (mM) at three sampling times after amendment of a sandy loam with 2% N-Viro Soil (dry w $w^{-1}$) and urea (0.25 g $L^{-1}$ dry soil)**

| Temperature (°C) | pH[a] | $NH_3 + NH_4^+$ (mM)[b] | $NH_3$ (mM)[b] |
|---|---|---|---|
| Day 0 | | | |
| 21 | 10.7 (±0.3) | 9.3 (±1.0) | 8.3 (±1.2) |
| 26 | 10.9 (±0.3) | 11.6 (±1.2) | 10.7 (±1.4) |
| 31 | 11.0 (±0.2) | 12.9 (±1.5) | 12.1 (±1.6) |
| Day 3 | | | |
| 21 | 10.3 (±0.4) | 28.8 (±6.1) a[c] | 12.4 (±1.5) a |
| 26 | 9.6 (±0.2) | 40.9 (±4.0) b | 23.7 (±1.3) b |
| 31 | 9.9 (±0.3) | 58.6 (±7.3) b | 40.0 (±4.5) c |
| Day 5 | | | |
| 21 | 10.2 (±0.4) | 36.8 (±9.4) a | 16.4 (±2.4) a |
| 26 | 9.2 (±0.1) | 57.7 (±3.9) b | 24.9 (±2.1) b |
| 31 | 9.0 (±0.1) | 49.1 (±4.4) ab | 18.5 (±2.1) ab |

[a] Soil solution pH was determined on a 1:5 dilution shaken for 15 min and allowed to settle for 15 min.
[b] Concentration of $NH_3 + NH_4^+$ in soil solution was determined by the Henderson-Hasselbach equation (Tenuta and Lazarovits, 2002b). Data was log transformed ($\log_{10}(x + 1)$) prior to analysis; non-transformed data shown.
[c] Means within a column and time followed by the same letter are not statistical different according to Tukey's adjustment for multiple comparison test ($P < 0.001$). Means are followed by the ±standard error and $N = 10$.

in final egg densities between the WHC treatments (Table 2). There was an effect of adding NVS plus urea to soil, with eggs $L^{-1}$ soil being more than 100× lower in NVS plus urea-amended soil compared to unamended soil. The initial percentage of WHC had no impact on plant growth. Rather the addition of nitrogen significantly ($P < 0.0001$) increased both root and shoot growth compared to that in unamended soil regardless of WHC. Shoot dry weights in NVS plus urea-amended soils ranged from 20.8–23.4 g compared to 12.4–14.4 g in unamended soils. In Trials 1 and 2, root wet weights were 4 and 2 times large in NVS plus urea-amended soils compared to unamended soils, respectively.

The moisture content of the soil (25, 50 or 75% of WHC) had no impact on maximum soil solution pH ($P > 0.05$) which increased immediately after the addition of NVS plus urea to soil (Table 1). The maximum pH to which nematode were exposed was strongly related ($r^2 = 0.79$, $P < 0.001$) to a reduction in nematode survival. After 42 days pH in NVS plus urea-amended soils was on average 8.5 (±0.1). Unamended soil maintained a soil pH in the range of 6.7–7.9 during the entire experiment. Soil moisture had a highly significant ($P < 0.001$) impact on maximum and cumulative $NH_3$ in soil (Table 1), with higher concentrations being present at the lowest percentage of WHC. Both maximum and cumulative $NH_3$ were related ($r^2 = 0.60$ and 0.62, respectively, $P < 0.001$) to a reduction in nematode survival. There was an immediate impact of percentage soil moisture on the presence of $NH_3$ in soil solution with a higher concentration of $NH_3$ present at the lowest WHC, 25%, compared to the higher percentages of WHCs (Fig. 3B). This trend continued during subsequent sampling times, and $NH_3$ concentration in soil actually continued to increase through day 5 at 25% of WHC, while it decreased in 50 and 75% of WHC.

