2020

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Technical Note

Applied Injected Air into Subsurface Drip Irrigation: Plant Uptake of Pharmaceuticals and Soil Microbial Communities

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Abstract: The growing global food security crisis is complicated by the need for increased crop production with less arable land and limited water resources. Reuse of treated wastewater for agricultural irrigation is becoming more common, often paired with other conservation measures such as subsurface drip irrigation (SDI). Passively injecting air into SDI systems increases crop yields and overcomes root zone wetting issues. However, when used with treated irrigation water, contaminants in the water might be taken up by the crops. This paper investigates the impact of air-injected water containing caffeine, carbamazepine, and gemfibrozil on plant uptake and soil microbial communities in Salanova lettuce (*Lactuca sativa*). Aerated lettuce yielded higher plant mass and root length. The use of air-injected water reduced the uptake of caffeine and gemfibrozil and increased the uptake of carbamazepine. Gemfibrozil and carbamazepine were primarily detected in leachate, while caffeine was observed in the soil samples. Injected air significantly impacted (p-value < 0.001) the fate and transport of gemfibrozil. Injection of pharmaceutically active compounds and the presence/absence of injected-air created a variation in soil microbial communities. **DOI: 10.1061/(ASCE)EE.1943-7870.0001655.**

Introduction

The world's supply of fresh water is a finite, limited resource. The increasing need for water resources is a consequence of demographic growth, economic development, and improvement of living standards, climate change, and pollution (FAO 2012). With the constant increase in population, there is a challenge to feed the people by producing crops on less arable land with limited water resources. Therefore, irrigation that is more efficient represents a potential solution.

The rapid growth of subsurface drip irrigation (SDI) has been observed around the world (Lamm et al. 2012) due to its ability to efficiently apply water and nutrients to crops (Camp et al. 2000; Ayars et al. 2015). SDI use increased by approximately 90% in the United States between 2003 and 2013 (Lamm 2016). SDI reduces or nearly eliminates surface evaporation, surface runoff, and deep percolation losses (Ayars et al. 2015; Bhattarai et al. 2004, 2005, 2008; Hanson and May 2004). SDI makes possible the use of degraded-quality water by increasing irrigation frequency, thus minimizing the matric and osmotic stress, and by reducing pathogen movement, odors, and animal and human contact (Ayars et al. 2015; Palacios-Díaz et al. 2009). SDI also affects plant growth, yield, and quality of the produce (Ayars et al. 2015; Palacios-Díaz et al. 2009). However, SDI requires a higher initial investment, has more clogging problems, and generates smaller wetting patterns than surface drip irrigation (Ayars et al. 2015).

Passively injected air into the SDI (using a commercial injector) can increase the root zone aeration and, consequently, wetting patterns and the yield of various crops (Abuarab et al. 2013; Goorahoo et al. 2001, 2007, 2008). Goorahoo et al. (2001, 2002), investigating the impact of injected-air on bell peppers, observed that bell pepper count and fresh weight from the aerated plots exceeded the nonaerated plots in both bell pepper count by 33% and 39%, respectively. Also, bell peppers receiving aerated water had greater dry weight and larger root mass compared to those receiving nonaerated water (Goorahoo et al. 2001, 2002). Similar results were also achieved with melons, tomatoes, strawberry (Goorahoo et al. 2007), and corn (Abuarab et al. 2013). The enhanced crop water use efficiency observed during air-injected irrigation may be due to a decrease in transpiration rates and an increase in net photosynthetic rate (Goorahoo et al. 2008). In contrast, the potential impact on the removal of pharmaceutically active compounds (PhACs) via plant uptake as well as on the soil microbial community is poorly understood. The environmental fate of PhACs is currently receiving increased attention due to advances in analytical instrumentation and the potential impact on the aquatic and terrestrial organism, ecosystems, and human health (Madureira et al. 2011; Pal et al. 2013; Bolong et al. 2009; Vajda et al. 2008).

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Note. This manuscript was submitted on March 20, 2019; approved on July 25, 2019; published online on December 11, 2019. Discussion period open until May 11, 2020; separate discussions must be submitted for individual papers. This technical note is part of the ***Journal of Environmental Engineering***, © ASCE, ISSN 0733-9372.

