Effects of Mycorrhizal Colonization on Nitrogen and Phosphorus Leaching from Nursery Containers

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Abstract. Our goal was to investigate the effects of mycorrhizal colonization on nitrogen (N) and phosphorus (P) leaching from plants grown in nursery containers. We compared the growth response and the content of nitrate (NO3), ammonium (NH4), and orthophosphate, in leachates collected from mycorrhizal (AM) and nonmycorrhizal (NonAM) plants of the fast-growing perennial, Encelia californica Nutt. (california sunflower), and the slow-growing woody shrub, Rhus integrifolia (Nutt.) Brewer & S. Watson (lemonade berry). Plants were grown for 8 weeks with no fertilizer or with 0.88 g (half rate) and 1.76 g (full rate) of 18N–2.6P–9.9K Osmocote (18–6–12, 6–7 month longevity at 26 °C). Mycorrhizal colonization reduced the fertilizer requirement to achieve maximum growth from AM and NonAM plants of the fast-growing perennial, Encelia californica Nutt. (california sunflower), and Rhus integrifolia (Nutt.) Brewer & S. Watson (lemonade berry). Mycorrhizal colonization contributed to reduce the content of NO3, NH4, and orthophosphate by up to 65% in leachates from E. californica grown with half rate of Osmocote and up to 70% to 80% in those from plants grown in full rates of Osmocote. In contrast, only the leachates from AM plants of R. integrifolia grown without fertilizer had generally lower nutrient content than those from NonAM plants. Leachates collected from AM plants grown in half rates of Osmocote had less P but no less N, and there were mostly no significant differences in the leachate content of NO3, NH4, and orthophosphate from AM and NonAM plants of R. integrifolia grown in full rates of Osmocote. However, mycorrhizal colonization reduced the fertilizer requirement to achieve maximum growth in both species. AM plants of E. californica and R. integrifolia grown with half rates of Osmocote had greater dry weight than the NonAM ones grown in full rates of Osmocote. Our study shows that mycorrhizal colonization can reduce N and P leaching either by increasing nutrient uptake or by allowing the use of lower fertilizer rates.

Mitigation of N and P runoff is a major goal of the nursery industry. Woody and herbaceous ornamental plant production may be a significant source of surface water and groundwater contamination (Mangiafico et al., 2009; Thompson et al., 2002). Plant production in containers is intensive, one acre of land can be occupied by thousands of containers, and nursery cultural practices such as the use of soilless mixes, high fertilizer rates, and frequent irrigation are highly conducive to nutrient leaching (Juntunen et al., 2002; Million et al., 2007).

A number of Best Management Practices have been proposed to maximize production and minimize water contamination from runoff and leaching losses. These practices vary with particular nursery conditions but most encompass proper irrigation and fertilizer programs to optimize nutrient use efficiency (Lea-Cox et al., 2010; Yeager et al., 2010).

Mycorrhizal technology can also be included as an important component of nurseries’ cultural programs to reduce nutrient runoff while maintaining plant quality and yield (Amaya-Carpio et al., 2009; Sousa et al., 2011). Arbuscular mycorrhizal fungi are a group of microorganisms that colonize the roots of most plants, establishing a mutually beneficial relationship. They develop an extra-radical mycelium that enhances the plant’s ability to acquire mineral nutrients and water (Liu et al., 2007). The hyphae of arbuscular mycorrhizal fungi take up substantial amounts of P and N from different sources in the soil (Atul-Nayyar et al., 2009; Govindarajulu et al., 2005; Hodge et al., 2001), although few studies have investigated the effects of mycorrhizal colonization on N and P leaching in nursery conditions (e.g., Zinati et al., 2007), Haines and Best (1976) showed decreased leaching of ammonium (NH4) and nitrate (NO3) from mycorrhizal plants of Liquidambar styraciflua L. grown in soil. Asghari et al. (2005) found decreased P leaching from mycorrhizal plants of Trifolium subterraneum L. grown in soil at low P levels but not at high P levels. Van der Heijden (2010) reported that grassland microcosms with mycorrhizal plants of Festuca ovina L., Anthoxanthum odoratum L., or Poa pratensis L. lost 60% less P, 7.5% less NH4, although not less NO3, than the nonmycorrhizal control microcosms. Asghari and Cavagnaro (2011) found lower levels of NO3, NH4, and P in soils and leachates collected from containers with mycorrhizal plants of Phalaris aquatica L. than in those collected from containers with nonmycorrhizal plants.

