

Fertilizer, Irrigation, and Natural Ericaceous Root and Soil Inoculum (NERS): Effects on Container-grown Ericaceous Nursery Crop Biomass, Tissue Nutrient Concentration, and Leachate Nutrient Quality

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Abstract. We tested the effects of using an inoculum containing natural ericoid roots and soil (NERS) with two fertilizer and irrigation rates on plant growth, shoot (stems and leaves) nutrient concentration, leachate quality, and mycorrhizal colonization of container-grown Coast Leucothoe [*Leucothoe axillaris* (Lam.) D. Don] and Japanese Pieris [*Pieris japonica* (Thunb.) D. Don ex G. Don]. Uniform rooted liners were grown in 10.8-L containers in a pine bark, peatmoss, and sand (8:1:1 by volume) substrate medium in a randomized complete block design with four replications. A controlled-release fertilizer, Polyon® Plus 14-16-8 (14N-7P-6.6K), was incorporated in the substrate medium at the 100% manufacturer's recommended fertilizer rate [representing high fertilizer rate (HF)] (56 g per container) to supply 7.84 g nitrogen (N) and at 50% the manufacturer's recommended rate [representing low fertilizer rate (LF)]. Plants were irrigated using a cyclic drip irrigation system at high (HI) and low (LD) irrigation rates calibrated to supply 25.2 L of water and 16.8 L per week, respectively. On average, NERS inoculation increased shoot growth of *Leucothoe* and *Pieris* by 56% and 60%, respectively. Shoots of *Leucothoe* inoculated with NERS had higher N, phosphorus (P), magnesium (Mg), and manganese (Mn) concentrations than non-inoculated plants. At LF, nitrous-N (NO_x-N) and orthophosphorus (PO₄-P) concentrations in the leachate were reduced by 53% from *Leucothoe* and 62% from *Pieris* compared with HF-treated plants. A reduction of 37% and 36% in PO₄-P concentration in leachates from *Leucothoe* and *Pieris*, respectively, were achieved at the reduced irrigation (LD) rate. The NERS inoculation reduced PO₄-P concentrations in leachate from *Leucothoe* by 26% and NO_x-N concentration by 33% in leachates from *Pieris* compared with non-inoculated plants. Compared with plants grown in the HI-HF treatment, the combination of LI-LF treatment reduced NO_x-N concentrations in leachates from *Leucothoe* by 60% ($P = 0.016$) and reduced PO₄-P leachate concentrations from *Pieris* by 72% ($P = 0.0096$). Decreasing the fertilizer rate to 50% of the recommended rate and the irrigation rate to 67% of the recommended rate in conjunction with the incorporation of NERS reduced leachate nutrient concentrations of two main water pollutants (NO_x-N and PO₄-P). Adopting the practice of adding NERS containing fungi and bacteria can be an effective system to increase shoot dry weight, allow reduction in fertilizer application, conserve water for irrigation, and minimize subsequent nutrient runoff in nursery operations.

Heightened environmental and economic concerns make it necessary to develop production systems for the nursery industry that enhance plant growth and nutrient availability through modifications of growing substrate medium's physical and chemical characteristics (Yu and Zinati, 2006), reducing the de-

pendency on chemical fertilizer (Zinati, 2006) and exploiting biological systems such as management of mycorrhizal fungi and addition of plant growth promoting bacteria (Azcon and Ocampo, 1981; Garbaye, 1991; Pinton et al., 2001) as options to reduce effluent pollution.

An inoculum contained natural ericoid roots and soil contained a mix of mycorrhizal fungi, rhizospheric and non-rhizospheric bacteria, and saprotrophic fungi. All of these microbial entities have the potential to enhance plant growth by improving nutrient uptake. Most members of Ericaceae, including species as *Calluna*, *Kalmia*, *Rhododendron*, *Pieris*, *Leucothoe*, and *Vaccinium*, are important plants in the nursery industry (Read, 1983). Ericoid mycorrhizae, associated with ericaceous plants (such as *Vaccinium*, *Rhododendron*), facilitate the transfer of nutrients to the host plant (Read and Kerley, 1999). Ericaceous plants have relatively low nitrate-reducing abilities (Johansson, 2000; Michelsen et al., 1999); thus, mycorrhizal colonization of ericaceous plants has been shown to improve access to mineral forms of N; other soluble inorganic, organic; and insoluble forms of N and P, which are largely unavailable to non-mycorrhizal plants (Bajwa and Read, 1986; Kerley and Read, 1995; Mitchell and Read, 1981; Read, 1978; Stribley and Read, 1974).

There is very limited research work published on the impact of ericoid inoculation on ericaceous plant growth, nutrient uptake, and root colonization. In micropropagation systems, Jansa and Vosátka (2000) showed that ericoid mycorrhiza fungal strains were found beneficial for the growth of micropropagated rhododendron (*Rhododendron* L.) 'Belle-Heller' plants when inoculated post-vitro after transplantation to peat-based substrate. Starrett et al. (2001) also, showed that mycorrhizal-inoculated micropropagated *Pieris floribunda* (Pursh) Benth. & Hook. f. grew more than non-inoculated microshoots during 1 month in vitro, but the inoculation did not stimulate root or shoot development during 3 months in a greenhouse. Scagel (2005) and Scagel and Yang's (2005) research results showed that mycorrhizal colonization of blueberry (*Vaccinium corybosum* L.) plants varied with production practices, cultivar, and fertilizer source and was negatively correlated to ammonium in soil. In field experiments, increased soil fertility depressed mycorrhizal colonization levels in blueberry seedlings (Golldack et al., 2001; Powell, 1982). High levels of N in particular decreased ericoid mycorrhizal colonization in *Rhododendron* spp. (Moore-Parkhurst and Englander, 1982) and increased concentrations of ammonium inhibited mycorrhizal colonization of *Vaccinium macrocarpon* Ait. (Stribley and Read, 1976). However, the mechanism behind the sensitivity of the ericoid-plant association to high fertility is unknown. Yang et al. (2002) reported that inoculation of 'Elliot' blueberry plants with ericoid mycorrhizal fungi enhanced plant growth, whereas others (Reich et al., 1982; Yang et al., 1998) reported that there was no change in blueberry plant growth when inoculated with a native, unidentified ericoid mycorrhizal fungal isolate. Mycorrhizal associations are expected to be most beneficial in nutrient-limited habitats (Allen, 1991).

Many companies sell commercial inoculants to nurseries and the landscape industry.

These mainly contain arbuscular or ectomycorrhizal fungi and also contain ingredients that act as fertilizers or growth regulators. However, commercial inoculum is at present expensive, difficult to use on a large scale, and does not always produce reliable results (Gaur et al., 1998; Rowe et al., 2007; Wiseman et al., 2009).

The goal of this study was to develop a system that could improve plant growth and reduce nutrient concentration in leachates to minimize the environmental impact from commercial production of container-grown woody ornamental ericaceous plants. The system will include 1) added soil from a mixed oak pine forest with an understory of blueberry (*Vaccinium vacillans* Kalm.) and huckleberry (*Gaylussacia baccata*) (Wangenh.) K. Koch, as an inoculum, that would contain ericoid mycorrhizae (fungal hyphae and colonized root fragments) and associated bacterial communities (Landesman and Dighton, 2010); 2) less irrigation water; and 3) lower fertilizer rate. The underlying hypothesis was that the primary beneficial factor in using a soil inoculum, collected from a natural site supporting ericaceous understory plants, would be the ericoid mycorrhizal component.

