Storage of Pine Tree Substrate Influences Plant Growth, Nitrification, and Substrate Properties

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ABSTRACT

Pine tree substrate (PTS) is a relatively new substrate for container crop production. There are no detailed studies that elucidate how storage time impacts PTS chemical, physical, and biological aspects. The objective of this research was to determine how PTS storage time influenced PTS chemical and physical properties, nitrification, and plant growth. Pine tree substrate was manufactured by hammer-milling chips of loblolly pine trees (*Pinus taeda* L.) through two screen sizes, 4.76 mm (PTS) and 15.9 mm amended with peat (PTSP). PTS and PTSP were amended with lime at five rates. A peat-perlite mix (PL) served as a control treatment. Prepared substrates were placed in storage bags and stored in an open shed in Blacksburg, Virginia. Subsamples were taken at 1, 42, 84, 168, 270, and 365 days. At each subsampling day, twelve 1-L containers were filled with each substrate. Six containers were left fallow and six were planted with marigold (*Tagetes erecta* L. 'Inca Gold') seedlings. Substrate was also collected from select treatments for Most Probable Number assays to estimate density of nitrifying microorganisms, and for chemical and physical property analyses. Pour-through extracts were collected from fallow containers at 0, 2, and 4 weeks, and from marigold containers at harvest for determination of pH, electrical conductivity, ammonium-N and nitrate-N. At harvest, marigold height, width, and dry weight were measured. At least 1 $kg \cdot m^{-3}$ lime for PTS, and 2 to 4 $kg \cdot m^{-3}$ lime for PTSP were needed to maintain pH values ≥ 5.5 for 365 days. Bound acidity of unlimed PTS increased but cation exchange capacity for unlimed PTS and

PTSP decreased over 365 days. Carbon to nitrogen ratio and bulk density values were unchanged over time in all treatments. There were minor changes in particle size distribution for limed PTS and unlimed and limed PTSP. Marigold growth in PTS and PTSP was greater than PL in all limed treatments, except at day 1. Nitrite-oxidizing microorganisms were present and nitrification occurred in PTS and PTSP at all subsampling days. Pine tree substrate is relatively stable in storage, but pH decreases and lime addition may be necessary to offset this decrease.

DEDICATION

This work is dedicated to my parents, George and Bessie Carr, and to my five children, Chester, Whitney, Olivia, Mark, and Ruth.

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Chapter 1

INTRODUCTION

The greenhouse and nursery industry in the United States has grown rapidly in the past four decades. A 2007 report from the USDA stated that growth in this industry, in terms of cash receipts, was four times that of all agricultural commodities (Strickland, 2007). Since the majority of ornamental plants are now produced in containers, i.e. not field grown, there has been an increased demand for suitable container substrates. Pine bark (or bark from other tree species) and peat moss have been the most commonly used substrates, but development of new substrates has been an area of active research for several reasons. Pine bark production is not expected to keep up with the increased demand for use as fuel, landscape mulch, and potting substrate for nursery and greenhouse production, as well as for retail substrate sale (Lu, 2006). The law of supply and demand, therefore, dictates that pine bark cost will increase. The cost of transportation of peat moss from its source, usually Canada, has increased with increasing fuel prices, and restrictions on mining peat are increasing as these wetlands are becoming more valued from a conservation standpoint (Poulin et al., 2004).

Wood substrates, mainly from conifers, have been the subject of considerable research in the past two decades. In Europe, Toresa® is a top-selling substrate manufactured from pine and spruce sawmill residue in the form of chips that are mechanically ground with a precise quantity of mineral nutrients (Schilling, 1999). These nutrients supply microorganisms present in the substrate with their nutrient needs, thus offsetting immobilization (microbial consumption and conversion of inorganic nitrogen, N, to organic N) that normally occurs in

uncomposted wood-based substrates. In the United States, wood-based substrate research is focused mainly on pine tree substrates. Ground pine chips from trunks (with bark; termed pine tree substrate, PTS) of approximately 15-year-old loblolly pine trees (*Pinus taeda* L.) have been found to make a suitable substrate (Wright and Browder, 2005; Wright et al., 2006). Entire shoot portions (trunk, limbs, needles, and cones; termed WholeTree) of 12-year-old loblolly pine trees, similarly manufactured, have also been shown to be a suitable container substrate (Fain et al., 2008a), as has residual pine material (trunk, limbs, needles and cones; termed clean chip residual, CCR) from 10-year-old loblolly pines left from in-field chipping operations for the paper industry (Boyer et al., 2007). Plant growth in these substrates was similar to that in pine bark or peat moss. Preliminary data [R. Wright, unpublished data] indicates that Eastern white pine (*Pinus strobus* L.) is also a potential source of wood for PTS. Studies by Fain et al. (2008b) using slash pine (*Pinus elliottii* Engelm.) and longleaf pine (*Pinus palustris* Mill.) as substrates have also shown positive results. Other pine species are likely candidates as well. Problems of cost, availability, and sustainability of container substrates may be considerably lessened by use of wood-based pine tree substrates since they are produced from species that are native to wide geographic ranges and can be grown, harvested, and replenished locally.

Research has focused on construction of conifer-based substrates to attain recommended physical properties for soilless substrates as outlined by Yeager et al. (2007), as well as determination of fertilizer rate (specifically N) to achieve desired growth rates. In general, results of this research show that using smaller hammer-mill sieve sizes to produce a substrate with relatively small particle size, or amending coarser-milled softwood-based substrates with pine bark, peat moss, sand, or finer milled substrate, will produce substrate that has physical

property values that fall within recommended ranges (Boyer et al., 2008; Fain et al., 2008a; Gaches, 2010; Gruda, 2004; Jackson et al., 2010). However, additional N is needed for plants grown in these wood-based substrates compared to plants grown in pine bark or peat moss (Fain et al., 2008a; Jackson et al., 2008a; Jackson et al., 2008b; Wright et al., 2008). The reason for this is thought to be immobilization of N due to the relatively high carbon to nitrogen ratio of wood-based substrates.

Several other substrate management aspects remain unexplored. These substrates may be stored by manufacturers and growers until needed, and little is known about the effects of storage time on uncomposted conifer-based substrate chemical and physical properties and plant growth. Gaches et al. (2011) found that plant growth was greater in WholeTree aged for approximately 3 and 6 months, than in freshly manufactured WholeTree. Differences in substrate physical properties (specifically air space and container capacity), immobilization rate, and the presence of an allelopathic chemical in recently manufactured substrate but not aged, were given as possible explanations. The pH value of PTS has been observed to decrease over storage time [R. Wright, unpublished data], necessitating lime addition either at the time of planting or at storage, but lime addition rates have not been determined.

Effective and efficient fertilizer management of nursery and greenhouse crops requires knowledge of N transformations in container substrates. Nitrification, the biological oxidation of reduced forms of N to nitrate, is a pH sensitive transformation. In general, plants grow best with a combination of ammonium-N and nitrate-N (Barker and Mills, 1980). If nitrification occurs in a substrate, less expensive ammonium- or urea-based fertilizer N sources can be used,

relying on the action of nitrifying microorganisms to supply nitrate and avoid ammonium toxicity. Nitrate is more apt to be leached from container substrate than ammonium. Nitrification rate, then, needs to be taken into consideration when managing fertilizer N form and application concentration to minimize production site run-off of nitrate. Nitrification occurs in pine bark (Niemiera and Wright, 1986) and peat moss (Elliott, 1986) and the rate of nitrate production has been estimated in both, but its occurrence in freshly manufactured, as well as stored, conifer wood-based substrates, is unknown.

The purpose of this research was two-fold. One focus was to determine how storage time affects chemical and physical properties, as well as plant growth, in PTS and PTS amended with peat (PTSP). The second focus was nitrification. The research question was: Does nitrification occur in PTS and PTSP? If so, is its occurrence affected by substrate storage time and pH changes in stored substrate?

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Chapter 2

Storage Time and Lime and Peat Amendments Affect Pine Tree Substrate Chemical and Physical Properties and Marigold Growth

Abstract

Pine tree substrate (PTS) is a relatively new alternative to the commonly used pine bark and peat-based substrates for container crop production. Physical and chemical properties of freshly manufactured PTS have been studied, however, this new substrate will sometimes be manufactured and stored for later use. The objective of this research was to determine the effects of storage of PTS, with and without lime and peat amendments, on substrate properties. Properties studied were pH, bound acidity, cation exchange capacity (CEC), electrical conductivity (EC), carbon to nitrogen ratio (C:N), bulk density (BD), particle size distribution and plant growth. PTS was manufactured by hammer-milling chips of approximately 15-year-old loblolly pine trees (*Pinus taeda* L.) through two screen sizes, 4.76 mm (for a 100% PTS) and 15.9 mm. The 15.9 mm was amended with peat (3 PTS: 1 peat, v:v, PTSP). PTS and PTSP were amended with lime at five rates: 0, 1, 2, 4, or 6 $kg \cdot m^{-3}$ and a peat-perlite mix (4 peat: 1 perlite, v:v, PL) was included as a control treatment, for a total of 11 treatments. Substrates were prepared, placed in 0.08 m^3 plastic storage bags and stored on shelves in an open shed in Blacksburg, Virginia. Subsamples were taken at 1, 42, 84, 168, 270, and 365 days after storage. At each subsampling day, twelve 1-L containers of each treatment were filled. Six of the 12 were left fallow and six were planted with two-week-old marigold (*Tagetes erecta* L. 'Inca Gold') seedlings and placed in a greenhouse. Substrate was also collected for analysis of bound

acidity, CEC, C:N, BD, and particle size distribution. Substrate solution pH and EC were measured from solution collected from fallow containers. After three weeks of growth, marigold dry weight, growth index, and substrate solution pH and EC were measured. At least 1 $kg \cdot m^{-3}$ lime was needed to maintain PTS pH ≥ 5.5 over the 365 day storage period (day 1 pH = 5.8) and 2 to 4 $kg \cdot m^{-3}$ was needed to maintain PTSP pH ≥ 5.5 for 365 days (day 1 pH = 5.2). Electrical conductivity measurements were highest at day 1 (1.02 to 1.21 $dS \cdot m^{-1}$) in all treatments with a significant decrease by day 42. Bound acidity of PTS (0.87 $cm \cdot L^{-1}$ at day 1) increased with storage and CEC decreased in unlimed PTS and PTSP. A quadratic relationship existed in limed PTS and PTSP over storage time. Carbon to nitrogen ratio and BD did not change over time in any treatment. There were minor changes in particle size distribution for limed PTS and unlimed and limed PTSP. Marigold growth in PTS and PTSP was equal to or greater than PL in all limed treatments, except at day 1 when growth in all PTS and PTSP lime treatments was less. Pine tree substrate and PTSP are relatively stable when stored as above, except for pH which can be easily adjusted with lime. The data suggest that a phytotoxic substance exists in freshly manufactured PTS. Substrate aging may be recommended before use to allow time for this substance to be degraded.

