Title

Management of Soluble and Gaseous Nitrogen Losses from Soilless Container Plant Nursery Systems

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Management of Soluble and Gaseous Nitrogen Losses from Soilless Container Plant Nursery Systems

By

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DISSERTATION

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DAVIS

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Abstract:

The Central Valley Regional Water Quality Control Board required agricultural producers to document nitrogen (N) inputs and outputs to complete Irrigation and Nitrogen Management Plans (INMP) for estimation of potentially leachable N. A system nitrogen balance was developed to reduce uncertainty in output N from soilless substrate-based production systems. The majority of input N either remained in the growing substrate (57%) at end of production cycle or was taken up by the plant shoots (5%). Nitrous oxide-N lost from the growing substrate and the bed was 1.5% and 0.01% of input N, respectively. Runoff and soil infiltration N accounted for 6.5% and 2.4% of input N, respectively. Unaccounted N was 27.7% of input N and is attributed to complete denitrification. Environmentally harmful discharges were identified as aqueous N and nitrous oxide (N_2O) lost from the substrate. Very little research has been conducted to understand N_2O emissions from soilless substrates. A Douglas fir (*Pseudotsuga menziesii*) bark-based substrate planted with Crepe Myrtle (*Lagerstroemia indica* 'Whitt II') had controlled release fertilizer incorporated with differing amounts of surface-applied fertilizer. Gas flux and pour-through extract samples were regularly collected. A regression model indicated that significant predictors of N_2O flux were pour-through extract ammonium and nitrate concentration, volumetric water content, and substrate temperature. The total California-scaled fir bark-based substrate production system N_2O-N emissions were greater

than for soil-grown California horticultural crops. Nitrous oxide emissions from soilless substrates are believed to be from heterotrophic denitrification but soilless substrates have physical and chemical properties that could promote nitrification- and denitrification-derived N_2O simultaneously. Fir bark, peat, and peat:fir bark substrates were fertilized with $^{15}NH_4NO_3$, $NH_4{}^{15}NO_3$, unenriched NH_4NO_3, or unfertilized to determine contribution of nitrification and denitrification to N_2O production in soilless substrates. Heterotrophic denitrification accounted for almost all the N_2O emitted from all three substrates but played a larger role in the fir bark and peat:fir bark substrates. Nitrification-derived N_2O emissions began on day 11 in the peat substrate and continued to increase until the experiment ended, contributing to 6% of total N_2O emission from this substrate. Fundamental research to understand N_2O emissions from soilless substrates must be conducted to develop best management practices to reduce global warming potential from soilless-substrate production systems.

Introduction:

The Central Valley Regional Water Quality Control Board required the completion of Irrigation and Nitrogen Management Plans (INMP) by agricultural producers within the Central Valley Basin. The INMP consist of documenting annual nitrogen (N) inputs and outputs to calculate potential N available for leaching into groundwater. Inputs are N existing in soil or organic amendments, and N applied as fertilizer or in irrigation water. Nitrogen output is based on the yield and N content of harvested products.

Performing the necessary INMP computations with actual grower N inputs and estimated N outputs could result in gross over-estimation of potentially leachable N because some uncertainty exists in the fate of the N fertilizer applied in container-plant production. The quantity of N in major crops, like almonds or table grapes, is readily available (Geisseler, 2016). However, container-grown nursery crops do not fit neatly into the INMP worksheet. The whole product, including the shoots, roots, and substrate, is "harvested" and shipped from the nursery grounds to customers. Nitrogen remaining in the container substrate at the time of harvest can range from 0-41% of applied N (Cabrera, 2003; Narvaez *et al.*, 2012, 2013). Nitrogen losses due to leaching and denitrification from soilless substrate can be significant (Cabrera, 2003; Ku and Hershey, 1997; Narvaez et al., 2012, 2013; Stewart *et al.*, 1981). Leaching losses could contaminate ground and surface waters or they could be captured and recycled to reduce N loss. Complete denitrification produces harmless dinitrogen but incomplete denitrification can contribute nitrous oxide (N_2O) to atmospheric N pollution (Myhre *et al.*, 2013).

To help ornamental plant growers fulfill the INMP requirement, a system N balance for production of a woody ornamental plant was developed and losses of applied N from the system were identified. Implementation of best management practices to address environmentally harmful N discharge from nurseries may be more effective than requiring INMP completion. Best management practices for the nursery industry are specific procedures that are most effective at reducing pollutant discharge to protect water resources from contamination with agrichemicals (Bilderback *et al.*, 2013). Best management practices to address water quality are designed to increase production efficiency and reduce environmental degradation.