Our specific objectives were to evaluate the effects of adding NERS with two fertilizer and irrigation rates on two ericaceous nursery plants to: 1) enhance plant growth; 2) increase plant nutrient concentration; and 3) improve leachate quality during the growing season. If successful, this combination of practices could subsequently be integrated into best management practices for sustainable production of ericaceous nursery crops.

Materials and Methods

An experiment was conducted under a simulated commercial nursery container production system at the Rutgers University Fruit and Ornamental Research and Extension Center, Cream Ridge (lat. 40°8'6" N, long. 74°31'28" W), NJ, from 7 June to 15 Oct. 2006. Two plant species were used as host plants: Coast Leucothoe (*Leucothoe axillaris*) and Japanese Pieris (*Pieris japonica*) subsequently referred to as *Leucothoe* and *Pieris*, respectively. Uniform rooted liners were transplanted into classic 1200 black containers #3 (10.8 L) to provide one plant per treatment per container using

a growing substrate mix that provided optimum physical and chemical properties (Yu and Zinati, 2006) composed of 8 pine bark:1 Canadian sphagnum peatmoss:1 masonry sand (by volume).

The experiment was a completely randomized design with four replicates for each plant species with a 2 × 2 × 2 factorial, including two rates of fertilizer, two rates of irrigation, and with and without NERS inoculum. The fertilizer, used in this study, was a controlled-released fertilizer, Polyon® Plus (Harrell's LLC., Lakeland, FL) 14-16-8 (14N-7P-6.6K) (6.44% nitrate N and 7.56% ammoniacal N) and commonly used by New Jersey's nursery growers for container production of *Leucothoe* and *Pieris* plants. Polyon Plus was incorporated into the substrate media at the time of planting to supply 7.84 g of N per container, 100% of the manufacturer's fertilizer rate (considered here as HF) or to provide 3.92 g of N per container, 50% the manufacturer's fertilizer rate (considered LF). A cyclic drip irrigation was used to supply 4.2 L of water per irrigation event, twice a day, three times a week, at the standard grower's irrigation rate (considered HI) or at LI to provide 2.8 L per irrigation event, twice a day, three times a week. The rate of irrigation was 140 mL·min⁻¹ per container. Weather data, including daily minimum, maximum, and average air temperatures (°C), solar radiation (kJ·m⁻²·d⁻¹), and rainfall (cm·d⁻¹) during the growing season, were collected from the New Jersey Weather and Climate Network (2010) and presented in Figure 1.

Method of inoculation of plants. In our study, the NERS inoculum, consisting of root fragments and soil, was freshly collected from understory blueberry and huckleberry in the Pinelands forest close to the Rutgers Pinelands Research Station, New Lisbon, NJ. The organic horizon of 60% organic matter, 3.00 ± 0.59 µg·g⁻¹ extractable N, 0.31 ± 0.01 µg·g⁻¹ extractable P, and fungal bacterial ratio of 1.5 [≈300 µg fungal biomass carbon (C)/g to 200 µg bacterial biomass C/g] (Adams-Krumins et al., 2009) and upper 10 cm of mineral horizon of this sandy podzolic soil were collected and homogenized. High densities of fine ericoid roots are found mainly in the organic horizon, which typically contains abundant (20% to 30% root colonization) mycorrhizal structures (hyphal coils) (Dighton et al., unpublished data). We did not determine the species composition of the mycorrhizal symbionts colonizing our roots (because that was not the objective of our study). Plants were inoculated with NERS at the time of transplanting (Yoshida and Allen, 1998) with an average of 225 g of natural inoculum placed under the root ball.

Plant growth measurements. At the termination of the 17-week experiment, plant shoots (stems and leaves) were harvested and separated from plant roots. Roots were carefully cleaned with water several times to remove substrate particles. Plant parts were dried in a forced-air oven at 65 °C. Dry weight for each plant part per replicate (n = 4) was recorded and root-to-shoot ratio was determined.

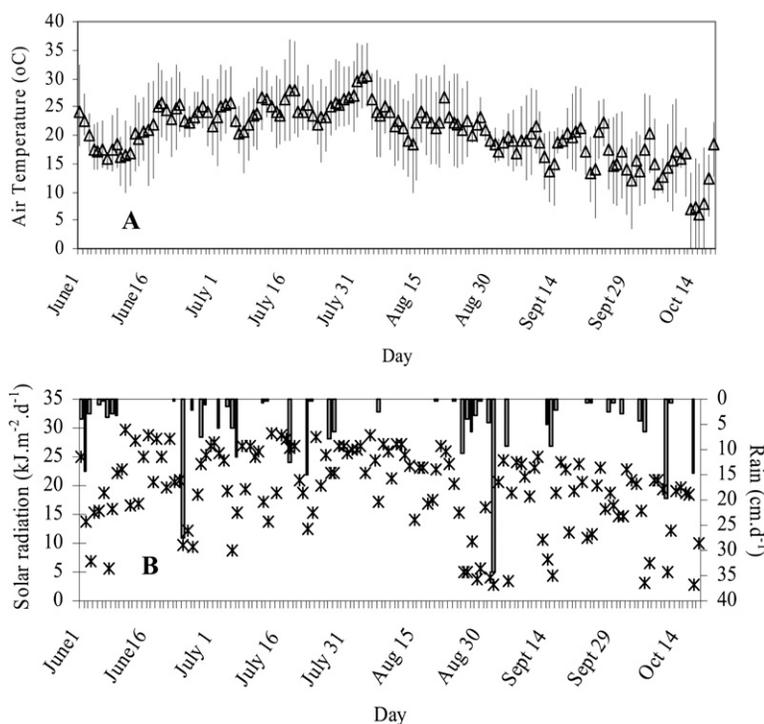


Fig. 1. Air temperature (A), solar radiation and rainfall (B) between June and Nov. 2006 on the experimental site at the Rutgers University Fruit and Ornamental Research and Extension Center, Cream Ridge, NJ. Data points and columns (A–B) represent daily mean values. Error bars (A) indicate daily range in air temperature.

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Shoot nutrient concentration. A homogeneous subsample, of shoot and leaf material combined, was taken from each of the shoot dry biomass replicates ($n = 4$), ground in a Wiley Mill to pass through a number 20 grid, and sent to the LSU AgCenter Soil Testing and Plant Analysis Laboratory (STPAL), Baton Rouge, LA, for analysis for the following nutrients: N, P, potassium (K), calcium (Ca), Mg, copper (Cu), Mn, iron (Fe), boron (B), and zinc (Zn). Tissue N concentration was determined by using dry combustion and analyzed with LECO TruSpec CN analyzer (LECO Corporation, St. Joseph, MI). For all other elements, the wet digestion (nitric acid and hydrogen peroxide) method was followed and analyzed on a SPECTRO-ARCOS EOP-ICAP analyzer (Kleve, Germany).

