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Nitrification in Pine Tree Substrate Is Influenced by Storage Time and Amendments

Linda L. Taylor[1], Alexander X. Niemiera, and Robert D. Wright
Department of Horticulture, Virginia Polytechnic Institute and State University, 301 Saunders Hall, 0327, Blacksburg, VA 24061

Gregory K. Evanylo and Wade E. Thomason
Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

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Abstract. **Pine tree substrate (PTS), for container plant production, is a relatively new alternative to the commonly used pine bark and peat substrates. Fertility management requires knowledge of nitrogen transformations in this new substrate. The objective of this study was to document the occurrence of nitrification in PTS and to determine if nitrification and density of nitrifying microorganisms are affected by substrate storage time and lime and peat amendments. Pine tree substrate was manufactured by hammermilling chips of ≈15-year-old loblolly pine trees (*Pinus taeda* L.) through two screen sizes, 4.76 mm (PTS) and 15.9 mm amended with peat (3PTS:1 peat, v:v, PTSP). Pine tree substrate and PTSP were amended with lime at five rates and a peat–perlite mix (4 peat:1 perlite, v:v, PL) served as a control treatment for a total of 11 treatments. Substrates were prepared, placed in plastic storage bags, and stored on shelves in an open shed in Blacksburg, VA. Subsamples were taken at 1, 42, 84, 168, 270, and 365 days after storage. At each subsampling day, each substrate was placed into 12 1-L containers. Six of the 12 were left fallow and six were planted with 14-day-old marigold (*Tagetes erecta* L. 'Inca Gold') seedlings; all containers were placed on a greenhouse bench. Substrates were also collected for most probable number (MPN) assays for nitrifying microorganism quantification. Substrate solution pH, electrical conductivity (EC), ammonium-N (NH_4-N), and nitrate-N (NO_3-N) were measured on fallow treatments. Marigold substrate solution pH, EC, NH_4-N, and NO_3-N were measured after 3 weeks of marigold growth. Nitrate-N was detected in fallow containers at low concentrations (0.4 to 5.4 mg·L^{-1}) in PTS in all limed treatments at all subsampling days, but in the non-limed treatment, only at Days 270 and 365. Nitrate-N was detected in the fallow containers at low concentrations (0.7 to 13.7 mg·L^{-1}) in PTSP in the 4- and 6-kg·m^{-3} lime rates at all subsampling days. Nitrite-oxidizing microorganisms were present in PTS at all subsampling days with the highest numbers measured at Day 1. Ammonium-to-nitrate ratios for the marigold substrate solution extracts for both PTS and PTSP decreased as pH increased. This study shows that nitrifying microorganisms are present and nitrification occurs in PTS and PTSP and is positively correlated to substrate pH.**

Nitrification, the biological oxidation of reduced forms of nitrogen (N) to nitrate (NO_3^-), affects the fertilizer management of nursery and greenhouse crop production. In general, plants grow best in a combination of NH_4-N and NO_3-N (Barker and Mills, 1980). The extent of nitrification in container substrate will influence fertilizer N choice. If nitrification does occur, less expensive NH_4^+ or urea-based fertilizers can be used. The occurrence of nitrification is also an environmental issue. Anionic NO_3^- is more easily leached from container substrates than NH_4-N forms (Stowe et al., 2010). The occurrence of nitrification impacts the amount of NO_3-N leached from containers, subsequently entering runoff from a production site, and contaminating waterways and groundwater. Furthermore, the production of nitrous and nitric oxide, either as byproducts of NH_4^+ oxidation or as intermediates in the process known as nitrifier denitrification, are gases that add to the greenhouse effect of the earth's atmosphere. Nitrification also acidifies the substrate (soil) and may affect nutrient form and availability and subsequently plant growth.

Autotrophic nitrification, thought to be responsible for the majority of NH_4^+ oxidation in most soils, is carried out by two distinct groups of chemolithotrophic bacteria, bacteria that derive their energy from oxidizing inorganic compounds and fix CO_2 to produce organic carbon. Ammonia-oxidizing bacteria (AOB) oxidize NH_4^+ to nitrite (NO_2^-) while nitrite-oxidizing bacteria oxidize NO_2^- to NO_3^-. Ammonia-oxidizing bacteria grow in a pH range of 5.8 to 8.5 and have growth optima in the range of 7.5 to 8.0 (Prosser, 1989). The generally accepted reason for this sensitivity is that pH determines the proportions of NH_4^+ and NH_3 present. The pKa value of the NH_4^+/NH_3 pair is 9.25; thus, NH_4^+ and NH_3 will be in equal proportions at pH 9.25. There will be more NH_4^+ than NH_3 below pH 9.25 and the converse will occur above pH 9.25. Ammonia (the actual substrate for the oxidizing enzyme) passively diffuses into bacterial cells, but NH_4^+ transport into cells is energy-dependent and, once inside, must be deprotonated for use as substrate (Prosser, 1989).

A wide variety of heterotrophic fungi and bacteria can oxidize NH_3 or reduced N from organic compounds to hydroxylamine, NO_2^-, and NO_3^-. No energy is derived from this conversion and rates are generally much lower than autotrophic nitrification (Prosser, 1989). This heterotrophic pathway is thought to occur in some acid forest soils (Brierley and Wood, 2001; Lang and Jagnow, 1986).

Nitrification has been verified in peat (Elliott, 1986) and pine bark (Niemiera and Wright, 1986b) substrates, two commonly used substrates in the greenhouse and nursery industries. Studies with these substrates have shown nitrification to be sensitive to pH, temperature, and concentration and form of supplied N. Nitrification rate increased with increasing pH (Niemiera and Wright, 1986a; Vetanovetz and Peterson, 1990) and with increasing temperature (Niemiera and Wright, 1987b). However, Walden and Wright (1995) found that temperatures greater than 46 °C had a negative impact on nitrification in a pine bark medium. Nitrification rate increased with increasing NH_4^+ fertilizer concentration in pine bark (Niemiera and Wright, 1987a). In peat-based substrate, nitrification activity was greater when a 1 NH_4-N:3 NO_3-N ratio was used than with either a 1:1 or a 3:1 ratio (Lang and Elliott, 1991).

Preliminary studies (L. Taylor, unpublished data) showed that nitrite-oxidizing microorganisms occur in recently manufactured and aged PTS, a relatively new alternative to pine bark and peat-based substrates (Wright and Browder, 2005; Wright et al., 2008), but nitrification in PTS has not been documented. Pine tree substrate is manufactured from trunks of ≈15-year-old loblolly pine trees (*Pinus taeda* L.) by chipping and hammermilling to a desired particle size. Like with other substrates, PTS is stored by manufacturers and growers for later sale or use. Recently manufactured PTS has a pH value within the recommended range for soilless substrates, 5.4 to 6.5 (Nelson, 2003), but pH decreases with storage time (Taylor et al., 2012). Pine tree substrate is often amended

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[1]To whom reprint requests should be addressed; e-mail lltaylor@vt.edu.

with peat (to improve water retention and cation exchange capacity) and, consequently, needs lime addition to increase substrate pH because of the acidifying nature of peat (Jackson et al., 2009). The objective of this study was to determine if nitrification occurs in PTS and PTS amended with peat and how nitrification and the density of nitrifying microorganisms are influenced by storage time and lime amendment.