This research hopes to improve our understanding of N cycling in soilless substrates used for plant production in containers, with the goal of identifying best management practices that mitigate N loss from soilless substrates and improve N use efficiency. The first chapter quantifies N_2O-N emissions, calculates emission factors, and identifies possible contributors to N_2O-N emissions from a fir bark soilless substrate. The second chapter describes an experiment to address knowledge gaps that container-plant growers face in accurately completing the INMP form. The third chapter discusses an experiment to determine the relative contribution of ammonia oxidation and heterotrophic denitrification to N_2O-N emissions from fir bark and peat soilless substrates.

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Chapter 1:

A nursery system nitrogen balance for production of a containerized woody ornamental plant

Abstract

To reduce nitrate contamination of groundwater in intensive agricultural production areas, crop producers should identify nitrogen (N) inputs and outputs to calculate potential N available for leaching into groundwater. However, poor understanding of N fate in container plant production may result in inaccurate estimation of potentially leachable N. To improve understanding of container-applied N fate, an experiment was conducted to measure N inputs and outputs from a woody ornamental plant (*Lagerstroemia indica* 'Whitt II') production system fertilized with controlled-release and surface-applied fertilizer. Two experimental bed types, polyethylene-lined and unlined, were installed at a production nursery in California. Measured N inputs included: the substrate, with fertilizer and roots, and irrigation water N. Outputs included: N remaining in the substrate and plant shoots at the end of the production cycle, nitrous oxide-N gas lost from the substrate and bed soil, and aqueous N lost in runoff during the production cycle. There was a significant difference in runoff N losses from the lined and unlined beds. The difference in runoff N lost between bed types was the amount of N infiltrating into the soil below the growing bed surface. The majority of input N either remained in the growing substrate (57%) at end of production cycle or was taken up by the plant shoots (5%). Nitrous oxide-N lost from the growing substrate and the bed was 1.5% and 0.01% of input N, respectively. Runoff and soil infiltration N accounted for 6.5% and 2.4% of input N, respectively. Unaccounted N was 27.7% of input N and is attributed to complete denitrification.

Future work should address the amount of aqueous N lost from the growing substrate to reduce surface and groundwater contamination.

Keywords: leaching; runoff; ammonium; nitrate; soilless substrate; *Lagerstroemia indica*

1. Introduction

As in many regions of the world, a large proportion of groundwater in the Central Valley of California is either contaminated with nitrate or at risk of contamination due to intensive agriculture (Harter and Lund, 2012). To reduce the risk of future nitrate contamination to groundwater, the California Central Valley Regional Water Quality Control Board required crop producers in the Central Valley Basin to implement nitrogen (N) management plans. The N management plans consisted of documenting yearly N inputs and outputs to develop an N balance sheet. Potential N available for leaching into groundwater is calculated by subtraction of N outputs from inputs. Inputs consist of the total N in the soil and applied as fertilizer, organic amendments, and irrigation water. Output is based on the harvested yield and N content of that material. The quantification of N in harvested yields of most major crops, like

almonds and table grapes, is readily available (Geisseler, 2016). However, some agricultural crops, like container-grown nursery crops, do not fit neatly into the N management plan balance sheet.

Container plant production is more complex than in-ground production of nut, fruit, vegetable, or cereal crops. Thousands of different plant taxa may be grown at a single nursery location in specially-formulated growing substrates and may receive multiple fertilizer applications throughout the production cycle. In most agricultural crops a plant part is seasonally harvested, whereas in container nurseries the whole plant, including the roots, soilless substrate, and container, is harvested year-round and shipped to customers. Use of controlled release fertilizers (CRF), rather than irrigation-applied soluble fertilizers, is recommended as a best management practice to reduce N leaching (Chen and Wei, 2018), and their use has become commonplace for N application in container plant production. Fertilizer salts that are not released from the CRF during the production cycle are shipped with the container-plant product. The complexity of nursery production systems makes it difficult for growers to accurately quantify N inputs and outputs required in the N management plans.