Our goal was to investigate the effects of mycorrhizal colonization on the content of N and P in leachates from plants grown in nursery conditions. We compared the growth response and the content of NO3, NH4, and orthophosphate in leachates collected from AM and NonAM plants of the fast-growing perennial, Encelia californica, and the slow-growing woody shrub, Rhus integrifolia, grown in a standard nursery mix with different levels of fertilizer. The following specific questions were addressed: 1) does mycorrhizal colonization significantly decrease N and P leaching? and if so, 2) is this response related to plant growth, to the extent of mycorrhizal colonization, and/or to the concentration of fertilizer in the growing medium?

Materials and Methods

Plant material

Two California native plants were selected based on their contrasting growth rates.

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Encelia californica (California sunflower) is an evergreen subshrub that grows quickly to 1.5 m high and wide. *Rhizomnora* (lentenerry) is a 1.5 to 3-m tall slow-growing woody perennial shrub or small tree with thick, dull green leaves that cover the plant densely. Both species are important components of the coastal sage scrub and chaparral communities and are widely propagated to be used as ornamentals and for ecological restoration (Newton and Claassen, 2003).

**Growth experiment**

To investigate the effects of mycorrhizal colonization on plant growth and N and P leaching, an experiment was conducted in a greenhouse at the Tree of Life Nursery in San Juan Capistrano, CA, from June to Aug. 2008. Average maximum and minimum temperatures were 29/18°C (day/night).

One week-old pre-germinated seedlings of *E. californica* and *R. integrifolia* were transplanted into 8.7 cm pots (McConkey, Garden Grove, CA) filled with a potting mix inoculated with one tea-spoon of mycorrhizal or nonmycorrhizal inoculants that had been previously incorporated in the planting hole.

**Growing medium**

The growing medium was a mixture of redwood bark, pine sawdust, calcined clay, and sand (1:2:1:1 by vol.) sterilized for 1 h on 2 consecutive days. It was amended and sand (1:2:1:1 by vol.) steam sterilized in the planting hole.

Growing medium

*Tagetes lemmonii* (Tree of Life Co., Marysville, OH). Before the incorporation of the fertilizer treatments, this soilless mix had 48, 44, and 112 mg kg–3 (1 lb/yd 3) of ferrous sulfate, and 0.59 kg m–3 (2 lb/yd 3) of dolomite, 0.59 kg m–3 (1 lb/yd 3) of ferrous sulfate, and 0.59 kg m–3 (1 lb/yd 3) of Sierra Micro-max® (Scott-Sierra Horticultural Products Co., Marysville, OH). Before the incorporation of the fertilizer treatments, this soilless mix had 48, 44, and 112 mg kg–1 of N, P, and potassium (K), respectively, and a pH of 6.8 (N and K were determined by sodium chloride extraction and P by sodium bicarbonate extraction at the Soil and Plant Laboratory, Inc., Orange County, CA).

**Mycorrhizal and nonmycorrhizal inoculum**

Mycorrhizal and nonmycorrhizal inoculants were propagated in pot cultures 4 months before the beginning of the experiment. Seeds of marigold (*Tagetes lemmonii Gray*) were planted in 1-gallon pots filled with a mixture of calcined clay with root pieces colonized by VAM 80™ (Tree of Life Nursery) or microwaved sterilized root pieces (for the mycorrhizal and nonmycorrhizal inoculum, respectively). VAM 80™ consists of at least 60 propagules per cubic centimetre of spores, hyphae, and/or root pieces colonized by a species of *Glomus* that sporulates intraradically and is propagated for large-scale inoculation of California native plants in containers at Tree of Life Nursery. After 4 months of growth, plant tops were removed and the substrate was allowed to dry. The roots were finely chopped and thoroughly mixed with the media to obtain homogenous inoculants.

**Fertilizer treatments**

One week after transplanting, 20 mycorrhizal and nonmycorrhizal pots per species were not fertilized or were top-dressed with 0.88 g (half-rate) or 1.76 g (full-rate) of 18N-2.6P-9.9K Osmocote (18-6-12, 6–7 month longevity at 26°C, Osmocote® controlled-release fertilizer; Scotts Co., Marysville, OH). The fertilizer prills were carefully mixed in the surface of the container and covered with an additional layer of potting mix. The amounts of Osmocote were calculated based on the recommended low rates of 4.15 kg m–3 (7 lb/yd 3). The primary plant nutrients derived from this product are ammonium nitrate, ammonium phosphate, calcium phosphate, and potassium sulfate (9.7% of the source of nitrogen is NO3 and 8.3% is NH4).