Materials and Methods

Preparation of substrates. Approximately 15-year-old loblolly pine trees growing in Blackstone, VA, were harvested and delimbed on 16 Apr. 2009 and chipped on 21 Apr. 2009 with a Bandit chipper (Model 200; Bandit Industries, Inc., Remus, MI). Resulting pine chips were then passed through a hammermill (Meadow Mills, Inc., North Wilkesboro, NC) on 23 and 24 Apr. using two screen sizes, 4.76 mm and 15.9 mm. The PTS produced with the 4.76-mm screen was used for a 100% PTS and the PTS milled with the larger screen size was amended with peat (PTSP; Premier Tech, Quebec, Canada; 3 PTS:1 peat, v:v). Initial air space (AS; % vol) and container capacity (CC; % vol) for PTS produced with the 4.76-mm screen size have been reported as 36.5% and 50.5%, respectively; initial AS and CC for PTSP produced with a 15.9-mm screen size have been reported as 34.1% and 53.1%, respectively (Jackson et al., 2010). These values are within or near the recommended ranges (10% to 30% for AS and 45% to 65% for CC; Yeager et al., 2007) for substrates used in container plant production. Values for pH and cation exchange capacity (CEC) for PTS and PTSP and carbon-to-nitrogen ratio (C:N) for PTS are given in the Results and Discussion section of this article.

A 4 peat:1 perlite substrate (v:v), similar to a conventional substrate for greenhouse-grown crops in terms of waterholding capacity and air porosity, was included as a control. Both PTS and PTSP were amended with pulverized dolomitic limestone (Pro pulverized limestone; Old Castle Stone Products, Atlanta, GA; calcium carbonate equivalency of 95%) at the rates of 0, 1, 2, 4, or 6 $kg \cdot m^{-3}$ for a total of 10 treatments; PL was amended with 6 $kg \cdot m^{-3}$ pulverized dolomitic limestone. Lime rates were chosen to ensure that pH of PTS and PTSP would be maintained, at least in one treatment, at an optimal pH for nitrification over the intended 365-d study period. All 11 substrate treatments were amended with 0.6 $kg \cdot m^{-3}$ calcium sulfate ($CaSO_4$; Espoma Organic Traditions, Millville, NJ), which has been shown to improve growth of herbaceous species in PTS (Saunders et al., 2005). After preparation, each substrate was placed in 85-L perforated plastic bags and stored on shelves in an open shed in Blacksburg, VA, for 365 d. Monthly high and low temperatures were recorded and average daily temperatures were calculated (Table 1).

Subsampling. At Days 1, 42, 84, 168, 270, and 365, substrate subsamples of each treatment were taken from bags. Subsamples were used to fill 12 1-L plastic containers. Six containers were left fallow and six were planted with ≈14-d-old marigold (*Tagetes erecta* L. 'Inca Gold') seedlings grown in a 144-cell plug tray using Fafard Superfine Germinating Mix (Conrad Fafard, Inc., Agawam, MA). Substrate was also collected for MPN studies that were initiated 2 to 3 d after subsampling.

Fallow containers. Fallow containers were arranged in a completely randomized experimental design on a greenhouse bench with average day and night temperatures of 24 and 19 °C, respectively. Each container was irrigated (beaker-applied) with 500 mL tap water; the next day (designated Week 0) substrate solution was extracted using the pour-through method (Wright, 1986). Substrate solution pH and EC were measured using a Hanna HI 9811 instrument (Hanna Instruments, Woonsocket, RI), and extracts were frozen for later NH_4-N and NO_3-N analysis. Immediately after extracts were collected, each container was fertilized with 500 mL of a 200 $mg \cdot L^{-1}$ N, 20N–4.4P–16.6K, fertilizer solution with N from ammonium sulfate [$(NH_4)_2SO_4$], phosphorus from phosphoric acid (H_3PO_4), potassium from potassium chloride (KCl) and micronutrients from Peters Special S.T.E.M (Peters Fertilizer Products, Allentown, PA; 15 $mg \cdot L^{-1}$). The fertilizer solution pH was adjusted to ≈6.2 using 2N sodium hydroxide (NaOH). At the end of Weeks 1 and 2, 250 mL of fertilizer solution was applied to each container; substrate solution was extracted 1 h after fertilizer addition at the end of Week 2. At the end of Week 3, containers were irrigated with 250 mL of tap water to prevent EC values from exceeding 1.9 $dS \cdot m^{-1}$. At the end of Week 4, containers were irrigated with 250 mL of fertilizer solution and 1 h later substrate solution was extracted. Extracts were analyzed for NH_4-N using an HNU ion-selective electrode (HNU Systems, Newton, MA) and NO_3-N using an Orion ion-selective electrode (Thermo Electron, Beverly, MA).

Containers with marigolds. Containers with marigolds were arranged in a completely randomized experimental design on a greenhouse bench adjacent to fallow pots. Each container was initially irrigated (beaker-applied) with 500 mL of a 300 $mg \cdot L^{-1}$ N (8% ammonium, 12% nitrate), 20N–4.4P–16.6K, complete fertilizer solution (Jack's Professional, Allentown, PA). The next day, 250 mL of fertilizer solution was applied. Until the time of harvest (3 weeks), all containers received 250 mL fertilizer solution when irrigation was needed with the exception that tap water was used to irrigate when substrate solution EC values exceeded 1.9 $dS \cdot m^{-1}$. Irrigation frequency was based on conventional greenhouse irrigation practices. After 3 weeks, 250 mL fertilizer solution was added to each container, substrate solution was extracted 1 h later, and extract was analyzed for pH, EC, NH_4-N, and NO_3-N as previously described. At Day 270 (Jan. 2010), plants were provided supplemental lighting using 400-W metal halide lamps from 0600 HR to 2000 HR daily.

Table 1. Monthly high, low, and average daily temperatures at the Urban Horticulture Center in Blacksburg, VA, where substrates were stored in plastic storage bags on shelves in an open shed.

	High (°C)	Low (°C)	Avg daily (°C)
Apr. 2009	30	–1	18
May 2009	27	1	16
June 2009	30	9	21
July 2009	29	10	20
Aug. 2009	30	14	21
Sept. 2009	29	7	18
Oct. 2009	29	–3	11
Nov. 2009	23	–3	8
Dec. 2009	15	–12	1
Jan. 2010	12	–13	–2
Feb. 2010	7	–8	–2
Mar. 2010	22	–6	6
Apr. 2010	29	–1	14

Data from fallow and planted containers were subjected to analysis of variance with mean separation by Tukey's honestly significant difference and regression analysis using JMP (Version 8; SAS Institute, Cary, NC).

Most probable number. Attempts were made to enumerate both ammonia-oxidizing microorganisms and nitrite-oxidizing microorganisms using a modified MPN technique (Alexander, 1982) as outlined by Schmidt and Belser (1994). The modification used deionized water instead of a phosphate buffer as a diluent because water has been shown to maximize oxidizer counts in substrates with low ammonium concentrations (Donaldson and Henderson, 1989). To estimate nitrifier population numbers present at subsampling Day 1, 10 cm^3 of air-dried substrate fine particles (less than 0.5 mm in diameter) from PTS without lime and PL without lime were each added to flasks containing 90 mL sterilized, deionized water and flasks were shaken vigorously by hand for 60 s (10^{-1} dilution). Ten milliliters of this suspension were immediately and aseptically drawn from the flask and transferred to a second flask containing 90 mL sterilized, deionized water (10^{-2} dilution). This process was repeated until a 10^{-7} dilution was established.

