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Comparison of Fertilizer Nitrogen Availability, Nitrogen Immobilization, Substrate Carbon Dioxide Efflux, and Nutrient Leaching in Peat-lite, Pine Bark, and Pine Tree Substrates

Brian E. Jackson[1,3] **and Robert D. Wright**[2]
Department of Horticulture, Virginia Polytechnic Institute and State University, 301 Saunders Hall, Blacksburg, VA 24061

Mark M. Alley[2]
Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

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Abstract. **The objective of this study was to compare substrate solution nitrogen (N) availability, N immobilization, and nutrient leaching in a pine tree substrate (PTS), peat-lite (PL), and aged pine bark (PB) over time under greenhouse conditions. Pine tree substrate was produced from loblolly pine logs (*Pinus taeda* L.) that were chipped and hammer-milled to a desired particle size. Substrates used in this study were PTS ground through a 2.38-mm hammer mill screen, PL, and aged PB. A short-term (28-d) N immobilization study was conducted on substrates fertilized with 150 or 300 mg·L^{-1} NO_3-N. Substrates were incubated for 4 days after fertilizing and NO_3-N levels were determined initially and at the end of the incubation. A second medium-term study (10-week) was also conducted to evaluate the amount of N immobilized in each substrate when fertilized with 100, 200, 300, or 400 mg·L^{-1} N. In addition to determining the amounts of N immobilized, substrate carbon dioxide (CO_2) efflux (μmol CO_2/m^{-2}·s^{-1}) was also measured as an assessment of microbial activity, which can be an indication of N immobilization. A leaching study on all three substrates was also conducted to determine the amount of nitrate nitrogen (NO_3-N), phosphorus, and potassium leached over 14 weeks under greenhouse conditions. Nitrogen immobilization was highest in PTS followed by PB and PL in both the short- and medium-term studies. Nitrogen immobilization increased as fertilizer rate increased from 100 mg·L^{-1} N to 200 mg·L^{-1} N in PL and from 100 mg·L^{-1} N to 300 mg·L^{-1} N for PB and PTS followed by a reduction or no further increase in immobilization when fertilizer rates increased beyond these levels. Nitrogen immobilization was generally highest in all substrates 2 weeks after potting, after which immobilization tended to decrease over the course of several weeks with less of a decrease for PTS compared with PL and PB. Substrate CO_2 efflux levels were highest in PTS followed by PB and PL at each measurement in both the short- and medium-term studies. Patterns of substrate CO_2 efflux levels (estimate of microbial populations/activity) at both fertilizer rates and over time were positively correlated to N immobilization occurrence during the studies. Nitrate leaching over 14 weeks was lower in PTS than in PB or PL through 14 weeks. This work provides evidence of increased microbial activity and N immobilization in PTS compared with PB and PL. Increased N immobilization in PTS explains the lower nutrient (primarily N) levels observed in PTS during crop production and justifies the additional fertilizer required for comparable plant growth to PL and PB. This work also provides evidence of less NO_3-N leaching in PTS compared with PL or PB during greenhouse crop production despite the higher fertilizer rates required for optimal plant growth in PTS.**

In recent years, several peat and pine bark (PB) alternative substrates have been developed and researched in the United States and throughout the world. The interest in new substrates is in response to the increasing cost and environmental issues surrounding the use of peatmoss and the cost and availability of PB substrates. Many of the substrates investigated are wood-based or plant debris-based materials that have been processed for use as a container substrate from plants, including chinese tung tree (*Aleurites fordi* Hemsl.; Gruszynski and Kämpf, 2004), paper bark tree (*Melaleuca quinquenervia* Cav.; Poole and Conover, 1985), forest gorse (*Ulex europaeus* L.; Iglesias et al., 2008), tree fern (*Dicksonia squarosa* Swartz.; Prasad and Fietje, 1989), and miscanthus (*Miscanthus sinensis* Anderss.; Carthaigh et al., 1997) to name a few. Evaluation of these and other wood/plant-based substrates has proven successful in the production of vegetables (Pudelski and Pirog, 1984; Schnitzler et al., 2004), foliage plants (Roeber and Leinfelder, 1997), bedding plants (Boyer et al., 2008a; Wright and Browder, 2005; Wright et al., 2009), poinsettias and mums (Jackson et al., 2008b; Wright et al., 2008), and woody shrubs and trees (Boyer, 2008; Jackson et al., 2008a; Wright et al., 2006). Much attention is now focused on pine tree substrates (PTS) produced from loblolly pine trees that are ground (with or without bark, limbs, needles, and so on) in a hammer mill and clean chip residual (CCR), which is produced from byproducts of the pine tree harvesting process. These substrates can be hammer-milled to a size acceptable for use as a container substrate (Boyer, 2008; Fain et al., 2008a; Jackson et al., 2007; Wright and Browder, 2005).

In contrast to peat and PB, plant production in substrates composed of wood, or large portions of wood, have a tendency to become nitrogen (N) -deficient as a result of high rates of N immobilization (Handreck, 1991, 1993; McKenzie, 1958). Wood contains large amounts of useable/degradable carbon (C) compounds but only a small amount of nutrients available for microorganisms, resulting in a draw on nutrient sources (primarily N) from the substrate solution (Gumy, 2001). The N extraction from the soil/substrate solution by microorganisms lowers available nutrient supplies to plants, which in turn leads to plant nutrient deficiencies if additional N is not added to correct the problem (Bodman and Sharman, 1993; Handreck, 1993). Successfully producing crops in wood substrates will require new strategies in N management so that the collective amounts of N required by microorganisms and by plants will be supplied in sufficient quantities to promote or maintain desired plant growth or to prevent nutrient deficiencies (Lunt and Clark, 1959; Worrall, 1985).

Several methods have been developed and used to reduce N immobilization in wood substrates and improve fertilizer management strategies during crop production: 1) composting wood materials has been shown to eliminate or significantly reduce the potential for N immobilization to occur during crop production by lowering the C:N ratio and allowing the initial breakdown, which requires high levels of N by micro-organisms (Gutser et al., 1983; Prasad, 1997); 2) a nutrient impregnation process used in the production of Toresa®, a commercial wood fiber substrate in Europe, mechanically grinds wood chips together with nutrient compounds in machines called retruders (Gumy, 2001; Schilling, 1999; Schmilewski, 2008; personal observation, Brian Jackson and Robert Wright at an Intertoresa AG Toresa® manufacturing facility in Hamburg, Germany, 13 Mar. 2007); 3) a technique called the Fersolin process impregnates wood material with sulfuric acid in the presence of hot gases (933 °C) resulting in a decrease in

decomposable cellulose, which results in lower microbial activity and need for N (Bollen and Glennie, 1961); and 4) a process for treating wood materials by pyrolysis (a form of incineration that chemically decomposes organic materials by heat in the absence of oxygen) has been evaluated as a method to break down unstable and toxic wood components into more stable and nontoxic components that are resistant to microbial decay, which retards microbial N demand (Bollen and Glennie, 1961). The methods described are often expensive, time-consuming, and nonpractical for many substrate companies and growers. As a more practical approach, a common method for supplying nutrients to counteract microbial N immobilization from a substrate is by the application of additional fertilizer during crop production. This is the most commonly used and preferred method of countering the effects of N immobilization on plant growth (Gruda, 2005; Gruda et al., 2000; Wright et al., 2008).