Leachate collection and analysis. Leachates were collected on 21 June, 7 July, 19 July, 7 Aug., 21 Aug., and 1 Sept. from three replicates. To ensure collection of enough leachate on those dates, plants were irrigated by drip irrigation as described previously before leachates were collected using the pour-through method described by Wright (1986). Leachate was transferred into high-density polyethylene white bottles and transported to the laboratory in a cold chest, stored in the refrigerator at 4 °C, and filtered through Whatman # 42 filter paper within 2 d from collection. Filtered leachate was frozen and sent to LSU AgCenter STPAL for nutrient analysis. Leachates were analyzed for ammonium, nitrate, nitrite, phosphate, pH, and electrical conductivity (EC). Leachate was analyzed for nitrate nitrogen ($\text{NO}_3\text{-N}$) and nitrite nitrogen ($\text{NO}_2\text{-N}$). We report leachate ($\text{NO}_3 + \text{NO}_2\text{-N}$ as $\text{NO}_x\text{-N}$). Ammonium N was determined using the SM-4500-NH₃-E method and determined on an Accumet® Ammonia probe (Fisher Scientific, Pittsburgh, PA). The anions were measured on an Ion Chromatogram ICS125 (Dionex Corporation, Sunnyvale, CA) using the EPA300.0 method. The pH and EC were determined on an Accumet® AR50, Dual pH/Conductivity meter (Fisher Scientific).

Staining and assessment of ericoid mycorrhizal root colonization. Fresh roots were randomly selected from each plant and gently teased apart in water and substrate media particles carefully removed. Root pieces were placed in 20-mL scintillation vials in water and refrigerated until processed (usually less than 2 d). Roots were cleared and stained using 0.05% Trypan blue in lactoglycerol using the procedure outlined in Brundrett et al. (1994) but replacing the heating steps by leaving the roots in both potassium hydroxide and the stain for 1 week at room temperature. After staining, 20 random 1-cm segments of root were placed onto microscope slides and observed under a compound microscope at 400× magnification. The presence or absence of mycorrhizal structures at 50 random locations on root segments was scored categorized into hyphae internal to the root cortex or hyphal coils (McGonigle et al., 1990). Root colonization was expressed as the percent of observations containing my-

corrhizal structures (hyphae or coils) and was reported here as root colonization.

Statistical data analysis. Data were statistically analyzed using analysis of variance (ANOVA) (SAS Institute Inc, 1999–2001) per each plant species. Comparison of means was done using Tukey's honestly significant difference test where the F-value was significant. Significant differences were accepted at $\alpha = 0.05$. Leachate data analyzed over time were reported as means overall treatments and analyzed by repeated-measures ANOVA. Root-to-shoot ratio data were arcsine transformed and log of colonization index taken to normalize before ANOVA.

Results

Plant growth. Our data indicated no significant shoot growth benefit for *Leucothoe* for either irrigation or fertilizer (22.22 ± 2.21 g/plant; mean \pm SE). Similarly, in *Pieris*, there were no significant differences in shoot dry weight with either irrigation or fertilizer (65.65 ± 4.85 g/plant) treatment. However, the inoculation of *Leucothoe* with NERS significantly increased shoot dry weight by 56% ($P < 0.01$) compared with non-inoculated plants (Fig. 2A). The inoculation of *Pieris* with NERS increased shoot dry weight by 60% ($P < 0.0001$) compared with non-inoculated plants (Fig. 2B).

Leucothoe root dry weight was not significantly influenced with irrigation, fertilizer, or NERS (5.11 ± 0.57 g/plant) treatment.

Similarly, there was no significant effect of irrigation or fertilizer (20.30 ± 2.03 g/plant) on *Pieris*' root growth. However, there was a significant ($P = 0.004$) increase in root dry weight in NERS-inoculated *Pieris* plants compared with non-inoculated plants (Fig. 2B).

Among the tested factors, NERS-treated *Leucothoe* plants had lower ($P = 0.0036$) root-to-shoot ratio than non-mycorrhizal plants (Fig. 2C). The impact of NERS inoculation on shoot dry weight was so great as to offset the increase in root dry weight in inoculated *Pieris* plants. Consequently, root-to-shoot ratio was lower ($P = 0.0177$) in NERS-treated plants compared with non-inoculated plants (Fig. 2C).

Nutrient concentration in shoots. The influence of irrigation in *Leucothoe* was to significantly increase the shoot tissue concentration of Ca ($P = 0.03$) and the trace elements Fe, B, and Zn ($P = 0.03$, $P = 0.006$, and $P = 0.02$, respectively) in the HI treatment compared with LI (Table 1). The only effect of fertilizer was to increase B tissue concentration ($P = 0.02$) in the HF level in *Leucothoe* (Table 1). The NERS inoculation significantly increased N, P, Mg, and Mn concentration in shoot tissue of *Leucothoe* ($P = 0.02$, $P = 0.006$, $P = 0.05$, and $P = 0.009$, respectively) (Table 1). The effect was just the opposite for B concentration, which was lower in NERS-treated plants ($P = 0.02$) (Table 1).

A significant ($P = 0.01$) irrigation (I) \times fertilizer (F) interaction was found in tissue B concentration in *Leucothoe*. In the presence

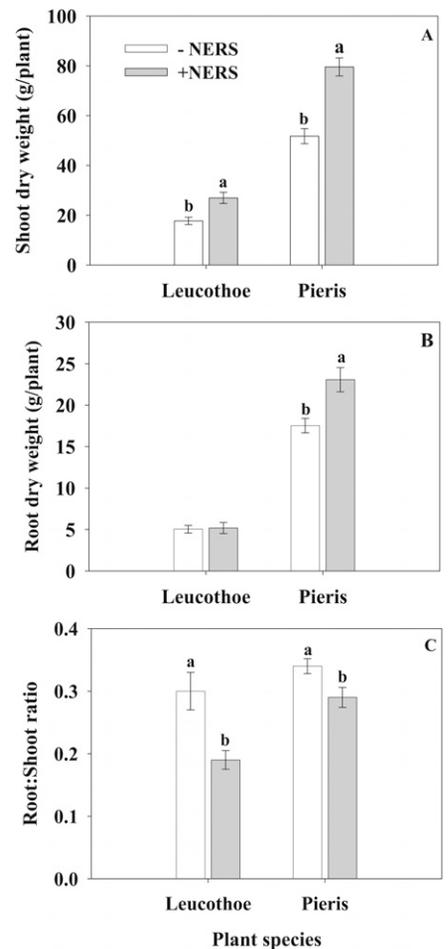


Fig. 2. Shoot (shoot and leaves) dry weight (A), root dry weight (B), and root-to-shoot ratio (C) of Coast *Leucothoe* [*Leucothoe axillaris* (Lam.) D. Don] and Japanese *Pieris* [*Pieris japonica* (Thunb.) D. Don ex G. Don] after 17 weeks of growth. Plants were inoculated (+NERS) or not (-NERS) with inoculum containing soil and roots from ericoid mycorrhizal plants. Columns with different letters denote significant difference ($\alpha = 0.05$) between treatments within a plant species (Tukey's honestly significant difference comparison of means). Error bars are SEs ($n = 16$).

of fertilizer, the increase in B concentration in *Leucothoe* tissue was more pronounced at HI (LF 26.86 ± 1.03 $\mu\text{g}\cdot\text{g}^{-1}$ versus HF 32.86 ± 3.06 $\mu\text{g}\cdot\text{g}^{-1}$) than at LI (LF 26.62 ± 0.76 $\mu\text{g}\cdot\text{g}^{-1}$ versus HF 26.40 ± 0.83 $\mu\text{g}\cdot\text{g}^{-1}$).

There also was a significant ($P = 0.02$) I \times NERS interaction for B concentration. In the presence of NERS, the decrease in B concentration in *Leucothoe* tissue was more pronounced at HI (-NERS 32.76 ± 2.98 $\mu\text{g}\cdot\text{g}^{-1}$ versus +NERS 26.96 ± 1.29 $\mu\text{g}\cdot\text{g}^{-1}$) than at LI (-NERS 26.52 ± 0.90 $\mu\text{g}\cdot\text{g}^{-1}$ versus +NERS 26.50 ± 0.70 $\mu\text{g}\cdot\text{g}^{-1}$).