From each dilution, a 1-mL aliquot was added aseptically to each of five sterile polystyrene tubes containing 4 mL of ammonia-oxidizer medium and five sterile polystyrene tubes containing 4 mL nitrite-oxidizer medium. Tubes were incubated in the dark at 25 ± 2 °C for 4 weeks and then checked for presence or absence of NO_2^- as outlined by Schmidt and Belser (1994), indicating oxidation of NH_4^+ in the ammonia oxidizer tubes and complete oxidation of NO_2^- to NO_3^- in the nitrite oxidizer tubes, respectively. Tubes were returned to the previous incubation setting and retested every 2 weeks until no change was detected for two successive testing periods. An estimate of the number of nitrite-oxidizing microorganisms was determined from the number of positive tubes per dilution and using the MPN table generated by Woomer (1994). Multiple attempts to enumerate ammonia-oxidizing microorganisms using varying media ammonium concentrations

resulted in no to very low counts; these attempts were considered unsuccessful because nitrite oxidation was observed and depends on nitrite generation in the ammonia oxidation step. MPN assays were performed on non-limed PTS, PTS with 6 kg·m^{-3} lime, and PL with 6 kg·m^{-3} lime at all subsequent subsampling days. The 6-kg·m^{-3} lime rate for PTS was chosen because substrate pH values would be the highest in this treatment over the experimental period, and AOB are reported to grow best in a near neutral environment (Prosser, 1989). There were three replications of each of the three substrates per subsampling day and mean oxidizer numbers and SEM were calculated using JMP (Version 8; SAS Institute, Inc.).

Results and Discussion

Fallow containers. Nitrate was detected in the substrate solution of PTS for each sampling day and the Week 0, 2, and 4 substrate solution extraction times with the exception of Day 42 when NO_3^- was absent at Week 2 (Table 2). Nitrate was also absent in non-limed PTS except at Week 4 of Day 270 and Week 4 of Day 365. For PTSP, NO_3^- was detected in the substrate solution for each sampling day and at the Week 0, 2, and 4 solution extraction times (Table 2). For Days 1, 270, and 365, NO_3^- was present in the 2-, 4-, and 6-kg·m^{-3} lime rates in PTSP, whereas NO_3^- was only detected at the two highest lime rates for Days 42, 84, and 168. PTS and PTSP-filled containers were fertilized with NH_4^+ as the sole N source; thus, the occurrence of nitrification was verified. There was a decrease in substrate solution NH_4-N concentration with lime addition at all subsampling days for PTS and PTSP at Weeks 2 and 4 (no $NH4^+$ was applied before Week 0) (Table 3). Addition of lime increased substrate solution pH in a quadratic fashion (Table 4), and the higher pH values of the limed treatments were more conducive to the activity and growth of nitrifying microorganisms, i.e., more NH_4^+ was oxidized to NO_3^-. This is supported by work of Niemiera and Wright (1986a), who showed that NO_3^- production in a pine bark substrate increased with increasing lime rate. Increasing lime would have a slight effect, if any, on NH_4^+ adsorption to substrate particles in PTS because CEC for PTS is low (~2.0 cmol·L^{-1}; Jackson et al., 2008) and the fertilizer solution supplied a relatively high NH_4-N concentration (300 mg·L^{-1}), enough to maintain exchange site saturation at all times. Additionally, calcium and magnesium from the lime, potassium, and other cations supplied by the fertilizer solution would have also adsorbed onto available exchange sites (substrate solution EC values were always between 1.5 and 2.4 ds·m^{-1}, data not shown). At Week 4 of subsampling Day 365, NH_4-N concentration in the non-limed PTS (pH 3.9) was the same as that for PTS at the 1-kg·m^{-3} lime rate (pH 5.7; Table 3). This also suggests that increased NH_4-N adsorption to substrate particles with increasing lime rate is not the

Table 2. Substrate solution extract nitrate-N (NO_3-N) of pine tree substrate (PTS), PTS:peat substrate (PTSP), and peat:perlite substrate (PL) with various rates of lime amendment.[z]

	NO_3-N (mg·L^{-1})																	
	Day 1			Day 42			Day 84			Day 168			Day 270			Day 365		
Substrate	Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)		
Lime rate (kg·m^{-3})	0	2[t]	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
PTS[w]																		
0	0.0 e[y]	0.0 e	0.0 d	0 d	0.0 b	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 b	0.0 d	0.0 c	0.0 d	1.1 bc	0.0 c	0.0 b	0.9 b
1	1.3 de	1.3 d	3.5 bc	0.7 cd	0.0 b	0.0 b	0.4 bc	0.0 b	0.0 b	0.8 bc	0.0 b	0.0 d	0.8 bc	0.9 bc	1.2 bc	0.6 bc	1.3 b	1.2 b
2	1.6 cd	1.5 cd	4.0 bc	1.0 bcd	0.0 b	0.0 b	0.7 bc	1.4 b	0.2 b	0.8 bc	1.6b	0.0 d	0.6 bc	1.2 bc	1.3 bc	0.9 bc	0.9 bc	0.2 b
4	2.7 abc	1.7 bc	5.4 b	1.5 abc	0.0 b	0.2 b	1.1 bc	1.6 b	1.1 b	1.1 b	2.2 b	1.2 cd	0.9 bc	1.2 bc	1.5 bc	1.2 b	1.5 b	1.4 b
6	3.6 a	2.0 b	3.8 bc	2.6 a	0.0 b	0.3 b	1.6 b	2.0 b	1.3 b	1.2 b	3.4 b	1.3 cd	1.4 b	1.3 bc	1.5 bc	1.6 b	1.7 b	1.4 b
Significance[x]	L***	Q***	L***	L***	NS	NS	L***	Q***	L***	Q**	L***	L***	L***	Q***	L***	L***	Q***	NS
r^2 value	0.80	0.83	0.69	0.64			0.33	0.83	0.83	0.35	0.85	0.84	0.39	0.77	0.41	0.48	0.68	
PTSP[v]																		
0	0.0 e	0.0 e	0.0 d	0.0 d	0.0 b	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 b	0.0 d	0.0 c	0.0 d	0.0 c	0.0 c	0.0 b	0.0 b
1	0.0 e	0.0 e	0.0 d	0.0 d	0.0 b	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 b	0.0 d	0.0 c	0.0 d	0.0 c	0.0 c	0.0 b	0.0 b
2	0.5 de	1.2 d	1.4 cd	0.0 d	0.0 b	0.0 b	0.0 c	0.0 b	0.0 b	0.0 c	0.0 b	0.0 d	0.0 c	0.7 cd	1.0 bc	0.0 c	0.4 b	0.0 b
4	1.8 bcd	1.5 cd	2.2 cd	1.0 bcd	0.0 b	0.0 b	1.1 bc	2.1 b	0.7 b	0.9 bc	7.5 b	32.8 b	1.2 b	1.6 bc	7.7 b	1.2 b	1.7 b	1.0 b
6	2.9 abc	1.8 bc	2.4 cd	2.1ab	0.89 b	0.7 b	1.0 bc	4.9 b	0.8 b	1.3 b	13.7 b	3.3 c	1.1 b	1.7 b	1.7 bc	1.5 b	1.8 b	1.2 b
Significance	L***	Q***	Q***	Q***	Q***	Q***	L***	Q***	L***	L***	L***	Q***	L***	L***	Q***	L***	L***	L***
r^2 value	0.79	0.89	0.91	0.74	0.70	0.50	0.44	0.94	0.46	0.71	0.86	0.40	0.58	0.83	0.52	0.59	0.77	0.59
PL[u]																		
6	3.0 ab	2.9 a	14.2 a	2.2 a	18.7a	37.4 a	5.7 a	85.5 a	135.7 a	8.0 a	123.8 a	152.3 a	7.0 a	2.7 a	71.1 a	7.3 a	12.6 a	162.3 a