Previous nursery N balance research has focused on the container-plant product, documenting N input and output to the individual plants and growing substrate (Cabrera, 2003; Ku and Hershey, 1997; Narvaez *et al.*, 2012, 2013; Stewart *et al.*, 1981). Plant N uptake was 6% to 50% for four woody and two herbaceous ornamental perennial species, depending on N fertilizer rate and application method (Cabrera, 2003; Ku and Hershey, 1997; Narvaez et al., 2012, 2013; Stewart et al., 1981). For plants fertilized with CRF, 5% to 20% of the N remained in the prills and 34% to 57% of CRF-released N remained in the substrate at the end of production (Narvaez

et al., 2012, 2013). A significant portion of both CRF- (Narvaez et al., 2012, 2013) and irrigation-applied N (Cabrera, 2003; Ku and Hershey, 1997; Stewart et al., 1981) was leached from the growing substrate. Unaccountable N could be a significant proportion of applied N (up to 62%), depending on fertilizer application method and rate, and has been attributed to denitrification (Cabrera, 2003; Ku and Hershey, 1997; Narvaez et al., 2012, 2013; Ristvey *et al.*, 2004; Stewart et al., 1981).

Performing the necessary N management plan computations with actual grower N inputs and estimated N outputs based on previous research may result in gross over-estimation of potentially leachable N because some uncertainty exists in the fate of the N fertilizer applied in container-plant production. A more robust determination of leachable N should include measuring aqueous N that leached into the soil below the growing bed. Previous research monitoring NO_3-N leaching into soil underlying a container-plant crop resulted in 51.8 – 405 kg NO_3-N ha^{-1} loading into the soil over two and half months to a year (Brand *et al.*, 1993; Colangelo and Brand, 1997; Colangelo and Brand, 2001). However, the soil NO_3-N loading studies had no runoff which is atypical of irrigation events, resulting in possible overestimation of soil NO_3-N loading at a container-plant nursery. Frequent irrigation to replace limited substrate moisture results in wet soil conditions that may encourage denitrification. Denitrification could reduce NO_3-N loading to the soil underlying the growing beds (Colangelo and Brand, 1997) and the rate of denitrification should be measured to accurately quantify N loading to this soil.

The authors are not aware of any research that has tried to develop a container-plant N balance that also documents N leaching into the underlying soil, N runoff from the container-plant

production area, and gaseous N losses from the substrate and underlying soil. To achieve a nursery production system N balance for a woody ornamental container-plant crop, all components of the N management plan and losses were documented during a typical production cycle at a California nursery. Nitrogen inputs that were measured included: substrate with CRF and roots, irrigation water, and surface-applied fertilizer (Figure 1.1). Harvested product N outputs included: the substrate, including roots and any remaining fertilizer, and plant shoots at end of the production cycle (Figure 1.1). Additional outputs included gaseous N emitted from the substrate and aqueous N that was leached from the substrate or applied as irrigation water and flowed off the growing beds during the production cycle (Figure 1.1). To more accurately estimate N accumulation in soil underlying growing bed, gaseous N emitted via denitrification from this soil was estimated. The outputs and inputs were used to develop a nursery system N balance for the production cycle of a woody container plant.

2. Materials and methods

2.1 Nursery site

The research was performed at an ornamental plant nursery near Sacramento, California, USA during an 81-day woody ornamental production cycle from 4 May 2018 – 24 July 2018. The nursery, which was located on a clay loam soil, grew a large variety of woody and herbaceous perennials for sale to retail garden centers and landscape contractors. During the experiment, the mean temperature and relative humidity were 21.5°C and 57%, respectively. Rain occurred on two days during the production cycle, 3 mm on May 16 and 13.4 mm on May 25.

2.2 Experimental bed construction

Four lined and four unlined experimental beds (4.6 x 12.2 m) were installed in a growing area at the nursery. The beds were oriented with length east-to-west and the width north-to-south. The bed types were randomly arranged in a row and adjacent to each other. Adjacent beds were separated with a 61-cm buffer, covered with 0.152-mm polyethylene sheeting (Home Depot, Atlanta, GA) and gravel, to convey irrigation and runoff water away from the experimental area. All the beds were sloped (<2%) with the lowest point in the southwest corner. An elevated border (Benda Board, Epic Plastics, Lodi, CA) was staked into the ground at the perimeter of each bed to prevent runoff water from flowing beyond the bed perimeter.