There was a significant F \times NERS interaction for *Leucothoe* tissue N, P, and Mn concentration ($P = 0.003$, $P = 0.0003$, and $P = 0.01$, respectively). Inoculation with NERS resulted in a greater increase in shoot N, P, and Mn concentration at the HF rate (N in -NERS 7.70 ± 0.31 $\text{mg}\cdot\text{g}^{-1}$ versus +NERS 10.71 ± 0.65 $\text{mg}\cdot\text{g}^{-1}$; P in -NERS 0.80 ± 0.04

mg·g⁻¹ versus +NERS 1.5 ± 0.008 mg·g⁻¹; and Mn in -NERS 82 ± 8.75 µg·g⁻¹ versus +NERS 206 ± 32.26 µg·g⁻¹) than at the LF rate (N in -NERS 9.496 ± 0.30 mg·g⁻¹ versus +NERS 9.03 ± 0.07 mg·g⁻¹; P in -NERS 1.20 ± 0.06 mg·g⁻¹ versus +NERS 1.20 ± 0.09 mg·g⁻¹, and Mn in -NERS 133 ± 11.64 µg·g⁻¹ versus +NERS 136 ± 21.64 µg·g⁻¹).

Similarly, there was a significant I × F × NERS interaction for *Leucothoe* shoot B concentration ($P = 0.0002$). At the LF rate, the inoculation with NERS did not impact tissue B concentration at either HI or LI (-NERS 25.78 ± 1.085 µg·g⁻¹ versus +NERS 27.69 ± 1.36 µg·g⁻¹). However, the inoculation with NERS at the HF rate resulted in

a greater decrease in tissue B concentration at HI (-NERS 39.79 ± 2.71 µg·g⁻¹ versus +NERS 25.93 ± 2.11 µg·g⁻¹) than at the LI rate (-NERS 27.23 ± 1.53 versus 25.57 ± 0.68 µg·g⁻¹).

Only shoot P was increased at the HF level in *Pieris* (Table 2). Irrigation treatments and NERS inoculation had no influence on nutrient concentrations in shoots of *Pieris* (Table 2) and there were no significant interactions between the main factors.

Nutrient concentration, electrical conductivity, and pH in leachates. The seasonal pattern of nutrient concentration in leachate for NH₄-N, NO_x-N, and PO₄-P were similar in both *Leucothoe* (Fig. 3) and *Pieris* (Fig. 4).

Nutrients, EC, and pH in leachate, collected from *Leucothoe* plants were significantly different by sampling date (Fig. 3). The overall mean concentration of PO₄-P in leachate increased up to 19 July and then plateaued for *Leucothoe* but subsequently declined in *Pieris* (Fig. 4). For both plant species, EC peaked at 7 July and declined thereafter, whereas pH started high and decreased to a lower level by 19 July and remained low.

Irrigation treatment had no significant effect on NH₄-N concentrations (LI 0.88 ± 0.14 mg·L⁻¹ versus HI 1.04 ± 0.16 mg·L⁻¹) and NO_x-N (LI 14.92 ± 1.88 mg·L⁻¹ versus HI 15.65 ± 1.52 mg·L⁻¹) in leachate from containers of *Leucothoe*. Leachate from containers

Table 1. Concentrations of nutrients in aboveground tissues of Coast *Leucothoe* [*Leucothoe axillaris* (Lam.) D. Don] inoculated or not with natural ericaceous roots and soil (NERS) and grown in containers for 17 weeks with two different rates of irrigation and fertilizer.

Treatment ^z	Mean tissue nutrient concn									
	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Copper	Manganese	Iron	Boron	Zinc
	(mg·g ⁻¹)					(µg·g ⁻¹)				
Irrigation (I)										
Low	9.2 ± 0.4 ^y	1.2 ± 0.06	8.6 ± 0.2	7.6 ± 0.3	2.0 ± 0.08	2.16 ± 0.2	135 ± 20	517 ± 23	26.51 ± 0.5	33.37 ± 1.6
High	9.3 ± 0.5	1.2 ± 0.08	8.4 ± 0.3	8.5 ± 0.3	1.8 ± 0.1	2.18 ± 0.2	143 ± 16	597 ± 28	29.86 ± 1.7	38.25 ± 1.5
Fertilizer (F)										
Low	9.3 ± 0.4	1.2 ± 0.05	8.6 ± 0.3	7.8 ± 0.2	2.0 ± 0.1	2.10 ± 0.1	134 ± 12	555 ± 33	26.74 ± 0.6	36.27 ± 1.7
High	9.2 ± 0.5	1.1 ± 0.08	8.3 ± 0.2	8.3 ± 0.5	1.9 ± 0.08	2.24 ± 0.2	144 ± 23	558 ± 22	29.63 ± 1.7	35.35 ± 1.6
NERS										
-	8.6 ± 0.3	1.1 ± 0.06	8.1 ± 0.2	8.4 ± 0.4	1.7 ± 0.08	2.08 ± 0.2	107 ± 10	564 ± 31	29.64 ± 1.7	35.32 ± 1.7
+	9.9 ± 0.5	1.3 ± 0.07	8.8 ± 0.3	7.7 ± 0.2	2.1 ± 0.1	2.26 ± 0.2	171 ± 21	549 ± 25	26.73 ± 0.7	36.30 ± 1.6
Significance ^x										
I	NS	NS	NS	*	NS	NS	NS	*	**	*
F	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
NERS	*	**	NS	NS	*	NS	**	NS	*	NS

^zIrrigation: plants irrigated with 4.2 L (HI) or 2.6 L (LI) of water, twice a day, 3 d a week. Fertilizer: 14N-7P-6.6K controlled-release fertilizer incorporated into growing substrate at planting for a total of 7.84 g N (HF) or 3.92 g N (LF). NERS = plants inoculated (+NERS) or not (-NERS) with ≈225 g of natural ericoid root fragments and soil (NERS) that was freshly collected from an understory of *Vaccinium* spp. in the Pinelands forest near Rutgers Pinelands Research Station, New Lisbon, NJ.

^yData represent mean values ± SE, n = 16.

^xNS Non-significant or significant at * $P \leq 0.05$, **0.01, or ***0.001, respectively.

Table 2. Concentrations of nutrients in aboveground tissues of Japanese *Pieris* [*Pieris japonica* (Thunb.) D. Don ex G. Don] inoculated or not with natural ericaceous roots and soil (NERS) and grown in containers for 17 weeks with two different rates of irrigation and fertilizer.