Introduction

Pine bark and peat moss are widely used substrates for container-grown crops in the greenhouse and nursery industries. Recently considerable research into developing other soilless substrates has been reported. This research stems from the increasing cost and decreasing availability of pine bark, and the cost and sustainability of peat moss mining. A wide

variety of materials has been investigated and wood-based substrates have been shown to be suitable substitutes. Several of these wood-based substrates are from coniferous species (softwoods) and are produced from chipped and ground trunks (with bark; termed pine tree substrate, PTS) (Wright et al., 2006), chipped and shredded trunks with low bark amounts (Gruda, 2004; Gumy, 2001), whole shoot portions (needles, limbs, bark and trunk; termed WholeTree®) (Fain et al., 2008a; Fain, 2006), or wood, bark, foliage and other materials (remains from in-field chipping operations for the paper industry; termed clean chip residual) (Boyer et al., 2008). Problems of cost, availability and sustainability of container substrates may be considerably lessened by using these alternatives to peat and pine bark. These new substrates can be produced from species that are native to wide geographic ranges, and can be grown specifically for this purpose, harvested and replenished locally.

Softwood-based substrates have been shown to be suitable for at least some horticultural crop species and produce plant growth that is similar to, or greater than plants grown in pine bark or peat moss (Boyer, 2009; Fain et al., 2008b; Gruda, 2004; Wright et al., 2008). In these studies, substrate particle size and effects of amendments such as lime, peat, pine bark, and sand were investigated. Fertilizer regimes have also been investigated because N immobilization decreases plant available N in uncomposted wood substrates. Research has shown that a higher N application rate is required to compensate for immobilized N (Gruda, 2000; Jackson et al., 2008a; Jackson et al., 2008b; Wright et al., 2008). In nearly all of these studies, substrates were manufactured just before use or, in a few cases, stored for up to 144 days. However, substrate manufacturers and growers may store these substrates for later sale or use, and research on the effects of wood-based substrate storage is needed.

Extensive research conducted in the 1960s and early 1970s by the paper industry addressed the effects of large pile storage on chipped wood. Most of these studies included at least one species of pine (*Pinus* spp.) (Feist et al., 1973; Feist, 1971; Feist, 1973; Hajny, 1966; Hatton, 1970, 1972; Lindgren, 1961; Springer, 1970). These studies researched the effects of storage on chipped wood extractives, pulp yield, and pH. There are few studies that investigated the effects of storage on wood-based substrate properties and plant growth. Kostov et al. (1991) investigated the decomposition of composted sawdust and pine bark on microorganism activity (CO_2 evolution, ammonification, nitrification) and density. Dickinson and Carlile (1995) conducted a study on combinations of composted pine and spruce bark, and chipboard and paper waste. Neither of these aforementioned studies used uncomposted and untreated wood constituents.

Gaches et al. (2011) found that dry weight, growth index, and bloom count of *Tagetes patula* L. 'Little Hero' marigold and *Petunia* x*hybrida* Vilm. 'Dreams White' petunia were higher in a ground pine tree, peat substrate (1:1, v:v) that was aged for 94 and 169 days than in those produced in the same substrate that was recently manufactured. The authors suggest differences in air space and container capacity, rate of N immobilization, and the presence of an allelopathic chemical in recently manufactured substrate as explanations for growth differences. Decrease in pH has been observed in both stored PTS and loblolly pine logs [R. Wright, unpublished data] indicating that PTS may need lime amendment. Lime amendment is suggested when PTS is amended with peat (Jackson et al., 2009), due to the acidifying nature of peat.

The objective of this work was to determine the effects of storage time on PTS chemical and physical properties and on plant growth. Specifically, the effect of storage on PTS pH, EC, CEC, C:N, bound acidity, particle size distribution and BD was studied. The influence of PTS storage time on marigold growth was also studied.

Materials and Methods

Preparation of substrates. Fifteen-year-old loblolly pine (*Pinus taeda* L.) trees growing in Blackstone, Va., were harvested and delimbed on 16 April 2009 and chipped on 21 April 2009 with a Bandit chipper (Model 200; Bandit Industries, Inc., Remus, MI). Resulting coarse pine chips were then passed through a hammer-mill (Meadow Mills, Inc. North Wilkesboro, N. C.) on 23 and 24 April, using a 4.76 mm and a 15.9 mm screen size. The PTS produced with the 4.76 mm screen was used to produce a 100% PTS, and the coarser PTS (15.9 mm screen) was amended with peat (Premier Tech, Quebec, Canada; 3 PTS: 1 peat, v:v, PTSP). Previous work (Jackson et al., 2008a, 2010) has shown that these screen sizes produce a PTS and a PTSP with container capacity and total air space values within or near the recommended range of 45 – 65% for container capacity and 10 – 30% for total air space (Yeager et al., 2007). A peat-perlite substrate (4:1, v:v, PL), similar to a conventional substrate for greenhouse-grown crops, 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 suit a wide range of pH change possibilities over the intended 365 day study period with 6 $kg \cdot m^{-3}$, the rate

used commonly in the very acidic peat, as the highest rate. All 11 substrate treatments were amended with 0.6 kg·m^{-3}calcium sulfate ($CaSO_4$; Espoma Organic Traditions, Millville, NJ). Calcium sulfate has been shown to improve growth of herbaceous species in PTS (Saunders et al., 2005). After preparation each substrate was placed in 0.08 m^3 perforated plastic bags and stored on shelves in an open shed in Blacksburg, Virginia for 365 d. Monthly high and low temperatures and average daily temperatures were monitored (Table 1).

Subsampling. At 1, 42, 84, 168, 270 and 365 days of storage, substrate subsamples from each treatment were transferred to twelve 1-L plastic containers. Six containers were left fallow and 6 were planted with 14-day-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). At day 1, approximately 4 liters each of PTS, PTSP, and PL without lime and gypsum amendments were placed in gallon freezer bags and stored in a freezer at -15 °C for future analysis of bound acidity, CEC, C, N, BD, and particle size distribution. For the remaining storage periods, approximately 4 liters of each of the 11 treatments were collected for same analyses as at day 1 and frozen as above.

Fallow containers. Fallow containers were placed in a completely randomized design on a greenhouse bench with average day and night temperatures of 24°C and 19°C, respectively. Each container was irrigated (beaker-applied) with 500 mL tapwater needed to insure thorough wetting, and the following day 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).

Containers with marigolds. Containers with marigolds were placed in a completely randomized design on a bench adjacent to the fallow pots. Each container was 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 following day 250 mL of fertilizer solution was applied, which resulted in approximately a 20% leaching fraction. Until the time of harvest (3 weeks), all containers received 250 mL fertilizer solution based on plant need for irrigation, with the exception that tapwater was used to irrigate when substrate solution EC values (measured weekly) were ≥ 2 $dS \cdot m^{-1}$. After 3 weeks, substrate solution was extracted using the pour-through technique, and analyzed for pH and EC as above. Growth Index (GI) was determined by dividing the sum of marigold height, greatest width and perpendicular width values were summed and divided by 3. Marigold dry weight (DW) was determined by cutting the stems at substrate surface, drying in an oven at 65°C for 4 d, and weighing. At day 270 (Jan 2010) plants were provided supplemental lighting using 400 watt metal halide lamps from 6 a.m. to 8 p.m. daily.

CEC, C:N, particle size distribution, and BD. Cation exchange capacity, C:N ratio, particle size distribution, and BD were determined for five treatments: unlimed PTS, unlimed PTSP, limed PTS (1 $kg \cdot m^{-3}$), limed PTSP (4 $kg \cdot m^{-3}$), and the PL control. These analyses were conducted for substrates incubated for 1, 168, and 365 days. The 1 and 4 $kg \cdot m^{-3}$ lime rates were chosen because the substrate solution pH of these lime treatments was maintained between 5.5 and 6.5, the recommended range for soilless greenhouse crops (Yeager et al., 2007). These analyses were conducted on the previously mentioned frozen subsamples with three replicates. Therefore, day 1 values for the limed PTS and PTSP will be the same as for day 1 unlimed PTS

and PTSP. Substrates were analyzed for CEC (A & L Eastern Laboratories, Richmond, VA) using the AOAC Official Method 973.09 for peat materials (Thorpe, 1973). This method determines CEC at a pH of 7.5, measuring sites pertinent at pH levels used in crop production. For C:N ratios, removal of any residual lime was necessary for accurate values of carbon in the limed treatments, thus a modified acid fumigation method of Harris et al. (2001) was used. Acid treated as well as non-acid treated samples, were analyzed for C and N (Univ. of Florida Soil and Water Science Department Wetland Biogeochemistry Laboratory using a Thermo Electron Flash EA 1112 Nitrogen/Carbon Analyzer with MAS 200 R autosampler; three replicates). Substrate BD was determined using the North Caroline State University Porometer Method (Fonteno and Hardin, 2003) for each of the five selected treatments with three replicates. For particle size distribution, approximately 40 g oven dried substrate was shaken for 10 min on a Fisher-Wheeler Sieve Shaker, Model #5 (700 vibrations per minute) with 14 sieves ranging in size from > 6.3 mm to 0.63 mm and a bottom collection pan. Weights of each particle size fraction were recorded including the fraction in the pan. For ease and clarity of presentation, particle size textural classes of coarse, medium and fine will be presented here. Coarse, medium and fine particles are segregated into ≥ 2 mm in diameter, < 2 mm but ≥ 0.5 mm in diameter, and < 0.5mm in diameter, respectively.

Substrate bound acidity. Substrate bound acidity was determined, using a modification of the conductometric titration method of Katz et al. (1984) for sulfite pulps, on unlimed PTS from day 1 and day 365 as well as PTS with 1 $kg \cdot m^{-3}$ lime from day 365. A 0.1 M sodium bicarbonate ($NaHCO_3$) solution was used as a titrant instead of sodium hydroxide to prevent phenolic hydroxyl groups from ionizing as well as carboxylic acid groups. By graphing conductance by

volume of $NaHCO_3$ added, the amount of $NaHCO_3$ needed to neutralize the protons of pertinent acid functional groups present was determined and used in the following equation by Fras et al. (2004): $X = (C_{HCO3} \cdot V_t)/m_{dry}$ (where C_{HCO3} is the concentration of the $NaHCO_3$ solution, V_t is the volume of $NaHCO_3$ solution consumed in neutralizing the carboxylic acid functional groups, and m_{dry} is the oven-dry weight of the sample) giving $mmol \cdot kg^{-1}$ acidic group, then converted to $cmol \cdot L^{-1}$.

Statistical analyses. Regression analysis (JMP 8, SAS Institute, Cary, NC) was used to describe data within substrates and analysis of variance (ANOVA) with Tukey's HSD means comparison was used to separate treatment means between and within substrates (JMP 8, SAS Institute, Cary, NC).

