Influence of Ammonium : Nitrate Ratio and Nitrogen Concentration on Nitrification Activity in Soilless Potting Media

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Abstract. Effects of medium, NH_4^+ : NO_3^- ratio, and N fertilizer rate on the development of NH_4^+ oxidation in soilless potting media were evaluated. In two separate experiments, NH_4^+ oxidizing activity increased to a maximum (4 to 6 weeks of cropping) and then dropped off sharply. Ammonium oxidation activity varied significantly among types of soilless potting media. Media fertilized with 1 NH_4^+ -N: 3 NO_3^- -N had higher rates of NH_4^+ oxidation than media fertilized with ratios of either 1:1 or 3:1. Nitrogen fertilization at 15 mM gave consistently higher oxidation rates than fertilization at 30 mM. In general, media samples that had been cropped with plants had higher nitrifying activity than unplanted samples. Ammonium oxidation rate over all observations was significantly correlated with medium pH ($r = 0.50$). pH values above 6.8 were necessary but not completely sufficient for relatively high rates of NH_4^+ oxidation. Rates of oxidation were insignificant with pH values < 5.6 .

Nitrification is defined as the oxidation of NH_4^+ to NO_3^- and is generally mediated by the activities of two groups of chemoautotrophic bacteria (Alexander, 1977). One group, the NH_4^+ oxidizers, initiates the process with the formation of NO_2^- , while another group, the NO_2^- oxidizers, completes the process by converting NO_2^- to NO_3^- . The nitrification process is sensitive to pH, water, and temperature and has an absolute requirement for O_2 (Haynes, 1986).

Until recently, little detailed work on the process of nitrification has been done with soilless potting media used in the production of horticultural crops (Elliott, 1986; Niemiera and Wright, 1987a, 1987b). These media are becoming popular substrates for horticulture production because they are lightweight, uniform, reproducible, and generally available (Nelson, 1985). Because these media may contain several essentially sterile ingredients, soilless media characteristically have a smaller beginning biological population than mineral field soils (Lang, 1990). Under unfavorable environmental conditions for plant growth, such as in acidic media, or under cool, cloudy days, low rates of nitrification may lead to NH_4^+ toxicity (Barker and Mills, 1980). Certain cultural practices, notably the application of N fertilizers and other chemicals, may also influence microbial activity and ultimately affect nitrification (Elliott and Lang, 1991). Hence, understanding and characterizing the nitrification process in these media is important in the effective regulation of N supply to plants.

The overall objective of this study was to characterize the influence of NH_4^+ -N : NO_3^- -N ratio and N concentration in fertilizer on the development of nitrification in soilless media.

Materials and Methods

Medium and ratio effects (Expt. 1). Rooted poinsettia cuttings (*Euphorbia pulcherrima* Wind. 'V-14 Pink') were planted on 10 Oct. in 500-cm³ plastic pots (one plant per pot) containing either Metro-Mix 220 (MM220) or Metro-Mix 360 (MM360) (W.R. Grace & Co., Fogelsville, Pa.). Exact composition of these media is proprietary information, but MM220 contains sphagnum peat, vermiculite, and perlite; MM360 contains peat-moss, vermiculite, processed bark ash, and sand. Plants were grown in a glass greenhouse for 8 weeks under natural daylength with heating and cooling setpoints of 18/24C. Plants were fertilized at each irrigation with a nutrient solution containing (in mM) 15 (210 ppm) total N with NH_4^+ -N : NO_3^- -N ratios of either 3:1 [4.5 KNO_3 , 1 $\text{NH}_4\text{H}_2\text{PO}_4$, 2.75 NH_4NO_3 , and 4 NaNO_3], 1:1 [4.5 KNO_3 , 1 $\text{NH}_4\text{H}_2\text{PO}_4$, 3 NH_4NO_3 , and 1.75 $(\text{NH}_4)_2\text{SO}_4$], or 1:3 [3.75 KNO_3 , 0.75 KH_2PO_4 , 0.25 $\text{NH}_4\text{H}_2\text{PO}_4$, and 5.5 $(\text{NH}_4)_2\text{SO}_4$]. All solutions contained the following nutrients and concentrations: P (2.3 mM), K (5.4 mM), Fe (20 μM), Mn (10 μM) B (20 μM), Zn (2.5 μM), Cu (1.5 μM), and Mo (1 μM). Sulfur concentration varied between the different treatment solutions. Plants were irrigated as needed using a plastic tube irrigation system. Pots were arranged in a randomized complete block design with two blocks, each containing four pots per medium-treatment combination. At 2-week intervals, media samples were collected, pots in each block combined, and pooled samples analyzed for pH and short-term NH_4^+ oxidation activity.