[z]Substrates were stored for 365 d (Apr. 2009 to Apr. 2010) in plastic storage bags in an open shed in Blacksburg, VA, and subsampled at Days 1, 42, 84, 168, 270, and 365. At the time of subsampling, substrates were placed in 1-L containers and substrate solution extracted at the time of filling (0 weeks) and at 2 and 4 weeks (n = 6).

[y]Means within columns separated by Tukey's honestly significant difference ($P \leq 0.05$).

[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001; L = linear, Q = quadratic response for lime rate at *, **, or ***.

[w]PTS was produced from ≈15-year-old loblolly pine trees harvested at ground level, chipped, and hammermilled to pass through a 4.76-mm screen size.

[v]PTSP was produced from ≈15-year-old loblolly pine trees harvested at ground level, chipped, and hammermilled to pass through a 15.9-mm screen size and amended with peat (PTS 3:peat 1, v:v).

[u]PL was produced from peat amended with perlite (peat 4:perlite 1, v:v).

[t]After Week 0, container substrates were fertilized weekly with a 20N–4.4P–16.6K fertilizer solution, nitrogen from $(NH_4)_2SO_4$, except at the end of Week 3 when tapwater was applied.

Table 3. Substrate solution extract ammonium-N (NH_4-N) of pine tree substrate (PTS), PTS:peat substrate (PTSP), and peat:perlite substrate (PL) with various rates of lime amendment.[z]

	NH_4-N ($mg \cdot L^{-1}$)																	
	Day 1			Day 42			Day 84			Day 168			Day 270			Day 365		
Substrate	Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)		
Lime rate ($kg \cdot m^{-3}$)	0	2[t]	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
PTS[w]																		
0	0.0 b[y]	55.2 b	57.4 b	0.0 b	82.7 a	96.3 a	0.0 b	95.4 ab	87.4 b	0.0 a	116.6 a	72.1 b	0.0 a	128.0 a	91.5 b	0.0 a	106.5 a	65.0 b
1	0.0 b	37.9 cd	45.5 cd	0.0 b	54.4 c	62.3 b	0.0 b	62.0 cd	55.2 c	0.0 a	65.4 e	38.5 cd	0.0 a	100.1 b	69.3 cd	0.0 a	78.8 bcd	60.7 b
2	0.0 b	37.5 cd	40.2 d	0.0 b	40.0 d	49.2 cd	0.0 b	52.2 cd	45.6 cd	0.0 a	68.6 e	36.1 cde	0.0 a	87.2 bcd	46.3 ef	0.0 a	54.8 ef	39.6 cd
4	0.0 b	36.7 cd	48.6 bcd	0.0 b	45.8 cd	57.6 bc	0.0 b	55.7 cd	52.5 c	0.0 a	68.4 e	38.6 cd	0.0 a	80.0 de	49.4 ef	0.0 a	64.8 de	46.0 c
6	0.0 b	34.5 cd	43.8 d	0.0 b	48.5 cd	57.6 bc	0.0 b	56.7 cd	53.0 c	0.0 a	67.3 e	42.4 cd	0.0 a	67.1 e	49.3 ef	0.0 a	72.3 bcd	44.0 c
Significance[x]	NS	Q**	Q*	NS	Q***	Q***	NS	Q***	Q***	NS	Q***	Q***	NS	Q***	Q***	NS	Q***	Q***
r^2 value		0.45	0.30		0.76	0.74		0.69	0.68		0.61	0.64		0.82	0.77		0.71	0.52
PTSP[v]																		
0	0.0 b	45.2 bc	54.8 bc	0.0 b	73.7 ab	100.1 a	0.0 b	110.0 a	104.3 a	0.0 a	108.3 ab	99.4 a	0.0 a	119.7 a	121.0 a	0.0 a	109.7a	102.5 a
1	0.0 b	37.9 cd	48.3 bcd	0.0 b	53.4 c	61.0 b	0.0 b	96.1 ab	87.7 b	0.0 a	96.4 bc	72.0 b	0.0 a	92.8 bcd	82.9 bc	0.0 a	86.1 b	63.3 b
2	0.0 b	28.4 d	40.6 d	0.0 b	44.5 cd	47.4 cde	0.0 b	83.6 b	56.1 c	0.0 a	88.6 c	46.1 c	0.0 a	96.1 bc	72.2 cd	0.0 a	81.7 bc	37.1 cd
4	0.0 b	27.3 d	38.5 d	0.0 b	45.3 cd	45.9 de	0.0 b	63.6 c	38.9 d	0.0 a	86.0 cd	33.9 de	0.0 a	82.8 cd	57.9 de	0.0 a	72.8 cd	30.8 de
6	0.0 b	29.6 d	40.3 d	0.0 b	41.0 d	41.3 de	0.0 b	47.0 de	38.5 d	0.0 a	72.4 de	27.8 e	0.0 a	85.3 cd	41.2 f	0.0 a	49.0 f	31.4 de
Significance	NS	Q***	Q***	NS	Q***	Q***		L***	Q***	NS	L***	Q***	NS	Q***	Q***	NS	L***	Q***
r^2 value		0.77	0.65		0.75	0.80		0.88	0.95		0.68	0.95		0.75	0.90		0.83	0.92
PL[u]																		
6	12.8 a	67.4 a	87.0 a	10.7 a	66.4 b	37.4 e	7.2 a	34.0 e	23.2 e	0.0 a	21.7 f	14.8 f	0.0 a	79.1 de	74.2 c	0.0 a	68.5 cde	22.9 e

[z]Substrates were stored for 365 d (Apr. 2009 to Apr. 2010) in plastic storage bags in an open shed in Blacksburg, VA, and subsampled at Days 1, 42, 84, 168, 270, and 365. At the time of subsampling, substrates were placed in 1-L containers and substrate solution extracted at the time of filling (0 weeks) and at 2 and 4 weeks (n = 6).

[y]Means within columns separated by Tukey's honestly significant difference ($P \leq 0.05$).

[x]NS (nonsignificant) or significant at $*P \leq 0.05$, **0.01, or ***0.001; L = linear, Q = quadratic response for lime rate at *, **, or ***.

[w]PTS was produced from ≈15-year-old loblolly pine trees harvested at ground level, chipped, and hammermilled to pass through a 4.76-mm screen size.