### 3.4. *Temperature experiment*

Among the different experiments, *M. incognita* survival and reproduction was the most variable in temperature experiments. Nematode reproduction was greater in Trial 1 (Table 2) than Trial 2 (data not shown), although the same trend occurred. There was only a difference in eggs $L^{-1}$ dry soil between the main factor of NVS plus urea-amended or unamended, with there being more eggs in unamended soils. The addition of NVS plus urea, not incubation temperature, influenced plant growth; in general, shoot and root biomass was greater in those soils that received NVS plus urea.

There was no difference in soil solution pH between the temperatures after amendment with NVS plus urea (Tables 1 and 3). A difference in maximum and cumulative $NH_3$ did occur among the different temperatures ($P < 0.001$), with nematodes being exposed to higher concentrations of $NH_3$ at 31 °C (Table 1). Similar to the moisture and open vs. closed experiments, there was a relationship between nematode survival and maximum $NH_3$ ($r^2 = 0.59$, $P < 0.001$) and cumulative $NH_3$ ($r^2 = 0.59$, $P < 0.001$). Since the concentration of $NH_3$ is dependent upon the temperature dependency of the $pK_a$ coefficient and $NH_3 + NH_4^+$ concentration in solution, $NH_3$ and $NH_3 + NH_4^+$ data are presented (Table 3). At day 0 there was no difference in the concentration of $NH_3 + NH_4^+$ in soil solution between the temperatures. At the later days there was a difference in $NH_3 + NH_4^+$ concentrations in soil solution with the 26 and 31 °C treatments having higher levels at day 3 than for the 21 °C treatment. There was a decrease in $NH_3 + NH_4^+$ and $NH_3$ at the highest temperature, 31 °C, compared to the other temperatures at day 5 (Table 3).

## 4. Discussion

Manipulation of the soil environment after the application of NVS to soil dramatically changed the dynamics of $NH_3$ in soil solution. While difference were not observed in *M. incognita* suppression when NVS plus urea was added to soil at different temperatures, moistures or incubation condition, this was probably due to the fact that high pH and toxic concentrations of $NH_3$ were always achieved in soil solution. The results of

this study do demonstrate that rates of NVS and supplemental nitrogen can be reduced when applied together under certain conditions to achieve nematode suppression.

It is desirable to reduce the rate of NVS necessary to achieve consistent and reliable nematode suppression from an environmental and economic perspective. The NVS rate chosen for this study was based upon our previous findings in laboratory, greenhouse and field experiments (Meyer et al., 2005; Zasada and Tenuta, 2004; Zasada et al., 2007). When NVS was applied alone to sand in small incubation containers a lethal concentration value necessary to kill 90% of *M. incognita* eggs was calculated as 2.6% (dry w $w^{-1}$) (Zasada and Tenuta, 2004). When *M. incognita* eggs, inoculated into a 1:5 compost:-sand mixture, were exposed to 1 and 3% NVS (dry w $w^{-1}$), the lower rate did not result in a reduction in nematode survival while the higher rate did, compared to an unamended control (Meyer et al., 2005). Five times as much $NH_3$ (5 mM after 1 h) was generated after the amendment to soil of 3% NVS compared to 1% NVS. In a field microplot experiment, NVS applied at a rate equivalent to 2.5% (dry w $w^{-1}$) to a loamy sand resulted in non-significant reduction in *M. incognita* juvenile population densities compared to an unamended control (Zasada et al., 2007). A 2% application rate was chosen for this study as a rate that would result in some nematode suppression but probably could not stand alone as a reliable and consistent nematode management practice.