The aim of this work was to investigate the impact of air-injected water containing PhACs on: (1) plant uptake, and (2) the soil microbial community. Plant growth, expressed in terms of weight and development of the roots, was also investigated. Three PhACs—caffeine, carbamazepine, and gemfibrozil—were the chemicals simultaneously added to the water to irrigate Salanova lettuce (*Lactuca sativa*).

Materials and Methods

Caffeine, carbamazepine, gemfibrozil, and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). All analytes had a purity greater or equal to 97%.

Pelleted seed of Salanova lettuce (*L. sativa*) (Johnny's Selected Seeds, Winslow, ME) were seeded into a 72-cell pak containing a seed germinating mix. After 21 days, plants were graded and transplanted into 11.4-L (3-gal.) pots containing a sandy loamy soil mix (40% sand, 40% vermiculite, and 20% soil). Temperatures were maintained at 15.6°C–18.3°C by day and 7.2°C–10.0°C by night by using an air-conditioned greenhouse. A total of twelve 11.4-L (3-gal.) pots were used during the study. The experimental setup consisted of two lines, irrigating six pots each. The first line received air-injected water during the plants' growth and PhACs' injection, while the second line received nonaerated water throughout the study. A Mazzei air injector (Mazzei Injector Company, LLC, Bakersfield, CA) was used to inject air into the first line. Once plants were well established, caffeine ($C_0 = 1.34\ mg\,L^{-1}$), carbamazepine ($C_0 = 1.25\ mg\,L^{-1}$), and gemfibrozil ($C_0 = 1.17\ mg\,L^{-1}$) were simultaneously added during the daily irrigation for 8 days. At the end of the study plants were sampled for dry weight.

Liquid samples and all extracts were analyzed using an Acquity Ultra Performance Liquid Chromatography-tandem Mass Spectrometer (UPLC/MS, Waters Corporation, Milford, MA) equipped with an electrospray ionization source in positive/negative mode to detect the pharmaceutical compounds. Pharmaceuticals were separated using a Waters Acquity BEH C18 column (50×2.1 mm, 1.7 μm particle size). Separation was achieved using water and acetonitrile as mobile phases. Additional details regarding the cone voltages, collision energy, and the gradient used during the analysis are given in Table S1. Leachate from each pot was collected during the PhACs' injection, and 1 mL was centrifuged at 10,000 rpm for 16 min before being analyzed on the UPLC/MS using 1-μL injection. Also, for each pot, a representative soil sample was collected every 2 cm throughout the pot (total mass: 15 g), mixed, ground with a pestle to break the aggregates, and sieved (<5.95 mm). A subsample (1 g) was weighed and carefully transferred into a 15-mL centrifuge tube. Five mL of acetonitrile were added into each tube to extract the PhACs. After that, each tube was sonicated for 30 min, vigorously agitated with a shaker for 30 min, and centrifuged at 3,000 rpm for 30 min. The supernatant was collected and run on the UPLC/MS using 1-μL injection. Lettuce tissue was harvested, weighed, and freeze-dried using a Labconco Freeze Dryer (Labconco Corporation, Kansas City, MO). Then, 0.25 g of finely ground plant material was added to a 15-mL centrifuge tube and extracted with a procedure similar to the one used for analyzing the soil samples. Results, reported as mean ± standard deviation, were statistically analyzed using T-test (SigmaPlot, Systat Software, Inc.).

Before DNA extraction, soil samples were randomly collected every 2 cm throughout each pot with a sterile spatula and mixed to provide representative samples. Total DNA was extracted from the soil samples using the MoBio Power Soil DNA Isolation Kit (MoBio, Laboratories Inc, Carlsbad, CA) following the manufacturer's instructions. The DNA was eluted in 100 μL and used as a template for amplicon pyrosequencing analysis, which was performed by MR DNA Laboratory (Shallow Water, TX) using universal eubacterial primers 515F and 806R in a single-step PCR, followed by Ion Torrent PGM sequencing (Dowd et al. 2008). Data were analyzed using a proprietary pipeline, and operational taxonomic units (OTUs) were assigned at 97%, using a curated database (Dowd et al. 2008).

Results and Discussion

The injection of air impacted the plants' growth. In fact, 98.62 g (±13.12 g) was the average weight for nonaerated lettuce, while 104.42 g (±13.27 g) was the average weight for aerated lettuce. Similarly, longer roots, 16.38 (±1.52) cm, occurred for aerated lettuce compared to nonaerated lettuce, 15.11 (±0.28) cm. Even if aerated pots yielded higher plant mass and root length, the difference between the two treatments was not significant ($p > 0.05$). A similar but more pronounced trend was observed using different crops (Abuarab et al. 2013; Goorahoo et al. 2001, 2002, 2007). For example, bell peppers grown in aerated plots exceeded the bell peppers from the nonaerated plots regarding number (+33%), total weight (+39%), and root mass (Goorahoo et al. 2001, 2002).