Results and Discussion

Substrate solution pH (fallow pot). On day 1, pH values of all treatments, with the exception of unlimed PTSP (pH value of 5.2) were within or slightly above the generally accepted range (5.5 – 6.5) for non-ericaceous crops (Fig. 1A and B, Table 2). Unlimed PTSP pH values were consistently lower than unlimed PTS pH values throughout the study, due to the acidifying effect of peat, which has been observed by others (Jackson et al., 2009; Wang, 1998; Wang and Konow, 2002). By day 42, pH values of all treatments had decreased and at least 1 and 4 $kg \cdot m^{-3}$ lime were needed to maintain PTS and PTSP pH values, respectively, at 5.5 and higher (Fig. 1A and B). The pH for unlimed PTS, when stored for six weeks, was 5.2, a pH value suitable for ericaceous crops (recommended pH values of 4.5 to 5.5, Yeager et al., 2007) but low for most other plant species production. For unlimed PTSP, pH value was 4.3 by day 42. For unlimed

PTS and unlimed PTSP, pH values remained relatively stable from day 42 until day 270 when a slight decrease was observed with no change thereafter (Fig. 1A and B, Table 2). By day 84 for limed PTS and limed PTSP treatments, pH values had increased (Fig. 1A and B). Values remained relatively stable throughout the remainder of the experiment for all limed PTS treatments with day 365 pH values higher than day 1 values (Fig. 1A, Table 2). For PTSP limed treatments, however, pH decreased from day 84 and all day 365 pH values were less than day 1 values (Fig. 1B, Table 2). In the 2, 4, and 6 $kg \cdot m^{-3}$ lime rates of PTS, but only in the 6 $kg \cdot m^{-3}$ lime rate of PTSP, pH values were higher than the suggested upper limit at one or more subsampling days. The PL control showed a similar trend as the PTS and PTSP treatments with the lowest value (6.2) at day 42 and all other values either 6.4 or 6.5 (Table 2).

Substrate solution EC (fallow pot). Day 1 EC values were between 1.02 and 1.21 $dS \cdot m^{-1}$ for all substrate and lime treatments (Table 2). By day 42, EC values decreased by 50% in PTS, 20 to 40% in PTSP, and 25% in PL. This decrease in substrate solution salts indicates salt tie-up, most likely by microorganisms present in the substrate. Salts in wood, the most abundant of which are calcium, potassium and magnesium, contributing to EC, are mainly from deposits in cell walls and lumina (Sjöström, 1993). Even though peat is considered stable, the addition of water and lime to this substrate at the initiation of storage, may have activated microorganisms present in peat (Carlile and Wilson, 1991), and the subsequent activity resulted in microbial uptake of solution salts. The salt contribution from irrigation water was minor since irrigation water EC value was 0.1 $dS \cdot m^{-1}$. The EC contribution from lime was also low since unlimed substrate EC values were similar to limed substrates. After day 42, EC values were about the same or higher throughout the rest of the experiment, but never as high as day 1 values.

CEC. For both unlimed PTS and PTSP there were small linear decreases in CEC over the 365 day period. Values decreased from 2.0 to 1.7 cmol·L^{-1} for unlimed PTS and from 5.7 to 4.6 cmol·L^{-1} for unlimed PTSP (Table 3). There were small changes in CEC in the limed PTS and PTSP (Table 3). Cation exchange capacity would not play a significant role in supplying plants with nutrients due to the relatively high nutrient amounts of commercial fertilizer regimes, and so these small changes would be inconsequential in conventionally fertilized plant production systems. The addition of peat to PTS (PTSP substrate) nearly tripled the CEC of PTS (Table 3). There was no change in PL CEC over the 365 day storage period (Table 3).

Bound Acidity. For solid wood substances, there are three categories of acidic functional groups (Pu, 1989). They are carboxylic acid groups, phenolic hydroxyl groups, and weakly acidic hydroxyl groups of polysaccharides with pKa values (the pH value at which 50% of sites are protonated) of 4.5, 10.2, and 13.7, respectively. The carboxylic acid functional group is found in pectin and hemicelluloses, the phenolic group is lignin associated, and the weakly acidic hydroxyl group is associated with the glucose subunits of cellulose (Pu, 1989). Of the three groups, only the carboxylic acid functional group would be of interest since it is the only group whose pKa value is in the range of the pH values of substrates used in container crop production.

Bound acidity for unlimed PTS, increased (P = 0.04) from 0.87 cmol·L^{-1} acid functional groups on day 1, to 0.98 cmol·L^{-1} on day 365, an increase of 12%. Storage of PTS amended with 1 kg·m^{-3} lime resulted in 1.27 cmol·L^{-1} acid functional groups on day 365, 30% more than that of unlimed PTS on day 365 (P = 0.0009). Differences in pH of the stored substrates at day 365 (5.0 and 6.3

for unlimed PTS and PTS at the 1 $kg \cdot m^{-3}$ lime rate, respectively) very likely resulted in changes in the microbial community, i.e., activity, density, or species (Carlile and Wilson, 1991; Fierer and Jackson, 2006). These changes may be responsible, in part or wholly, by differential saccharification of substrate components (cellulose, hemicellulose, pectin and lignin), for increased carboxylic acid groups. An increase in bound acidity would represent an increase in the number of acid reserve sites, and would explain the gradual decrease in substrate solution pH observed after day 42. Even though bound acidity increased over storage time in unlimed PTS, (i.e., potentially more cation exchange sites were produced), CEC, as measured earlier, decreased. In theory, bound acidity should represent all potential cation exchange sites for PTS within the pH limits of interest. A likely possibility is that CEC could be less than measured bound acidity if any of the measured acid sites sterically inhibited cations larger than a hydrogen ion, such as ammonium or calcium. Why CEC is greater than bound acidity in this case is unclear. Measuring bound acidity is possibly a more accurate method of determining PTS CEC. When determining CEC of pine bark, Daniels and Wright (1988) found that CEC varied with the displacing cations used. They also hypothesized that internal sites of particles were present as well as external sites. This hypothesis was based upon the occurrence of a sharp drop in the reaction vessel pH soon after the cessation of base addition (1.25 $mL \cdot min^{-1}$) during a potentiometric titration. The conductometric titration used here to determine bound acidity, and potentially CEC in the future, used a much slower titration speed (from 0.1 to 0.5 $mL \cdot 5\ min^{-1}$), to allow for diffusion into particle internal spaces, and has been developed specifically for wood.

C:N. The C:N ratios for PTS without lime and for the 1 kg·m^{-3} lime treatment ranged from 155:1 to 179:1 (Table 4). Ratios were the same for limed and unlimed substrates at sampling days 1, 168 and 365, and were the same for limed and unlimed PTS at each of these sampling dates. C:N ratio of unlimed PTS was 179:1 at day 1, much lower than 550:1 previously reported (Jackson et al., 2008b) for PTS. The C:N ratios for PTSP with and without lime ranged from 88:1 to 92:1. Similar to PTS, the PTSP C:N ratios for the with and without lime treatments were the same over time and ratios were the same for lime and without lime at days 1, 168, and 365 (Table 4). Amending PTS with peat (25% by vol.) decreased the C:N ratio by 50%. The C:N ratio for PL ranged from 51:1 to 54:1 over time and was unaffected by storage time. Because the peat C:N ratio (53:1) is considerably lower than the PTS ratio, the PTSP C:N ratio was expectedly lower than PTS. The peat C:N ratio found in this study is consistent with other reported peat C:N values (Marrush, 2007; Tripepi, 2008). Since C:N ratios in all substrates, regardless of lime rate, were the same over time, we infer that there was no appreciable decomposition of PTS and PTSP during 365 days of storage. Although this demonstrates substrate stability, the maintenance of a relatively high ratio over time will result in N immobilization when these stored substrates are used, and higher than conventional N application rates will be required for crop production. Jackson et al. (2009a) showed that N immobilization is substantially greater in PTS than in PL. These authors have also shown that plants grown in PTS and PTSP required more N than plants grown in PL (Wright et al., 2008).

The C:N ratios for limed PTS of acid-treated and non-acid-treated samples were the same at day 168 and day 365 (data not shown). The same result was also true for limed PTSP. Theoretically, the amount of C contributed by 1 kg·m^{-3} lime to PTS was 1.1 g·kg^{-1} of substrate, a relatively

insignificant contribution to the C:N ratio. The amount of C contributed by 4 $kg \cdot m^{-3}$ lime to PTSP would be 4.3 $g \cdot kg^{-1}$. Although there were no differences between non-acidified and acidified C values for the 4 $kg \cdot m^{-3}$ lime rate PTSP, total C of non-acidified samples were 6 and 11 $g \cdot kg^{-1}$ higher than acidified samples for day 168 and day 365, respectively. Acid fumigation of samples for C analysis seems warranted with this rate of lime addition. Regardless of lime amendment rate, acid fumigation would dissolve lime aggregates present in the substrate, and result in a more accurate value than without acid fumigation.

Particle Size Analysis. Studies have shown that the fine particle texture group has a major influence on the physical characteristics of potting substrate, e.g., air space, container capacity, and ease of water release (Handreck, 1983). Particle size fractions for unlimed and limed (1 $kg \cdot m^{-3}$ treatment) PTS were unaffected over the 365 day storage period with the exception of an increase over time in medium sized particles of the limed treatment (Table 5). Coarse particle size fractions were the same over time for unlimed and limed PTSP and fine particle size fractions were the same in limed (4 $kg \cdot m^{-3}$ treatment) PTSP over time (Table 5). There was a decrease in the fraction of medium size particles over time for unlimed PTSP and an increase in the fraction of medium size particles for limed PTSP. There were also increases in the fraction of fine particles over time for the unlimed PTSP treatment. For PTS and PTSP the changes in the fraction of particle size over 365 days was not higher than 6%. Thus, particle size is relatively stable over a one year storage period regardless of lime rates. For PL, there were major decreases in the fractions of coarse and medium particle sizes over time and a major increase in the fine particle size fraction over time (Table 5). The decrease in coarse and medium particle size fractions was at least 13% and the increase in fine particle size fraction

was 27%. Thus, storage time has a major influence on the limed PL particle size distribution. Lime addition to peat caused a major pH increase (from approximately 3.8 to 6.5). Microbial flora was undoubtedly altered (Fierer and Jackson, 2006), as was microbial activity, and hence peat decomposition was apparently appreciable. Handling of this substrate during the experimental period could also be a contributing factor in the observed particle size changes.

Bulk Density. Bulk densities (BD) remained relatively unchanged over 365 days of storage for all substrates. Bulk density values for PTS, unlimed and limed, and PL were approximately 0.11 $g \cdot cc^{-1}$ while values for PTSP, unlimed and limed, were 0.12 $g \cdot cc^{-1}$.

Marigold Growth. A relationship was absent between marigold shoot DW and substrate solution pH for PTS or PTSP stored for 1 day (Fig. 2A). The pH values for PTS ranged from 6.0 to 6.7, within or near the recommended 6.0 to 6.6 range recommended for marigold culture (Whipker, 2000), while pH values for PTSP ranged from 5.1 to 6.7. By day 42, there was a positive relationship between DW and pH for both PTS and PTSP (Fig. 2B). For PTS, pH values ranged from 5.8 to 7.0, while for PTSP pH ranged from 4.8 to 6.8. Positive relationships between DW and pH were also found for both PTS and PTSP for the duration of the storage period (Fig. 2C - F) except for PTS at day 365. For PTS, minimum pH values for these last four subsampling days ranged from 4.3 to 5.3 while maximum pH values ranged from 6.5 to 7.0. For PTSP, minimum pH values ranged from 3.9 to 4.4 while maximum pH values ranged from 6.3 to 6.8. Comparison of PTS and PTSP coefficients of determination (r^2 values) (Fig. 2B - F) consistently showed a closer relationship between marigold DW and pH in PTSP than in PTS. Low r^2 values for PTS indicate that other factor(s) were significant in influencing marigold dry

weight. Absence or presence of relationships between growth index (GI) and pH for PTS and PTSP were the same as those of DW and pH (data not shown).