Free $NH_3$ is nematicidal, whereas $NH_4^+$ is not. Many factors influence $NH_4^+/NH_3$ dynamics, including pH and temperature. According to the Henderson-Hasselbalch equation, at a pH of 9.3 ($pK_a$) the ratio of N present as $NH_4^+$:$NH_3$ is 1:1, with more $NH_3$ being generated as pH increases. We applied approximately 138 mg N $kg^{-1}$ when NVS was combined with urea, and observed substantially *M. incognita* mortality when soil solution pH was elevated. When applied alone urea supplied 58 mg N $kg^{-1}$ and there was no reduction in nematode survival; NVS supplied 80 mg N $kg^{-1}$ and nematode reduction was intermediate between urea alone and NVS plus urea. The application of both 70 and 700 ppm N as urea to a soil with a pH of 6.2 decreased *Pratylenchus penetrans* population densities (Walker, 1971). The 700 ppm N treatment resulted in $NH_3$ levels above 300 ppm N after 5 days. Urea applied to soil, pH 8.3, at rates >100 mg N $kg^{-1}$ reduced plant-parasitic nematode population densities compared to an untreated control (Akhtar, 1998). Decreased viability of *M. incognita* eggs was observed when ≥200 mg N $kg^{-1}$ soil (soil pH 6.3) was applied as urea (Crow et al., 1996). Urea also suppressed several nematode species, including *Meloidgyne* spp., when applied at rates above 300 kg N $ha^{-1}$ (Rodríguez-Kabána, 1986). In general, previous research demonstrated that very high rates of ammonical N were necessary to control nematodes at soil pHs ranging from 6.3 to 8.3. The goal of combining reduced rates of NVS with an additional N source is to rapidly manipulate soil pH to ≥10.0 to promote the majority of $NH_3 + NH_4^+$ to exist as gaseous and nematicidal $NH_3$. Similar to our findings, Oka et al. (2006) demonstrated that the nematicidal activities of chicken litter or ammonium sulphate (50 mg N $kg^{-1}$) were enhanced when combined with cement kiln dust which resulted in an increase in soil pH to >10.0. This was similar to their previous experiments (Oka and Pivonia, 2003) where a nitrification inhibitor was added to soil and kept $NH_4^+$ concentrations and soil pH values higher for longer periods of time compared to untreated soil. It is important to note that a labile source of nitrogen will have to be used to take advantage of the rapid rise in pH facilitated by the application of NVS to soil, promoting the conversion of $NH_4^+$ to $NH_3$.

We also evaluated the influence of temperature on $NH_3 + NH_4^+$ dynamics in soil and nematode suppression. The $pK_a$ of $NH_3$ is temperature dependent; therefore, the $NH_3/NH_4^+$ equilibrium should be shifted toward $NH_3$ at higher temperatures. The effect of temperature on $pK_a$ and $NH_3$ at the high soil pH achieved with addition of NVS would be small. At day 0, the effect of temperature on $pK_a$ and $NH_3$ concentration was estimated to increase $NH_3$ concentration by 3 and 1% for the 26 and 31 °C treatments over the 21 °C treatment, respectively. In comparison elevated $NH_3 + NH_4^+$ concentration at day 0 was 25 and 11% higher for the 26 and 31 °C treatments over the 21 °C treatment, respectively. At day 3 the influence of increased accumulation of $NH_3 + NH_4^+$ with temperature was more striking. On this day the effect of temperature on $pK_a$ and $NH_3$ concentration was estimated to decrease $NH_3$ concentration by 21% and increase it by 23% for the 26 and 31 °C treatments over the 21 °C treatment, respectively. In contrast, higher $NH_3 + NH_4^+$ concentration would result in 42 and 43% higher $NH_3$ concentration for the 26 and 31 °C treatments over the 21 °C treatment, respectively. Our results indicate that increased $NH_3$ concentrations in soil were achieved by increased concentration of $NH_3 + NH_4^+$ being present rather than temperature lowering $pK_a$.

Urease activity has been shown to be remarkably stable in soil with rapid drying of soil over a temperature range of 30–60 °C resulting in no loss of activity (Zantua and Bremner, 1977). Further, urease activity in non-dried soil increased with temperature to at least 37 °C (Gould et al., 1973). It seems in this study, urea hydrolysis increased with higher temperatures resulting in greater accumulation of $NH_3 + NH_4^+$. At the high pH of soil following NVS addition, a decrease in $pK_a$ with temperature would increase $NH_3$ accumulation slightly. However, greater urea hydrolysis at high pH imparts that most liberated $NH_3 + NH_4^+$ from urea fertilizer would be present in soil in the $NH_3$ form.