**Harvests**

Ten randomly selected plants per treatment were harvested 4 and 8 weeks after transplanting. Shoots were separated from roots and were oven-dried at 70°C to record shoot dry weight. Roots were air-dried, weighed, and a subsample of the root system was used to assess mycorrhizal colonization. Root pieces were cleared and stained using the technique of Koske and Gemma (1989) and 50 1-cm-long root pieces were mounted in polyvinyl alcohol lactoglycerol on microscope slides. The percentage of mycorrhizal colonization was determined in 100 intersections by the magnified intersection method of McConigle et al. (1990). The concentration of N and P in shoots obtained at the second harvest was determined at the University of California Davis Division of Agriculture and Natural Resources Analytical Laboratory. Total N and P shoot content was calculated by multiplying the concentration by the shoot dry weight at the second harvest.

**Leachate collection and analysis**

To retain the leachates, 10 replicate plants randomly designated for the final harvest were placed in plastic cups. Leachates from containers were collected weekly, on the same day each week. After hand watering with 138 mL of tap water that contained 0.70 dS m–1. Leachate total volumes were recorded and 20-mL sample aliquots were collected in vials and immediately frozen until ready to be analyzed. Solutions were analyzed for NO3, NH4, and orthophosphate with an Astoria Analyzer (Astoria-Pacific International, Clackamas, OR). Nitrate was analyzed using the colorimetric automated, hydrzone reduction method (Kamphake et al., 1967), NH4 using the colorimetric, automated phenate method, and orthophosphate by the colorimetric, automated ascorbic acid reduction method (U.S. Environmental Protection Agency, 1979). The content of NO3, NH4, and orthophosphate in leachates was calculated by multiplying the concentration of each ion by the total volume of water collected during 8 weeks.

**Experimental design and data analysis**

The study was designed as a 2 x 3 factorial with 20 replicates for two harvests per plant species. The two levels of the inoculum were mycorrhizal and nonmycorrhizal, and the three levels in the fertilizer treatment were unfertilized or fertilized with half or full rates of Osmocote. Replicates of each treatment were randomly arranged on a greenhouse bench.

Two-way analysis of variance (ANOVA) with inoculum and fertilizer treatments as factors was performed on plant growth (root, shoot, total dry mass, and root:shoot). The percentages of mycorrhizal colonization were arcsine-square root transformed before statistical analysis with one-way ANOVA. Mean contrasts were performed using Fisher’s protected least significant difference with P ≤ 0.05 as the level of significance. The content of NO3, NH4, and orthophosphate obtained during 8 weeks was analyzed by two-way repeated-measures ANOVA. When interactions between fertilizer and inoculum treatments were significant, one-way ANOVA was used to analyze significant differences among fertilizer rates in each inoculum treatment, and the Student’s t test was used to analyze differences between mycorrhizal and nonmycorrhizal plants in each fertilizer treatment (Statview; SAS Institute, Inc., Cary, NC).

**Results**

**Effects of different fertilizer rates on mycorrhizal colonization**

*Encelia californica.* Plants grown without fertilizer had significantly lower percentages of mycorrhizal colonization than those grown with half rate of Osmocote at the first harvest (P = 0.05) and than those grown with half and full rates of Osmocote at the second harvest (P < 0.0001). The percentages of mycorrhizal colonization of plants grown with no fertilizer and half and full rates of Osmocote were 18.6 ± 3.2, 35.2 ± 6.9, and 26 ± 6.0, respectively, 4 weeks after transplanting; and 2 ± 0.6, 30 ± 3.6, and 26 ± 4.4, respectively, 8 weeks after transplanting.

*Rhus integrifolia.* There were no significant differences in the percentages of mycorrhizal colonization of *R. integrifolia* obtained in the different fertilizer treatments 4 and 8 weeks after transplanting. The percentages of mycorrhizal colonization of plants grown with no fertilizer and half and full rates of Osmocote were 34.5 ± 5.9, 30.5 ± 5.5, and 21.4 ± 4.5, respectively, at the first harvest, and 53.9 ± 5.7, 57.3 ± 5.4, and 45 ± 3.3, respectively, at the second harvest.

**Effects of mycorrhizal colonization on plant growth**

*Encelia californica.*

**First harvest.** The two-way ANOVA indicated significant effects of fertilizer in all the growth response variables and of inoculum in root dry weight and root:shoot ratio 4 weeks after transplanting (F ratios of fertilizer effects were 13.30, P ≤ 0.0001; 3.67, P ≤ 0.05; and 8.21, P ≤ 0.001, for shoot, root, and total dry weight, respectively;
Fertilization with half and full rates of Osmocote significantly reduced the root:shoot ratio of NonAM plants. Mycorrhizal colonization reduced the root:shoot ratio of plants grown without fertilizer but there were no significant differences in the root:shoot ratios of AM and NonAM plants grown in half and full rates of Osmocote at the second harvest (Table 1).