The unlined beds represented a typical commercial bed, with plants placed directly on gravel-covered soil. A 20-cm deep below-grade trench was dug along the inside of the elevated border of the unlined beds. Trenches were lined with 0.152-mm polyethylene sheeting. Soil was placed on top of the polyethylene sheeting and compacted with a vibrating soil compactor to ensure a good seal. Lined beds and their elevated borders were covered with two layers of 0.152-mm polyethylene sheeting that was sandwiched between non-woven filter fabric (Ground Cover Industries, Inc., Santa Rosa Beach, FL). All beds were covered with 2-cm crushed gravel to maintain a uniform surface. A 208-L plastic tank was installed below grade in the southwest corner of each bed to capture irrigation runoff water. For the unlined beds, a concrete skirt was constructed around the opening of the tank to prevent infiltration of the runoff water into the back-filled hole. For the lined beds, a hole was cut in the polyethylene sheeting over the tank to allow water to drain into it.

A submersible pump (PE-2H, Franklin Electric Co., Inc., Fort Wayne, IN) and float switch (LV612-P, Omega Engineering, Inc., Stamford, CT) were installed in each runoff capturing tank. Water was pumped out of each tank through a separate flowmeter (M25 Nutating Disk Meter Model RCDL, Badger Meters, Madison, WI) connected to a pulse recording datalogger (Pulse101A, Madgetech, Inc., Warner, NH). The datalogger recorded pulses per minute; one pulse equaled 3.785 L of water. The water was pumped downslope from the experimental beds through 13-mm polyethylene drip tube.

2.3 Irrigation

All beds were located within a single irrigation zone that applied water overhead through rotary stream sprayers (MP-3000, Hunter Industries, San Marcos, CA) mounted on shrub sprinkler bodies pressure regulated at 275 kPa (PROS-00-PRS40, Hunter Industries, San Marcos, CA). The water used for irrigation was from a groundwater aquifer. Mean precipitation rate tests were conducted for each bed. Twenty-four Texas A&M University catch cans (College Station, TX) were placed in a consistent pattern within each bed. Irrigation was applied for ten minutes, cup volume was manually read, and mean precipitation rate was calculated. There was no significant difference ($p > 0.1$) in mean precipitation rate (9.8 mm hr^{-1}) applied to individual beds (data not shown). Beds were irrigated for 50 minutes twice per day resulting in mean total irrigation of 1,112 mm over 81 days to all beds.

2.4 Planting

The soilless fir bark growing substrate consisted of 7:1 (v:v) Douglas fir (*Pseudotsuga menziesii*) bark:washed sand that was mechanically incorporated with 3.47 kg Apex 9-2-0 sulfur-coated

urea (J.R. Simplot Co., Boise, ID) and 6.16 kg Osmocote Plus 15-9-12 controlled release fertilizer (CRF) (Scotts MiracleGro, Marysville, OH) m^{-3} substrate. The Osmocote Plus consisted of 6.6% NO_3-N and 8.4% NH_4-N, which resulted in 0.41 kg NO_3-N and 0.52 kg NH_4-N m^{-3} substrate. On day three after planting, 35 g of 20-9-9 fertilizer (Loveland Products, Loveland, CO) was applied to the substrate surface of each container. The N source in this surface-applied fertilizer was urea-formaldehyde. Surface-applied 20-9-9 fertilizer provided 0.31 kg N m^{-3} substrate. The soilless substrate was 24.4-cm deep in the #3 (14-L) containers. The substrate volumetric water content was 39.6% and air-filled porosity was 44.8% at container capacity. Substrate bulk density was 0.43 g cm^{-3}, the organic matter component constituted 45%, and the mineral component was 55% by weight. For information about electrical conductivity and pH of growing substrate solution, please refer to Pitton et al. (2021).

On day zero, *Lagerstroemia indica* 'Whitt II' plants in #1 (3-L) containers were individually transplanted into 14-L containers filled with the fir bark substrate. *Lagerstroemia* plants were selected because they require an average amount of N fertilizer compared to other ornamental plants (Evans, 2014). Prior to transplanting, all plants were pruned to approximately one meter in height. Mean shoot dry biomass of *Lagerstroemia* plants was 14.64 and 97.54 g at beginning and end of the experiment, respectively. A portion (<20%) of the plants were planted off-center in the pot to allow for installation of a 10-cm diameter static chamber for gas sampling (Pitton *et al.*, 2021). All plants were randomly placed in the experimental area by nursery staff on 61-cm centers. The experimental beds had 143 to 155 plants equivalent to 24,062 plants per ha.