Shoot DW for PL (6 $kg \cdot m^{-3}$) at 1 day of storage was higher than DW in PTS and PTSP regardless of lime rate (Table 6). After day 1, DW in PTS and PTSP were the same as or higher than DW in PL, with a few exceptions. These exceptions were unlimed PTSP (pH 5.0) at day 42, unlimed PTS (pH 5.4) and unlimed PTSP (pH 4.0) at day 270, and unlimed PTSP (pH 4.3) at day 365. Substrate solution pH values for PTS and PTSP on day 1 were within or slightly below the recommended values for marigold culture and higher than at later subsampling days where there were no differences in DW between PTS and PTSP versus PL. This result suggests that there may be some inhibitory substance(s), or phytotoxin(s), at day 1 that is no longer present or present in a relatively low concentration by day 42. Phytotoxic compounds are wood extractives, extractives simply defined as the nonstructural constituents of wood that are soluble in neutral organic solvents or water (Sjöström, 1993). This possibility of phytotoxic compounds in PTS inhibiting plant growth is supported by work of Gruda et al. (2009) who found that lettuce and tomato seed radical length values were lower when sown on germination paper saturated with hot or cold water extracts of PTS as compared to germination paper soaked with distilled water. Radical length, when seeds were sown on paper saturated with water extracts taken from PTS that was previously leached with cold or hot water, was longer than from non-leached PTS, but was less than PL. In this same study marigold DW in a freshly manufactured unlimed PTS was less than in a PL control, but DW was the same as PL when the PTS was previously washed or leached.

Gaches et al. (2011) have also speculated that some chemical is present in recently manufactured WholeTree that inhibits plant growth. Jackson et al. (2009) reported that marigold (*Tagetes erecta* L. 'Inca Gold') DW for PTS and PTSP was equal to or higher than that of PL, and the PTS and PTSP (3PTS: 1peat, v:v) used, were manufactured 105 and 135 days prior to planting. Thus, this time lag in planting may have been responsible for the similar DWs by allowing enough time for the phytotoxic compounds in the PTS to be degraded. These extractives may also explain the decrease in pH seen in all PTS and PTSP treatments by day 42 (Fig. 1). Extractives are readily attacked by microorganisms and their degradation leads to production of organic acids and a decrease in pH (Crawford, 1983; Gray et al., 1971; Tuomela et al., 2000). Apparently the majority of these extractives were degraded by day 42, because pH values after this day decrease slowly compared to the day 1 to day 42 period. This would also be a possible explanation for the finding that marigold DW of PTS and PTSP at all lime rates, except unlimed PTSP, are similar to PL by day 42. Complete degradation of phytotoxins may also help explain why there was no relationship between pH and DW in PTS at day 365.

Substrate solution EC values, after three weeks of marigold growth, were higher for PL than for all other treatments (Table 6) at all subsampling days, except at day 1 when EC values were the same for all treatments. Higher values in PL are attributed to a lower rate of N immobilization than in PTS and PTSP and a higher contribution of nutrient ions from the more numerous cation exchange sites compared to PTS and PTSP. Several other studies have also shown substrate solution EC values to be higher in peat than in 100% PTS (Gruda et al., 2009; Jackson et al., 2008a; Jackson et al., 2009; Wright et al., 2008).

In conclusion, results of this work have shown that the pH of PTS and PTSP decreases during storage, with the majority of the decrease occurring by 6 weeks of storage. The likely mechanism behind this initial decrease is the microbial degradation of easily accessible extractives resulting in production of organic acids. The addition of 1 $kg \cdot m^{-3}$ lime to PTS before storage, maintained the pH of this substrate within recommended pH limits for soilless substrates, whereas at least 2 to 4 $kg \cdot m^{-3}$ lime addition was needed to maintain PTSP pH within recommended limits. Results of the marigold growth experiment support the hypothesis for the presence of phytotoxic extractives in freshly manufactured PTS. We propose that the initial decrease in PTS and PTSP pH could be avoided by leaching PTS with water prior to use. This would also leach phytotoxic extractives from the substrate. An alternative treatment would be to store the substrate at least 6 weeks to allow time for degradation of extractives and then adjust pH with lime if desired. Pine tree substrates are relatively stable over storage time, at least when stored as described here. Substrate storage does not mitigate the degree of immobilization and additional N will continue to be necessary for crop production compared to other conventional container substrates.

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Table 1. 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)	Average Daily (°C)
April 2009	30	-1	18
May 2009	27	1	16
June 2009	30	9	21
July 2009	29	10	20
August 2009	30	14	21
September 2009	29	7	18
October 2009	29	-3	11
November 2009	23	-3	8
December 2009	15	-12	1
January 2010	12	-13	-2
February 2010	7	-8	-2
March 2010	22	-6	6
April 2010	29	-1	14

Table 2. Pine tree substrate (PTS), PTS: peat substrate (3:1, v:v, PTSP), and peat: perlite substrate (4:1, v:v, PL) solution extract pH values and electrical conductivity (EC) levels at varying lime rates after 1, 42, 84, 168, 270, and 365 days of storage (n = 6).

Substrate	Day 1		Day 42		Day 84		Day 168		Day 270		Day 365	
Lime rate ($kg \cdot m^{-3}$)	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)	pH	EC ($dS \cdot m^{-1}$)
PTS												
0	5.8 e[z]	1.10 bc	5.2 g	0.55 de	5.3 e	0.69 d	5.2 f	0.73 abc	5.0 g	0.73 ab	5.0 g	0.75 ab
1	6.2 cd	1.18 ab	5.8 e	0.62 e	6.3 c	0.74 cd	6.4 d	0.70 bc	6.2 e	0.73 ab	6.3 d	0.72 abc
2	6.3 bc	1.20 a	6.0 d	0.63 cd	6.7 b	0.79 bcd	6.6 c	0.71 c	6.6 c	0.69 ab	6.5 b	0.68 bc
4	6.5 ab	1.15 ab	6.3 b	0.58 de	6.8 b	0.82 bc	6.8 a	0.72 abc	6.8 b	0.74 ab	6.7 a	0.62 c
6	6.6 a	1.21 a	6.5 a	0.58 de	7.0 a	0.81 bc	6.9 a	0.70 c	7.0 a	0.69 ab	6.8 a	0.71 abc
Significance[y]	Q***	Q*	Q***	NS	Q***	Q***	Q***	NS	Q***	NS	Q***	Q**
r^2 value	0.84	0.24	0.94		0.89	0.44	0.88		0.92		0.89	0.29
PTSP												
0	5.2 f	1.09 bc	4.3 i	0.71 b	4.4 g	0.80 bcd	4.4 h	0.74 abc	4.1 i	0.70 ab	4.1 i	0.64 bc
1	5.7 e	1.15 ab	5.0 h	0.70 bc	5.1 f	0.76 bcd	5.1 g	0.70 c	4.8 h	0.72 ab	4.7 h	0.65 bc
2	6.1 d	1.13 ab	5.4 f	0.71 b	5.7 d	0.78 cd	5.7 e	0.72 bc	5.5 f	0.69 ab	5.4 f	0.64 bc
4	6.4 ab	1.02 c	6.1 c	0.82 a	6.4 c	0.81 bc	6.4 d	0.82 a	6.2 e	0.80 a	6.1 e	0.70 abc
6	6.6 a	1.14 ab	6.4 a	0.86 a	6.8 b	0.86 b	6.7 b	0.79 abc	6.6 c	0.80 a	6.4 c	0.80 a
Significance	Q***	NS	Q***	L***	Q***	Q**	Q***	L**	Q***	L***	Q***	Q***
r^2 value	0.98		1.00	0.72	0.99	0.36	1.00	0.27	1.00	0.39	1.00	0.52
PL												
6	6.5 a	1.15 ab	6.2 bc	0.87 a	6.5 c	1.02 a	6.4 d	0.81 ab	6.4 d	0.74 ab	6.4 c	0.71 abc

[z]Means within columns across substrates separated by Tukey's HSD ($P \leq 0.05$).

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

Table 3. Cation exchange capacity (CEC) of unlimed and limed pine tree substrate (PTS), PTS: peat substrate (3:1, v:v, PTSP), and peat: perlite substrate (4:1, v:v, PL) after 1, 168, and 365 d of storage (n = 3).

Substrate	CEC ($cmol \cdot L^{-1}$)			
Lime rate ($kg \cdot m^{-3}$)	Day 1	Day 168	Day 365	Significance[z]
PTS				
0	2.0	1.9 b[y]	1.7 b	L***, $r^2 = 0.90$
1	2.0	2.5 a	2.5 a	Q*, $r^2 = 0.80$
PTSP				
0	5.7	5.4 a	4.6 a	L*, $r^2 = 0.93$
4	5.7	5.3 a	5.7 a	Q**, $r^2 = 0.82$
PL				
6	13.1	12.2	12.3	NS

[y]Means separated within columns (between lime rates within each substrate) by Tukey's HSD, $P \leq 0.05$.

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

Table 4. Carbon to nitrogen ratio (C:N) of unlimed and limed pine tree substrate (PTS), PTS: peat substrate (3:1, v:v, PTSP), and peat: perlite substrate (4:1, v:v, PL) after 1, 168, and 365 d of storage (n = 3).

Substrate	C:N			
Lime rate ($kg \cdot m^{-3}$)	Day 1	Day 168	Day 365	Significance[y]
PTS				
0	179	178 a[z]	177 a	NS
1	179	155 a	169 a	NS
PTSP				
0	90	91 a	92 a	NS
4	90	88 a	90 a	NS
PL				
6	53	51	54	NS

[y] NS = nonsignificant at $P \leq 0.05$.

[z] Means separated within columns (between lime rates within each substrate) by Tukey's HSD ($P \leq 0.05$).

Table 5. Particle size analysis of limed and unlimed pine tree substrate (PTS), PTS: peat substrate (3:1, v:v, PTSP), and peat: perlite substrate (4:1, v:v, PL) after 1, 168, and 365 days of storage (n = 3).

Substrate		Texture group (% mass)											
Lime rate ($kg \cdot m^{-3}$)		coarse				medium				fine			
		Day 1	Day 168	Day 365	Significance[z]	Day 1	Day 168	Day 365	Significance	Day 1	Day 168	Day 365	Significance
PTS													
	0	5.0	3.9 a[y]	3.3 a	NS	71.9	70.9 b	70.5 b	NS	23.5	25.2 a	26.2 a	NS
	1	5.0	3.7 a	3.5 a	NS	71.9	77.7 a	77.1 a	Q**, $r^2 = 0.91$	23.5	20.5 b	21.4 b	NS
PTSP													
	0	22.9	24.9 a	24.5 a	NS	61.6	53.5 b	55.6 b	Q*, $r^2 = 0.76$	16.8	22.8 a	20.5 a	Q***, $r^2 = 0.90$
	4	22.9	21.3 a	21.4 a	NS	61.6	62.2 a	63.1 a	L*, $r^2 = 0.45$	16.8	18.1 b	17.3 b	NS
PL													
	6	39.2	23.8	24.9	Q***, $r^2 = 0.95$	47.5	34.6	34.5	Q***, $r^2 = 0.96$	13.8	42.8	41.3	Q***, $r^2 = 1.00$

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

[y]Means separated within columns (between lime rates within each substrate) by Tukey's HSD ($P \leq 0.05$).

Table 6. Pine tree substrate (PTS), PTS: peat substrate (3:1, v:v, PTSP), and peat: perlite substrate (4:1, v:v, PL) solution extract pH and EC, and marigold dry weight (DW) at harvest (3 weeks); n = 6.