**Rhus integrifolia.**

**First harvest.** Four weeks after transplanting, the two-way ANOVA indicated significant effects of fertilizer on shoot height (F = 10.04, P = 0.0001), root dry mass (F = 3.33, P = 0.05), and root:shoot (F = 3.10, P = 0.05), but only significant effects of inoculum in shoot height (F = 4.33, P = 0.05). Addition of fertilizer reduced the root:shoot ratio of AM and NonAM plants grown with either half or full rates of Osmocote, but there were no significant differences between inoculum treatments regardless of fertilizer rate. The root:shoot ratios of AM plants grown without fertilizer and with half and full rates of Osmocote were 0.46 ± 0.04, 0.37 ± 0.05, and 0.37 ± 0.02, respectively, and of AM plants 0.46 ± 0.04, 0.37 ± 0.04, and 0.38 ± 0.03, respectively.

**Second harvest.** Eight weeks after transplanting, the two-way ANOVA indicated significant effects of fertilizer on shoot height but there were no significant differences in shoot and total dry weight of AM and NonAM plants grown without fertilizer (Table 2). Nevertheless, increasing the amount of Osmocote did not stimulate the growth of AM plants. There were no significant differences in shoot and total dry weight of AM plants grown with half and full rates of Osmocote.

Mycorrhizal colonization decreased the root:shoot ratio of plants grown with half and full rates of Osmocote, but there were no significant differences in the root:shoot ratios of AM and NonAM plants grown without fertilizer (Table 2).

**Effects of mycorrhizal colonization on shoot tissue nitrogen and phosphorus.**

Encelia californica. The two-way ANOVA indicates that there were interactions between fertilizer and inoculum in the content of N in shoot tissue and in the content and concentration of P. The percentage of N significantly increased as the fertilizer rate increased in both

### Table 1. Effect of fertilizer rate and mycorrhizal colonization on the growth of Encelia californica 8 weeks after transplanting.

| Treatment | Shoot dry wt (g) | Root dry wt (g) | Total dry wt (g) | Root:shoot |
|-----------|-----------------|----------------|-----------------|-----------|
|           | NonAM AM | NonAM AM | NonAM AM | NonAM AM |
| No Osmocote | 0.19 ± 0.03 a | 0.22 ± 0.02 a | 0.12 ± 0.01 a | 0.08 ± 0.01 a |
| Half rate   | 0.72 ± 0.09 b | 1.17 ± 0.08 b | 0.32 ± 0.05 b | 0.61 ± 0.06 b |
| Full rate   | 1.12 ± 0.09 c | 1.66 ± 0.11 c | 0.37 ± 0.05 b | 0.58 ± 0.04 b |
| Significance* | *** | *** | *** | ** |
| **Fertilizer** |      |      |      |      |
| **Inoculum** |      |      |      |      |
| **Fertilizer × inoculum** |      |      |      |      |

1 Plants were grown with no fertilizer or with 0.88 g (half rate) and 1.76 g (full rate) of 18N–2.6P–9.9K Osmocote (18-6-12, 6–7 mo. longevity at 26 °C). Osmocote® controlled-release fertilizer; Scotts Co., Marysville, OH) and were not inoculated (NonAM = nonmycorrhizal) or were inoculated with VAM 80® (AM = mycorrhizal).

2 Data represent the mean ± se of 4 replicates. Means within a column followed by the same letter are not significantly different at α = 0.05 as determined by Fisher’s protected least significant difference.

3 Values in bold indicate significant differences between NonAM and AM plants at each fertilizer treatment (across rows) according to the Student’s t-test at P ≤ 0.05.

*ns, *, **, and *** indicate non-significant or significant differences at P = 0.05, 0.001, and 0.0001, respectively.

### Table 2. Effect of fertilizer rate and mycorrhizal colonization on the growth of Rhus integrifolia 8 weeks after transplanting.

| Treatment | Shoot dry wt (g) | Root dry wt (g) | Total dry wt (g) | Root:shoot |
|-----------|-----------------|----------------|-----------------|-----------|
|           | NonAM AM | NonAM AM | NonAM AM | NonAM AM |
| No Osmocote | 0.14 ± 0.02 a | 0.29 ± 0.03 a | 0.12 ± 0.009 a | 0.11 ± 0.01 a |
| Half rate   | 0.45 ± 0.06 a | 0.65 ± 0.06 b | 0.14 ± 0.01 a | 0.12 ± 0.01 a |
| Full rate   | 0.43 ± 0.06 a | 0.75 ± 0.05 b | 0.12 ± 0.01 a | 0.13 ± 0.01 a |
| Significance* | *** | *** | *** | ** |
| **Fertilizer** |      |      |      |      |
| **Inoculum** |      |      |      |      |
| **Fertilizer × inoculum** |      |      |      |      |

1 Plants were grown with no fertilizer or with 0.88 g (half rate) and 1.76 g (full rate) of 18N–2.6P–9.9K Osmocote (18-6-12, 6–7 mo. longevity at 26 °C). Osmocote® controlled-release fertilizer; Scotts Co., Marysville, OH) and were not inoculated (NonAM = nonmycorrhizal) or were inoculated with VAM 80® (AM = mycorrhizal).