[v]PTSP was produced from ≈15-year-old loblolly pine trees harvested at ground level, chipped, and hammermilled to pass through a 15.9-mm screen size and amended with peat (PTS 3:peat 1, v:v).

[u]PL was produced from peat amended with perlite (peat 4:perlite 1, v:v).

[t]After Week 0, container substrates were fertilized weekly with a 20N–4.4P–16.6K fertilizer solution, nitrogen from $(NH_4)_2SO_4$, except at the end of Week 3 when tapwater was applied.

case or is not the major phenomenon responsible for lower NH_4^+ concentrations in limed substrates. Niemiera and Wright (1986a) demonstrated, with the use of a nitrification inhibitor, that NH_4-N depletion in a pine bark substrate was mainly an effect of nitrification and not adsorption. Immobilization of NH_4^+ must also be considered. The higher pH values of all limed treatments were also more suitable for the growth and activity (Gray and Williams, 1971; Tate, 2000) of a more diverse bacterial community in general (Fierer and Jackson, 2006) than the lower pH values in the non-limed PTS. This would result in higher immobilization of ammonium in the limed PTS and PTSP treatments.

At subsampling Day 1, NO_3-N concentrations ranged from 0.5 to 3.6 $mg \cdot L^{-1}$ in the Week 0 substrate solution extracts of all treatments except for non-limed PTS (pH 5.8), non-limed PTSP (pH 5.2), and the PTSP 1 $kg \cdot m^{-3}$ lime (pH 5.7) treatments (Table 2). Because this Week 0 measurement was taken before the addition of any fertilizer, the NO_3^- could have originated from one of three sources or any combination of the three. The NO_3^- could have 1) already been in the substrate at the time of manufacture; 2) been in the tap water used initially to saturate the substrate and then later used for the pour-through analysis (Blacksburg, VA, tap water contains less than 1 $mg \cdot L^{-1}$ NO_3-N); and/or 3) the product of nitrifying microorganisms that oxidized NH_4^+ that was indigenous to wood cells present at the time of PTS manufacture or released through mineralization during the initial 24-h incubation period. Because the PTS and PTSP treatments were prepared from the same wood source at the same time and because the tap water and amount used was the same for all treatments, the differences observed in NO_3^- concentration would most likely be the result of different rates of nitrification, i.e., the higher the lime rate, the higher the pH value and the higher the rate of nitrification.

Nitrification, however, could not be ruled out in treatments with no measurable NO_3-N. The C:N ratios of the PTS and PTSP were ≈179:1 and 90:1 (Taylor et al., 2012), respectively, and the likelihood exists that some, if not most, of NO_3^- produced was immobilized. For PTS, the highest (greater than 2 $mg \cdot L^{-1}$) Day 1 Week 0 NO_3-N values occurred at the 4- and 6-$kg \cdot m^{-3}$ lime rates (2.7 and 3.6 $mg \cdot L^{-1}$, respectively); for PTSP, NO_3-N concentration was 2.9 $mg \cdot L^{-1}$ at the 6-$kg \cdot m^{-3}$ lime rate (Table 4). The Week 0 NO_3-N concentration of PL, a conventionally used substrate in which nitrification is known to occur, was 3.0 $mg \cdot L^{-1}$ (pH 6.5). At all subsequent subsampling days, in almost all cases, NO_3-N was detectable in PTS at a lower lime rate compared with PTSP. This emphasizes the acidifying effect of peat and its influence on nitrification. A relatively high NO_3-N concentration (32.8 $mg \cdot L^{-1}$) was detected in PTSP at Week 4 in the 4-$kg \cdot m^{-3}$ lime rate (pH 6.0) at Day 168 (Table 4). The reason for this is not understood because such an increase was not observed in the 6-$kg \cdot m^{-3}$ lime rate treatment with a higher pH (6.4) that

Table 4. Substrate solution extract pH values of pine tree substrate (PTS), PTS:peat substrate (PTSP), and peat:perlite substrate (PL) with various rates of lime amendment.[z]

	pH																	
	Day 1			Day 42			Day 84			Day 168			Day 270			Day 365		
Substrate	Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)			Time (weeks)		
Lime rate (kg·m^{-3})	0	2[t]	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
PTS[w]																		
0	5.8 e[y]	4.7 f	4.2 g	5.2 g	4.6 h	4.4 g	5.3 e	4.6 g	4.5 g	5.2 f	4.8 f	4.2 i	5.0 g	4.5 g	4.0 i	5.0 g	4.4 h	3.9 j
1	6.2 cd	6.1 d	6.7 b	5.8 e	5.7 f	5.9 d	6.3 c	6.0 d	6.1 c	6.4 d	5.8 d	5.7 e	6.2 e	5.8 e	5.6 f	6.3 d	5.7 e	5.7 e
2	6.3 bc	6.4 c	6.9 a	6.0 d	6.0 e	6.2 c	6.7 b	6.4 c	6.4 b	6.6 c	6.1 c	6.1 c	6.6 c	5.9 d	6.0 d	6.5 b	5.8 d	6.0 d
4	6.5 ab	6.6 b	6.9 a	6.3 b	6.4 c	6.4 b	6.8 b	6.6 b	6.6 a	6.8 a	6.3 b	6.3 b	6.8 b	6.2 c	6.2 c	6.7 a	6.0 c	6.3 b
6	6.6 a	6.7 a	6.9 a	6.5 a	6.6 a	6.5 a	7.0 a	6.7 a	6.7 a	6.9 a	6.4 b	6.4 a	6.9 a	6.3 b	6.3 b	6.8 a	6.4 a	6.4 a
Significance[x]	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***
r^2	0.84	0.86	0.78	0.94	0.94	0.89	0.89	0.89	0.87	0.88	0.92	0.89	0.91	0.87	0.89	0.9	0.85	0.89
PTSP[v]																		
0	5.2 f	4.6 f	4.0 h	4.3 i	4.2 i	4.0 h	4.4 g	4.2 h	4.2 h	4.4 h	4.2 g	3.9 j	4.0 i	3.7 h	3.7 h	4.1 i	3.9 i	4.0 i
1	5.7 e	5.2 e	5.0 f	5.0 h	5.0 g	4.7 f	5.1 f	5.0 f	4.9 f	5.1 g	4.9 f	4.6 h	4.8 h	4.5 g	4.4 h	4.7 h	4.5 g	4.5 h
2	6.1 d	6.0 d	5.9 e	5.4 f	5.7 f	5.4 e	5.7 d	5.8 e	5.5 e	5.7 e	5.6 e	5.3 g	5.5 f	5.4 f	5.3 g	5.4 f	5.3 f	5.2 g
4	6.4 ab	6.5 bc	6.4 cd	6.1 c	6.2 d	6.1 c	6.4 c	6.4 c	6.3 b	6.4 d	6.4 b	6.0 d	6.2 e	6.4 b	6.1 d	6.1 e	6.1 b	6.1 c
6	6.6 a	6.7 a	6.5 c	6.4 a	6.5 b	6.3 b	6.8 b	6.6 ab	6.6 a	6.7 b	6.5 a	6.4 a	6.6 c	6.6 a	6.4 a	6.4 c	6.4 a	6.4 a
Significance	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***	Q***
r^2	0.98	0.99	0.99	1.00	0.99	1.00	0.99	0.99	1.00	1.00	1.00	0.99	0.99	0.99	0.99	0.99	0.99	0.99
PL[u]																		
6	6.5 a	6.5 bc	6.3 d	6.2 bc	6.3 d	5.8 d	6.5 c	5.9 d	5.8 d	6.4 d	5.8 d	5.6 f	6.5 d	6.2 c	5.7 e	6.4 c	6.1 b	5.6 f

[z]Substrates were stored for 365 d (Apr. 2009 to Apr. 2010) in plastic storage bags in an open shed in Blacksburg, VA, and subsampled at Days 1, 42, 84, 168, 270, and 365. At the time of subsampling, substrates were placed in 1-L containers and substrate solution extracted at the time of filling (0 weeks) and at 2 and 4 weeks (n = 6).