Treatment ^z	Mean tissue nutrient concn									
	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Copper	Manganese	Iron	Boron	Zinc
	(mg·g ⁻¹)					(µg·g ⁻¹)				
Irrigation (I)										
Low	7.4 ± 0.3 ^y	0.92 ± 0.04	5.5 ± 0.09	6.8 ± 0.2	1.8 ± 0.05	2.90 ± 0.06	188 ± 6	233 ± 11	17.24 ± 0.5	27.20 ± 1
High	7.0 ± 0.2	0.88 ± 0.04	5.3 ± 0.1	7.5 ± 0.2	1.9 ± 0.06	2.93 ± 0.09	169 ± 15	240 ± 16	17.43 ± 0.5	24.46 ± 1
Fertilizer (F)										
Low	6.9 ± 0.1	0.83 ± 0.03	5.4 ± 0.1	7.3 ± 0.3	1.8 ± 0.06	2.85 ± 0.07	164 ± 11	225 ± 8	17.13 ± 0.5	25.32 ± 1
High	7.5 ± 0.3	0.96 ± 0.04	5.4 ± 0.1	7.0 ± 0.2	1.9 ± 0.05	2.98 ± 0.08	193 ± 18	247 ± 19	17.54 ± 0.4	26.34 ± 1
(NERS)										
-	7.4 ± 0.3	0.94 ± 0.05	5.5 ± 0.1	7.0 ± 0.2	1.8 ± 0.04	2.88 ± 0.9	175 ± 14	232 ± 10	16.85 ± 0.3	26.03 ± 0.9
+	7.0 ± 0.2	0.80 ± 0.03	5.4 ± 0.09	7.3 ± 0.3	1.9 ± 0.06	2.95 ± 0.07	182 ± 17	240 ± 17	17.82 ± 0.6	25.63 ± 1
Significance ^x										
I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
NERS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^zIrrigation: plants irrigated with 4.2 L (HI) or 2.8 L (LI) of water, twice a day, 3 d a week. Fertilizer: 14N-7P-6.6K controlled-release fertilizer incorporated into growing substrate at planting for a total of 7.84 g N (HF) or 3.92 g N (LF). NERS = plants inoculated (+NERS) or not (-NERS) with ≈225 g of natural ericoid root fragments and soil (NERS) that was freshly collected from an understory of *Vaccinium* spp. in the Pinelands forest near Rutgers Pinelands Research Station, New Lisbon, NJ.

^yData represent mean values ± SE, n = 16.

^xNS Non-significant or significant at * $P \leq 0.05$, **0.01, or ***0.001, respectively.

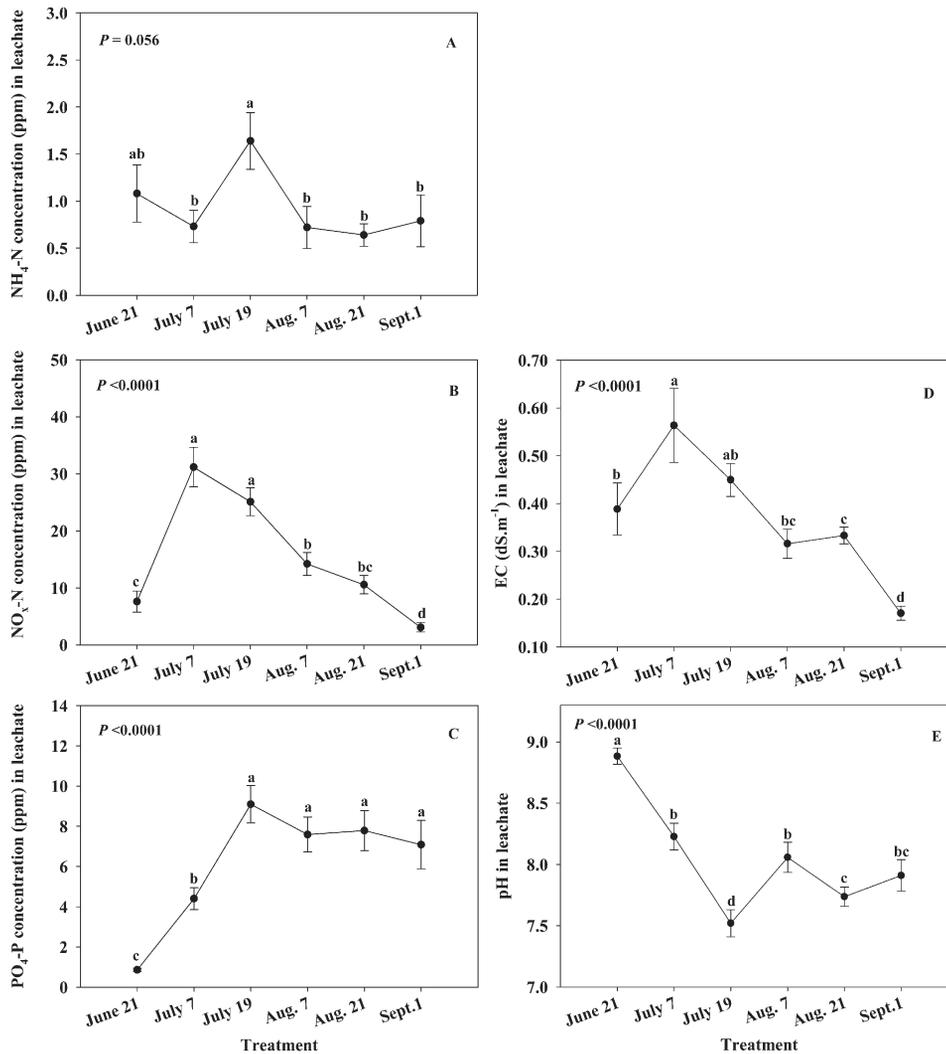


Fig. 3. Mean concentrations across all treatments of NH₄-N (A), NO_x-N (B), PO₄-P (C), electrical conductivity (EC) (D), and pH (E) in leachate collected during the 17 weeks of growth from containers containing Coast Leucothoe [*Leucothoe axillaris* (Lam.) D. Don] plants. *P* values shown are from analysis of variance of the nutrient concentration, EC, and pH in leachate over time. Means with different letters denote significant difference ($\alpha=0.05$) between dates (Tukey's honestly significant difference comparison of means). Error bars are *SES* ($n=24$).

of *Leucothoe* in the HI treatment had a greater ($P < 0.05$) PO₄-P concentration (7.46 ± 0.66 mg·L⁻¹) than leachate from the LI treatment (4.80 ± 0.45 mg·L⁻¹). Similarly, leachate from containers of *Pieris* in the HI treatment had greater ($P < 0.01$) concentrations of PO₄-P (7.09 ± 0.86 mg·L⁻¹) than in the LI treatment (4.87 ± 0.53 mg·L⁻¹). Additionally, leachate from containers of *Pieris* in the HI treatment had lower ($P < 0.05$) EC (0.33 ± 0.03 dS·m⁻¹) and pH (7.88 ± 0.10) than leachate from the LI treatment (EC, 0.39 ± 0.02 dS·m⁻¹; pH 8.18 ± 0.07). The influence of treatments on leachate composition was similar at each measurement date.

For both *Leucothoe* and *Pieris*, increased fertilizer rate significantly increased leachate EC and concentrations of NH₄-N, NO_x-N, and PO₄-P and decreased pH (Table 3).

The inoculation with NERS did not affect either NH₄-N concentration (–NERS 0.99 ± 0.16 mg·L⁻¹ versus +NERS 0.87 ± 0.14 mg·L⁻¹) in leachate from *Leucothoe* or the NO_x-N concentration (–NERS 15.89 ± 1.80 mg·L⁻¹ versus +NERS 14.66 ± 1.61 mg·L⁻¹).

However, inoculation of *Leucothoe* with NERS reduced ($P = 0.0008$) the PO₄-P concentration in leachate (Fig. 5A). The inoculation of *Pieris* plant roots with NERS decreased ($P = 0.018$) leachate NO_x-N concentrations (Fig. 5B) and increased ($P = 0.0004$) leachate pH (8.20 ± 0.08 mg·L⁻¹) compared with non-mycorrhizal (7.90 ± 0.09 mg·L⁻¹).