2 Data represent the mean ± se of 4 replicates. Means within a column followed by the same letter are not significantly different at α = 0.05 as determined by Fisher’s protected least significant difference.

3 Values in bold indicate significant differences between NonAM and AM plants at each fertilizer treatment (across rows) according to the Student’s t-test at P ≤ 0.05.

*ns, *, **, and *** indicate non-significant or significant differences at P = 0.05, 0.001, and 0.0001, respectively.
AM and NonAM plants. However, mycorrhizal colonization only increased the concentration of N in plants grown without fertilizer. The total content of N of AM plants grown in half and full rates of Osmocote was significantly higher than the N content of NonAM plants, because AM plants had greater biomass (Table 3).

AM plants had significantly larger shoot P concentrations and content than NonAM plants at all fertilizer rates (Table 3).

Rhus integrifolia. The two-way ANOVA indicated significant interactions between fertilizer and inoculum in the content and concentration of N and P in shoot tissue. AM plants had significantly greater concentrations and content of shoot tissue N and P than NonAM plants at all fertilizer levels (Table 4). AM plants grown with half and full rates of Osmocote had approximately double the content of N and up to four times more P than NonAM plants.

Increasing the fertilizer rate from half to full rate of Osmocote increased the content of N of AM plants but not of NonAM plants. There were no significant differences in the shoot tissue N content of NonAM plants grown with half and full rates of Osmocote, but AM plants grown with a full rate of Osmocote had a higher content of N than those grown with the half rate (Table 4).

Effects of mycorrhizal colonization on leachate content of nitrogen and phosphorus

Encelia californica. The two-way ANOVA for repeated measures indicated significant interactions between time and inoculum on the content of NO₃, NH₄, and orthophosphate in leachates collected from plants grown without fertilizer and half and full rates of Osmocote (Table 5).

Nitrate-N. Leachates collected from AM plants grown without fertilizer and with half rates of Osmocote had significantly lower content of NO₃ than those from NonAM plants during the 8 weeks of the experiment. Nitrate content in leachates from mycorrhizal plants grown with half rates of Osmocote was also significantly lower, except in Weeks 1, 3, and 8 (Fig. 1A–C).

The content of NO₃ increased from Week 2 to Week 6 from 0.17 mg to 0.41 mg in leachates from NonAM plants grown without fertilizer (Fig. 1A) and from 0.35 mg to 0.78 mg in those grown with half rates of Osmocote (Fig. 1B). However, the maximum content of NO₃ in leachates collected from AM plants grown without fertilizer and with half rate of Osmocote was 0.045 mg and 0.47 mg, respectively, also at Week 6 (Fig. 1A–B).

Nitrate content in leachates collected from NonAM plants grown with full rate of Osmocote increased from 0.36 mg at Week 2 to 2.11 mg at Week 4 and then declined to 0.63 mg at Week 8. In leachates from AM plants, NO₃ content fluctuated from 0.19 mg at Week 2 to up to 0.82 mg at Week 5 and decreased to 0.18 mg at Week 8 (Fig. 1C).

Ammoniacal-N. Leachates collected from AM plants grown without fertilizer had less NH₄ than those collected from NonAM plants, except in Weeks 2, 5, and 6 (Fig. 2A). Leachates from AM plants grown with half rates of Osmocote also had lower NH₄ content from Week 3 to Week 7. The content of NH₄ in leachates from NonAM plants increased from 0.049 mg at Week 2 to 0.111 mg at Week 6, whereas the content of NH₄ in leachates collected from AM plants only fluctuated from 0.025 mg at Week 2 to 0.036 mg at Week 6 (Fig. 2B). Ammonium content in leachates collected from AM plants grown in full rates of Osmocote was significantly lower than in those from NonAM plants during the first 4 weeks of the experiment. The maximum NH₄ content obtained from NonAM and AM plants was 0.29 mg versus 0.07 mg, respectively, at Week 3 (Fig. 2C).

Orthophosphate. Leachates collected from AM plants grown without fertilizer had lower content of orthophosphate than those collected from NonAM plants except at Week 2.