	Subsampling Day																	
Substrate	1			42			84			168			270			365		
Lime rate (kg·m^{-3})	pH	EC	DW (g)	pH	EC	DW (g)	pH	EC	DW (g)	pH	EC	DW (g)	pH	EC	DW (g)	pH	EC	DW (g)
PTS																		
0	6.1 d[z]	1.61 a	2.47 d	6.0 d	1.43 b	2.12 cd	5.1 g	1.63 b	2.60 abcd	4.5 g	1.97 b	1.21 cd	5.4 g	0.86 e	1.44 bc	5.1 g	0.73 c	3.30 ab
1	6.5 ab	1.67 a	2.63 cd	6.8 a	1.06 de	2.56 bc	6.3 de	1.28 e	3.11 a	5.7 d	1.61 cd	1.38 abcd	6.4 d	1.08 bcd	1.67 abc	6.3 d	0.90 bc	3.74 a
2	6.7 a	1.70 a	2.51 d	6.9 a	1.00 e	2.94 ab	6.5 bc	1.35 de	3.12 a	5.9 c	1.56 d	1.49 ab	6.6 c	1.02 d	1.72 abc	6.5 c	0.90 bc	3.63 a
4	6.6 ab	1.54 a	2.52 d	6.9 a	1.11 de	2.87 ab	6.6 ab	1.32 de	3.08 ab	6.2 ab	1.54 d	1.52 ab	6.8 ab	1.06 bcd	1.77 ab	6.7 b	0.94 bc	3.59 a
6	6.6ab	1.72 a	2.59 cd	6.9 a	1.14 cde	2.83 ab	6.6 ab	1.37 cde	3.00 abc	6.3 a	1.45 d	1.58 a	6.9 a	1.06 bcd	1.77 ab	6.9 a	0.85 bc	3.63 a
PTSP																		
0	5.5 e	1.68 a	2.82 cd	5.0 f	1.43 b	1.71 d	4.5 h	1.6 b	2.00 e	4.2 h	1.88 bc	1.14 d	4.0 i	1.15 b	1.25 c	4.3 i	1.08 b	2.71 b
1	5.7 e	1.73 a	3.00 bc	5.8 e	1.32 bc	2.38 bc	5.0 g	1.51 bc	2.59 bcd	4.8 f	1.68 bcd	1.29 bcd	4.8 h	1.02 cd	1.81 b	4.9 h	0.90 bc	3.34 ab
2	6.1 d	1.61 a	3.21 b	6.2 c	1.32 bc	2.99 ab	5.7 f	1.63 b	2.39 de	5.4 e	1.60 cd	1.37 abcd	5.6 f	1.14 bc	1.66 abc	5.7 f	0.82 c	3.39 ab
4	6.4 bc	1.62 a	2.94 bcd	6.6 b	1.14 cde	3.28 a	6.2 e	1.42 cde	2.81 abcd	6.1 bc	1.72 bcd	1.51 ab	6.5 cd	1.16 b	1.94 a	6.5 cd	0.93 bc	4.05 a
6	6.5 ab	1.58 a	3.03 bc	6.8 a	1.21 cd	3.28 a	6.4 cd	1.47 bcd	2.67 abcd	6.2 ab	1.62 cd	1.44 abc	6.7 b	1.07 bcd	1.90 ab	6.7 b	0.92 bc	3.59 a
PL																		
6	6.2 cd	1.72 a	3.99 a	6.1 cd	2.17 a	2.72 abc	5.7 f	2.13 a	2.49 cde	5.3 e	2.57 a	1.38 abcd	6.1 e	1.42 a	1.97 a	5.9 e	1.70 a	3.53 a

[z]Means separated within columns across substrates using Tukey's HSD ($P \leq 0.05$).

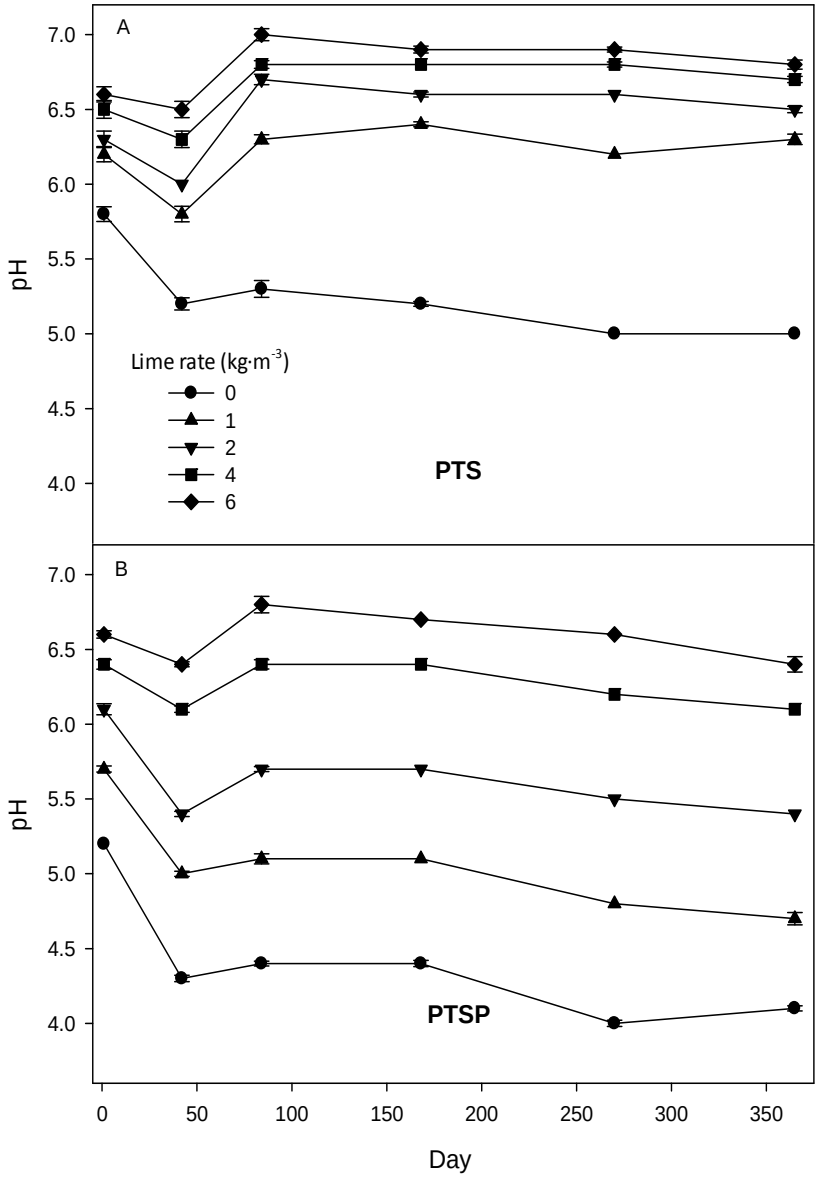

Fig. 1. **(A)** Pine tree substrate (PTS) and **(B)** pine tree substrate amended with peat (3:1, v:v, PTSP) solution extract pH at five lime rates after 1, 42, 84, 168, 270, and 365 d of storage (April 2009 to April 2010) in plastic storage bags in an open shed in Blacksburg, Virginia. Each point represents the mean of six replicates. Error bars indicate ± SE when larger than symbols.

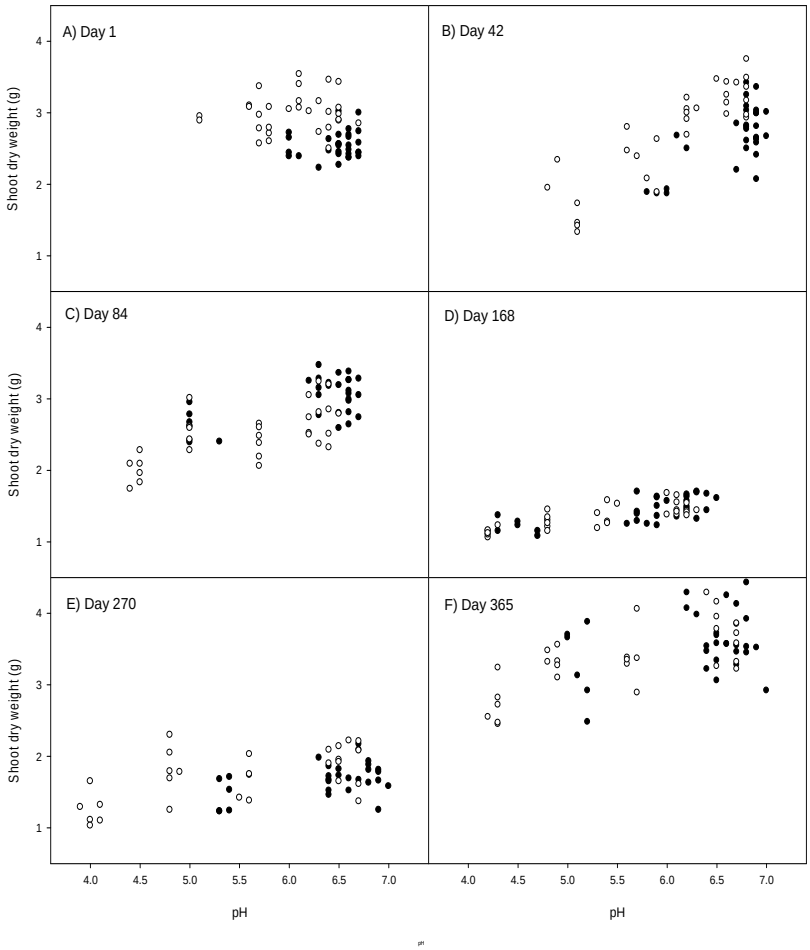

Fig. 2. Marigold dry weight by substrate solution pH at six subsampling days from April 2009 to April 2010 for pine tree substrate (•, PTS) and PTS: peat (3:1, v:v;) substrate (o, PTSP). **(A)** Day 1 (no significant differences for PTS or PTSP); **(B)** Day 42 (PTS: y = -2.6624 + 0.7970x, r^2 = 0.44, *P* < 0.0001 and PTSP: y = -2.7281 + 0.8974x, r^2 = 0.76, *P* < 0.0001); **(C)** Day 84 (PTS: y = 1.2056 + 0.2862x, r^2 = 0.32, *P* = 0.0011 and PTSP: y = 0.7305 + 0.3170x, r^2 = 0.40, *P* = 0.0002); **(D)** Day 168 (PTS: y = 0.3616 + 0.1873x, r^2 = 0.46, *P* < 0.0001 and PTSP: y = 0.4567 + 0.1672x, r^2 = 0.59, *P* < 0.0001); **(E)** Day 270 (PTS: y = 0.3873 + 0.2016x, r^2 = 0.23, *P* = 0.0092 and PTSP: y = 0.5465 + 0.2124x, r^2 = 0.34, *P* = 0.0010); **(F)** Day 365 (PTS: no significant differences and PTSP: y = 1.1625 + 0.4029x, r^2 = 0.48, *P* < 0.0001).