Media samples were dried under a high-efficiency particulate adsorption (HEPA)-filtered air stream for 12 to 36 h at 25 to 30C and sifted through a 2-mm mesh screen to remove plant roots and to provide a uniform sample. The pH of the media was measured in a saturated paste (Rhoades, 1982). Ammonium oxidation rate was determined in 15-cm³ samples to which were added 20 ml of 1 mM $(\text{NH}_4)_2\text{SO}_4$ and 10 mM NaClO_3 in 1 mM phosphate buffer (pH 7.0) (Elliott, 1988). Chlorate was added to inhibit NO_2^- oxidation (Belser and Mays, 1980; Elliott, 1988; Less and Simpson, 1957). After incubation in the dark at 25C for 6 and 17 h, three random subsamples of each medium-treatment combination were removed. The aqueous phase was filtered and the extracts frozen until analyzed. Nitrite was determined calorimetrically (Keeney and Nelson, 1982). Rates of NH_4^+ oxidation were calculated from the difference in NO_2^- concentration between 6 and 17 h of incubation. Nitrite formation

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within this period is linear (Elliott, 1988; Lang, 1990). Analysis of variance was calculated using SAS General Linear Model and treating $\text{NH}_4\text{-N} : \text{NO}_3\text{-N}$ ratio, media type, harvest time, and blocking as classification variables.

Medium, ratio, and N concentration effects (Expt. 2). Rooted cuttings of chrysanthemum (*Dendranthema grandiflora* (Ramat.) Kitamura 'Bright Golden Anne') were planted on 31 Oct. in 500-cm³ pots (1 plant per pot) containing either MM220, Metro-Mix 300 (MM300), or Metro-Mix 350 (MM350). MM350 is composed of sphagnum peat, vermiculite, perlite, and bark ash; MM300 contains peatmoss, vermiculite, perlite, sand, bark ash, and composted pine bark. Plants were grown in a glasshouse for 11 weeks under natural daylength with heating and cooling setpoints of 18/24C. Plants were fertilized at each irrigation with a nutrient solution containing a $\text{NH}_4\text{-N} : \text{NO}_3\text{-N}$ ratio of either 3:1, 1:1, or 1:3 as in Expt. 1. Fertilizer was applied at either 15 or 30 mM N. Pots containing medium but without plants were fertilized similarly. Pots were arranged in a split-split plot design, four pots per medium-treatment combination, with pots serving as the main plot, harvest dates serving as the subplots, and planting/no planting serving as the sub-subplots. At 4, 8, and 11 weeks, media samples from replicate pots were collected and analyzed individually for pH and NH_4 oxidation activity. Media pH was determined in a saturated paste extract. Ammonium oxidation rate was determined as in Expt. 1, except that samples were incubated for 6 and 18 h. Statistical analysis was performed on the data again using the general linear model.

Results and Discussion

Main effect means are presented over all observations for the treatment factors of medium type, $\text{NH}_4\text{-N} : \text{NO}_3\text{-N}$ ratio, N concentration, planting, and time of cropping. Except for the interaction between N concentration and planting, interactions between other treatment factors were nonsignificant.

