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

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Abstract. Effects of medium, NH₄ : NO₃ ratio, and N fertilizer rate on the development of NH₄ oxidation in soilless potting media were evaluated. In two separate experiments, NH₄-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 NH₄-N : 3 NO₃-N had higher rates of NH₄ 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 NH₄ oxidation. Rates of oxidation were insignificant with pH values <5.6.

Nitrification is defined as the oxidation of NH₄ to NO₃ and is generally mediated by the activities of two groups of chemoheterotrophic bacteria (Alexander, 1977). One group, the NH₄ oxidizers, initiates the process with the formation of NO₂, while another group, the NO₂ oxidizers, completes the process by converting NO₂ to NO₃. The nitrification process is sensitive to pH, water, and temperature and has an absolute requirement for 0\(^2\), (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 NH₄ 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 NH₄-N : NO₃-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-cm\(^3\) 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 day-length with heating and cooling setpoints of 18/24°C. Plants were fertilized at each irrigation with a nutrient solution containing (in mM) 15 (210 ppm) total N with NH₄ concentrations between 6 and 17 h of incubation. Nitrite formation was determined calorimetrically (Keeney and Nelson, 1982). Rates of oxidation were calculated from the difference in NO₂ 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 NH₄ oxidation activity.

Media samples were dried under a high-efficiency particulate adsorption (HEPA)-filtered air stream for 12 to 36 h at 25 to 30°C 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-cm\(^3\) samples to which were added 20 ml of 1 mM (NH₄)\(_2\)SO₄ and 10 mM NaCIO₃ in 1 mM phosphate buffer (pH 7.0) (Elliott, 1988). Chlorate was added to inhibit NO₃ oxidation (Belser and Mays, 1980; Elliott, 1988; Less and Simpson, 1957). After incubation in the dark at 25°C 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 colorimetrically (Keeney and Nelson, 1982). Rates of NH₄ oxidation were calculated from the difference in NO₃ concentration between 6 and 17 h of incubation. Nitrite formation
Results and Discussion

Main effect means are presented over all observations for the treatment factors of medium type, \( \text{NH}_4^- : \text{NO}_3^- \) 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, \( \text{NH}_4^- \)-oxidizing activity increased up to a maximum and then sharply decreased (Fig. 1). In Expt. 1, the maximum \( \text{NH}_4^- \)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 \( \text{NH}_4^- \)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 \( \text{NH}_4^- \)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 \( \text{NH}_4^- \)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 \( \text{NH}_4^- \)oxidizing bacteria are characteristically less sensitive than \( \text{Nitrobacter} \) to high \( \text{NH}_4^- \)concentrations (Nakos and Wolcott, 1972). Although \( \text{NH}_4^- \)in the media was not measured in this study, it is very likely that in these small contained volumes, retention of \( \text{NH}_4^- \)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 \( \text{pH} \) (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. 1. Overall rates were lower, however, in Expt. 2 because of the additional treatments of no planting and increased fertilizer rate.

Medium type significantly affected \( \text{NH}_4^- \)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 \( \text{NH}_4^- \)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 \( \text{NH}_4^- \)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).

![Chart](chart.png)  
**Fig. 1.** Development of \( \text{NH}_4^- \)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 mean effect of 144 observations. Error bars, shown if larger than the dimensions of the symbol, represent the standard error of the mean.

| Medium type | \( \text{NO}_2^- \) formed (nmol·cm\(^{-2}\)·h\(^{-1}\)) | Medium pH |
|-------------|-------------------------------|-----------|
| **Expt. 1** |                               |           |
| MM220       | 2.03 a*                       | 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 | **                           |           |

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

*\( \text{pH} \) was determined in media by reading directly in the saturated paste (Expt. 1) or in the filtered saturated paste extract (Expt. 2).

*Mean separation by Duncan’s multiple range test at \( P = 0.05 \).

**Significant at \( P = 0.05 \) or 0.01, respectively, using analysis of variance.

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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 NH₄ 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 NH₄ oxidation. Relatively small differences in media pH may result in significant changes in NH₄ 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 (Nie-miera and Wright, 1986).

Ammonium oxidation rates decreased as the level of NH₄-N in the fertilizer solution increased (Table 2). Pots fertilized with 1 NH₄-N : 3 NO₃-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 NH₄ oxidation rate between media fertilized with solutions containing NH₄-N : NO₃-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 NH₄ oxidation rate between pots fertilized with different NH₄ levels can be partially explained by pH. In media fertilized with solutions containing 3 NH₄-N : 1 NO₃-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 NH₄-N : NO₃-N ratios of 1:1 and 3:1. The process of NH₄ oxidation by autotrophic bacteria also decreases the pH and may lead to a self-induced inhibition of activity.

Increasing N fertilizer concentration decreased NH₄ 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, however, 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 NH₄ 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 NH₄ 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 NH₄ 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 NH₄ 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 NH₄ 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 NH₄ oxidation, although it appears that, in these cases, the environment becomes modified so that the organisms can either become better established or oxidize NH₄ more efficiently. Release of CO₂ and other compounds from the root system may contribute to this increased activity. Planted media may also contain lower substrate-inhibiting NH₄ levels because of plant uptake of NH₄.

It appears that medium pH is a major factor controlling NH₄ oxidation in soilless potting media. When NH₄ 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 (r²) for the model was 0.25, explaining some of the variation in NH₄ oxidizing activity between treatments. In general, values above pH 6.8 are necessary for NH₄ oxidation; however, this is not a sufficient condition since many samples with near-neutral pH had low rates of NH₄ 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 NH₄ oxidation rates in this study were performed using a pH 7.0 buffer. Consequently, any organisms that oxidize NH₄ 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 NH₄-N : NO₃-N fertilizer ratio on nitrite formation and pH of soilless potting media.

| NH₄-N : NO₃-N ratio | NO₂ formed (nmol·cm⁻²·h⁻¹) | Medium pH* |
|---------------------|-----------------------------|------------|
| Expt. 1             |                             |            |
| 1:3                 | 2.86 a                      | 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        | **                          | **         |

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

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

| Treatment     | N level (mM) | NO₂ formed (nmol·cm⁻²·h⁻¹) | Medium pH |
|---------------|--------------|-----------------------------|-----------|
| Initial       | ---          | 0.67 ± 0.17a                | 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 |
| **Planted × N level** | **NS**       |                             |           |

*Indicates standard error of the mean.
**NS:** Nonsignificant or significant at P = 0.01, respectively, using analysis of variance.
Fig. 2. Short-term NH₄⁺ 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 NH₄⁺ oxidation over the course of the experiment and should be taken into consideration when growing plants that are sensitive to high levels of NH₄⁺. 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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