[y]Means within columns separated by Tukey's honestly significant difference ($P \leq 0.05$). n = 6.

[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001; L = linear, Q = quadratic response for lime rate at *, **, or ***.

[w]PTS was produced from ≈15-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammermilled to pass through a 4.76-mm screen.

[v]PTSP was produced from ≈15-year-old loblolly pine trees harvested at ground level, chipped, and hammermilled to pass through a 15.9-mm screen size and amended with peat (PTS 3:peat 1, v:v).

[u]PL is produced from peat amended with perlite (peat 4:perlite 1, v:v).

[t]After Week 0, container substrates were fertilized weekly with a 20N–4.4P–16.6K fertilizer solution, nitrogen from $(NH_4)_2SO_4$, except at the end of Week 3 when tapwater was applied.

would have presumably been more conducive to nitrification. By Day 270, NO_3-N values were significantly less and more similar to Day 84 subsampling day values.

Most probable number. At subsampling Day 1, the number of nitrite oxidizers in non-limed PTS (pH 5.8) was approximately half that of PL (Table 5). Although attempts to enumerate ammonia-oxidizing microorganisms were unsuccessful, their presence is strongly supported, because the existence of viable populations of nitrite-oxidizing microorganisms implies viable populations of ammonia oxidizers. When NO_2^- is found in soils, NH_4^+ oxidation is the presumed precursor reaction (Tate, 2000). By Day 42, the number of nitrite oxidizers estimated in both non-limed PTS and PTS with 6 kg·m^{-3} lime (23 organisms per mL of substrate for both) was considerably less than Day 1 PTS. An influence other than pH was responsible for the decline in numbers, at least in the limed treatment, because the pH of this limed PTS was 6.5. Competition for available N with other more robust microbes is a likely possibility. Furthermore, because these surviving nitrite oxidizers are poor competitors (Prosser, 1989), the lack of NO_3-N at Week 2 of Day 42 (Table 2), regardless of lime rate, in PTS is understandable as is the lack of measurable NO_3-N in any but the highest lime rate of PTSP because there was a sharp decrease in the number of nitrite oxidizers in PL by Day 42. The C:N of PL (53:1; Taylor et al., 2012) is much lower that the approximate 179:1 value of PTS (Taylor et al., 2012) and this may explain why, although nitrite oxidizer numbers are low at Day 42 in PL, NO_3-N concentration increased from Week 0 to Weeks 2 and 4. A lower C:N ratio implies a lesser amount of immobilization in PL than in PTS and PTSP and therefore more NO_3-N would be present in a PL substrate solution than a PTS substrate solution. Nitrite oxidizer numbers remained steady at ≈23 organisms per cm^3 of substrate at all remaining subsampling days for PTS and PTS with 6 kg·m^{-3} lime, as did NO_3-N values in the fallow containers. Nitrite oxidizers, and presumably ammonia oxidizers, were able to survive in storage over 365 d. Interestingly, PL nitrite oxidizer numbers increased substantially from Day 42 through Day 365 and by Day 365, there was an estimated 7755 organisms per cm^3 of substrate. PL NO_3-N values likewise increased and were measured at over 100 mg·L^{-1}, except at subsampling Day 270 when NO_3-N was 71.1 mg·L^{-1}.

Table 5. Most probable number (MPN) estimates of nitrite-oxidizing microorganisms present in non-limed and limed pine tree substrate (PTS) and peat–perlite substrate (PL) after 1, 42, 84, 168, 270, and 365 d of storage in plastic bags in an open shed in Blacksburg, VA.

	MPN (organisms/cm^3)					
Substrate	Day					
Lime rate (kg·m^{-3})	1	42	84	168	270	365
PTS,[y] 0	119 (56)[z]	23 (0)	23 (0)	23 (0)	26 (3)	23 (0)
PTS, 6	—[w]	23 (0)	23 (0)	23 (0)	23 (0)	23 (0)
PL,[x] 6	230 (0)	8 (2)	348 (75)	1719 (515)	3454 (802)	7755 (2897)

[z]SEM, n = 3.

[y]PTS is produced from ≈15-year-old loblolly pine trees harvested at ground level, chipped, and hammermilled to pass through a 4.76-mm screen size.

[x]PL is produced from peat amended with perlite (peat 4:perlite 1, v:v).

[w]MPN was not determined and assumed to be the same as non-limed PTS.

Marigold substrate solution extract studies. Variation in substrate pH values within lime rate was greater in the marigold studies than in the fallow pot studies (especially in the weakly buffered PTS; data not shown), most likely as a result of plant–soil interactions. Results of NH_4-N and NO_3-N values (NH_4:NO_3 ratios) will therefore be presented on a pH rather than a lime rate basis. As substrate pH increased, NH_4:NO_3 ratios decreased in the substrate solution extracts taken at harvest (Week 3; 21-d growing period) at all subsampling days

and in both PTS and PTSP (Fig. 1). For all but one subsampling day in PTS (Day 270) and 1 d in PTSP (Day 1), the r^2 value describing the decreasing NH_4:NO_3 ratios with increasing pH was 0.57 or greater and 0.81 or greater, respectively. These trends in NH4-N:NO_3-N ratios support the occurrence of nitrification in PTS and PTSP because nitrification generally increases as substrate pH increases in the 5.0 to 7.0 pH range. This relationship could have been a result of 1) preferential immobilization of NH_4^+ over NO_3^- as pH increased; 2) preferential root uptake of NH_4^+ over NO_3^- as pH increased; 3) increased adsorption of NH_4^+ to substrate particles as pH increased; and 4) increase in nitrification rate as pH increased. The preference by microorganisms as well as by plants for NH_4^+ over NO_3^- occurs when microbial metabolic energy is limited because energy is necessary to reduce NO_3^- to NH_4^+ for subsequent incorporation into amino acids (Sylvia et al., 2005). However, in this study, metabolic energy was not limiting for either the microorganisms or plants because both groups were supplied with essential nutrients, water, and energy in the form of carbon–hydrogen bonds (substrate- or plant-derived) for microorganisms and sunlight (auxiliary lighting supplied on Day 270) for plants. Furthermore, a study by El Jaoual and Cox (1998) showed that NO_3^- (and not NH_4^+) was preferentially absorbed for the first 50 d of marigold growth (*Tagetes erecta* L. 'First Lady'). As mentioned earlier, increased adsorption of NH_4^+ to substrate particles is expected to be negligible as lime rate increases in a PTS- and PTS-based substrate. An increase in nitrification, therefore, seems to be the most plausible explanation. The relatively low NH_4-N:NO_3-N ratios at Days 270 and 365 at all lime rates in PTS suggested the occurrence of nitrification at low pH values as well as the higher values. In support of this, there was measurable NO_3-N in the non-limed PTS in the fallow container study at 4 weeks at both