2.5 Data collection

2.5.1 Water sampling

Excess applied irrigation and substrate leachate water flowed off the beds into the buried water collection tanks and was pumped through the 13-mm polyethylene tube connected to the flowmeter. Downstream from the flowmeter, a short length of 6-mm polyethylene tubing with a point-source drip emitter (SW10, Rain Bird Corp., Azusa, CA) was connected to the 13-mm tubing. The 4-L h^{-1} drip emitter delivered water into a plastic bucket where it was held for collection. A 250-mL aliquot was collected from each plastic bucket on days 1, 4, 5, 7, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, and 81 after planting. The remainder of runoff from the plastic bucket was poured downstream where it would not enter any beds. To capture runoff samples from irrigation events in the afternoon and following morning, the drip emitter was placed in the collection bucket before the afternoon irrigation cycle on the day prior to water sample collection. On each water sample collection day, four irrigation water samples were also collected, while the irrigation system was running, to quantify total N concentration in applied irrigation water.

The NO_3^- and NH_4^+ ion concentration in water samples was determined by diffusion-conductivity method (Carlson *et al.*, 1990). The concentration of total Kjeldahl nitrogen (TKN) in water samples was determined by sulfuric acid digestion, distillation, and titration (EPA Method 350.2). Weekly N mass load in runoff water from each bed was calculated as the product of weekly N concentration of each N species and total weekly flow rate centered on the sampling day.

2.5.2 Gas sampling

Nitrous oxide (N_2O) emissions from substrate surface and soil below gravel on unlined beds were collected 14 and 15 times, respectively, over an 81-day period. They were both measured on days 1, 4, 5, 7, 11, 18, 32, 39, 46, 53, 60, 67, 74, and 81 after planting, while N_2O flux from soil below gravel on unlined experimental beds was collected on day 25 after planting as well. Substrate N_2O-N flux sampling was conducted according to Pitton et al. (2021). A total of eight potted plants were randomly selected from across all the beds for substrate-to-atmosphere N_2O flux measurements on each sampling day. Eight bed locations were randomly selected for bed soil-to-atmosphere N_2O flux measurements on each sampling day. At least 24 hours before each sampling event, a PVC base for the gas flux chamber was installed into each selected potted plant or bed location. On the sampling day, all bases installed in bed soil were filled to the top with water, and little to no water was lost via infiltration. Four of these bases were filled with acetylene-saturated water to inhibit N_2O reductase and prevent the conversion of N_2O to N_2 (Ryden *et al.*, 1979) and the other four were filled with irrigation water. It was assumed that all N_2O measured from bed soil with acetylene-saturated water would have been converted to N_2, therefore N_2O flux samples from bed soil treated with acetylene represents complete denitrification. Gas flux samples from bed soil without acetylene represent incomplete denitrification.

Gas flux sampling began three hours after acetylene-saturated water application. Gas fluxes were measured using the static chamber method (Hutchinson and Livingston, 1993) with insulated, vented round PVC chambers that were installed onto the PVC bases. Effective chamber height was 17 cm for substrate gas flux samples and 15 cm for bed flux samples. Gas samples were collected at each of four 10-minute time intervals and stored in a laboratory

cabinet prior to analysis using a Shimadzu gas chromatograph (Model 2014, Shimadzu Corp., Kyoto, Japan) linked to a Shimadzu auto sampler (Model AOC-5000).

The gas chromatograph was equipped with a ^{63}Ni electron capture detector for N_2O. The gas chromatograph was calibrated daily using analytical grade carbon dioxide and N_2O gas standards (Airgas Inc., Sacramento, CA) prepared on the same day that samples were collected. Chamber gas concentrations were converted to mass per volume using the ideal gas law and chamber air temperature, measured by a temperature datalogger (Onset Corporation, Bourne, MA).

Gas fluxes were calculated with 'gasfluxes' package (Fuss, 2019) for R using chamber volume, surface area, and rate of change in chamber gas concentration (Hutchinson and Mosier, 1981). Kappa.max was used to select gas flux from the different gas flux calculation schemes (Huppi *et al.*, 2018). The gas fluxes measured each sampling day were used to estimate cumulative plant container-scaled and cumulative bed area gas emissions with trapezoidal integration of daily-sampled flux, under the assumption that the measured flux represented the daily mean flux and the mean daily flux changed linearly between measurements (Zhu-Barker *et al.*, 2015). For the plant containers, it was assumed that N_2O-N efflux escaped solely from the top of the substrate in the plastic container.