The most frequently used and accepted method for determining N immobilization in soilless substrates is the nitrogen drawdown index (NDI) procedure developed by Handreck (1992a, 1992b). The NDI procedure involves saturating or "charging" a substrate with a KNO_3 fertilizer solution containing 75 mg·L^{-1} N and then incubating the substrate at 22 °C for 4 d. Substrate solution nitrate nitrogen (NO_3-N) levels are determined immediately after saturation on Day 0 and then again after Day 4 (the incubation period). The NDI is then calculated by the following formula (NO_3-N measured on Day 4/NO_3-N measured on Day 0 × 100). The resulting index is a value between 1.0 and 0.0 with a value of 1.0 representing no N loss during the 4-d incubation and an index value of 0.0 indicating complete N loss after 4 d. Substrates composed of large amounts of wood materials (high C:N ratio) will immobilize all, or nearly all, of the N during the 4-d incubation when using 75 mg·L^{-1} N, making it impossible to determine the maximum amount used by micro-organisms. Handreck (1992b) has recommended that the N concentration in the saturating solution be 150 mg·L^{-1} N when substrates with a high demand for N are being tested or that the incubation time be decreased to obtain measurable amounts of N remaining in the substrate after incubation. Similarly, Sharman and Whitehouse (1993) suggest that saturating solutions with concentrations of 150, 200, or 300 mg·L^{-1} N be used in N immobilization tests on materials with high C:N ratios.

Nitrogen immobilization in soils and organic materials results from microbial assimilation of ammonium nitrogen (NH_4-N) and NO_3-N into proteins, nucleic acids, and other organic complexes contained within microbial cells (Davet, 2004). Carbon dioxide (CO_2) release represents the final stage of oxidation of organic substrates (Davet, 2004). Because root respiration is also a source of CO_2 in the soil/substrate, it is important to take into account that the CO_2 measured is not solely a result of microbial respiration. Soil CO_2 efflux is influenced by a number of factors, including soil/substrate quality and organic matter content, temperature, soil moisture, root biomass, nutrient availability, and microbial activity and biomass (Casadesus et al., 2007; Fog, 1988; Wang et al., 2003).

The estimation of microbial populations (e.g., bacteria, fungi, protozoa) in soils or soilless substrates may be accomplished by several methods, for example by counting the population (by either microscopy or plating on agar), chloroform fumigation procedure, quantifying carbon mineralization, or by assaying some unique component of biomass such as ATP, extracellular dehydrogenase, or by measuring the metabolic activity of the population (Blagodatsky et al., 2000; Boyer et al., 2008b; Carlile and Dickinson, 2004; Henriksen and Breland, 1999; Needelman et al., 2001; Turner and Carlile, 1983; Vance et al., 1987). Measuring the metabolic activity of a microbial population (respiratory activity) involves monitoring CO_2 evolution or O_2 consumption. Techniques for monitoring CO_2 evolution from soil were pioneered by Waksman (1932) and are still widely used in studies of microbial activity in soils and soilless substrates (Gough and Seiler, 2004; Jackson et al., 2008a; Pronk, 1997; Söderstrom et al., 1983; Turner and Carlile, 1983). Microbial activity (estimated by CO_2 efflux from soils) increases in response to N fertilization in N limiting soils (Zhang and Zak, 1998) and to phosphorus (P) fertilization in P-limiting soils (Gallardo and Schlesinger, 1994). Microbial activity has also been reported to decrease in response to high rates of N fertilization of forest soils (Smolander et al., 1994; Thirukkumaran and Parkinson, 2000). Less work has been completed on soilless substrates compared with field or forest soils using CO_2 efflux to monitor/estimate microbial activity.

In addition to N immobilization, nutrient leaching in PTS has been proposed as a possible reason for the lower electrical conductivity and nutrient levels observed in PTS compared with peat-lite (PL) or PB during plant production (Jackson, 2008; Wright and Browder, 2005; Wright et al., 2008). Nutrients such as NO_3-N and orthophosphate anions (P) have been shown to leach from horticulture crop production areas and are a major concern for growers and environmental agencies. Although P is considered rather immobile in many soils, it is more readily leached from soilless container media (Broschat, 1995; Yeager and Wright, 1982). Limited information is available on nutrient leaching from wood substrates, and no information is available on nutrient leaching in PTS during crop production. This is an important issue in light of the higher fertilizer requirements reported for PTS (Jackson et al., 2008a; Jackson and Wright, 2009; Wright et al., 2008), which increases the potential for nutrient leaching.

Most nursery and greenhouse producers base their fertility management on previous growing experiences with PL and PB substrates. These fertility practices may not be applicable when growing crops in PTS in light of the higher fertilizer requirements, limited understanding of N immobilization timing and rate, and its unknown leaching potential. Determining the extent and timing of N immobilization and nutrient leaching in PTS therefore needs to be determined for more accurate nutrient management (application timing and rates) strategies when producing plants in this substrate. The objective of these studies was to compare N immobilization, substrate CO_2 efflux, and nutrient leaching rates in PL, PB, and PTS over time under greenhouse conditions.

Materials and Methods

Short-term (4-week) N immobilization. Pine tree substrate used in this study was produced from loblolly pine trees (≈25-cm basal diameter) that were harvested at ground level and delimbed on 25 Apr. 2006 in Warsaw, VA. Trees were then chipped (including bark) with a Morbark Chipper (Winn, MI) operated by Wood Preservers Inc. (Warsaw, VA) on 26 Apr. 2006. Wood chips (2.5 cm × 2.5 cm × 0.5 cm) were further ground in a hammer mill (Meadows Mills, Inc., North Wilkesboro, NC) on 27 Apr. 2006 to pass through a 2.38-mm screen. Pine tree substrate (≈90% wood and 10% bark) was used fresh (uncomposted) and amended with 0.6 kg·m^{-3} calcium sulfate ($CaSO_4$) because Saunders et al. (2005) reported improved growth of herbaceous species when $CaSO_4$ was incorporated. Samples of PTS were tested for pH before potting and not amended with lime as a result of the relatively high pH (≈6.0) observed, which has been previously reported in freshly ground pine wood (Wright et al., 2008). Other substrates used in this study were an aged PB and PL [composed of 80% peat (Premier Tech, Quebec, Canada) and 20% perlite (v/v)]. Pine bark and PL were preplant-amended with dolomitic lime at a rate of 3.6 kg·m^{-3} and $CaSO_4$ at the rate of 0.6 kg·m^{-3}. Substrates were prepared on 1 May 2006 and moistened to 50% moisture content (based on the substrate's waterholding capacity) to facilitate lime reactions (in the peat and PB) and provide adequate moisture for

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[1]Graduate Research Assistant.
[2]Professor.
[3]To whom reprint requests should be addressed; e-mail brian_jackson@ncsu.edu.

microbial activity in all substrates. The municipal water source (Blacksburg, VA) used to moisten the substrates had an alkalinity of 5.8 mEq·L^{-1} and pH of 6.8. Substrates were stored in closed containers for 8 d (after wetting) before the initiation of the experiment as suggested by Handreck (1992a).