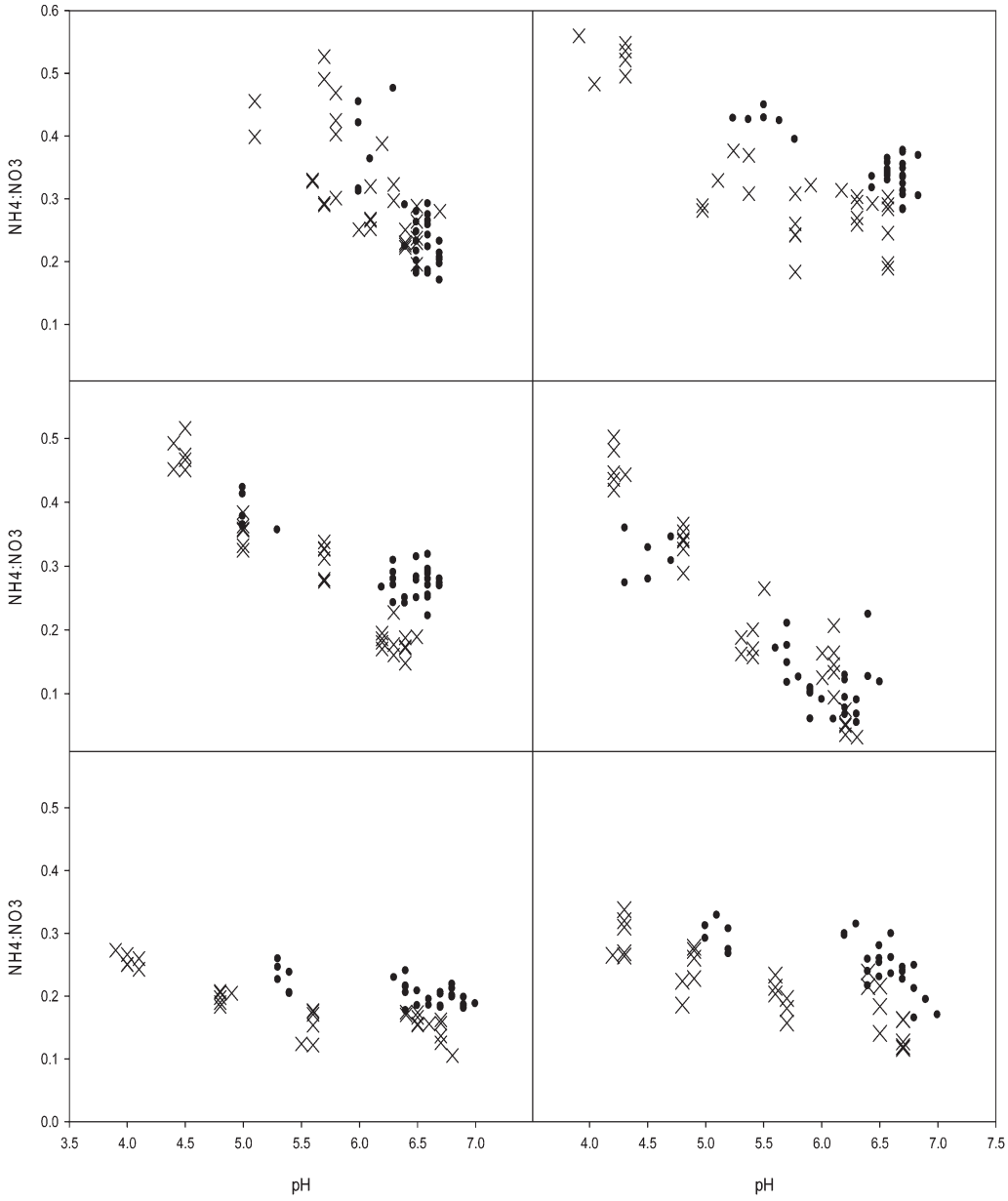

Fig. 1. Marigold substrate solution extract NH_4:NO_3 ratio after 3 weeks of fertilization with a complete 20–10–20 fertilizer solution at 6 subsampling days from Apr. 2009 to Apr. 2010 for pine tree substrate (•, PTS) and pine tree:peat (3:1, v:v) substrate (× , PTSP). (**A**) Day 1 (PTS: y = 1.9580–0.2624x, $r^2 = 0.57$, $P < 0.0001$ and PTSP: y = 1.1869–0.1439x, $r^2 = 0.46$, $P < 0.0001$); (**B**) Day 42 [PTS: y = 1.0340–0.1025x, $r^2 = 0.69$, $P < 0.0001$ and PTSP: y = 0.9151–0.1040x + 0.1014$(x - 6.0767)^2$, $r^2 = 0.81$, $P < 0.0001$]; (**C**) Day 84 [PTS: y = 0.4422–0.0269x + 0.0555$(x - 6.21)^2$, $r^2 = 0.78$, $P < 0.0001$ and PTSP: y = 1.1269–0.1487x, $r^2 = 0.93$, $P < 0.0001$]; (**D**) Day 168 [PTS: y = 0.6466–0.0902x + 0.0448$(x - 5.7333)^2$, $r^2 = 0.78$, $P < 0.0001$ and PTSP: y = 1.2344–0.1870x, $r^2 = 0.91$, $P < 0.0001$]; (**E**) Day 270 [PTS: y = 0.3637–0.0248x, $r^2 = 0.43$, $P < 0.0001$ and for PTSP: y = 0.3482–0.0334x = 0.0187$(x - 5.5167)^2$, $r^2 = 0.83$, $P < 0.0001$]; (**F**) Day 365 [PTS: y = 0.8548–0.0909x – 0.0653$(x - 6.2679)^2$, $r^2 = 0.63$, $P < 0.0001$ and for PTSP: y = 0.5285–0.0559x, $r^2 = 0.69$, $P < 0.0001$].

sampling Days 270 and 365 (Table 2), whereas none had been detected in previous subsampling days.

The acidifying effect of nitrification would be expected to cause pH values to decrease over the 3-week growing period. For subsampling Days 0 and 42, pH values remained the same or increased for all PTS and PTSP lime treatments (Table 6). A pH increase of non-limed PTS in plant production after the addition of an acid-forming fertilizer has been noted previously (Gruda et al., 2009) and the reason for this is unclear. The possibility exists that the preferential uptake of NO_3^- by marigolds resulted in an increase in rhizosphere pH and, therefore, substrate pH by symport of hydrogen with NO_3^- absorption. In the limed treatments, this increase can be explained by the action of lime. A pH decrease did not occur until subsampling Day 84 (in July; Table 6) when warmer temperatures prevailed. Greater pH decreases occurred at subsampling Day 168 (October) compared with July. By subsampling Days 270 (January) and 365 (April), pH values began increasing again after 3 weeks of marigold growth (Table 6).