In both experiments, NH_4 -oxidizing activity increased up to a maximum and then sharply decreased (Fig. 1). In Expt. 1,

Fig. 1. Development of NH_4 -oxidizing activity over time for all media. (●—●) Experiment 1, development over 8 weeks with sampling every 2 weeks. Each point represents the mean of 36 observations. (■—■) Experiment 2, development over 11 weeks with sampling at 4, 8, and 11 weeks. Each point represents the main effect mean of 144 observations. Error bars, shown if larger than the dimensions of the symbol, represent the standard error of the mean.

the maximum NH_4 oxidation rate occurred after 6 weeks of cropping, but then dropped off significantly between 6 to 8 weeks. Experiment 2 showed a similar trend, but the decrease in rate of NH_4 oxidation between 4 and 8 weeks was more gradual than in Expt. 1.

It has been documented that flushes of nitrifying activity can be stimulated by the addition of NH_4 fertilizers (Belser, 1979; Haynes, 1986). Under ideal conditions of growth, however, one would expect that activity would level off after an initial increase (Belser, 1979). In this study, it appears that either the growth of NH_4 oxidizers was suppressed or their activity was severely inhibited.

High levels of microbial substrate can inhibit the growth and activity of nitrifying organisms (Boon and Laudelout, 1962; Malhi and McGill, 1982). It has also been shown that NH_4 oxidizing bacteria are characteristically less sensitive than *Nitrobacter* to high NH_4 concentrations (Nakos and Wolcott, 1972). Although NH_4 in the media was not measured in this study, it is very likely that in these small contained volumes, retention of NH_4 in media might have increased over time due to the high rates of fertilization (Niemiera and Wright, 1987b). This, along with a lowering of the pH (data not shown), may have contributed to the sharp decline in activity at 8 weeks.

Direct comparison of critical values between experiments is not possible since samples were not taken at 2 or 6 weeks for Expt. 2. Overall rates were lower, however, in Expt. 2 because of the additional treatments of no planting and increased fertilizer rate.

Medium type significantly affected NH_4 oxidizing activity (Table 1). In both experiments, MM220 had the highest activity, although the difference was less in Expt. 1 than in Expt. 2.

Elliott (1986) showed that uncropped, nontreated, commercial soilless media vary considerably in nitrifying activity. The fact that MM220 had higher rates than other media tested has generally been observed in other studies in our laboratory (Elliott, 1988). More recent work, however, has shown that this is not always the case, as MM350 may exhibit NH_4 oxidation rates as high as or higher than MM220 (Elliott and Lang, 1991). However, MM300, a mixture of peat, vermiculite, perlite, sand, and composted pine bark, gave the lowest rate of NH_4 oxidation (Expt. 2). This result is in contrast to previous experiments where bark or bark-containing media exhibited higher nitrifying activity (Elliott, 1986; Niemiera and Wright, 1986).

Table 1. Effect of medium type on nitrite formation and pH of soilless potting media.

Medium type ^z	NO_2^- formed ($\text{nmol} \cdot \text{cm}^{-3} \cdot \text{h}^{-1}$)	Medium pH ^y
Expt. 1		
MM220	2.03 a ^x	6.30 a
MM360	1.73 b	5.88 b
Significance	*	**
Expt. 2		
MM220	1.50 a	6.74 a
MM350	0.63 b	6.23 b
MM300	0.14 c	5.55 c
Significance	**	**

^zMedium type MM220, MM360, MM350, and MM300 represent Metro-Mix 220, 360, 350, and 300, respectively.

^ypH was determined in media by reading directly in the saturated paste (Expt. 1) or in the filtered saturated paste extract (Expt. 2).

^xMean separation by Duncan's multiple range test at $P = 0.05$.

^{**}Significant at $P = 0.05$ or 0.01 , respectively, using analysis of variance.