On 8 May 2006, 12 cm tall × 15 cm square (1.7-L) plastic containers were filled with the three substrates and placed on raised benches in the Virginia Tech (Blacksburg, VA) Greenhouse Facility (glass-covered) with average day and night temperatures of 24 and 19 °C, respectively. Containers were irrigated with 500 mL of nutrient solution (beaker-applied) at the rates of 0, 150, or 300 mg·L^{-1} N as potassium nitrate (KNO_3; Handreck, 1992a), which consequently supplied 420 mg·L^{-1} potassium (K) at the 150 mg·L^{-1} N rate and 840 mg·L^{-1} K at the 300 mg·L^{-1} N rate. Phosphorus was also supplied at 45 mg·L^{-1} P as phosphoric acid (H_3PO_4) as a result of published studies reporting the requirement (and immobilization) of P by microbial populations in soils and wood substrates (Gallardo and Schlesinger, 1994; Handreck, 1996). The 0 mg·L^{-1} N rate was added as a control to observe the effect of fertilizer on substrate CO_2 efflux compared with a nonfertilized treatment. Fertilizer treatments were applied to substrates on Day 0 (day of potting) and every 7 d thereafter for 28 d for a total of five applications. After fertilizer applications (every 7 d), three replications of each treatment were removed from the greenhouse and analyzed for N immobilization using the NDI incubation and extraction procedure as described previously. Substrate solutions extracted before and after incubation at each sampling date were frozen and later analyzed for NO_3-N with an Orion ion selective electrode (Thermo Electron, Beverly, MA) on 17 Aug. 2006. Substrate solution N levels were determined on Day 0 (initial) and on Day 4 (final) and the amount of N immobilized was calculated by determining the difference between Day 4 and Day 0. Total N loss (mg N per L of substrate) was then calculated for the total 4 d incubation. Nitrogen immobilization data from the 0 mg·L^{-1} N rate in all substrates were excluded from data analysis as a result of no N immobilization occurring at that rate. Between fertilizer/irrigation applications, containers were kept uncovered on open greenhouse benches and substrate moisture was determined by weighing representative containers of each substrate and applying tap water (same water source as previously described) to readjust substrate moisture to 70% of their waterholding capacities (determined before the initiation of this experiment).

Substrate CO_2 efflux levels were determined on all substrates and at all fertilizer rates as an estimate of microbial activity and potential N immobilization (Wang et al., 2003). Substrate CO_2 efflux (μmol CO_2/m^{-2}·s^{-1}) was determined each week on three container replications of each substrates at each fertilizer rate using a LI-COR 6250 (LI-COR, Lincoln, NE) infrared gas analyzer (IRGA) equipped with a closed chamber constructed from a polyvinyl chloride pipe end cap designed to take nondestructive CO_2 measurements from the substrate-filled containers. The chamber was placed on the substrate and pressed firmly to the surface before CO_2 efflux measurements were taken. A gas sampling and return air port (constructed from 0.6-cm plastic tubing) from the chamber allowed air to be circulated from the chamber to the IRGA. Soil CO_2 efflux rates were determined by measuring change in CO_2 concentration (ΔC) over a 30-s period. The LI-COR 6250 was recalibrated before each sampling date and the system zeroed between treatment replications. Substrate CO_2 efflux was measured at about the same time of day for each sampling date.

The experimental design was completely randomized with three substrates, three fertilizer rates, and 15 replications per substrate for a total of 135 containers. Nitrogen immobilization and substrate CO_2 efflux data were tested using the analysis of variance procedures and correlation analysis of SAS (Version 9.1; SAS Institute, Inc., Cary, NC) with treatment means separated by Duncan's multiple range test ($\alpha = 0.05$). Data were also subjected to regression analysis using SigmaPlot (Version 9.01; SPSS, Inc., Chicago, IL).

Medium-term (10-week) immobilization. Pine tree substrate used in this study as well as the leaching experiment (described next) was produced from loblolly pine trees that were harvested at ground level and delimbed on 15 July 2007 in Blackstone, VA. Trees were then chipped (including bark) with a Bandit Chipper (Model 200; Bandit Industries, Inc., Remus, MI) on 17 July 2007. Wood chips were further ground in a hammer mill on 18 July 2007 to pass through a 2.38-mm screen. Other substrates used in this study included aged PB and PL. All substrates were amended similarly as described in the short-term experiment.

On 21 Aug. 2007, 12 cm tall × 15 cm square (1.7-L) plastic containers were filled with the three substrates and placed on raised benches in the Virginia Tech (Blacksburg, VA) Greenhouse Facility (glass-covered) with average day and night temperatures of 24 and 19 °C, respectively. Fertilizer solutions were beaker-applied (500 mL) every 2 weeks (14 d) to the substrates at the rates of 100, 200, 300, or 400 mg·L^{-1} N as KNO_3, which consequently supplied 280 mg·L^{-1} K for each 100 mg·L^{-1} N rate increase. Phosphorus was also supplied at 45 mg·L^{-1} as H_3PO_4. After fertilizer application and drainage for 1 h, three single container replications of each treatment were removed from the greenhouse and analyzed for N immobilization using the NDI incubation and extraction procedure (as described in the short-term experiment) on Week 0 (day of potting) and again every 14 d for 10 weeks for a total of six sampling dates. Substrate solutions extracted before and after incubation at each sampling date were frozen and later analyzed for NO_3-N with an Orion ion selective electrode on 22 Jan. 2008. Nitrogen immobilization data were calculated in this study as described previously in the short-term experiment. Between fertilizer/irrigation applications, containers were kept uncovered on open greenhouse benches and substrate moisture was maintained at 70% in all substrates as described in the short-term experiment. Substrate CO_2 efflux was determined every 2 weeks (alternate weeks of fertilizer applications) on three container replications of each substrates at each fertilizer rate using a LI-COR 6250 as previously described previously.

The experimental design was completely randomized with three substrates, four fertilizer rates, and 24 replications per substrate for a total of 288 containers. Data were tested using the analysis of variance procedures and correlation analysis of SAS (Version 9.1; SAS Institute, Inc.). Data were also subjected to regression analysis using SigmaPlot (Version 9.01; SPSS, Inc.).

Nutrient leaching. Pine tree substrate, PL, and PB used in this experiment were prepared as described previously in the medium-term experiment and amended similarly. On 21 Aug. 2007, 12 cm tall × 15 cm square (1.7-L) plastic containers were filled (by vol) with the three substrates. Fertilizer solutions were beaker-applied (500 mL) every 2 weeks (14 d) to the substrates at the rates of 100 or 300 mg·L^{-1} N prepared from KNO_3, which consequently supplied 280 mg·L^{-1} K at the 100 mg·L^{-1} N rate and 840 mg·L^{-1} K at the 300 mg·L^{-1} N rate. Phosphorus was supplied at 45 mg·L^{-1} P as H_3PO_4 to both the rates (100 and 300 mg·L^{-1} N). Fertilizer applications were made to all substrates every other week through the conclusion of the study (14 weeks).