2.5.3 Initial and final plant harvest

Twelve plants were randomly selected from all the experimental beds at both the beginning and end of the experiment. The plant shoots were cut off at the crown and dried at 60°C. Leaves and stems from each plant were separated, weighed, and ground until they could pass

through a 2-mm mesh before TKN, NO_3-N, and NH_4-N analysis. Plant total Kjeldahl nitrogen analysis was via boric acid titration method (Gavlak *et al.*, 2005) and NO_3-N and NH_4-N analyses were conducted by the diffusion-conductivity method (Carlson et al., 1990). Plant shoot N from the beginning of the experiment was subtracted from the plant shoot N at conclusion of experiment to calculate plant N uptake during the production cycle.

Each plant's substrate and root ball were dried at 60°C and ground. A 50-g sample of the dried substrate plus roots was submitted to Perry Labs (Watsonville, CA) for TKN, NO_3-N, and NH_4-N analysis. Saturated paste extracts were prepared from the remaining substrate plus roots. In addition, saturated paste extracts were prepared from dried and ground samples of unused, unfertilized fir bark:sand substrate. The substrate extracts were analyzed for concentration of NO_3-N and NH_4-N via diffusion-conductivity method (Carlson et al., 1990). Extracts were analyzed for concentration of TKN by digestion and subsequent analysis with diffusion-conductivity method (Carlson et al., 1990).

2.6 Calculations

2.6.1 Bed scale nitrogen estimation

The total amount of applied N in substrate and 20-9-9 fertilizer per bed was calculated as the total amount of N in the soilless substrate, as estimated from substrate TKN, NO_3-N, and NH_4-N analysis at experiment initiation, and the 35 g of 20-9-9 surface-applied fertilizer multiplied by the number of plants in that bed. The total N applied in irrigation water was calculated as the mean concentration of all irrigation water samples multiplied by the total volume of water applied to each bed. Total plant shoot N uptake per bed was measured as the mean total N

mass uptake by the plants from day 0 to 81 after planting multiplied by the number of plants in that bed. Total N in the substrate on day 81 after planting was calculated as the mean total amount of N in the substrate per container from twelve plants multiplied by the number of plants in that bed. Total N_2O-N loss from the substrate was measured as total cumulative N_2O-N emission per pot for the length of the nursery production cycle (Pitton et al., 2021) and multiplied by the number of plants in that bed. Total N mass load in runoff water for each bed during length of experiment was sum of weekly mass loads.

2.6.2 Nursery system nitrogen balance estimation

The total input N to the nursery system N balance was calculated as the sum of 1) N in the substrate and the surface-applied fertilizer, multiplied by plants ha^{-1}, and 2) applied N in irrigation water ha^{-1}. The total amount of N at experiment completion, when plants were ready for sale, was calculated as the sum of the remaining N in the soilless substrate and plant N uptake per pot from day 0 to 81, multiplied by plants ha^{-1}, and will hereafter be referred to as "sold N". The quantity of N_2O-N lost from the substrate was the total N_2O-N emitted per pot (Pitton et al., 2021) multiplied by the number of plants ha^{-1}. Bed runoff N was calculated as the mean runoff N from the lined beds multiplied by the number of beds ha^{-1}. Bed infiltration N was calculated using the difference in mean runoff N from the lined and unlined beds. Bed denitrification N (with and without acetylene) was calculated as total N_2O-N emitted ha^{-1}. Unaccounted N was the difference in total input N and all other fates.

2.7 Statistics

To improve normality of the residual errors and homoscedasticity, the number of plants and the amounts of N input, utilized by plant shoots, and remaining in the substrate was Box-Cox transformed. Optimal lambda (λ) was 39.0 for the plants per bed data and 36.7 for the other transformed data. A Student's T-test was performed to determine significant differences (α = 0.05) between different N variables measured for the lined and unlined beds. All statistical analyses were conducted in R statistical software.

3. Results

There was no significant difference ($p > 0.05$) in mean number of plants per bed for the lined and unlined beds (Table 1.1). The total input N, total shoot uptake N, total substrate N, and total N_2O-N emissions were based on the number of plants in each bed. Therefore, there were no significant difference ($p > 0.05$) seen between the lined and unlined beds for total N fertilizer applied, total plant shoot uptake, total substrate N, N_2O-N emissions, or unaccountable N (Table 1.1). The total irrigation applied N was also the same between beds. Runoff N from lined beds was greater ($p = 0.015$) than from unlined beds.