Autotrophic nitrifying bacteria are strongly affected by pH, with values lower than 7.0 beginning to inhibit their growth and metabolism (Focht and Verstraete, 1977). One possible explanation for the difference in NH_4 oxidation between media is the different pH values of the three media. MM220 maintained a significantly higher pH than either MM360, MM350, or MM300 (Table 1). The higher pH may have modified the micro-ecological environment of MM220 during treatment, allowing greater rates of NH_4 oxidation. Relatively small differences in media pH may result in significant changes in NH_4 oxidation. This result parallels those of other nitrification studies with pine bark media, where relatively small pH changes due to lime additions caused relatively large changes in nitrate accumulation (Niemiera and Wright, 1986).

Ammonium oxidation rates decreased as the level of $\text{NH}_4\text{-N}$ in the fertilizer solution increased (Table 2). Pots fertilized with 1 $\text{NH}_4\text{-N}$: 3 $\text{NO}_3\text{-N}$ had significantly higher rates than pots fertilized with a 1:1 ratio, which had significantly higher rates than those fertilized with a 3:1 ratio. The difference in NH_4 oxidation rate between media fertilized with solutions containing $\text{NH}_4\text{-N}$: $\text{NO}_3\text{-N}$ ratios of 1:1 and 3:1, however, was less pronounced in Expt. 1 than in Expt. 2.

As with media type, the decrease in NH_4 oxidation rate between pots fertilized with different NH_4 levels can be partially explained by pH. In media fertilized with solutions containing 3 $\text{NH}_4\text{-N}$: 1 $\text{NO}_3\text{-N}$, values dropped to below 6.00 (pH 5.61 in Expt. 1 and pH 5.89 in Expt. 2). Highest pH values were associated with media fertilized with solutions containing a 1:3 ratio (pH 6.60 in Expt. 1 and pH 6.51 in Expt. 2). It is well documented that fertilization of plants with high ammonium-containing fertilizers will result in a lowering of the medium pH (Barker and Mills, 1980), and this may have contributed to lower pH levels for the media fertilized with solutions containing $\text{NH}_4\text{-N}$: $\text{NO}_3\text{-N}$ ratios of 1:1 and 3:1. The process of NH_4 oxidation by autotrophic bacteria also decreases the pH and may lead to a self-induced inhibition of activity.

Increasing N fertilizer concentration decreased NH_4 oxidation rates (Table 3). Fertilization with 30 mM N decreased oxidation rates in media by approximately one-half compared to similar media fertilized with 15 mM N. The higher fertilization rate also caused a significant decrease in pH of the media, with the mean value nearly 0.5 units lower.

Media in which plants were grown had higher oxidation rates than unplanted fertilized media (Table 3). Medium pH, how-

Table 3. Nitrite formation and pH of planted and unplanted soilless potting media fertilized with two rates of nitrogen.

Treatment	N level (mM)	NO_2 formed (nmol·cm ⁻³ ·h ⁻¹)	Medium pH
Initial	---	0.67 ± 0.17 ^z	6.65 ± 0.04
Planted	15	1.32 ± 0.22	6.41 ± 0.07
	30	0.62 ± 0.11	5.99 ± 0.09
Unplanted	15	0.68 ± 0.12	6.40 ± 0.07
	30	0.38 ± 0.08	5.87 ± 0.08
Planting × N level		**	NS

^zIndicates standard error of the mean.

NS, **Nonsignificant or significant at $P = 0.01$, respectively, using analysis of variance.

ever, was similar in both planted and unplanted media. In both treatments; pH decreased from the initial value for untreated, unplanted media (pH 6.65).

Significant interaction between planting and fertilizer rate revealed that planted samples were more affected by fertilizer rate than unplanted samples (Table 3). Planted samples decreased more in NH_4 oxidation rate than unplanted samples as fertilizer N concentration was increased from 15 to 30 mM. This suggests that the plant contributes to the inhibition of NH_4 oxidation more as fertilizer N concentration within media is increased. This does not appear, however, to be a consequence of H^+ extrusion, since pH was similar or slightly lower in the unplanted samples.