Leaching of NO_3-N, P, and K was monitored on six replicates of each treatment. Leachate collection devices were made by cutting 15-cm circular holes in the lids of 4.5-L plastic buckets. Lids were then securely fitted on buckets and fallow substrate-filled containers were inserted halfway through the lids into the buckets. Buckets (with containers inserted) were then placed on raised benches in the Virginia Tech (Blacksburg, VA) Greenhouse Facility (glass-covered) with average day and night temperatures of 24 and 19 °C, respectively. This system allowed only the leachate passing through the fallow containers to be collected after irrigations. After applying fertilizer solutions, containers were allowed to completely drain (1 h) before containers were removed from buckets for leachate collection. Leachate volume was determined and an aliquot was taken and subsequently frozen and later analyzed for NO_3-N with an Orion ion selective electrode on 17 Jan. 2008, and P and K concentrations were analyzed on 31 Jan. 2008 with a Spectro Ciros Vision ICP (Spectro Analytical Instrument, Mahwah, NJ). Between fertilizer/irrigation applications, containers were kept uncovered on open benches in a greenhouse and substrate moisture was maintained at 70% as described in the medium-term experiment.

The experimental design was completely randomized with three substrates, two fertilizer rates, and six replications per substrate for a total of 36 containers. Data were tested using the analysis of variance procedures of SAS (Version 9.1; SAS Institute, Inc.).

Results and Discussion

Short-term (4-week) N immobilization. At each measuring date and at both fertilizer rates, the amount of N immobilization was highest in PTS compared with PB and PL, and the amount of immobilization in PB was higher at all dates and fertilizer rates compared with PL (Table 1). At both fertilizer rates (150 mg·L^{-1} and 300 mg·L^{-1} N), N immobilization occurred in the first days after potting (Day 0) and increased with each measurement date through the end of the study (28 d) in all substrates except PL at the high fertilizer rate (Table 1). The initial immobilization in all substrates explains the need for starter charge fertilizers that are typically added to commercial substrate mixes. Quick-release starter charge fertilizers supply N to the substrate on planting, which can offset the N immobilized by microbes. Immobilized N was equal at both fertilizer rates for PL and PB at all five measuring dates, whereas PTS had a higher amount of N immobilization at the 300 mg·L^{-1} N rate through Day 21 compared with the 150 mg·L^{-1} N rate (Table 1). On Day 28, N immobilization was the same (15 and 16 mg per L substrate) at both fertilizer rates in PTS. Immobilization amounts were at least as high at Day 28 (end of the experiment) at both fertilizer rates and in all substrates compared with Day 0 indicating the continuous occurrence of N immobilization over the course of 28 d during crop production (Table 1). However, at Day 28, more than twice the amount of N was immobilized in PTS compared with PB and more than five times the amount immobilized in PL (Table 1).

Substrate solution NO_3-N levels, reflective of N immobilization, were lower in PB and PTS compared with PL after the 4-d incubation at both fertilizer rates at all measurement dates with levels in PTS being the lowest of all the substrates (Table 2). The levels of NO_3-N recovered in PTS at the low fertilizer rate were very low at the first two measurements (less than 1.0 mg·L^{-1} N) but increased by the last measurement date (9.3 mg·L^{-1} N; Table 2). At all measuring dates (0, 7, 14, 21, and 28), the NO_3-N levels after incubation in PTS remain lower at both fertilizer rates than PB and PL (Table 2). The lower substrate solution NO_3-N levels in PTS (resulting from more N immobilization) compared with PB have also been reported even when plant growth was similar (Jackson et al., 2008a) and in PL (Jackson et al., 2008b; Wright et al., 2008). The reason for higher initial (Day 0) substrate solution N levels at all measuring dates (Table 2) in PB and PL compared with PTS at the 300 mg·L^{-1} N rate can be explained by the already existing N in the substrates from previous fertilizer applications that remained in the substrate as a result of the lower amount of N immobilization occurring in those substrates each week (Table 1).

Short-term fast-growing crops (e.g., bedding plants) grown in PL or PB are commonly fertilized at rates between 100 and 200 mg·L^{-1} N, which is unacceptable for plants growing in PTS (Jackson and Wright, 2009). Based on the increased N immobilization in PTS compared with PB and PL at both fertilizer rates (Table 1) and as a result the higher NO_3-N levels remaining in substrate solution in PB and PL after 4 d compared with PTS (Table 2), it is clear why decreased plant growth is usually observed in PTS at relatively low (100 to 200 mg·L^{-1} N) fertilizer rates. Similar to these results, Sharman and Whitehouse (1993) observed less available N in substrate solution at low fertilizer rates (75 and 150 mg·L^{-1} N) in wood-based substrates, but at higher fertilizer rates (300 mg·L^{-1} N), N levels in substrate solution were higher and closer to levels in peat. Work by

Table 1. Nitrate nitrogen (NO_3-N) immobilized (milligrams) in peat-lite, pine bark, and pine tree substrate (PTS) at five sampling dates over 28 d (short-term experiment) in containers fertilized with two rates of N from potassium nitrate (KNO_3).

Substrates	NO_3-N immobilized[z]					
N rate (mg·L^{-1})[y]	Day 0	Day 7	Day 14	Day 21	Day 28	Significance[x,w]
Peat-lite[v]						
150	2.4 d[u]	3.0 d	3.2 d	3.0 d	2.9 c	L** Q*
300	2.2 d	2.5 d	2.8 d	2.8 d	2.4 c	NS
Pine bark						
150	6.1 c	7.3 c	7.5 c	9.3 c	6.2 b	L* Q**
300	5.6 c	7.0 c	7.9 c	8.6 c	6.4 b	L*** Q**
PTS[t]						
150	9.8 b	11.3 b	13.2 b	14.4 b	15.0 a	L*** Q*
300	11.4 a	16.2 a	18.1 a	20.0 a	16.1 a	L*** Q**
Substrate (S)	0.0001	0.0001	0.0061	0.0052	0.0010	
Fertilizer (F)	0.0002	0.0012	0.0001	0.0007	0.0021	
S × F	0.0031	0.0001	0.0043	0.0214	0.1021	

[z]NO_3-N immobilized = milligrams of NO_3-N immobilized per L substrate during a 4-d incubation.
[y]1 mg·L^{-1} = 1 ppm.
[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[w]L = linear; Q = quadratic response across measurement dates (28 d) at *, **, or ***.
[v]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[u]Means separated within columns using Duncan's multiple range test ($P \leq 0.05$; n = 3).
[t]PTS was produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

Table 2. Substrate solution nitrate nitrogen (NO_3-N) concentrations after 0 d incubation and after 4-d incubation in peat-lite, pine bark, and pine tree substrate (PTS) over 28 d (short-term experiment) when fertilized with two rates of N from potassium nitrate (KNO_3).[z]

Substrates	NO_3-N concn (mg·L^{-1})[y]									
	Day 0		Day 7		Day 14		Day 21		Day 28	
N rate (mg·L^{-1})	0	4	0	4	0	4	0	4	0	4
Peat-lite[x]										
150	31.9	23.0	39.3	29.0	41.7	31.0	54.0	44.2	65.0	55.3
300	68.0	60.7	80.1	71.7	103.3	94.0	118.7	109.0	171.0	163.0
Pine bark										
150	31.0	11.1	34.7	10.3	48.7	23.7	63.0	32.0	74.0	53.0
300	69.6	51.0	84.7	61.7	115.0	88.6	127.0	98.6	167.0	146.3
PTS[w]										
150	33.3	0.7	38.0	0.4	45.3	1.4	53.0	5.3	58.6	9.3
300	59.8	21.7	70.1	16.0	87.3	27.0	102.6	36.3	147.0	94.0
Substrate (S)	0.0701	0.0011	0.2071	0.0001	0.0031	0.0001	0.0027	0.0001	0.0330	0.0041
Fertilizer (F)	0.0001	0.0003	0.0020	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
S × F	0.0601	0.0001	0.0041	0.0001	0.0034	0.0001	0.0401	0.0001	0.0501	0.0001

[z]Substrate solution extracted using a nitrogen drawdown procedure for determining nitrogen immobilization in substrates (Handreck, 1992a).
[y]1 mg·L^{-1} = 1 ppm.
[x]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[w]PTS produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

Browder et al. (2006) showed that when PTS was amended with 40% peatmoss (v/v), plant growth was similar to plants grown in 100% peat at 200 mg·L^{-1} N. Additionally, Fain et al. (2008b) reported improved plant growth at lower fertilizer starter charge rates when 20% to 50% peatmoss (v/v) was amended to the PTS (ground whole pine trees, including limbs and needles) used in their study. These previous studies suggest that the presence of peat (having less N immobilization) allowed more N to remain in substrate solution for plant use.