A significant I × F interaction occurred for NO_x-N in *Leucothoe*. The addition of HF increased ($P = 0.016$) NO_x-N concentration more in leachate under LI than HI (Fig. 6A). In *Pieris*, a significant I × F interaction showed that there was a greater loss of PO₄-P under HF and HI than under low rates of fertilizer and irrigation (Fig. 6B). A greater ($P = 0.0289$) decrease in pH with fertilizer addition at HI compared with LI occurred, but the difference may be biologically insignificant (Fig. 6C).

In *Pieris*, there was also a significant I × NERS interaction in which NERS inoculation resulted in no change of pH at LI (–NERS 8.20 ± 0.10 versus +NERS 8.20 ± 0.11) but increased pH at HI from 7.60 ± 0.15

in the absence of NERS to 8.10 ± 0.11 in the presence of inoculum. A significant F × NERS interaction showed that there was a decrease in EC (–NERS 0.50 ± 0.05 dS·m⁻¹) of leachate on inoculation of *Pieris* with NERS (0.38 ± 0.03 dS·m⁻¹) at HF. At the LF rate, although not significantly, EC in non-mycorrhizal (0.27 ± 0.02 dS·m⁻¹) was lower than in mycorrhizal plants (0.29 ± 0.02 dS·m⁻¹).

Mycorrhizal root colonization. Root colonization by mycorrhizal fungi at the end of the 17-week experiment was not significantly different between NERS-inoculated and non-inoculated *Leucothoe* or *Pieris*. There were no significant effects of fertilizer or irrigation on the abundance of mycorrhizal structures in roots in either plant species. Across all treatments, average mycorrhizal colonization of *Leucothoe* was 3.50 ± 0.57 and average colonization of *Pieris* was 7.84 ± 0.92 .

Discussion

To our knowledge, this is the first report of describing the combined effects of NERS

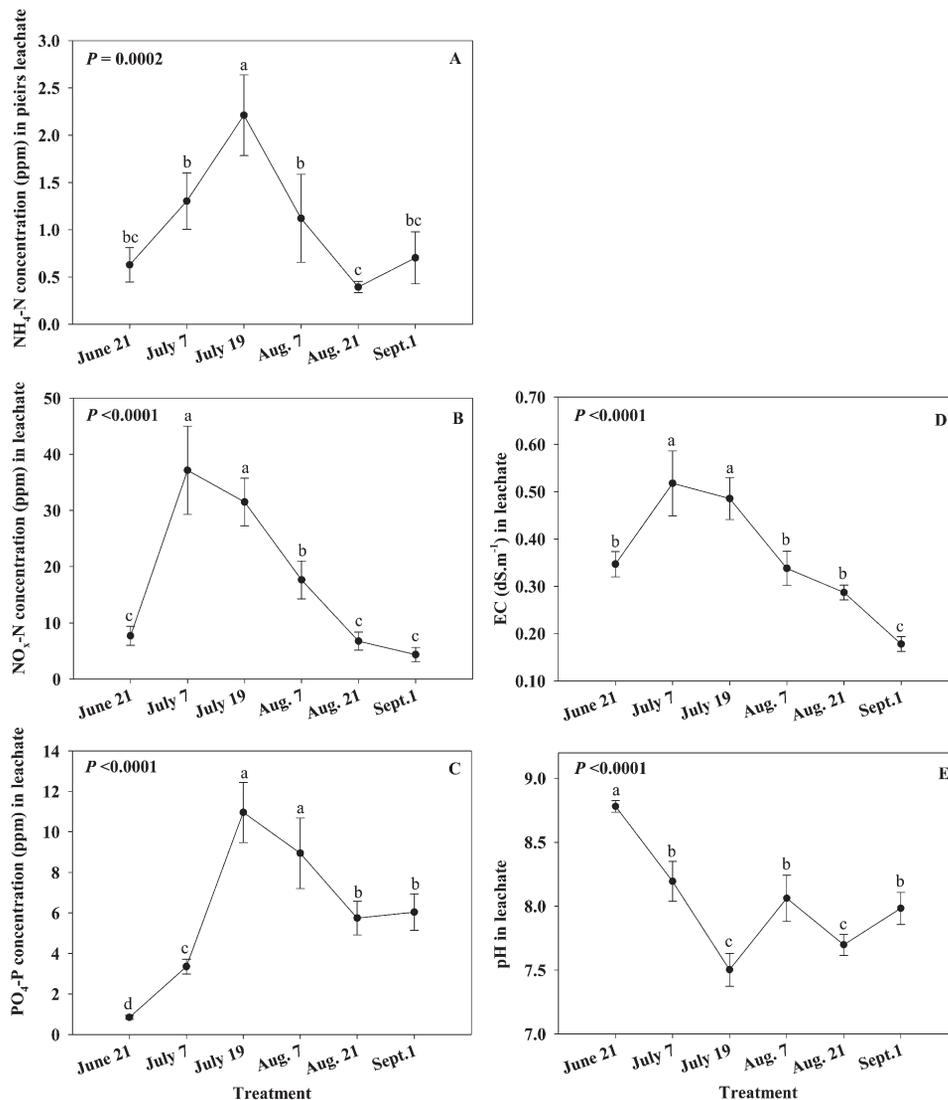


Fig. 4. Mean concentrations across all treatments of NH₄-N (A), NO_x-N (B), PO₄-P (C), electrical conductivity (EC) (D), and pH (E) in leachate collected during the 17 weeks of growth from containers containing Japanese Pieris [*Pieris japonica* (Thunb.) D. Don ex G. Don] as affected by sampling date. *P* values shown are from analysis of variance of the nutrient concentration, EC, and pH in leachate over time. Means with different letters denote significant difference ($\alpha = 0.05$) between dates (Tukey's honestly significant difference comparison of means). Error bars are *SES* ($n = 24$).

Table 3. Mean \pm SE concentrations of NH₄-N, NO_x-N, PO₄-P, pH, and electrical conductivity (EC) in leachates² collected from Coast *Leucothoe* [*Leucothoe axillaris* (Lam.) D. Don] and Japanese Pieris [*Pieris japonica* (Thunb.) D. Don ex G. Don] plants as affected by fertilizer treatments.

Measurement	Coast <i>Leucothoe</i>			Japanese Pieris		
	HF ^y	LF ^x	<i>P</i>	HF	LF	<i>P</i>
NH ₄ -N (mg·L ⁻¹)	1.48 \pm 0.19	0.385 \pm 0.06	<0.0001	1.70 \pm 0.24	0.412 \pm 0.06	<0.0001
NO _x -N (mg·L ⁻¹)	20.7 \pm 1.96	9.79 \pm 1.08	<0.0001	25.5 \pm 3.24	9.45 \pm 1.69	<0.0001
PO ₄ -P (mg·L ⁻¹)	8.35 \pm 0.65	3.91 \pm 0.35	<0.0001	8.64 \pm 0.85	3.32 \pm 0.36	<0.0001
EC (dS·m ⁻¹)	0.429 \pm 0.03	0.311 \pm 0.02	0.0003	0.438 \pm 0.03	0.279 \pm 0.02	<0.0001
pH	7.80 \pm 0.08	8.31 \pm 0.06	<0.0001	7.70 \pm 0.09	8.37 \pm 0.06	<0.0001

²Leachates were collected on 21 June, 7 July, 19 July, 7 Aug., 21 Aug., and 1 Sept. from three replicates. ^{y,x}A controlled-release fertilizer, Polyon® Plus 14-16-8 (14N-7P-6.6K), was incorporated into the substrate medium at planting for a total of 7.84 g N (HF) or 3.92 g N (LF), respectively, $n = 72$.

inoculation and irrigation and fertilizer application on growth of container-grown ornamental ericaceous plants and irrigation water runoff quality.