After day 1, the total N loss rate in runoff water increased steadily for about 25 to 30 days, then decreased (Figure 1.2). During the first 30 days, the rate of total N loss from lined beds was approximately 50% greater than from unlined beds (Figure 1.2). The lined beds continued to lose N at a higher rate than unlined beds until day 52, after which the rate of loss was similar for both bed types (Figure 1.2). Nitrate, the predominant N species in runoff, was lost from lined and unlined beds at similar rates for the first 38 days (Figure 1.2). For the next 7 days, lined beds continued to lose NO_3-N at the same rate, while the rate of loss from unlined beds

decreased (Figure 1.2). After day 45, both bed types lost NO_3-N at similar rates. There were significantly more cumulative NH_4-N (p = 0.0002) and total N (p = 0.015) in runoff from lined beds (Table 1.2).

Nitrous oxide emissions from the growing bed soil during the 81-day production cycle were 32.9 ± 9.6 and 9.1 ± 4.3 mg N m^{-2} with or without acetylene-saturated water application, respectively. Total N_2O-N loss from the soil below the growing bed was significantly greater (p = 0.026) for the acetylene-treated than untreated gas flux samples.

4. Discussion

An N balance for the *L. indica* production system was calculated using the results obtained from the lined and unlined beds based on density of 24,062 plants ha^{-1} spaced on 61-cm centers (Table 1.3). The finished plant ready for wholesale retained 62% of total input N (Table 1.3) with 5% of input N partitioned in the plant shoot while the substrate retained 57% of the total input N (Table 1.3). Nitrogen retained in the substrate at the end of production cycle included organic N in plant roots or immobilized in the fir bark substrate, and inorganic N from surface-applied fertilizer and CRF. An N reserve in growing substrate ensures that the plants will continue to be aesthetically appealing at the time of purchase by the consumer.

Substrate N_2O-N loss was only 1.5% of total input N, but it was estimated that N_2O-N emitted and subsequent global warming potential from nursery production in California exceeded four other horticultural crops (Pitton et al., 2021). Nitrogen losses in bed runoff, estimated as the mean runoff N value from unlined beds, represented 6.5% of the total input N (Table 1.1). Capture and recycling of runoff is common at nurseries in California (Pitton *et al.*, 2018), and

reuse of N-laden runoff could partially offset additional fertilizer needs (Raudales *et al.*, 2017). Nitrogen that infiltrated into the soil below the growing bed, which amounted to 2.4% of total input N, has the potential to contaminate underlying aquifers (Harter and Lund, 2012). Although nearly 23 kg N ha^{-1} infiltrated during the woody ornamental production cycle of 81-days, total nursery acreage in California is small compared to that of other horticultural systems (Pitton et al., 2021), which limits the overall impact. Total N gas emission from the growing bed soil was approximately one percent of total N infiltration below growing bed (Table 1.3) indicating that denitrification does not mitigate the N loading to the soil underlying growing beds and groundwater. Nearly 28% of total input N was unaccounted for (Table 1.3) which is comparable to other N balance studies (Cabrera, 2003; Ristvey et al., 2004; Stewart et al., 1981). Like other studies, we attribute most of this to N loss as N_2 following complete denitrification in soilless substrate.

The large NO_3-N mass runoff rate for both bed types during majority of the experiment (Figure 1.2) was to be expected (Cox, 1993; Narvaez et al., 2012) due to negative charge of substrate and NO_3-N. The discrepancy in total runoff N between bed types was predominantly due to the difference in amount of NH_4-N in the runoff (Figure 1.2 and Table 1.2). The clay loam soil, with high cation exchange capacity, underlying the gravel of the unlined beds reduced the amount of NH_4-N in runoff compared to the lined beds. However, the NH_4-N held by the soil below the gravel bed could leach as NO_3-N into groundwater when nitrification occurs (Colangelo and Brand, 2001).

Both bed styles had high N runoff rates early in the experiment (Figure 1.2), which may indicate an excess of broken CRF prills, a lack of root establishment in growing substrate to utilize

available N, and low N demand of young plants. A study by Huett and Morris (1999) found that damaged Osmocote prills resulted in 3-15 times greater leaching of N than undamaged prills. Other studies indicate that greater N leaching occurred before plant roots became established in the container (Broschat, 1995; Cox, 1993; Hershey and Paul, 1982; Huett and Morris, 1999) which is consistent with results from this study. The total N in runoff, as a percentage of total fertilizer N, was 21.2% and 13.3% for the lined and unlined beds, respectively. For a variety of woody and herbaceous ornamental plants, 4 – 60% of applied CRF N was leached from containers (Broschat, 1995; Cox, 1993; Hershey and Paul, 1982; Narvaez et al., 2012) and leaching losses depended on amount of applied N (Hershey and Paul, 1982; Narvaez et al., 2012) and application method (Broschat, 1995; Cox, 1993). Differences among studies in percentage of applied N that leached may be due to variation in leaching fraction (Huett and Morris, 1999; Tyler *et al.*, 1996).