Substrate CO_2 efflux increased as fertilizer rates increased in all substrates and at each measurement date and was highest in PTS and lowest in PL with PB being intermediate (Table 3), similar to results reported by Jackson and Wright (2007). There was a substrate × fertilizer rate interaction for substrate CO_2 efflux at each measurement date with PTS having a more pronounced CO_2 efflux increase as fertilizer rate increased compared with PB or PL (Table 3). Higher substrate CO_2 efflux in PTS indicates a higher level of microbial activity, which is supported by the aforementioned higher amount of N immobilized in PTS compared with PB and PL (Table 1). Substrate CO_2 efflux levels across measuring dates did not change in PL at any of the fertilizer rates (Table 3) in this study. The low microbial activity/presence in peat substrates has been reported by several authors in previous studies (Boyer et al., 2008b, Carlile and Wilson, 1991; Jackson, 2008). No change in substrate CO_2 efflux over time was also observed in PB at the 0 mg·L^{-1} N fertilizer rate (Table 3). Substrate CO_2 efflux did increase over the experiment in PB at the 150 and 300 mg·L^{-1} N rates and in PTS at all fertilizer rates (Table 1). There was a positive correlation ($R^2 = 0.72$) between substrate CO_2 efflux levels for the three substrates and the corresponding amount of N immobilized in the substrates (data not shown). A positive correlation between N immobilization and soil/substrate CO_2 efflux has been reported by other authors (Qui et al.,

Table 3. Substrate carbon dioxide (CO_2) efflux measured weekly in peat-lite, pine bark, and pine tree substrate (PTS) over 28 d (short-term experiment) in containers when fertilized with three rates of nitrogen (N) from potassium nitrate (KNO_3).

Substrates	CO_2 efflux (μmol CO_2/m^{-2}·s^{-1})[z]					
N rate (mg·L^{-1})[y]	Day 0	Day 7	Day 14	Day 21	Day 28	Significance[x,w]
Peat-lite[v]						
0	0.7 d[u]	0.6 d	0.8 e	0.5 f	0.6 f	NS
150	1.1 cd	0.9 c	0.9 e	1.1 ef	0.8 ef	NS
300	1.4 c	1.1 c	1.5 d	1.5 e	1.3 e	NS
Significance[ts]	L** Q*	L** Q**	L* Q**	L*** Q**	L*** Q*	
Pine bark						
0	0.7 d	0.5 d	0.8 e	0.7 f	0.7 f	NS
150	1.6 c	2.9 b	2.4 cd	2.5 d	2.1 d	L** Q**
300	1.7 c	3.4 b	3.6 c	3.6 c	3.4 c	L* Q**
Significance	L** Q**	L*** Q***	L*** Q***	L*** Q***	L** Q***	
PTS[r]						
0	0.6 d	0.6 d	1.0 e	0.8 f	1.1 e	L* NS
150	2.6 b	3.6 ab	6.5 b	5.7 b	5.9 b	L*** Q**
300	3.9 a	4.3 a	8.4 a	8.0 a	7.7 a	L** Q***
Significance	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	
Substrate (S)	0.0081	0.0021	0.0240	0.0061	0.0005	
Fertilizer (F)	0.0001	0.0001	0.0001	0.0001	0.0001	
S × F	0.0021	0.0010	0.0073	0.0260	0.0007	

[z]CO_2 efflux measured on undisturbed fallow containers with a LI-COR 6200 infrared gas analyzer (n = 3).
[y]1 mg·L^{-1} = 1 ppm.
[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001, respectively.
[w]L = linear; Q = quadratic response across measurement dates (28 d) at *, **, or ***.
[v]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[u]Means separated within columns using Duncan's multiple range test ($P \leq 0.05$; n = 3).
[ts]NS or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[s]L = linear; Q = quadratic response for fertilizer rate at *, **, or ***.
[r]PTS produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

Table 4. Nitrate nitrogen (NO_3-N) immobilization in peat-lite, pine bark, and pine tree substrate (PTS) at five sampling dates when fertilized with four N rates from potassium nitrate (KNO_3) over 10 weeks (medium-term experiment).

Substrates	NO_3-N immobilized[z]						
N rate (mg·L^{-1})[y]	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Significance[x,w]
Peat-lite[v]							
100	2.4 f[u]	3.4 f	2.6 g	1.3 g	2.1 f	1.5 g	L*
200	3.1 f	4.2 e	3.9 f	3.6 f	3.2 e	3.7 e	NS
300	4.0 e	4.3 e	2.9 g	3.6 f	2.8 e	3.1 f	L** Q**
400	1.8 g	4.0 e	3.2 f	3.2 f	2.0 f	2.9 f	L* Q*
Significance[ts]	L** Q**	L** Q***	L** Q**	L* Q**	L* Q**	L*** Q**	
Pine bark							
100	4.1 e	7.0 d	5.5 e	6.7 e	2.4 ef	3.0 f	L* Q**
200	5.7 d	8.8 c	5.9 de	8.6 d	7.5 c	6.6 d	L*** Q**
300	7.0 cd	7.4 d	6.7 d	11.2 c	6.5 cd	6.1 d	L*** Q**
400	4.9 e	7.3 d	5.9 de	6.8 d	5.3 d	5.3 de	L** Q*
Significance	L*** Q***	L* Q***	L** Q**	L* Q*	L*** Q**	L** Q***	
PTS[r]							
100	8.7 c	9.3 c	10.2 c	10.4 c	7.4 c	8.2 c	L** Q**
200	12.4 b	15.9 b	12.5 b	12.0 bc	10.4 b	10.2 b	L* NS
300	14.1 a	19.1 a	19.1 a	17.4 a	15.6 a	11.3 a	L** Q***
400	12.6 b	16.1 b	12.6 b	15.0 b	11.3 b	10.2 b	L* Q**
Significance	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L** Q***	
Substrate (S)	0.0001	0.0121	0.0029	0.0401	0.0001	0.0055	
Fertilizer rate (F)	0.0001	0.0001	0.0001	0.0001	0.0432	0.0001	
S × F	0.0171	0.0210	0.0158	0.0107	0.0024	0.0241	

[z]NO_3-N immobilization = milligrams of NO_3-N immobilized per L substrate during a 4-d incubation.
[y]1 mg·L^{-1} = 1 ppm.
[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[w]L = linear; Q = quadratic response across measurement dates (10 weeks) at *, **, or ***.
[v]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[u]Means separated within columns using Duncan's multiple range test ($P \leq 0.05$; n = 3).
[ts]NS or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[s]L = linear; Q = quadratic response for fertilizer rate at *, **, or ***.
[r]PTS produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

2008; Turner and Carlile, 1983). There was also a positive correlation between substrate CO_2 efflux level and fertilizer rate for PB ($R^2 = 0.82$) and PTS ($R^2 = 0.96$).