There were no fertilizer or irrigation effects on plant shoot growth for both plant species. However, inoculating with NERS increased shoot growth of *Leucothoe* and *Pieris*, in-

creased root growth of *Pieris*, and decreased the root-to-shoot ratio of both cultivars. Reduction of root:shoot ratio has been an observed result of mycorrhizal colonization of roots (Smith and Read, 1997).

Results from our study strongly suggest that the fertilizer and irrigation rates we evaluated play a less important role in the

growth of container-grown *Leucothoe* and *Pieris* than does inoculation with NERS. We can invoke the possible role of soil microbiota on plant growth. Either mycorrhizae could increase plant growth, as shown by Jansa and Vosátka (2000), Scagel (2005), and Scagel et al. (2005) or growth-promoting bacteria in the inoculum (Azcon and Ocampo, 1981; Garbaye, 1991; Pinton et al., 2001) could also contribute to the observed growth enhancement of NERS-treated plant. However, our results were in contrast to those by Starrett et al. (2001), who showed mycorrhizal-inoculated micropropagated *Pieris floribunda* did not stimulate root or shoot development during 3 months in a greenhouse.

Although not measured in this study, one could speculate that for *Leucothoe* and *Pieris*, shoot and root growth enhancement in NERS treatments could be the result of an increase in carbon assimilation (photosynthate production), a lower respiration rate, or both (Stabler et al., 2001). Changes in the carbon allocation

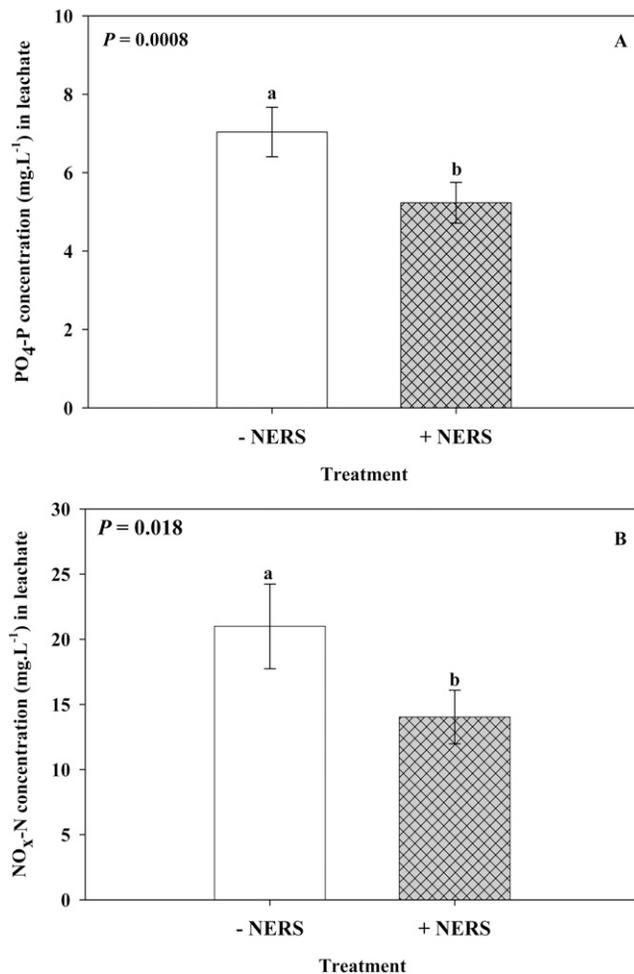


Fig. 5. Mean concentration of PO₄-P in leachate collected during the 17 weeks growth from containers containing Coast *Leucothoe* [*Leucothoe axillaris* (Lam.) D. Don] (A) and concentration of NO_x-N in leachate collected from containers containing Japanese *Pieris* [*Pieris japonica* (Thunb.) D. Don ex G. Don] (B) as affected by the addition of inoculum containing soil and roots from ericoid mycorrhizal plants (NERS), inoculated (+NERS) or not (-NERS). *P* values shown are from analysis of variance of the nutrient concentration in leachate over NERS. Columns with different letters denote significant difference ($\alpha=0.05$) within treatment (Tukey's honestly significant difference comparison of means). Error bars are SES ($n=72$).

pattern could account for our observed decrease in root-to-shoot ratio of *Leucothoe* and *Pieris* by NERS and can be ascribed to increased shoot growth relative to root growth (Biermann and Linderman, 1983).

Our results showed that the overall increase in plant growth was accompanied with an increase in shoot N, P, and Mn concentration when *Leucothoe* plants were inoculated with NERS. This response could be attributed to effect of pH on solubility of P or the inoculation with mycorrhizal fungi may hydrolyze organic phosphates in growing medium (Dighton, 1983). The mobilization and transfer of these nutrients to the shoot is improved by the natural mycorrhizal fungi symbiosis (Chen et al., 2003).

Because there were no significant differences in *Leucothoe* shoot N and P concentrations resulting from reductions in irrigation and fertilizer rates in +NERS-treated plants (Table 1), it would be beneficial to try similar LI systems with *Leucothoe* plants inoculated with natural mycorrhizae. In the presence of NERS, *Leucothoe* tissue Mn concentration

increased under LF rate and was reduced under HF.

For *Pieris*, the increase in plant shoot biomass in inoculated plants was accompanied with a slight difference in shoot N, Ca, Mg, Cu, Fe, and B concentration and a decrease in P, Mn, and Zn concentration (Table 2). Azaizeh et al. (1995) and Kothari et al. (1991) indicated that a reduction in host plant Mn absorption after arbuscular mycorrhizal (AM) fungi colonization is common when uptake of other nutrients has increased. The LI rate induced higher N, P, K, Cu, Mn, Fe, and Zn concentration in *Pieris* plants than at commonly used recommended rate of irrigation (HI) for nursery plant production. Thus, reducing irrigation rate could still benefit NERS-inoculated *Pieris* plant nutrient uptake and enhance its growth.

Across treatments, the overall mean NH₄-N concentration exceeded the U.S. Environmental Protection Agency-recommended level of NH₄-N (0.5 mg.L⁻¹) in potable water (National Academy of Sciences, 1973), whereas NO_x-N concentration increased to three times the maximum contamination levels

of nitrate in drinking water [10 mg.L⁻¹; U.S. Environmental Protection Agency (EPA), 1982] around 7 July. Although there are no federal limits on P concentrations in fresh water, PO₄-P in leachate exceeded the U.S. EPA-recommended total phosphates and total P levels (0.05 mg.L⁻¹) in streams entering lakes and reservoirs and for total P (0.1 mg.L⁻¹) in flowing waters (U.S. EPA, 1986). The high air temperature (Fig. 1) during the period 16 June to 19 July could be a contributing factor to increases in nutrient releases (Cabrera, 1997) and nutrient concentrations in leachate. The NO_x-N and PO₄-P leachate losses from plants were significantly higher under HF treatment than in LF for both plant species and for PO₄-P under HI treatment for both species. Under the tested irrigation treatments, PO₄-P concentrations in leachate from containers in either *Leucothoe* or *Pieris* exceeded the U.S. EPA-recommended levels of P in pond and stream water. Our results showed that NERS inoculation significantly reduced leachate PO₄-P concentrations in *Leucothoe* and NO_x-N concentrations in *Pieris*. The addition of NERS also significantly reduced EC in *Pieris* with the increased addition of fertilizer.