### Table 3. Effect of fertilizer rate and mycorrhizal colonization on the concentration (%) and content (mg) of nitrogen (N) and phosphorus (P) in shoot tissue of *Encelia californica* 8 weeks after transplanting.

| Treatment | N (%) | N (mg/plant) | P (%) | P (mg/plant) |
|-----------|-------|--------------|-------|--------------|
|           | NonAM | AM | NonAM | AM | NonAM | AM | NonAM | AM | NonAM | AM | NonAM | AM |
| No Osmocote | 0.88 ± 0.04 a** | 1.41 ± 0.03 a | 2.77 ± 1.08 a | 2.99 ± 0.17 a | 0.08 ± 0.00 a | 0.57 ± 0.04 a | 0.23 ± 0.08 a | 1.21 ± 0.07 a |
| Half rate | 1.77 ± 0.18 b | 1.90 ± 0.08 b | 10.93 ± 1.32 b | 19.95 ± 1.02 b | 0.11 ± 0.01 a | 0.27 ± 0.01 b | 0.71 ± 0.09 b | 2.82 ± 0.16 b |
| Full rate | 2.46 ± 0.09 c | 2.44 ± 0.11 c | 24.81 ± 2.27 c | 36.85 ± 1.92 c | 0.16 ± 0.01 b | 0.22 ± 0.01 b | 1.53 ± 0.18 c | 3.32 ± 0.21 c |
| Significance | ** | ** | *** | *** | | | | |

*Plants were grown with no fertilizer or with 0.88 g (half rate) and 1.76 g (full rate) of 18N–2.6P–9.9K Osmocote (18-6-12, 6–7 mo. longevity at 26 °C, Osmocote® controlled-release fertilizer; Scotts Co., Marysville, OH) and were not inoculated (NonAM = nonmycorrhizal) or were inoculated with VAM 80® (AM = mycorrhizal).

*Data represent the mean ± se of 10 replicates. Means within a column followed by the same letter are not significantly different at α = 0.05 as determined by Fisher’s protected least significant difference.

*Values in bold indicate significant differences between NonAM and AM plants at each fertilizer treatment (across rows) according to the Student’s t test at P ≤ 0.05.

**ns, *, **, and *** indicate non-significant or significant differences at P ≤ 0.05, 0.001, and 0.0001, respectively.

### Table 4. Effect of fertilizer rate and mycorrhizal colonization on the concentration (%) and content (mg) of nitrogen (N) and phosphorus (P) in shoot tissue of *Rhus integrifolia* 8 weeks after transplanting.

| Treatment | N (%) | N (mg/plant) | P (%) | P (mg/plant) |
|-----------|-------|--------------|-------|--------------|
|           | NonAM | AM | NonAM | AM | NonAM | AM | NonAM | AM | NonAM | AM | NonAM | AM |
| No Osmocote | 0.61 ± 0.02 a** | 0.73 ± 0.02 a | 2.09 ± 0.11 a | 2.46 ± 0.14 a | 0.06 ± 0.002 a | 0.18 ± 0.007 a | 0.21 ± 0.011 a | 0.62 ± 0.04 a |
| Half rate | 0.92 ± 0.05 b | 1.27 ± 0.04 b | 4.31 ± 0.34 b | 7.44 ± 0.53 b | 0.06 ± 0.006 a | 0.19 ± 0.005 b | 0.27 ± 0.02 ab | 1.15 ± 0.08 b |
| Full rate | 1.05 ± 0.03 c | 1.41 ± 0.03 c | 4.66 ± 0.40 b | 9.97 ± 0.61 c | 0.07 ± 0.006 a | 0.18 ± 0.004 a | 0.30 ± 0.027 b | 1.28 ± 0.08 b |
| Significance | ** | ** | *** | ** | | | ** | ** |

*Plants were grown with no fertilizer or with 0.88 g (half rate) and 1.76 g (full rate) of 18N–2.6P–9.9K Osmocote (18-6-12, 6–7 mo. longevity at 26 °C, Osmocote® controlled-release fertilizer; Scotts Co., Marysville, OH) and were not inoculated (NonAM = nonmycorrhizal) or were inoculated with VAM 80® (AM = mycorrhizal).

*Data represent the mean ± se of 10 replicates. Means within a column followed by the same letter are not significantly different at α = 0.05 as determined by Fisher’s protected least significant difference.

*Values in bold indicate significant differences between NonAM and AM plants at each fertilizer treatment (across rows) according to the Student’s t test at P ≤ 0.05.