Medium-term (10-week) N immobilization. Nitrogen immobilization increased from the 100 mg·L^{-1} N rate to the 200 mg·L^{-1} N rate in PL (with Week 0 being the only exception) through all measurement dates (Table 4). During half of the measurement dates (Weeks 0, 4, and 8), immobilization decreased in PL from the 300 to the 400 mg·L^{-1} N rate (Table 4). There was no difference in N immobilized over the 10-week experiment (across dates) in PL at the 200 mg·L^{-1} N rate (Table 4), which is similar to the results from the short-term experiment over 4 weeks (Table 1). Immobilization of N in PB increased from 100 mg·L^{-1} N to 200 mg·L^{-1} N at all but one measuring dates (Table 4). Immobilization of N in PTS increased as fertilizer rate increased from 100 mg·L^{-1} N to 300 mg·L^{-1} N at all dates and was lower at the 400 mg·L^{-1} N rate compared with the 300 mg·L^{-1} N rate at all dates. Pine tree substrate had higher amounts of N immobilized at each fertilizer rate and at each week compared with PB and PL (Table 4). Similar to the short-term experiment discussed previously, there was a substrate × fertilizer rate interaction in this study for each measuring date with higher increases in N immobilization for PTS in response to fertilizer rate at each date compared with PB and PL (Table 4).

Nitrogen immobilization at low fertilizer rates observed in this study is also a common occurrence in traditional commercial substrates during early crop production and is associated with the undecomposed carbonaceous materials (wood fragments, bark, and so on) found in substrates that are being broken down by microbes (Bunt, 1988). The decrease in immobilization often seen at the high fertilizer rates (400 mg·L^{-1} N) may be in response to high salt levels, which may be toxic to microbes (Maas and Adamson, 1972), thereby limiting or decreasing their populations.

Substrate solution NO_3-N levels after incubation were lowest in PTS compared with PB or PL at each measuring date (Table 5). Nitrate levels generally increased through the weeks until the end of the experiment with levels from Week 0 being the lowest in all substrates at all fertilizer rates (Table 5). Similar to the data discussed in the short-term study (Table 2), the NO_3-N levels in PTS after incubation remain low (less than 25 mg·L^{-1} N) compared with PB and PL through Week 4 at the 100 and 200 mg·L^{-1} N fertilizer rates, but NO_3-N levels in solution were above 90 mg·L^{-1} at the 300 mg·L^{-1} N fertilizer rate at Week 4 (28 d). Solution NO_3-N levels in PTS at the 300 mg·L^{-1} N were closer to the levels reported for PB and PL at the 200 mg·L^{-1} N rate during several of the dates (Weeks 0, 2, 4, 6; Table 5), which supports the reason for an additional 100 mg·L^{-1} N required for comparable plant growth in PTS compared with PB or PL (Wright et al., 2008).

Table 5. Substrate solution nitrate nitrogen (NO_3-N) concentrations after 0 d incubation and after 4 d incubation in peat-lite, pine bark, and pine tree substrate (PTS) over 10 weeks (medium-term experiment) when fertilized with four rates of N from potassium nitrate (KNO_3).[z]

	NO_3-N concn (mg·L^{-1})[y]											
	Week 0		Week 2		Week 4		Week 6		Week 8		Week 10	
Substrates N rate (mg·L^{-1})	0	4	0	4	0	4	0	4	0	4	0	4
Peat-lite[x]												
100	23.3	15.3	32.7	21.7	36.0	27.3	36.3	32.0	53.0	46.0	28.3	23.3
200	44.3	34.0	59.0	45.7	90.0	77.3	74.7	62.7	89.7	79.0	73.7	61.3
300	78.0	64.7	126.0	111.0	166.0	157.0	131.0	119.0	221.0	212.0	108.0	97.7
400	75.0	69.0	147.0	134.0	210.0	199.0	172.0	162.0	210.0	204.0	223.0	213.0
Significance[wv]	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L** Q***	L*** Q**	L*** Q***	L*** Q***
Pine bark												
100	18.3	4.7	34.3	11.1	32.0	14.0	35.7	13.3	59.0	51.0	34.7	24.7
200	33.3	14.7	71.0	41.7	83.0	64.0	99.7	71.3	168.0	143.0	159.0	137.0
300	58.7	35.7	136.0	111.0	151.0	129.0	205.0	167.0	237.0	215.0	255.0	234.0
400	57.0	40.7	159.0	135.0	195.0	175.0	174.0	152.0	244.0	226.0	247.0	229.0
Significance	L** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L* Q**	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q**
PTS[u]												
100	29.3	0.3	32.3	1.3	36.7	2.67	38.7	4.17	37.3	12.8	35.3	8.1
200	42.7	1.3	55.0	2.0	67.3	22.7	87.0	47.0	71.0	36.7	61.0	27.0
300	72.3	25.3	115.0	51.0	158.0	94.7	115.0	57.0	166.0	114.0	93.0	55.7
400	72.7	30.7	159.0	105.0	174.0	132.0	175.0	125.0	172.0	134.0	161.0	127.0
Significance	L*** Q**	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q***	L*** Q**	L*** Q***	L*** Q***	L*** Q***
Substrate (S)	0.0471	0.0021	0.0502	0.0017	0.0720	0.0002	0.1320	0.0001	0.0209	0.0061	0.0001	0.0001
Fertilizer (F)	0.0051	0.0004	0.0001	0.0001	0.0001	0.0001	0.0031	0.0001	0.0001	0.0023	0.0001	0.0001
S × F	0.0621	0.0371	0.1601	0.0131	0.0369	0.0071	0.0201	0.0081	0.0008	0.0101	0.0151	0.0001

[z]Substrate solution extracted using a nitrogen (N) drawdown procedure for determining N immobilization (Handreck, 1992a; n = 3).
[y]1 mg·L^{-1} = 1 ppm.
[x]PL composed of 80% peatmoss/20% perlite (v/v).
[w]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[v]L = linear; Q = quadratic response for fertilizer rate at *, **, or ***.
[u]PTS produced from 12-year-old loblolly pine trees harvested, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

There was a substrate × fertilizer rate interaction for substrate CO_2 efflux at each measurement date (except Week 10) with PTS having a more pronounced CO_2 efflux increase as fertilizer rate increased compared with PB or PL. Substrate CO_2 efflux levels in all substrates were somewhat erratic, but the general response showed increasing CO_2 levels with increasing fertilizer rate followed by a decrease at the higher fertilizer rates (Table 6). The CO_2 efflux decrease at the 400 mg·L^{-1} N rate to at or below the levels recorded at the 100 mg·L^{-1} N rate in all substrates indicates a reduction in microbial activity at the highest fertilizer rate. The decrease in substrate CO_2 efflux at the highest fertilizer rate (400 mg·L^{-1} N) in PTS is positively correlated (R^2 = 0.81) to the decrease in N immobilization that was reported at the same fertilizer rate (Table 4). There was no positive correlation between substrate CO_2 efflux and N immobilization for PB and PL. Similar to the short-term study, there was also a positive correlation between substrate CO_2 efflux level and fertilizer rate for PTS (R^2 = 0.84). Lower CO_2 substrate efflux rates have also been reported at relatively high fertilizer rates by Jackson et al. (2008a) and Maas and Adamson (1972), even after increases in CO_2 efflux were observed in the same studies after the addition of low fertilizer rates.