The behavior of the three selected PhACs varied during the study (Fig. 1). Higher concentration levels of carbamazepine (up to 160 $\mu g\,L^{-1}$) and gemfibrozil (up to 563 $\mu g\,L^{-1}$) were detected in the leachate, while only small traces (up to 7 $\mu g\,L^{-1}$) of caffeine were observed in the leachate [Figs. 1(a–c)]. Among the three selected PhACs, caffeine showed the lowest relative occurrence (<0.6%), followed by carbamazepine (<13%) and gemfibrozil (<48%) [Figs. 1(a–c)]. The PhACs' exposure had a contrasting impact on the occurrence of the three PhACs throughout the study. In fact, a negligible impact ($p > 0.05$) was observed on the occurrence of caffeine, while a significant impact ($p < 0.001$) was observed in terms of carbamazepine and gemfibrozil. The occurrence of gemfibrozil in the leachate increased over time, increasing from 20% (after 1 day of exposure) to 44% (after 8 days of exposure). Even if the usage of air-injected water reduced the occurrence of PhACs in leachate samples, the difference between the two treatments was not statistically significant ($p > 0.05$) [Figs. 1(a–c)].

Residuals of PhACs, at the $ng\,g^{-1}$ parts per trillion (ppt) concentration levels, were detected in soil samples [Fig. 2(a)]. Among the three PhACs, regardless of the presence/absence of air, gemfibrozil (up to $8.72 \pm 2.49\ ng\,g^{-1}$) and caffeine (up to $253.94 \pm 126.53\ ng\,g^{-1}$) showed the lowest and highest occurrence in soil samples, respectively. The soil in aerated pots showed lower residual concentrations of caffeine ($228.50 \pm 98.76\ ng\,g^{-1}$ versus $253.94 \pm 126.94\ ng\,g^{-1}$), carbamazepine ($112.45 \pm 14.09\ ng\,g^{-1}$ versus $125.80 \pm 38.61\ ng\,g^{-1}$), and gemfibrozil ($5.47 \pm 2.37\ ng\,g^{-1}$ versus $8.72 \pm 2.49\ ng\,g^{-1}$) than those observed in nonaerated pots [Fig. 2(a)]. Overall, the usage of air-injected water reduced the occurrence of PhACs in soil samples. However, the difference between the two treatments was only statistically significant in terms of gemfibrozil ($p < 0.05$).

Lettuce was capable of residual PhACs uptake at the $ng\,g^{-1}$ (ppt) (caffeine and gemfibrozil) and at the $\mu g\,g^{-1}$ (ppb) (carbamazepine) concentration levels [Fig. 2(b)]. Among the three PhACs, regardless of the presence/absence of air, carbamazepine (up to $6.62 \pm 22.83\ \mu g\,g^{-1}$) and caffeine (up to $0.20 \pm 0.09\ \mu g\,g^{-1}$) showed the highest and the lowest uptake, respectively [Fig. 2(b)]. The usage of air-injected water reduced the uptake of caffeine ($0.20 \pm 0.09\ \mu g\,g^{-1}$ to $0.14 \pm 0.04\ \mu g\,g^{-1}$) and gemfibrozil ($0.64 \pm 0.20\ \mu g\,g^{-1}$ to $0.36 \pm 0.08\ \mu g\,g^{-1}$), while the uptake of carbamazepine