**ns, *, **, and *** indicate non-significant or significant differences at P ≤ 0.05, 0.001, and 0.0001, respectively.
The content of orthophosphate in leachates collected from AM plants grown in half rates of Osmocote was also generally lower than from NonAM plants except during the first and eighth weeks. Orthophosphate content in leachates from AM plants fluctuated from 0.002 mg at Week 2 to 0.003 mg at Week 5, reached its maximum value of 0.013 mg at Week 6, and then declined to 0.006 mg at Week 8. The content of orthophosphate in leachates from NonAM plants increased from 0.006 mg at Week 2, to 0.039 mg at Week 6, and then declined to Weeks 7 and 8 up to 0.008 mg (Fig. 3B).

The content of orthophosphate in leachates collected from NonAM plants grown with full rates of Osmocote increased from 0.009 mg at Week 1 to 0.026 mg at Week 5, and then declined to 0.019 at Week 7. Orthophosphate content in leachates from AM plants increased from 0.004 mg at Week 1 to 0.013 mg at Week 6 and the decreased to 0.01 mg at Week 7. At Week 8, there were no significant differences in orthophosphate content recovered from AM and NonAM plants grown in half rates of Osmocote (Fig. 3C).

The content of orthophosphate in leachates collected from NonAM plants grown with full rates of Osmocote increased from 0.009 mg at Week 1, to 0.026 mg at Week 5, and then declined to 0.019 at Week 7. Orthophosphate content in leachates from AM plants increased from 0.004 mg at Week 1 to 0.013 mg at Week 6 and the decreased to 0.01 mg at Week 7. At Week 8, there were no significant differences in orthophosphate content recovered from AM and NonAM plants grown in full rates of Osmocote (Fig. 3C).

Table 5. F ratios of two-way analysis of variance with repeated measurements of effects of mycorrhizal colonization on the content (mg) of nitrate (NO₃), ammonium (NH₄), and orthophosphate in leachates collected from plants of *Encelia californica* during 8 weeks.

| Fertilizer treatment | Inoculum | Week | Inoculum × week |
|----------------------|----------|------|-----------------|
| NO₃                  | No Osmocote | 25.919*** | 26.397*** | 20.418*** |
|                      | Half rate  | 4.103 | 102.19*** | 26.201*** |
|                      | Full rate  | 2.70  | 26.09*** | 2.67*    |
| NH₄                  | No Osmocote | 8.645* | 26.895*** | 10.312*** |
|                      | Half rate  | 4.357* | 24.189*** | 15.726*** |
|                      | Full rate  | 6.537* | 19.723*** | 11.276*** |
| Orthophosphate       | No Osmocote | 17.410** | 53.752*** | 65.65*** |
|                      | Half rate  | 11.03* | 46.6*** | 19.96*** |
|                      | Full rate  | 17.286* | 30.13*** | 7.91*** |

*There were two levels in the inoculum treatment (mycorrhizal and nonmycorrhizal) and three levels in the fertilizer treatment [no fertilizer, half rate (88 g), or full rate (1.76 g) of 18N–2.6P–9.9K Osmocote (18-6-12, 6–7 mo. longevity at 26 °C, Osmocote® controlled-release fertilizer; Scotts Co., Marysville, OH)]. *, **, and *** indicate significant differences at P ≤ 0.05, P ≤ 0.001, and P ≤ 0.0001, respectively.

Rhus integrifolia. The two-way ANOVA with repeated measures indicated significant interactions between time and inoculum on the content of NO₃, NH₄, and orthophosphate in leachates collected from plants grown without fertilizer and half and full rates of Osmocote (Table 6).

Nitrate-N. Leachates collected from AM plants of *R. integrifolia* grown without fertilizer had significantly lower content of NO₃ at all weeks except at Week 6. The content of NO₃ declined from 1.052 mg to 0.42 mg in leachates collected from NonAM plants and from 0.269 to 0.025 mg in those collected from AM plants (Fig. 4A). There were no significant differences in the content of NO₃ recovered from AM and NonAM plants grown in half rates of Osmocote, except at Week 5, when leachates collected from AM plants had significantly lower NO₃ content than those from NonAM plants (0.74 versus 0.39 mg, respectively), and at Week 8, when leachates recovered from AM plants had significantly higher content of NO₃ than those from NonAM plants (0.46 mg versus 0.77 mg, respectively) (Fig. 4B). There were no significant differences in the content of NO₃ in leachates from AM and NonAM plants grown in full rates of Osmocote (Fig. 4C).
and Weeks 6 and 7, increasing from 0.006 to 0.072 mg in those from NonAM plants at Weeks 3 and 4. The content of orthophosphate in leachates from NonAM plants at Week 1 and from Weeks 4 to 6. Orthophosphate content increased from 0.003 to 0.007 mg in those from AM plants grown in half rates of Osmocote until Week 8, when leachates collected from AM plants had significantly more orthophosphate than those collected from NonAM plants (0.014 versus 0.071 mg) (Fig. 6C).