Similar to these results, Boyer et al. (2008b) reported significantly higher microbial respiration from CCR (40% pine wood, 50% bark, and 10% needles) than from PB or peatmoss and noted an increase in respiration as N rate (0, 1, 2, 3 mg per 20 g substrate sample) increased in CCR. The authors suggest that N immobilization should not be a factor in CCR during crop production compared with PB based on similar plant growth at the same fertilizer rate in previous studies. The lower wood content (and higher bark content) of CCR compared with the PTS used

Table 6. Substrate carbon dioxide (CO_2) efflux measured every 2 weeks on peat-lite, pine bark, and pine tree substrate (PTS) when fertilized with four rates of nitrogen (N) from potassium nitrate (KNO_3) for 10 weeks (medium-term experiment).

Substrates	CO_2 efflux (μmol CO_2/m^{-2}·s^{-1})[z]						
N rate (mg·L^{-1})[y]	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Significance[x,w]
Peat-lite[v]							
100	0.7 de[u]	1.3 e	1.5 e	1.3 cd	1.3 cd	1.0 d	L* NS
200	1.2 d	1.7 de	1.6 de	1.8 c	1.2 d	1.0 d	NS
300	1.1 d	1.4 e	1.6 de	1.9 c	1.5 c	1.4 c	L** Q*
400	0.7 de	1.3 e	1.2 e	0.8 d	1.0 d	0.9 d	NS
Significance[t]	NS NS	NS Q*	L** NS	L* Q**	L** Q**	NS NS	
Pine bark							
100	2.1 c	2.0 d	1.7 d	1.8 c	1.9 b	1.5 c	L* Q**
200	2.5 bc	2.9 c	1.9 d	2.9 b	1.5 c	1.5 c	L** Q**
300	3.1 b	3.0 c	4.0 bc	2.6 bc	2.1 b	1.4 c	L*** Q*
400	2.1 c	2.0 d	1.9 d	1.7 c	1.5 c	1.5 c	NS Q*
Significance	L*** Q*	L** Q**	L* Q**	L** Q**	L** NS	NS NS	
PTS[s]							
100	2.7 bc	3.4 c	3.1 c	2.7 b	3.5 a	2.5 b	NS Q*
200	3.3 b	5.1 b	5.4 ab	4.5 a	3.1 a	3.2 a	L* Q*
300	5.3 a	6.9 a	6.3 a	4.5 a	3.6 a	2.6 b	L** Q***
400	2.9 b	1.9 d	2.0 d	2.2 c	1.9 b	1.7 c	L** Q**
Significance	L*** Q***	L*** Q***	L*** Q***	L* Q**	L** Q*	L* Q**	
Substrate (S)	0.0001	0.0001	0.0001	0.0001	0.0401	0.0001	
Fertilizer rate (F)	0.0401	0.0061	0.0273	0.703	0.0503	0.1820	
S × F	0.0012	0.0001	0.0021	0.0037	0.0170	0.0510	

[z]CO_2 efflux measured with a LI-COR 6200 infrared gas analyzer equipped with a soil CO_2 efflux chamber (n = 3).
[y]1 mg·L^{-1} = 1 ppm.
[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[w]L = linear; Q = quadratic response across measurement dates (10 weeks) at *, **, or ***.
[v]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[u]Means separated within columns using Duncan's multiple range test ($P \leq 0.05$; n = 3).
[t]L = linear; Q = quadratic response for fertilizer rate at *, **, or ***.
[s]PTS produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

Table 7. Nitrate nitrogen (NO_3-N) leached (nutrient leaching experiment) from peat-lite, pine bark, and pine tree substrate (PTS) over 14 weeks when fertilized with two rates of N from potassium nitrate (KNO_3).[z]

Substrates	NO_3-N leached per container (mg)								
N rate (mg·L^{-1})[y]	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Significance[x,w]
Peat-lite[v]									
100	2.5 c[u]	3.8 e	20.4 c	29.9 d	14.3 c	28.7 d	20.4 d	36.7 c	L*** Q***
300	4.7 c	10.3 cd	72.2 b	98.0 a	53.5 a	116.3 a	79.6 b	143.4 a	L*** Q***
Pine bark									
100	8.1 b	11.7 bc	21.5 c	22.9 d	15.4 c	18.7 d	18.9 d	37.5 c	L*** Q***
300	19.8 a	49.5 a	85.0 a	86.8 b	59.9 a	86.0 b	86.1 a	150.7 a	L*** Q***
PTS[t]									
100	2.2 c	5.6 de	9.0 d	7.2 e	4.0 d	4.0 e	6.2 e	10.6 d	L** Q**
300	8.4 b	16.8 b	62.5 b	65.5 c	35.1 b	69.8 c	68.7 c	109.1 b	L** Q***
Substrate (S)	0.0001	0.0001	0.0011	0.0001	0.0222	0.0001	0.0001	0.0021	
Fertilizer rate (F)	0.0041	0.0061	0.0001	0.0001	0.0001	0.0082	0.0001	0.0001	
S × F	0.0011	0.0029	0.0135	0.0210	0.0040	0.0753	0.0541	0.0003	

[z]Leaching data collected on six fallow containers of each substrate.
[y]1 mg·L^{-1} = 1 ppm.
[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[w]L = linear; Q = quadratic response across measurement dates (14 weeks) at *, **, or ***.
[v]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[u]Means separated within columns by Duncan's multiple range test ($P \leq 0.05$).
[t]PTS produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

in this study (90% wood) is likely the reason for the difference in expected N immobilization and N requirements of these substrates.

Nutrient leaching. The amount of N leached in all substrates generally increased as fertilizer rate increased from 100 to 300 mg·L^{-1} N at each sampling date (Table 7). At both fertilizer rates, more N leached from PB than from PTS at each sampling date through the duration of the 14-week experiment (Table 7). More N leached in PL than in PTS at each fertilizer rate beginning in Week 4 and continuing through the end of the study. Pine bark and PL had the same amounts of N leached at the low fertilizer rate beginning in Week 4 and continuing every sampling date thereafter (Table 7). Despite PB having higher amounts of N immobilization than PL (Table 4), similar N leaching could be in response to the higher saturated hydraulic conductivity of PB compared with PL as observed in unpublished studies of these authors (Brian Jackson and Robert Wright). The lower amounts of N leached from PTS than from PB or PL during most weeks of the study can reasonably be attributed to higher N immobilization rates in PTS compared with PB or PL (Tables 1 and 4). The amount of N leaching from PB and PL increased through the experiment more so than from PTS likely as a result of accumulated N in those substrates from previous fertilizations (a result of less N immobilization).

The amount of P leached in all substrates increased as fertilizer concentration applied increased from 100 to 300 mg·L^{-1} N in all 14 weeks of this study (Table 8). All substrates generally leached equal amounts of P at the low fertilizer rate throughout the study with only a couple of minor exceptions (Table 8). At the higher fertilizer rate, PB leached more P than PL or PTS through Week 2, equal amounts to PL and PTS through Week 8, and then less than PL and PTS from Week 10 through 14 (Table 8).