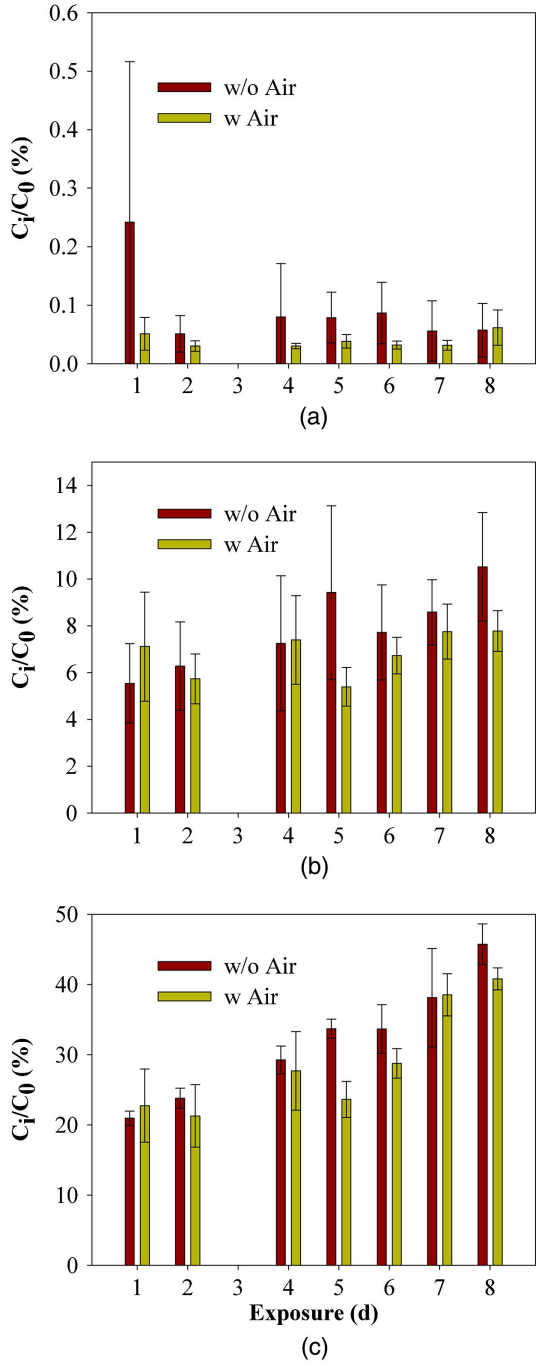

Fig. 1. Average detection, with relative standard deviation ($n = 6$), of (a) caffeine; (b) carbamazepine; and (c) gemfibrozil in the leachate of the two lines (with and without air) during the injection of the three pharmaceutical compounds. Values were normalized to the pharmaceuticals' starting concentration present in the feed water.

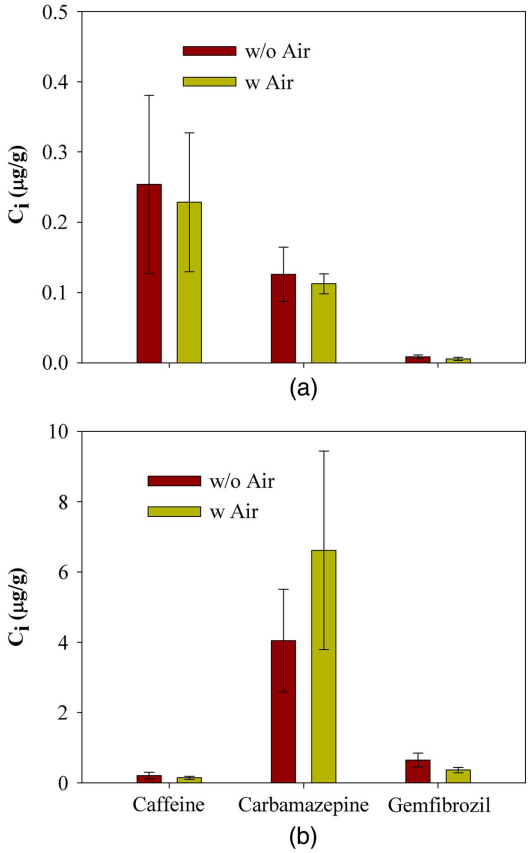

Fig. 2. Detection of caffeine, carbamazepine, and gemfibrozil in (a) soil; and (b) lettuce at the end of the pharmaceuticals' injection ($n = 6$).

increased (4.04 ± 1.46 $\mu g\,g^{-1}$ to 6.62 ± 2.83 $\mu g\,g^{-1}$) [Fig. 2(b)]. The difference between the two treatments was statistically significant in terms of gemfibrozil ($p < 0.05$). Among the different PhACs investigated, caffeine is being sorbed by the solid phase and then either not taken up by the lettuce or degraded within the lettuce. Gemfibrozil appears to have a low sorption affinity for the solid phase but can be degraded in the soil while water is not flowing and can be degraded within the lettuce. Carbamazepine can be sorbed by the solid phase, but it does not appear to be degraded in either the soil or the lettuce once it is taken up. The increase of carbamazepine in the presence of injected air may be related to the increased roots observed in the presence of injected air, which resulted in extraction of water from an overall larger volume of the pot and to the lack of degradation in the plants in contrast with gemfibrozil and caffeine.