Chapter 3

Nitrification in Pine Tree Substrate is Influenced by Storage Time and Lime and Peat Amendments

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 is affected by storage time and lime and peat amendments. Pine tree substrate was manufactured by hammer-milling chips of approximately 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: 0, 1, 2, 4, or 6 $kg \cdot m^{-3}$ and a peat-perlite mix (4 peat: 1 perlite, v:v, PL) was added as a control treatment, for a total of 11 treatments. Substrates were prepared, placed in 0.08 m^3 plastic storage bags and stored on shelves in an open shed in Blacksburg, Virginia. Subsamples were taken at 1, 42, 84, 168, 270, and 365 days after storage. At each subsampling day, twelve 1-L containers of each treatment were filled. Six of the 12 were left fallow and six were planted with two-week-old marigold (*Tagetes erecta* L. 'Inca Gold') seedlings and placed on a greenhouse bench. Substrate was also collected for Most Probable Number (MPN) assays for nitrifying microorganisms (for only PTS, PTS with 6 $kg \cdot m^{-3}$ lime, and PL) that were initiated after subsampling. Substrate solution pH, electrical conductivity (EC), ammonium-N (NH_4-N) and nitrate-N (NO_3-N) were measured on fallow

treatments at 0, 2, and 4 weeks after containers were filled. Marigold substrate solution pH, EC, NH_4-N and NO_3-N were measured after three weeks of marigold growth. Nitrate-N was detected in the fallow containers at low concentrations (0.4 to 5.4 $mg \cdot L^{-1}$) in PTS at 0, 2, and/or 4 weeks in all limed treatments at all subsampling days, but in the unlimed treatment, only at days 270 and 365 at week 4. Nitrate-N was detected in the fallow containers at low concentrations (0.7 to 13.7 $mg \cdot L^{-1}$) in PTSP in the 4, and 6 $kg \cdot m^{-3}$ lime rates at all subsampling days at 0, 2, and/or 4 weeks. An exception occurred at day 168 at the 4 $kg \cdot m^{-3}$ lime rate where NO_3-N was 32.8 $mg \cdot L^{-1}$ at week 4. Nitrite-oxidizing microorganisms were present in unlimed PTS and PTS with 6 $kg \cdot m^{-3}$ lime 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 nitrification occurs in PTS and PTSP and is positively correlated to substrate pH, except in PTS at day 365 when NO_3-N was detected in all treatments at the same concentration.

Introduction

Nitrification, the biological oxidation of reduced forms of N to nitrate, affects the fertilizer management of nursery and greenhouse crop production. In general plants grow best in a combination of ammonium-N (NH_4-N) and nitrate-N (NO_3-N) (Barker and Mills, 1980). The extent of nitrification in container substrate will influence how much NH_4 will be converted to NO_3. If nitrification does occur, less expensive ammonium or urea-based fertilizers can be used. Nitrification can also affect the amount of NO_3-N leached from containers, subsequently entering run-off from a production site, and contaminating waterways and groundwater. The

production of the greenhouse gases nitrous and nitric oxide, either as by-products of ammonia oxidation or as intermediates in the process known as nitrifier denitrification, is also an affect worthy of consideration. Nitrification is an acidifying process and, therefore, may affect nutrient form and availability, and subsequently, plant growth.

Autotrophic nitrification, thought to be responsible for the majority of ammonia oxidation in most soils, is carried out by two distinct groups of chemolithotrophic bacteria that derive their energy from oxidizing inorganic compounds and fix CO_2 to produce organic carbon. Ammonia-oxidizing bacteria (AOB, oxidize ammonia to nitrite via a hydroxylamine intermediate, while nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate. 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).

Autotrophic microorganisms other than AOB are also capable of oxidizing ammonia to nitrite (Francis et al., 2007; Prosser and Nicol, 2008; Treusch et al., 2005; Yao et al., 2011). These are the ammonia oxidizing archaea (AOA), prokaryotes that are fundamentally different from bacteria. They are found in great abundance in marine and fresh waters as well as in soil, and a debate exists as to which group, the AOB or the AOA, is responsible for oxidation of ammonia in

agricultural soils (Gubry-Rangin et al., 2010; Zhongjun and Conrad, 2009). A study by Nicol et al. (2008) suggests that both are responsible. Each has its own subgroups capable of growth and activity at different pH values; however, AOA ammonia mono-oxygenase gene and transcript abundance were found to decrease as pH increased in contrast to AOB ammonia mono-oxygenase transcript abundance which increased with increasing pH. These findings offer an explanation for the observation that nitrification proceeds in acidic environments at pH values well below those given for growth and activity of AOB.

A wide variety of heterotrophic fungi and bacteria can oxidize ammonia or reduced N from organic compounds to hydroxylamine, nitrite and nitrate. 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). 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 NH4-N: 3 NO3-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 pine tree substrate (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 approximately 15-year-old loblolly pine trees (*Pinus taeda* L.) by chipping and hammer-milling to a desired particle size. As 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, 5.5 to 6.5 (Yeager et al., 2007), but pH decreases with storage time [R. Wright, L. Taylor, unpublished data]. Pine tree substrate is often amended 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, if it occurs, is 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 April 2009 and chipped on 21 April 2009 with a Bandit chipper (Model 200; Bandit Industries, Inc., Remus, MI). Resulting pine chips were then passed through a hammer-mill (Meadow Mills, Inc. North Wilkesboro, NC) on 23 and 24 April, 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). A 4 peat: 1 perlite substrate, (PL; v: v), similar to a conventional substrate for greenhouse-grown crops, 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 0.08 m^3 perforated plastic bags and stored on shelves in an open shed in Blacksburg, Virginia for 365 days. 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 twelve 1-L plastic containers. Six containers were left fallow and six were planted with approximately 14-day-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 Most Probable Number (MPN) studies that were initiated two to three days 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°C and 19°C,

respectively. Each container was irrigated (beaker-applied) with 500 mL tapwater, and the following day week 0 substrate solution was extracted using the pour-through method (Wright, 1986). Substrate solution pH and electrical conductivity (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 [$(NH_4)_2SO_4$], P from phosphoric acid (H_3PO_4), K 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 approximately 6.2 using a 2 N sodium hydroxide (NaOH) solution. At the end of weeks 1 and 2, 250 mL of fertilizer solution was applied to each container, with a pour-through extract obtained one hour after fertilizer addition at the end of week 2. At the end of week 3, containers were irrigated with 250 mL of tapwater 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 one hour later substrate solution was extracted using the pour-through method. 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 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 following 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 tapwater was used to irrigate when substrate solution EC values exceeded 1.9 $dS \cdot m^{-1}$. Irrigation frequency was based on apparent plant need for water. After 3 weeks, 250 mL fertilizer solution was added to each container, substrate solution was extracted one hour later using the pour-through technique, and extract was analyzed for pH, EC, NH_4-N, and NO_3-N as previously described.

Data from fallow and planted containers were subjected to analysis of variance with mean separation by Tukey's HSD, 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 Most Probable Number (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 ammonia concentrations (Donaldson and Henderson, 1989). To estimate nitrifier population numbers present at subsampling day 1, 10 cm^3 of air-dried substrate fine particles (< 0.5 mm in diameter) from PTS without lime and peat without lime were each added to flasks containing 90 mL sterilized, deionized water and flasks were shaken vigorously by hand for 60 seconds (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 5 sterile polystyrene tubes containing 4 mL of ammonia-oxidizer medium and 5 sterile polystyrene tubes containing 4 mL-nitrite oxidizer medium. Tubes were incubated in the dark at 25°C ± 2°C for 4 weeks and then checked for presence or absence of nitrite, as outlined by Schmidt and Belser (1994), indicating oxidation of ammonia in the ammonia oxidizer tubes and oxidation of nitrite to nitrate in the nitrite oxidizer tubes, respectively. Tubes were returned to the previous incubation setting and retested every two 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 ammonia concentrations resulted in no to very low counts and were therefore considered unsuccessful. MPN assays were performed on unlimed PTS, PTS with 6 $kg \cdot m^{-3}$ lime, and PL with 6 $kg \cdot m^{-3}$ lime at all subsequent subsampling days. The 6 $kg \cdot m^{-3}$ 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 standard error of the mean were calculated using JMP (Version 8, SAS Institute, Inc., Cary, NC).

Results and Discussion

Fallow containers. Nitrate was detected in the substrate solution of PTS and PTS amended with peat containers receiving only NH_4 as a N source (Table 2). Thus, the occurrence of nitrification was verified. There was a decrease in NH_4-N with lime addition at all subsampling days for PTS and PTSP at weeks 2 and 4 (Table 3). Addition of lime increased pH (Table 4), and the higher pH of the limed treatments was 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 nitrate 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. Cation exchange capacity for PTS is low (approximately 2.0 $cmol \cdot L^{-1}$) (Jackson et al., 2008) and the fertilizer solution supplied a relatively high NH_4-N concentration (300 $mg \cdot L^{-1}$), 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 $ds \cdot m^{-1}$, data not shown). At week 4 of subsampling day 365, NH_4-N concentration in the unlimed PTS (pH 3.9) was the same as that for PTS at the 1 $kg \cdot m^{-3}$ lime rate (pH 5.7) (Table 3). This suggests that increased NH_4-N adsorption to substrate particles with increasing lime rate is not the 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 unlimed 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 unlimed PTS (pH 5.8), unlimed 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 nitrate could have originated from one of three sources or any combination of the three. The nitrate could have 1) already been in the substrate at the time of manufacture 2) been in the tapwater used initially to saturate the substrate and then later used for the pour-through analysis (Blacksburg, Virginia tapwater contains < 1$mg \cdot L^{-1}$ NO_3-N), and/or 3) the product of nitrifying microorganisms that oxidized ammonium that was indigenous to wood cells present at the time of PTS manufacture or released via 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 tapwater and amount used was the same for all treatments, the differences observed in nitrate 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 carbon to nitrogen (C:N) ratios of the PTS and PTSP were approximately 179:1 and 90:1 [L. Taylor, unpublished data], respectively, and the likelihood exists that some, if not most, of nitrate produced was immobilized. For PTS, the highest (> 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, nitrate 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, NO3-N was detectable in PTS at a lower lime rate compared to PTSP. This emphasizes the acidifying effect of peat and its influence on nitrification

In general, throughout the 365 day study, when NO_3 was detectable in any subsampling day for week 0 analysis, it was also detectable at the week 2 and 4 analyses. An exception to this occurred at day 42 for PTS. Nitrate-N was not detected at week 2 regardless of lime rate, and was measured only in very low concentrations at week 4 in the 4 and 6 $kg \cdot m^{-3}$ lime rates (0.2 and 0.3 $mg \cdot L^{-1}$ NO_3-N, respectively, Table 4). Also, in PTSP, for day 42, only the 6 $kg \cdot m^{-3}$ lime rate treatment had measurable NO_3-N at both 2 and 4 weeks (0.8 and 0.7 $mg \cdot L^{-1}$ NO_3-N, respectively) whereas at week 0 NO_3-N was detectable in the 4 $kg \cdot m^{-3}$ lime rate treatment. In both PTS and PTSP, pH values at weeks 2 and 4, where NO_3 was no longer detectable, were higher than or equal to pH values in those lime treatments with measurable NO_3-N at week 0. Thus, pH did not appear to be the reason for the absence of NO_3-N. The PTS manufacturing process (hammer-milling pine chips) most likely caused the release of highly degradable compounds resulting in an increase in microbial activity and growth (Crawford, 1983; Gray et al., 1971; Tuomela et al., 2000). The lack of detectable nitrates at day 42 in weeks 2 and 4 may be related to the inability of nitrifying microorganisms to compete with these heterotrophic neighbors, especially after the addition of fertilizer to the substrate. Both ammonia- and nitrite-oxidizers are poor competitors with heterotrophic microorganisms (Prosser, 1989).