In general, higher amounts of K were leached from PB and PTS than from PL in the first 4 weeks at the low fertilizer rate, and by Week 8, there were no differences in milligrams of K leached in all substrates at the low fertilizer rate (Table 9). At the high fertilizer rate, higher amounts of K were leached from PB and PTS than from PL through Week 8. Potassium leaching in PL equaled the amounts leached in PB and PTS beginning in Week 10, possibly as a result of the cation exchange capacity (CEC) of PL becoming saturated with K ions (from fertilizations in Weeks 0, 2, 4, and 6) at which point K was being released (leached) from the substrate by Week 10. Peat-lite has been reported to have a higher CEC than PB or PTS (Wright et al., 2008). In addition to the lower CEC of PB and PTS, higher amounts of K leaching is expected initially based on the numerous reports of higher K concentrations in wood materials and subsequently in substrate solution of PB and PTS and other wood

Table 8. Phosphorus (P) leached (nutrient leaching experiment) from peat-lite, pine bark, and pine tree substrate (PTS) over 14 weeks when fertilized with two rates of nitrogen (N) from potassium nitrate (KNO_3) and 45 mg·L^{-1} P from H_3PO_4.[z]

Substrates	P leached per container (mg)								
N rate (mg·L^{-1})[y]	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Significance[x,w]
Peat-lite[v]									
100	0.8 d[u]	1.0 d	3.4 b	7.0 b	4.8 b	10.2 d	7.3 c	11.5 d	L*** Q***
300	2.1 c	2.4 bc	15.3 a	21.0 a	16.7 a	37.9 a	23.8 a	41.4 a	L** Q***
Pine bark									
100	3.3 b	2.0 cd	4.3 b	5.2 bc	5.2 b	7.6 de	6.1 c	9.8 de	L*** Q**
300	8.9 a	8.7 a	16.0 a	18.3 a	16.7 a	22.4 c	18.2 b	29.4 c	L** Q**
PTS[t]									
100	1.8 cd	1.0 d	2.6 b	2.9 c	2.4 b	5.6 e	4.3 d	7.2 e	L* Q**
300	4.0 b	3.2 b	12.2 a	17.1 a	15.7 a	30.3 b	23.3 a	35.5 b	L*** Q**
Substrate (S)	0.0001	0.0001	0.0156	0.0201	0.0126	0.0001	0.0001	0.0001	
Fertilizer rate (F)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
S × F	0.0001	0.0108	0.1429	0.2229	0.2965	0.0001	0.0001	0.0001	

[z]Leaching data collected on six fallow containers of each substrate.
[y]1 mg·L^{-1} = 1 ppm
[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[w]L = linear; Q = quadratic response across measurement dates (14 weeks) at *, **, or ***.
[v]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[u]Means separated within columns by Duncan's multiple range test ($P \leq 0.05$).
[t]PTS produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

Table 9. Potassium (K) leached (nutrient leaching experiment) from peat-lite, pine bark, and pine tree substrate (PTS) over 14 weeks when fertilized with two rates of nitrogen (N) from potassium nitrate (KNO_3).[z]

Substrates	K leached per container (mg)								
N rate (mg·L^{-1})[y]	Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Significance[x,w]
Peat-lite[v]									
100	5.1 d[u]	1.9 e	9.4 d	16.1 e	12.4 d	23.5 c	20.5 d	33.4 b	L** Q*
300	5.0 d	4.5 de	30.3 b	48.2 c	40.3 c	94.5 b	98.0 b	126.0 a	L*** Q***
Pine bark									
100	23.0 c	10.6 c	20.4 c	22.4 d	18.8 d	27.0 c	21.6 d	40.1 b	L** Q*
300	33.7 a	35.5 a	57.4 a	68.3 b	57.2 b	84.6 b	74.8 c	128.3 a	L*** Q***
PTS[t]									
100	25.0 c	7.0 cd	18.2 c	19.2 de	13.3 d	31.3 c	21.5 d	35.0 b	L* Q**
300	30.0 b	15.9 b	58.5 a	77.9 a	74.4 a	132.0 a	107.3 a	130.8 a	L*** Q***
Substrate (S)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0218	
Fertilizer rate (F)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
S × F	0.0002	0.0037	0.0001	0.0001	0.0001	0.0001	0.0001	0.1688	

[z]Leaching data collected on six fallow containers of each substrate.
[y]1 mg·L^{-1} = 1 ppm.
[x]NS (nonsignificant) or significant at *$P \leq 0.05$, **0.01, or ***0.001.
[w]L = linear; Q = quadratic response across measurement dates (14 weeks) at *, **, or ***.
[v]Peat-lite composed of 80% peatmoss/20% perlite (v/v).
[u]Means separated within columns by Duncan's multiple range test ($P \leq 0.05$).
[t]PTS produced from 12-year-old loblolly pine trees harvested at ground level, delimbed, chipped, and hammer-milled to pass through a 2.38-mm screen.

substrates compared with peat (Jackson et al., 2008a; Prasad, 1980; Wright et al., 2008).

Conclusions

These studies demonstrate that more N is immobilized in PTS compared with PB or PL under greenhouse conditions. Pine bark was also shown to immobilize more N than PL over the course of these studies. It is important to also note the fact that N immobilization begins within days of potting and continues throughout 10 weeks of production, indicating the need for continual applications of higher levels of N during crop production. To counter/overcome the high amounts of N immobilization in PTS reported in this study, it is possible to amend PTS with 25% peatmoss or use larger particle sizes of PTS, which will likely decrease N immobilization and reduce PTS production costs (Jackson et al., 2008b). Nitrogen immobilization was positively correlated with substrate CO_2 efflux levels that were measured on all substrates. Evidence of the relationship between substrate CO_2 efflux (microbial activity) and N immobilization supports numerous other studies by these authors of the potential for N immobilization and its role in lower substrate nutrient levels observed in PTS during crop production. The lower substrate nutrient levels and N availability in PTS compared with PL or PB reported in this study once again justify the need for higher fertilizer applications to maintain nutrient levels recommended for optimal plant growth. The higher fertilizer amounts required for crop growth (herbaceous and woody plants) in PTS do not lead to higher nutrient leaching amounts from PTS compared with PB or PL based on other results reported in this article. The lower levels of NO_3-N leaching in PTS compared with PB or PL is important in the face of environmental pressures to reduce nutrient (particularly NO_3) leaching from agricultural/horticultural operations.

Despite increased N immobilization and microbial activity in PTS, no visual difference in substrate shrinkage was observed in PTS compared with PL or PB in any of these studies. In support of these observations, other works have demonstrated that substrate shrinkage of PTS during short-term (14 weeks) greenhouse crop production (Jackson et al., 2008b) or during long-term (70 weeks) nursery crop production (Jackson et al., 2009) is no different from shrinkage in PL or PB. Like all other organic substrates, decomposition (particle size reduction) of PTS does occur over time, but it is unaffected by fertilizer rate, and the degree of decomposition is not severe enough to be detrimental or inhibitive to plant growth in containers (Jackson et al., 2008a, 2009). New production and nutrient management practices will continue to be developed for PTS in the future to evaluate the most cost-effective and efficient methods of using PTS as a container substrate in greenhouse and nursery crop production.

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