It has been proposed that root colonization by mycorrhizal fungi decreases with increasing P availability in the medium (Koide et al., 1999), because plants can meet their nutrient requirements through direct nutrient uptake by their own roots without the extra C expenditure required to support mycorrhizal fungi (Aerts and Chapin 2000). However, in this study, root colonization was not significantly higher in soil-inoculated ericaceous plants even at LF, suggesting that even LF levels applied may be high enough to suppress mycorrhizal formation, or ericoid colonization was low as a result of specific cultural conditions used in the experiment that inhibited (or reduced) mycorrhizal colonization. Corkidi et al. (2004) examined the infectivity of several commonly available commercial mycorrhizal inoculants in a soil-based medium and in two soil-less mixes used in standard nursery practices. Their results showed that the growing medium influenced the colonization percentage of infective inoculants. Redwood shavings, some barks, certain peats, and soils with high organic matter content can have inhibitory effects on root colonization by AM fungi (Biermann and Linderman, 1983; Graham and Timmer, 1984; Johnson and Hummel, 1986). Scagel and Yang (2005) reported that mycorrhizal colonization varied with production practices, cultivar, and was negatively correlated to ammonium in soil. Although mycorrhizal colonization index of roots was low in our study, mycorrhiza may be functional for nutrient acquisition.

Enhanced phosphate uptake by birch associated with ectomycorrhizae was shown to occur even before classical mycorrhizal structures (sheath and Hartig net) were formed (Frankland and Harrison, 1985).

Our experience has shown that natural inoculum of NERS, containing soil, root fragments, and their associated mycorrhizal fungi,

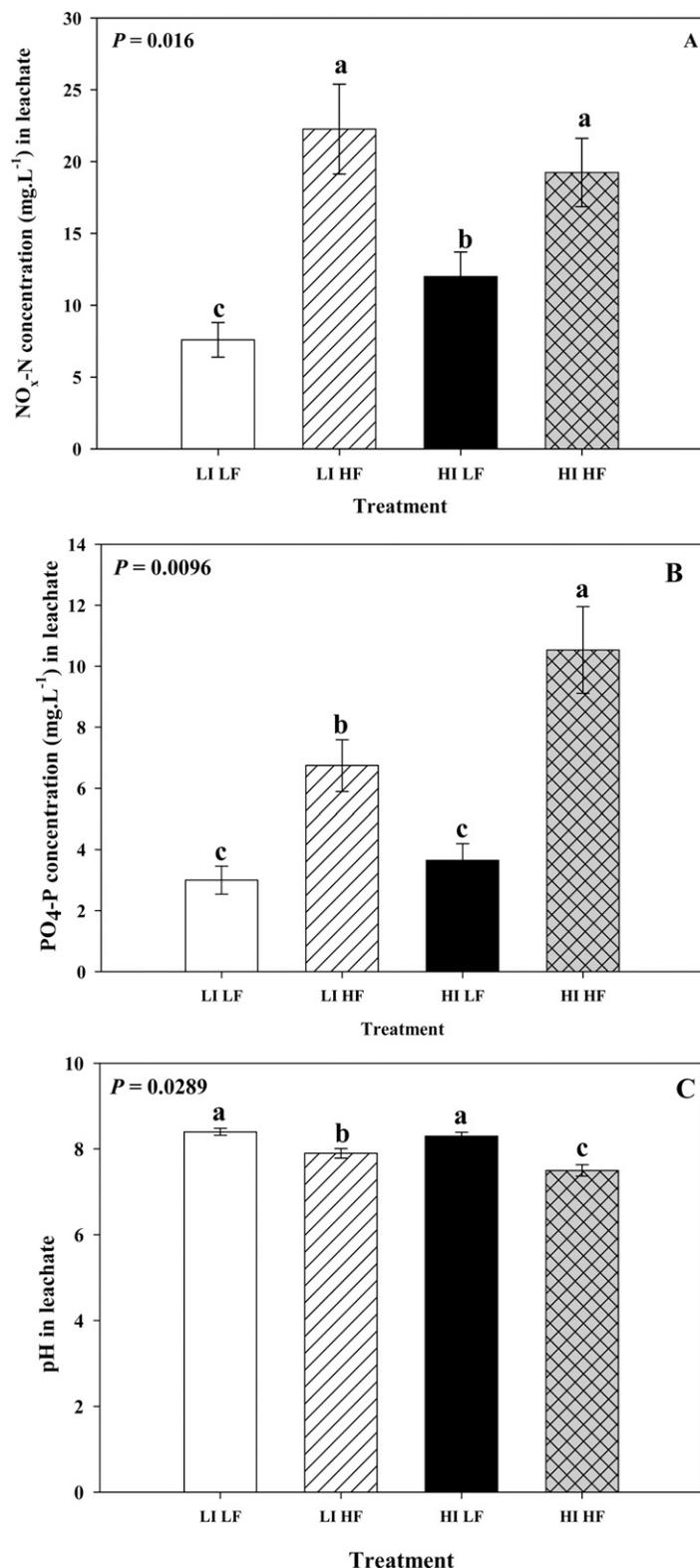


Fig. 6. Mean concentration of NO_x-N concentration in leachate during the 17 weeks plant growth from Coast *Leucothoe* [*Leucothoe axillaris* (Lam.) D. Don] (A), concentration of PO₄-P and pH in leachate collected from Japanese *Pieris* [*Pieris japonica* (Thunb.) D. Don ex G. Don] (B and C, respectively) as affected by the interaction of irrigation and fertilizer treatments. LI (low irrigation; 2.8 L per irrigation, twice a day, three times a week) and HI (high irrigation; 4.2 L per irrigation, twice a day, three times a week). A controlled-release fertilizer Polyon® Plus 14-16-8 (14N-7P-6.6K) was incorporated into the substrate medium at planting for a total of 7.84 g nitrogen (N) (HF) or 3.92 g N (LF). *P* values shown are from analysis of variance of the nutrient concentration and pH in leachate over fertilizer and irrigation treatments. Columns with different letters denote significant difference ($\alpha = 0.05$) within treatment (Tukey's honestly significant difference comparison of means). Error bars are SES ($n = 36$).

together with rhizospheric microbial communities is beneficial to plant growth in *Leucothoe* and *Pieris* and enhances macronutrient concentrations in shoots of *Leucothoe*. Decreasing the fertilizer rate to 50% of the recommended rate and the irrigation rate to 67% of the recommended rate in conjunction with the incorporation of natural soil inoculum reduced leachate nutrient concentrations of two main water pollutants (NO_x-N and PO₄-P). Adopting this practice may allow reduction in fertilizer application, conserve water for irrigation, and help to minimize subsequent nutrient runoff in nursery operations. In our study, we collected the natural soil inoculum of NERS from a forest site where healthy ericaceous plants were naturally growing and have not received any pesticides or runoffs. A cultural practice we recommend for the users of the natural soil inoculum of NERS is to select the source of soil natural inoculum of NERS from managed soil sites that have been screened to limit potential introduction of soil-dwelling plant pathogens and not impacted by water runoff that may be contaminated by pollutants.

In terms of plant production practices in the nursery, our work suggests reducing irrigation volume and lowering fertilizer rates in conjunction with the addition of natural soil inoculum could provide plants of equal market value with reduced potential nutrient leaching loss. However, we have seen considerable differences in responses of these two plant species from the same family, indicating that species variability is significant and that more work should be done to evaluate a larger range of woody ornamental plants to provide a more generic management plan.

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