The observation that planting of the media significantly increased rates of NH_4 oxidation during treatment agrees with work by Elliott (1986). Earlier studies by Herlihy (1972) reported that nitrifying bacteria exist in low concentrations in uncultivated peat, but may increase up to 1000-fold after cultivation and planting. As mentioned earlier, the greater NH_4 oxidation activity in planted samples, as compared with unplanted samples, cannot be entirely explained as a pH phenomenon. Unplanted samples had pH readings similar to planted samples, demonstrating that the organisms responsible for oxidizing NH_4 are contributing more to the lowering of the medium pH than previously thought. There is no clear explanation why planted media characteristically show greater rates of NH_4 oxidation, although it appears that, in these cases, the environment becomes modified so that the organisms can either become better established or oxidize NH_4 more efficiently. Release of CO_2 and other compounds from the root system may contribute to this increased activity. Planted media may also contain lower substrate-inhibiting NH_4 levels because of plant uptake of NH_4 .

It appears that medium pH is a major factor controlling NH_4 oxidation in soilless potting media. When NH_4 oxidation rate was plotted against medium pH for all observations in Expt. 2 (total observations = 432), a significant relationship was evident (Fig. 2). The simple coefficient of determination (r^2) for the model was 0.25, explaining some of the variation in NH_4 oxidizing activity between treatments. In general, values above pH 6.8 are necessary for NH_4 oxidation; however, this is not a sufficient condition since many samples with near-neutral pH had low rates of NH_4 oxidation. Examination of the scatter plot also revealed that there was no activity in samples with pH below 5.6. The assay conditions for determining NH_4 oxidation rates in this study were performed using a pH 7.0 buffer. Consequently, any organisms that oxidize NH_4 better at lower pHs, such as selective heterotrophs (Lang and Jagnow, 1986; Tate, 1977; Verstraete and Alexander, 1972) or acidophilic autotrophs (Hankinson and Schmidt, 1988), which do not tolerate high pH levels, would not have been detected in this study.

Table 2. Effect of $\text{NH}_4\text{-N}$: $\text{NO}_3\text{-N}$ fertilizer ratio on nitrite formation and pH of soilless potting media.

$\text{NH}_4\text{-N}$: $\text{NO}_3\text{-N}$ ratio	NO_2 formed (nmol·cm ⁻³ ·h ⁻¹)	Medium pH ^z
Expt. 1		
1:3	2.86 a ^y	6.60 a
1:1	1.50 b	6.05 b
3:1	1.26 c	5.61 c
Significance	**	**
Expt. 2		
1:3	1.06 a	6.51 a
1:1	0.77 b	6.11 b
3:1	0.44 c	5.89 c
Significance	**	**

^zpH was determined in media by reading directly in the saturated paste (Expt. 1) or in the filtered saturated paste extract (Expt. 2).

^yMean separation by Duncan's multiple range test at $P = 0.05$.

**Significant at $P = 0.01$ using analysis of variance.

Fig.2. Short-term NH_4 oxidation rate vs. pH for all observations in Expt. 2 (total observations = 432). pH was determined in the filtered extract of a saturated paste.

In summary, medium type, N fertilizer rate and formulation, and the presence of plants strongly influenced the nitrifying activity of soilless potting media. These factors significantly affected NH_4 oxidation over the course of the experiment and should be taken into consideration when growing plants that are sensitive to high levels of NH_4 . It also appears that much, but not all, of the treatment effects are explained by medium pH. Consequently, cultural factors that affect pH may also affect nitrification and, possibly, the growth of plants. It is likely, however, that pH is simply one among several environmental factors that influence microbial population dynamics and the process of nitrification.

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