Results of this study support the occurrence of nitrification in PTS and PTSP. MPN assays demonstrated that nitrite-oxidizing microorganisms were present throughout the 365 d of the experiment in PTS. Nitrate was measurable in NH_4^+-fertilized fallow pots with a positive correlation between substrate solution pH and NO_3-N. Ammonium-N to NO_3-N ratios decreased with increasing pH as a result of liming rate, which was expected if nitrification rate was greater at the higher pH values than at lower pH values. However, in PTS, there was evidence that nitrification proceeded in low pH situations, especially after storage for 270 d. Whether the nitrifying microorganisms involved had adapted in some way to acid conditions or whether different nitrifying species had become established is unclear.

Although nitrification is supported in PTS and PTSP, the contribution it makes to plant available nitrate appears to be small, at least for the first 3 to 4 weeks of plant production, which, for some crop species, would be the entire production cycle. Nitrifying microorganisms are poor competitors and when C:N ratios are as high as in PTS and PTSP, they are no match for heterotrophic microorganisms; NO_3^- production, then, is low and any NO_3^- produced is immobilized. Nitrate-N would need to be incorporated in the fertilizer to supply nitrate and protect against NH_4 toxicity. Other solutions may be to lower the C:N ratio by composting, or preplant incorporation of N, as demonstrated by Gruda and Schnitzler (1999) with shredded spruce wood chippings. There is evidence (L. Taylor, unpublished data) that nitrifiers build up populations over relatively long periods of time in PTS container-grown plant production (e.g., more than six to 12 months as is the case in some large nursery stock); hence, nitrification may supply enough nitrate to render the NH_4:NO_3 ratio suitable for most plant species.

Table 6. Substrate solution extract pH values at time of planting, marigold substrate solution extract pH values at harvest (3 weeks), and marigold substrate solution electrical conductivity (EC) at harvest of pine tree substrate (PTS), PTS:peat substrate (PTSP), and peat:perlite substrate (PL) with various rates of lime amendment.[z]

	Day																	
	1			42			84			168			270			365		
Substrate	pH[x] (SE)	pH (SE)	EC (dS·m^{-1})	pH (SE)	pH (SE)	EC (dS·m^{-1})	pH (SE)	pH (SE)	EC (dS·m^{-1})	pH (SE)	pH (SE)	EC (dS·m^{-1})	pH (SE)	pH (SE)	EC (dS·m^{-1})	pH (SE)	pH (SE)	EC (dS·m^{-1})
Lime rate (kg·m^{-3})	(planting)	(harvest)	(harvest)	(planting)	(harvest)	(harvest)	(planting)	(harvest)	(harvest)	(planting)	(harvest)	(harvest)	(planting)	(harvest)	(harvest)	(planting)	(harvest)	(harvest)
PTS																		
0	5.8 (0.05)	6.1 (0.05)	1.61	5.2 (0.02)	6.0 (0.06)	1.43	5.3 (0.06)	5.1 (0.05)	1.63	5.2 (0.02)	4.5 (0.07)	1.97	5.0 (0.10)	5.4 (0.02)	0.86	5.0 (0.00)	5.1 (0.04)	0.73
1	6.2 (0.05)	6.5 (0.02)	1.67	5.8 (0.02)	6.8 (0.03)	1.06	6.3 (0.03)	6.3 (0.03)	1.28	6.4 (0.02)	5.7 (0.03)	1.61	6.2 (0.00)	6.4 (0.02)	1.08	6.3 (0.08)	6.3 (0.05)	0.90
2	6.3 (0.05)	6.7 (0.05)	1.70	6.0 (0.00)	6.9 (0.02)	1.00	6.7 (0.03)	6.5 (0.05)	1.35	6.6 (0.02)	5.9 (0.02)	1.56	6.6 (0.00)	6.6 (0.04)	1.02	6.5 (0.05)	6.5 (0.03)	0.90
4	6.5 (0.06)	6.6 (0.03)	1.54	6.3 (0.02)	6.9 (0.04)	1.11	6.8 (0.03)	6.6 (0.02)	1.32	6.8 (0.02)	6.2 (0.04)	1.54	6.8 (0.02)	6.8 (0.03)	1.06	6.7 (0.05)	6.7 (0.02)	0.94
6	6.6 (0.05)	6.6 (0.03)	1.72	6.5 (0.02)	6.9 (0.02)	1.14	7.0 (0.04)	6.6 (0.02)	1.37	6.9 (0.02)	6.3 (0.04)	1.45	6.9 (0.02)	6.9 (0.03)	1.06	6.8 (0.08)	6.9 (0.03)	0.85
Significance[y]			NS			Q***			Q***			Q***			Q***			Q***
r^2 value						0.52			0.48			0.50			0.42			0.42
PTSP																		
0	5.2 (0.00)	5.5 (0.14)	1.68	4.3 (0.02)	5.0 (0.05)	1.43	4.4 (0.02)	4.5 (0.02)	1.60	4.4 (0.02)	4.2 (0.02)	1.88	4.0 (0.02)	4.0 (0.03)	1.15	4.1 (0.02)	4.3 (0.02)	1.08
1	5.7 (0.02)	5.7 (0.04)	1.73	5.0 (0.02)	5.8 (0.06)	1.32	5.1 (0.03)	5.0 (0.00)	1.51	5.1 (0.00)	4.8 (0.00)	1.68	4.8 (0.00)	4.8 (0.02)	1.02	4.7 (0.02)	4.9 (0.02)	0.90
2	6.1 (0.04)	6.1 (0.03)	1.61	5.4 (0.02)	6.2 (0.02)	1.32	5.7 (0.02)	5.7 (0.00)	1.63	5.7 (0.00)	5.4 (0.03)	1.60	5.5 (0.00)	5.6 (0.02)	1.14	5.4 (0.00)	5.7 (0.02)	0.82
4	6.4 (0.03)	6.4 (0.03)	1.62	6.1 (0.02)	6.6 (0.03)	1.14	6.4 (0.02)	6.2 (0.02)	1.42	6.4 (0.00)	6.1 (0.02)	1.72	6.2 (0.00)	6.5 (0.02)	1.16	6.1 (0.00)	6.5 (0.02)	0.93
6	6.6 (0.02)	6.5 (0.04)	1.58	6.4 (0.02)	6.8 (0.00)	1.21	6.8 (0.02)	6.4 (0.03)	1.47	6.7 (0.00)	6.2 (0.03)	1.62	6.6 (0.00)	6.7 (0.03)	1.07	6.4 (0.02)	6.7 (0.00)	0.92
Significance[y]			NS			Q***			L**			NS			NS			Q*
r^2 value						0.47			0.25									0.28
PL																		
6	6.5 (0.03)	6.2 (0.00)	1.72	6.2 (0.02)	6.1 (0.04)	2.17	6.5 (0.02)	5.7 (0.02)	2.13	6.4 (0.02)	5.3 (0.00)	2.57	6.5 (0.02)	6.1 (0.02)	1.42	6.4 (0.00)	5.9 (0.05)	1.7

[z]Substrates were stored for 365 d (Apr. 2009 to Apr. 2010) in plastic storage bags in an open shed in Blacksburg, VA, and subsampled at Days 1, 42, 84, 168, 270, and 365 (n = 6).
[y]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001; L = linear, Q = quadratic response for lime rate at *, **, or *** .
[x]pH values at planting are from Week 0 fallow container solution extracts.

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