**Discussion**

Mycorrhizal colonization increased the growth and nutrient uptake of both *E. californica* and *R. integrifolia* but was more effective at decreasing nutrient leaching from plants of *E. californica*. Leachates collected from AM plants of *E. californica* had significantly less N and P than those collected from NonAM plants at all fertilizer rates. Mycorrhizal colonization contributed to the reduction of NO₃, NH₄, and orthophosphate by up to 65% in leachates from *E. californica* grown with half rate of Osmocote and up to 70% to 80% in those from plants grown in full rates of Osmocote. In contrast, the effects of mycorrhizal colonization on N and P leaching from nursery containers (Amaya-Carpio et al., 2005).

Plant growth promotion and increased nutrient absorption could have influenced the effects of mycorrhizal colonization on decreasing N and P leaching from containers with *E. californica* but did not guarantee reductions in nutrient losses from those with *R. integrifolia* at all the fertilizer rates tested. The effects of mycorrhizal colonization on decreasing N and P leaching in different plant species might be limited by plant nutrient demand and inherent growth response to nutrient supply. *E. californica* and *R. integrifolia* are two species with contrasting growth rates and responses to nutrient availability. *E. californica* is a perennial shrub with a fibrous root system (Hellmers et al., 1955), which exhibited the typical plastic response of fast-growing species when subjected to increasing nutrient supply (Gray and Schlesinger, 1983). The growth of *E. californica* increased as the fertilizer rate increased in both AM and NonAM plants. In contrast, *R. integrifolia* is a woody shrub that showed the pattern of slow-growing perennials with limited responses to increased nutrient availability (Gray and Schlesinger, 1983; Lambers et al., 1983).
Addition of fertilizer did not increase the growth of NonAM plants of *R. integrifolia*. Half and full rates of Osmocote doubled the growth of AM plants compared with those grown without fertilizer. However, there were no significant differences in the total dry weight of plants grown with half and full rates of fertilizer. Nutrient concentration in the growing medium with Osmocote could have exceeded the capacity of nutrient uptake of even the AM plants of *R. integrifolia*, and therefore mycorrhizal colonization reduced N and P leaching in plants grown without fertilizer but not in those grown with half and full rates of Osmocote.

Asghari et al. (2005) also found decreased P leaching from plants of *Trifolium subterraneum* grown in low P levels but not in high P levels. However, in their case, high P decreased mycorrhizal colonization and the density of extramatrical fungal hyphae.

We did not measure mycorrhizal fungi extramatrical hyphal length, but full rates of Osmocote did not decrease the percentages of mycorrhizal colonization for either *E. californica* or *R. integrifolia*. In fact, plants of *R. integrifolia* had higher percentages of mycorrhizal colonization than those of *E. californica* at all fertility levels, yet mycorrhizal colonization was more effective at reducing nutrient losses in the latter.

Plant growth and/or nutrient uptake increases and nutrient leaching decreases were not synchronous in *E. californica*. Leachates collected from AM plants of *E. californica* grown in half and full rates Osmocote generally had lower N and P contents during the first 5 weeks of the experiment, although there were no significant differences between
the growth of AM and NonAM plants at these fertility rates, and AM plants grown with full rates of Osmocote had lower root:shoot ratios than NonAM plants at the first harvest. Moreover, mycorrhizal colonization increased the total dry mass of E. californica grown in half rates of Osmocote by 60% and in the full rate of Osmocote by 68% at the second harvest. However, there were no significant differences between N and P content in leachates collected from AM and NonAM plants at Weg. This shows that mycorrhizal colonization in nutrient leaching is critical early in the production cycle, because nutrient release of controlled-release fertilizers is relatively high during the first 10 weeks and then decreases (Merhaut et al., 2006).

Although the effects of mycorrhizal colonization were not always reflected in decreased content of N and P in leachates from R. integrifolia at all fertility rates, the potential still exists to use mycorrhizal inoculation to reduce N and P leaching for the propagation of both species, E. californica and R. integrifolia, without affecting their plant growth. As shown by other studies (Amaya-Carpio et al., 2005; Sousa et al., 2011), mycorrhizal colonization reduced the fertilizer requirement to achieve maximum growth in both species. AM plants of E. californica and R. integrifolia grown with half rates of Osmocote had greater dry mass than the NonAM ones grown in full rates of Osmocote. Our study shows that mycorrhizal colonization can reduce N and P leaching either by increasing plant nutrient uptake or by allowing the use of lower fertilizer rates.

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