Eighty percent of the small amount of energy generated from ammonia and nitrite oxidation reactions, relative to the breaking of carbon to carbon bonds by heterotrophs, is spent on the production of reducing power to fix CO_2 (Kelly, 1978). Another possible explanation for the lack of detectable NO_3 is increasing immobilization of any NO_3 produced by increasing populations of heterotrophic microorganisms. The addition of NH_4 in the fertilizer solution to the substrates undoubtedly caused an increase in microbial activity and density because the containers have essentially become N enriched compost bins. There were only three other exceptions to the generality that when NO_3 is detected at week 0, it is also detected at weeks 2 and 4. These all occurred with PTS, where immobilization would have been greater than in PTSP due to the higher C: N ratio of PTS. These exceptions occurred on day 84 at the 1 $kg \cdot m^{-3}$ lime rate for both weeks 2 (pH 6.0) and 4 (pH 6.1), and on day 168 at the 1 $kg \cdot m^{-3}$ lime rate at weeks 2 (pH 5.8) and 4 (pH 5.7), and 2 $kg \cdot m^{-3}$ lime rate at week 4 (pH 6.1).

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 since such an increase was not observed in the 6 $kg \cdot m^{-3}$ lime rate treatment with a higher pH (6.4) that 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.

There were two occurrences, one in PTS and one in PTSP, where NO_3-N was not measurable at week 0 analyses, but was detected by weeks 2 and 4. In both cases this occurred at days 270 and 365, and interestingly occurred at low pH values. For PTS, this occurred at week 4 in the unlimed treatment where NO_3-N had not been detected in any previous subsampling period.

Nitrate concentrations were 1.1 $mg \cdot L^{-1}$ (pH 4.0) and 0.9 $mg \cdot L^{-1}$ (pH 3.9) for days 270 and 365, respectively. For PTSP, this occurred after 2 and 4 weeks in PTSP with 2 $kg \cdot m^{-3}$ lime, where NO_3-N had been undetected after day 1. At day 270, NO_3-N concentrations were 0.7 $mg \cdot L^{-1}$ (pH 5.4) and 1.0 $mg \cdot L^{-1}$ (5.3) at weeks 2 and 4, respectively. At week 2 of day 365, NO_3-N concentrations were 0.4 $mg \cdot L^{-1}$ (pH 5.3) but NO_3-N was not present at week 4 (pH 5.2). There are several proposed mechanisms to explain nitrification at relatively low pH values. De Boer et al. (1988) proposed diffusion of NH_3 from an ammonifying microorganism directly into an adjacent ammonia-oxidizing microorganism as one mechanism. Another is the direct absorption of urea by the microorganism where urea is converted to NH_3 and CO_2 by urease in the cytoplasm (De Boer and Laanbroek, 1989; De Boer et al., 1989). Other proposals stem from observations that nitrification occurs in low pH when nitrifying organisms exist in aggregates or in biofilms (Allison and Prosser, 1993; De Boer et al., 1991). The specific mechanisms that these arrangements provide for suitable nitrification conditions are unclear. In the present study, nitrifying microorganisms may have adapted to lower pH environments after 168 days, by any of the above mentioned mechanisms, and were making more substantial contributions to solution NO_3-N values by weeks 2 and 4. The possibility exists that entirely new populations became established that were better suited for the acidic conditions, i.e., archaea or heterotrophic nitrifiers such as fungi. Subsampling days 270 and 365 occurred in January and April, respectively, during and after freezing conditions (ice crystals were observed in the stored substrates at day 270; Table 1). These low temperature conditions may have altered the species composition of the substrate. Another explanation is that NO_3 immobilization may have been lower at weeks 2 and 4 than at other subsampling days because of reduced

heterotrophic microbial populations brought about by freezing temperatures, or reduced microbial activity brought about by the low pHs, or both.

Most Probable Number. Nitrite-oxidizing microorganisms were present at all subsampling days. At day 1, the number of nitrite-oxidizers in unlimed PTS (pH 5.8) was approximately half that of peat, with 119 and 230 organisms per cm^{-3} substrate for PTS and peat, respectively (Table 5). Although attempts to enumerate ammonia-oxidizing microorganisms were unsuccessful, their presence is strongly supported, as the existence of viable populations of nitrite-oxidizing microorganisms implies viable populations of ammonia oxidizers. When nitrite (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 unlimed PTS and PTS with 6 $kg \cdot m^{-3}$ lime (23 organisms per mL of substrate for both) was considerably less than at day 1. An influence other than pH was responsible for the decline in numbers, at least in the limed treatment, since the pH of this limed PTS was 6.5. Competition for available N with other more robust microbes is a likely possibility. Further, since these surviving nitrite oxidizers are poor competitors, the lack of NO_3-N at day 42, week 2, at any lime rate in PTS is understandable as is the lack of measurable NO_3-N in any but the highest lime rate of PTSP. There was also a sharp decrease in the number of nitrite-oxidizers in PL by day 42, however, PL NO_3-N values increased by weeks 2 and 4. The C:N of PL (53:1, L. Taylor, unpublished data) is much lower that the approximate 179:1 value of PTS (L. Taylor, unpublished data) and this may explain why, even though nitrite-oxidizer numbers are low, NO_3-N concentration increased from week 0 to weeks 2 and 4. Immobilization would be less in PL than in PTS and PTSP and more NO_3-N would be present in substrate solution. Nitrite-oxidizer numbers remained steady at approximately 23 organisms per cm^3 of substrate

at all remaining subsampling days for PTS and PTS with 6 $kg \cdot m^{-3}$ lime, as did NO_3-N values. Nitrite-oxidizers, and presumably ammonia-oxidizers, were able to survive in storage over 365 days. 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 $mg \cdot L^{-1}$, except at subsampling day 270 where NO_3-N was 71.1 $mg \cdot L^{-1}$

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 due to 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. Ammonia-N to NO_3-N ratios support the occurrence of nitrification in PTS and PTSP. As pH increased, NH_4:NO_3 ratios decreased in the substrate solution extracts taken at harvest (week 3; 21 day growing period) at all subsampling days, and in both PTS and PTSP (Figure 1). This 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 NH4 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. Both groups were supplied with essential nutrients, water, and energy in the form of carbon-carbon bonds (substrate or plant derived) or reduced compounds for microorganisms, and sunlight (auxiliary lighting supplied on

day 270) for plants. Further, a study by El Jaoual (1998) showed that NO_3 (and not NH_4) was preferentially absorbed for the first 50 days 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. As mentioned earlier, there was measurable NO_3-N in the unlimed PTS in the fallow container study at 4 weeks at both sampling days.

The acidifying effect of nitrification would be expected to cause pH values to decrease over the three 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 unlimed PTS in plant production 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 (H) with NO_3 absorption or by antiport of hydroxyl (OH^-) or bicarbonate (HCO_3^-) 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) when warmer temperatures prevailed. Greater pH decreases occurred at subsampling day 168 (October), compared to July. By subsampling days 270 (January) and 365 (April) pH values began increasing again after 3 weeks of marigold growth.

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 days 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 due to 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 days. 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 three to four 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 container-grown plant production (e.g., more than 6 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.

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Table 1. 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)	Average Daily (°C)
April 2009	30	-1	18
May 2009	27	1	16
June 2009	30	9	21
July 2009	29	10	20
August 2009	30	14	21
September 2009	29	7	18
October 2009	29	-3	11
November 2009	23	-3	8
December 2009	15	-12	1
January 2010	12	-13	-2
February 2010	7	-8	-2
March 2010	22	-6	6
April 2010	29	-1	14

Table 2. Substrate solution extract nitrate-N (NO_3-N) at 0, 2, and 4 weeks, of pine tree substrate (PTS) and PTS: peat substrate (PTSP) amended with 0, 1, 2, 4, or 6 kg·m-3 lime, and peat: perlite substrate (PL) amended with 6 kg·m-3 lime when stored in plastic storage bags, placed on shelves in an open shed in Blacksburg, Virginia, and subsampled at 1, 42, 84, 168, 270, and 365 days.

	NO_3-N ($mg \cdot L^{-1}$)																	
	Day 1			Day 42			Day 84			Day 168			Day 270			Day 365		
Substrate	Time (wks)			Time (wks)			Time (wks)			Time (wks)			Time (wks)			Time (wks)		
Lime rate ($kg \cdot m^{-3}$)	0	2[u]	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
PTS[x]																		
0	0.0 e[z]	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[y]	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[w]																		
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[v]																		
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]Means within columns separated by Tukey's HSD ($P \leq 0.05$).

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

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

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

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

[u]After week 0, container substrates were fertilized weekly with a 20N-4.4P-16.6K fertilizer solution, N 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) at 0, 2, and 4 weeks, of pine tree substrate (PTS) and PTS: peat substrate (PTSP) amended with 0, 1, 2, 4, or 6 kg·m-3 lime, and peat: perlite substrate (PL). amended with 6 kg·m-3 lime when stored in plastic storage bags, placed on shelves in an open shed in Blacksburg, Virginia, and subsampled at 1, 42, 84, 168, 270, and 365 days.

	NH_4-N ($mg \cdot L^{-1}$)																	
	Day 1			Day 42			Day 84			Day 168			Day 270			Day 365		
Substrate	Time (wks)			Time (wks)			Time (wks)			Time (wks)			Time (wks)			Time (wks)		
Lime rate ($kg \cdot m^{-3}$)	0	2[u]	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
PTS[x]																		
0	0.0 b[z]	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[y]	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[w]																		
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	109.7 a	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[v]																		
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]Means within columns separated by Tukey's HSD ($P \leq 0.05$).

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

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

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

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

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

Table 4. Substrate solution extract pH values (at 0, 2, and 4 weeks) of pine tree substrate (PTS) and PTS: peat substrate (PTSP) amended with 0, 1, 2, 4, and 6 kg·m-3 lime, and peat: perlite substrate (PL) amended with 6 kg·m-3 lime when stored in plastic storage bags, placed on shelves in an open shed in Blacksburg, Virginia, and subsampled at 1, 42, 84, 168, 270, and 365 days.

	pH																	
	Day 1			Day 42			Day 84			Day 168			Day 270			Day 365		
Substrate	Time (wks)			Time (wks)			Time (wks)			Time (wks)			Time (wks)			Time (wks)		
Lime rate (kg·m^{-3})	0	2[u]	4	0	2	4	0	2	4	0	2	4	0	2	4	0	2	4
PTS[x]																		
0	5.8 e[z]	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[y]	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[w]																		
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[v]																		
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]Means within columns separated by Tukey's HSD ($P \leq 0.05$). n = 6.

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

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

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

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

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

Table 5. Most Probable Number (MPN) estimates of nitrite-oxidizing microorganisms present in unlimed and limed pine tree substrate (PTS) and peat-perlite substrate (PL) after 1, 42, 84, 168, 270, and 365 d of storage.