Ammonia volatilization after urea application was highest after 2 days and corresponded with daily maximum temperatures (Clay et al., 1990). Although there was no difference in nematode suppression between incubation temperatures in this study, temperature has been shown to improve the ability of chicken litter to control *M. incognita* (López-Pérez et al., 2005). At 30 °C, chicken litter consistently reduced egg numbers and root galling compared to an unamended control and to results from 20 and 25 °C. While a low concentration of $NH_3$ will be sustained at lower temperatures, to take advantage of the rapid NVS-facilitated increase in soil pH and generate lethal concentrations of $NH_3$ at minimal application rates, soil temperature should be elevated. This may require tarping of the soil or the application of amendments during a warm time of year.

Identifying a specific concentration of $NH_3$ in soil solution needed to kill nematodes is hard to pinpoint because of the complexity of the soil environment. We calculated the maximum and cumulative $NH_3$ concentrations to which nematodes were exposed to over a 5-day period. Our data

indicates that nematodes need to be exposed to at least a maximum concentration of 11.4 mM $NH_3$ or to a cumulative $NH_3$ concentration of 43.4 mM to achieve a 50% reduction in *M. incognita* egg survival. For the plant pathogen *Verticillium dahliae*, the accumulation of >35 mM $NH_3$ was necessary to result in microsclerotia death in a loam soil (Tenuta and Lazarovitis, 2002a). It is not clear from this study if nematode mortality is a function of a target maximum concentration of $NH_3$ being present or a constant exposure to accumulated $NH_3$, or both.

In addition to applying reduced rates of NVS with an additional N source, it may be possible to manipulate the soil environment into which amendments are being added to maximize their efficacy. Bags containing amended soil were left open or closed to simulate tarping. Closing the bags during incubation substantially increased the maximum and cumulative concentration of $NH_3$ in soil solution to which nematodes were exposed. Sealing $NH_4OH$-treated soil reduced the amount of N necessary to control nematodes from 200 to 75 mg N $kg^{-1}$ (Oka and Pivonia, 2002). Compacting beef cattle farmyard manure, then covering with a plastic sheet significantly reduced $NH_3$ emissions from manure by over 90% during the first year of storage (Chadwick, 2005). Tarping of NVS-amended soil should be advocated to minimize $NH_3$ loss to the environment, reduce application rates of amendments and to enhance nematode suppression.

The volatilization of $NH_3$ has been shown to increase with decreasing water content. Adding water to soil after the application of urea decreased $NH_3$ losses by 15% (Black et al., 1987). In addition, heavy rainfall was a major determinant in a model predicting the transfer of $NH_3$ across the soil/air interface (Roelle and Aneja, 2002). Tenuta and Lazarovits (2004) confirmed that $NH_3$ toxicity to *V. dahliae* occurred in soils amended with meat and bone meal with low moisture levels. The lowest percentage of WHC tested in this study increased the amount of $NH_3$ in soil solution, and compared to any of the others factors tested sustained this increase through 5 days. From a plant-parasitic management perspective, while low soil moisture increases $NH_3$ volatility, it stands to reason that the concentration of $NH_3$ in solution would be less dilute, therefore increasing the concentration to which nematodes are exposed. Conversely, although high soil moisture minimizes volatility, it also makes the concentration of $NH_3$ in solution more dilute.

It is important to manage the environment into which amendments are incorporated to maximize nematode suppression. We have demonstrated that the application rate of NVS can be reduced when combined with an additional nitrogen source for the rapid production of toxic concentrations of $NH_3$ in soil. Once incorporated into soil, management of soil temperature and moisture will also improve the efficacy of NVS as a plant-parasitic nematode management practices. Future research should strive to implement NVS in a site-specific manner only into environments and production systems where the soil environment can be altered.

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