The proteobacteria (γ- > β- ≥ α-) were the predominant class of bacteria present in the soil samples throughout the study (Fig. 2). The injection of PhACs as well as the presence/absence of injected-air created a variation in soil microbial communities. Before PhACs' injection, γ-Proteobacteria (40%), β-Proteobacteria (22%), and α-Proteobacteria (14%) were the predominant subclasses observed during the lettuce's growth. After the PhACs' injection, the proportion of the γ-Proteobacteria decreased from 40% to 27% with a corresponding increase in α-Proteobacteria from 14% to 27%. There was also a proportional increase in Actinobacteria (+6%) (Fig. 3). The injection of PhACs had a similar impact on the genus level. *Pseudomonas* (33%) and *Delftia* (19%) were the two predominant species before and after the PhACs' injection in the soil receiving nonaerated water. *Pseudomonas* is a ubiquitous stress-tolerant aerobic environmental bacteria, with a number of plant-associated species. *Delftia* is also an aerobic bacterium. Both decreased (−10%) after the PhACs' injection, while *Methylobacterium* (+10%) and *Methylophilus* spp. (+7%) increased. The low, almost absent, occurrence of caffeine in the leachate may be related to the occurrence of *pseudomonas* in the soil. *Pseudomonas* is known to degrade caffeine (D'Alessio et al. 2015; Gokulakrishan et al. 2005; Yamoaka-Yano and Mazzafera 1999).

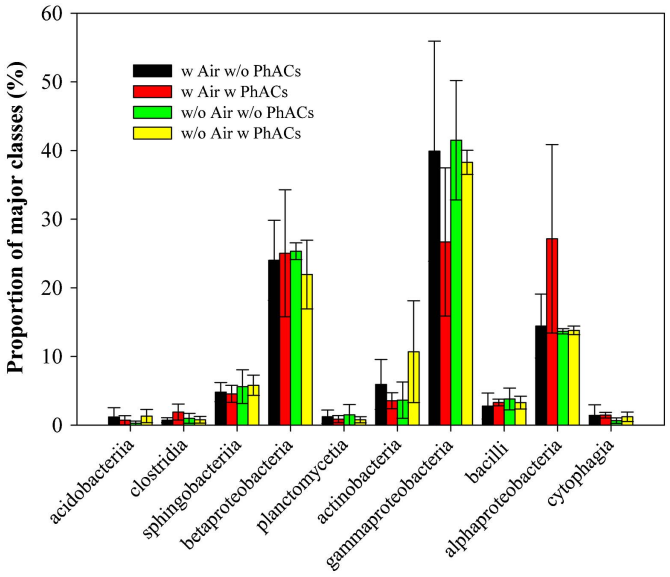

Fig. 3. Most abundant classes at the end of the three pharmaceutical (PhACs) compounds' injection.

Conclusions

Injected air impacted the plants' growth as well as on the removal of PhACs. Average weight and average root length increased by approximately 8% in the presence of injected air. Even if aerated pots yielded higher plant mass and root length, the difference between the two treatments was not significant. The behavior of the three selected PhACs varied during the study. Gemfibrozil (up to 563 $\mu g\,L^{-1}$) showed the highest concentration in leachate samples, while caffeine (up to 253.94 $ng\,g^{-1}$) and carbamazepine (up to 6.62 $\mu g\,g^{-1}$) showed the highest concentrations in soil samples and plant samples, respectively. Except for the plant uptake of carbamazepine, injected air decreased the PhACs' occurrence in leachate, soil samples, and plant uptake. Proteobacteria (γ- > β- ≥ α-) was the predominant class of bacteria present in the soil samples throughout the study. The injection of PhACs as well as the presence/absence of injected-air created a variation in soil microbial communities.

Data Availability Statement

All data, models, and code generated or used during the study appear in the published article.

Acknowledgments

We would like to acknowledge John Petrosso (Mazzei Injector Company, LLC) for proving the Mazzei air injector and his technical support during the study, and Dr. Dave Goorahoo (Fresno State University) for his guidance during the study.

Supplemental Data

Table S1 is available online in the ASCE Library (www.ascelibrary.org).

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