			MPN (organisms · cm^{-3})			
Substrate,			Day			
Lime rate ($kg \cdot m^{-3}$)	1	42	84	168	270	365
PTS[y], 0	119 (56)[z]	23 (0)	23 (0)	23 (0)	26 (3)	23 (0)
PTS, 6	–	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]SE of the mean, n = 3.

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

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

Table 6. Substrate solution extract pH values at time of planting, marigold substrate solution extracts at harvest (3 weeks), and marigold substrate solution electrical conductivity (EC) at harvest, of pine tree substrate (PTS) and PTS: peat substrate (PTSP) amended with 0, 1, 2, 4, or 6 kg·m-3 lime, and peat: perlite substrate (PL) amended with 6 kg·m-3 lime after substrate storage of 1, 42, 84, 184, 270, and 365 days in plastic storage bags placed on shelves in an open shed in Blacksburg,Virginia.

	Day																	
	1			42			84			168			270			365		
Substrate	pH[y] (SE)	pH (SE)	EC ($dS \cdot m^{-1}$)	pH (SE)	pH (SE)	EC ($dS \cdot m^{-1}$)	pH (SE)	pH (SE)	EC ($dS \cdot m^{-1}$)	pH (SE)	pH (SE)	EC ($dS \cdot m^{-1}$)	pH (SE)	pH (SE)	EC ($dS \cdot m^{-1}$)	pH (SE)	pH (SE)	EC ($dS \cdot m^{-1}$)
Lime rate ($kg \cdot m^{-3}$)	(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 (0.03)	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[z]			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[z]			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]Nonsignificant (NS)or significant at $P \leq 0.05$ (*), 0.01 (**), or 0.001 (***); L = linear, Q = quadratic response for lime rate at *, **, or *** .

[y] pH values at planting are from week 0 fallow container solution extracts.

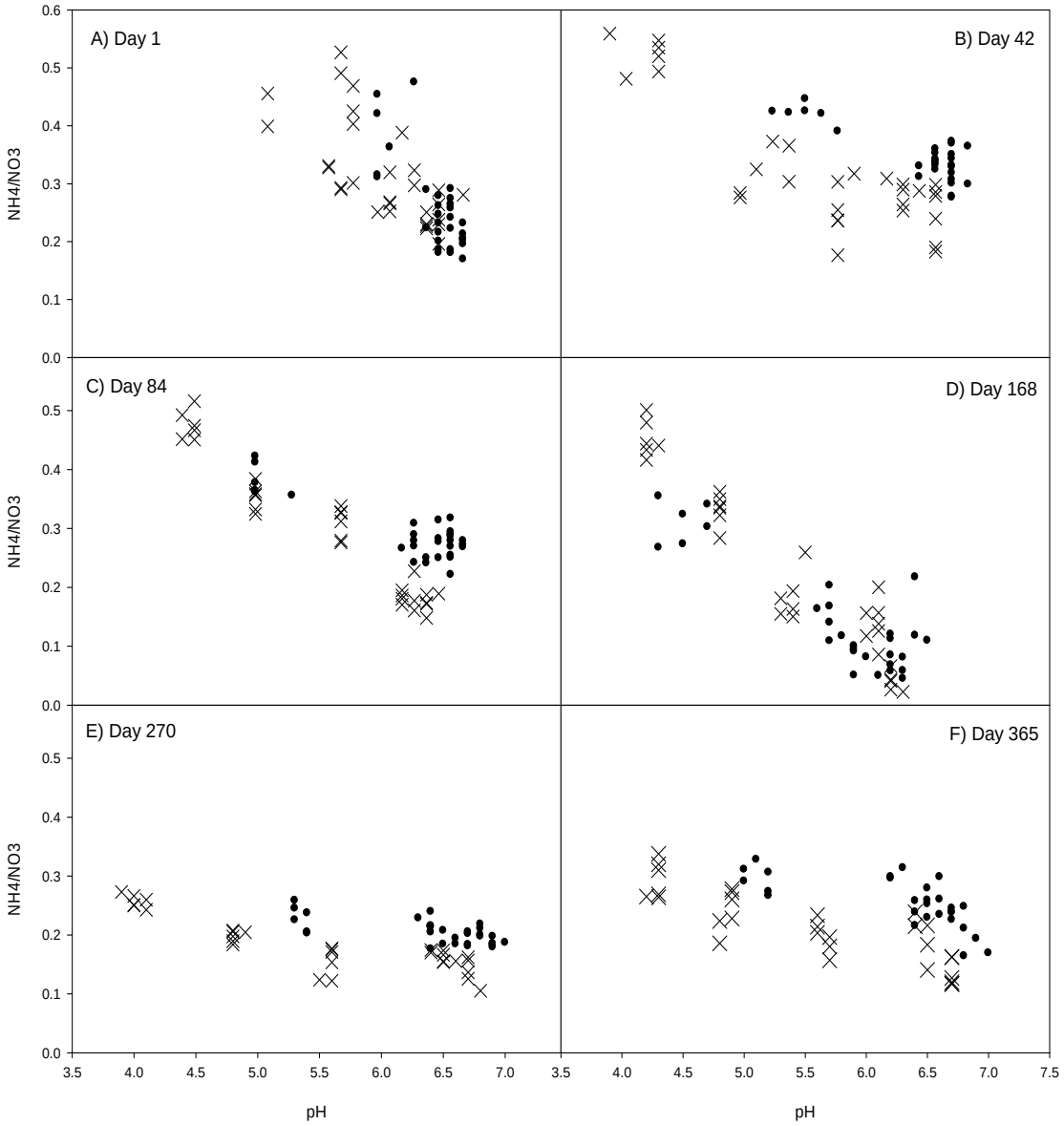

Fig. 1. Marigold substrate solution extract NH_4:NO_3 ratio after 3 weeks of fertilization with a complete 20N-4.4P-16.6K fertilizer solution at 6 subsampling days from April 2009 to April 2010 for pine tree substrate (•, PTS) and pine tree: peat (3:1, v:v) substrate (X, PTSP). **(A)** Day 1 (PTS: y = 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 = 03637 – 0.0248x, r^2 = 0.43, *P* < 0.0001 and 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 PTSP: y = 0.5285 – 0.0559x, r^2 = 0.69, *P* < 0.001).

Chapter 4

Summary

A one year storage period of unlimed and limed pine tree substrate (PTS) and PTS amended with peat (PTSP) had no or little effect on physical properties of these substrates. Particle size distribution was unchanged for unlimed PTS over 365 days, but in limed PTS there was an increase in medium-sized particles by day 168. For unlimed PTSP, there was a decrease in medium-sized particles and an increase in fine-sized particles, but in limed PTSP, there was only a slight increase in medium-sized particles. Changes did not exceed 6% and had no effect of bulk density. Bulk density values for PTS, unlimed and limed, were 0.11 $g \cdot cc^{-1}$, and values for PTSP, unlimed and limed, were 0.12 $g \cdot cc^{-1}$ throughout the 365 day storage period.

The most notable change in PTS chemical properties was substrate solution pH. All day 1 pH values were within or slightly above the recommended pH range for soilless substrates (5.5 to 6.5), except for unlimed PTSP (pH 5.2). From subsampling day 1 to day 42, substrate solution pH decreased in all treatments, and this was thought to be due to degradation of substrate extractives in PTS and PTSP. To maintain pH values for 365 days within recommended limits, 1 $kg \cdot m^{-3}$ lime was needed for PTS and 2 to 4 $kg \cdot m^{-3}$ lime was needed for PTSP. After 365 days, substrate bound acidity increased (0.11 $cmol \cdot L^{-1}$) in unlimed PTS, and bound acidity was 0.29 $cmol \cdot L^{-1}$ higher in limed PTS after 365 days than in unlimed PTS. An increase in bound acidity would represent an increase in the number of acid reserve sites. Substrate solution EC values were highest at day 1 and decreased at least 20% in all treatments by day 42. This indicates salt utilization by microorganisms which would be expected with degradation of extractives

released at the time of substrate manufacture. Cation exchange capacity (CEC) decreased in unlimed PTS (from 2.0 to 1.7 $cmol \cdot L^{-1}$) and PTSP (from 5.7 to 4.6 $cmol \cdot L^{-1}$) but the decreases would be inconsequential in conventionally fertilized plant production systems. There were different CEC data trends for limed PTS and PTSP, but changes were small and as for unlimed substrates, these changes would be inconsequential in a commercial production system. The carbon to nitrogen ratios (C:N) for PTS with and without lime (155:1 to 179:1) were unchanged over the 365 days and were the same for unlimed and limed treatments at subsampling days 1, 168, and 365. The C:N ratios for limed and unlimed PTSP (88:1 to 92:1) were also the same over time, and were the same for unlimed and limed treatments at subsampling days 1, 168, and 365.

Marigold growth in all PTS and PTSP treatments with lime was the same as growth in the control substrate peat-lite treatment (PL), at all subsampling days after day 1, except at day 270 in PTSP with 1 $kg \cdot m^{-3}$ lime. There was evidence to support the hypothesis that the presence of a phytotoxic substance in PTS and PTSP inhibited plant growth in freshly manufactured PTS. After day 42, this substance was partially or completely degraded since after this date, marigold growth in PTS, unlimed (except at day 270) and limed, was comparable to that in PL.

Nitrification occurred in PTS and PTSP. Nitrite oxidizers were present throughout the study and nitrate was measured at all subsampling days at 0, 2, and 4 weeks after containers were filled with substrates (fallow) and fertilized with an ammonium-based fertilizer. There was a positive relationship between lime rate and nitrate production for PTS and PTSP at all subsampling days except day 365 in PTS, when nitrate was measurable at the same concentrations in the

unlimed, relatively acidic PTS treatment, as in the limed treatments. Further, in unlimed PTS at day 270, nitrates were first detected, even though a positive relationship still existed between lime rate and nitrate production. Nitrate-N concentrations were detected again in the PTSP 2 $kg \cdot m^{-3}$ lime rate treatment at day 270 (undetected after day 1). This indicates either an adaptation to acidic conditions by the nitrifiers or establishment of different populations of microorganisms suited to this environment. Ammonia to nitrate ratios also supported the occurrence of nitrification in PTS and PTSP. Ratios decreased with increasing pH (lime rate) which would be expected with an increasing nitrification rate at higher pH values. Ratios were relatively low at all pH values at days 270 and 365 compared to previous days, supporting the occurrence of nitrification even at low pH values at these subsampling days.

The information gathered in this research will be helpful to the greenhouse and nursery industry in several ways. First, PTS and PTSP were shown to be relatively stable when stored for up to 365 days under the conditions of this study, i.e., in plastic bags, in an open shed in USDA Plant Hardiness Zone 6a. Second, the only significant change is the major decrease in pH that occurs between day 1 and day 42. This change can possibly be avoided by leaching substrate with water before storage to remove acid-forming extractives. Third, nitrification occurs in PTS and PTSP. For short term crop production, the amount of plant available nitrate produced by this microbial process may be limited by a high rate of N immobilization. Nitrates will need to be incorporated into the fertilizer formula to avoid ammonium toxicity, and ensure the presence of NO_3-N, a form that may be preferred by individual crop species, either throughout the life cycle, or at certain life cycle stages. However, for long term crop species receiving fertilizer, nitrate production may be substantial as the C:N ratio drops, immobilization

rate decreases, and nitrifier numbers increase. The less expensive ammonium and urea based N sources for fertilizer can be used in larger proportions.