Estimated N loading to the soil underlying the *L. indica* plant production area (Table 1.3) was 6 – 25% of NO_3-N loading measured for studies with CRF-fertilized ornamental plants that had a similar production time (Brand et al., 1993; Colangelo and Brand, 1997). However, no N runoff, either leachate or irrigation water, occurred in their experiments. The sum of N in runoff and bed infiltration from this study was similar to N loading to soil underlying growing areas reported by Colangelo and Brand (1997), but was still much less than reported by Brand et al. (1993). Colangelo and Brand (1997) irrigated plants between every two to five days, while the plants in this study were irrigated twice each day. Frequent irrigation in this study maintained soil moisture level near saturation, which facilitated runoff and reduced N leaching into soil underlying the growing beds.

Nitrous oxide-N emissions from the growing bed soil below the gravel were very low during the entire study period. Acetylene is known to inhibit N_2O reductase (Balderston *et al.*, 1976; Yoshinari and Knowles, 1976) and acetylene-saturated water was successfully used to inhibit N_2O reductase in saturated soil conditions (Bragan *et al.*, 1997). It was assumed that all N_2O-N emitted from the acetylene-saturated water treatment would have been converted to N_2 via N_2O reductase if not inhibited. Acetylene-saturated water significantly increased the total N_2O-N emitted from the growing bed soil. The estimated N_2 emitted from complete denitrification in the soil underlying the growing beds was an order of magnitude less than the lowest reported N_2 emissions from other California agricultural soils (Rolston *et al.*, 1976; Rolston *et al.*, 1982; Rolston *et al.*, 1978). Low N_2O-N flux, both acetylene-inhibited and uninhibited, from the growing bed soil may be due to standing water in the chamber base that was present during every sampling event. In a laboratory incubation of nine clay soils with entire pore space filled with water, soils with 3-cm standing water had significantly lower mean maximum and daily N_2O-N flux than soils without standing water (Bandibas *et al.*, 1994). Low N_2O-N emission rates from waterlogged soils are consistent with undetectable denitrification enzyme activity in permanently saturated riparian soils (Hill, 1996). It is not surprising that the growing bed soil was waterlogged because the bed was irrigated twice daily and the infiltration rate was 1 mm hr^{-1}, which may be partially due to intentional compaction of the bed soil during nursery construction. If the growing bed soil had been permitted to dry between irrigation events, more denitrification may have occurred.

Previously reported N balances for woody or herbaceous ornamental plant production could not account for 4% to 62% of applied N, whether from controlled-release or irrigation-applied

fertilizer (Cabrera, 2003; Ku and Hershey, 1997; Narvaez et al., 2012, 2013; Ristvey et al., 2004; Stewart et al., 1981). All these studies attributed unaccounted N to denitrification from the growing substrate (Cabrera, 2003; Ku and Hershey, 1997; Narvaez et al., 2012, 2013; Ristvey et al., 2004; Stewart et al., 1981). We attribute unaccounted N in our study to complete denitrification and conversion to N_2 because, unlike previous researchers, we were able to account for N_2O-N emission from the growing substrate. Ammonia volatilization is an unlikely contribution to unaccounted N in this study because the pH of fir bark substrate solution was likely acidic and ammonia volatilization occurs more readily at alkaline pH (pH >7.5) (Freney and Denmead, 1992). Although the pH of fir bark substrate solution was not measured for this experiment, the pour-through extract pH for a greenhouse experiment with the same fir bark substrate, fertilizer type, and rate was acidic throughout that experiment (Pitton et al., 2021).

Shoot N uptake during the 81-day production cycle was 9.7% of total applied fertilizer N. *Lagerstroemia indica* leaves in this experiment had similar concentrations of total N, NO_3-N, and NH_4-N (data not shown) as leaves of *Lagerstroemia* x 'Tonto' plants at optimal irrigation-applied N concentration (Cabrera, 2003) and the lower N utilization in this study indicates that the plants were over fertilized. Excessive N application to woody ornamentals may be common among plant producers due to low cost of N fertilizer and desire to eliminate risk of N deficiency. Surplus N application may not be exclusive to commercial ornamental growers as some researchers applied excessive N to woody ornamentals during N balance experiments (Narvaez et al., 2012; Stewart et al., 1981).

Approximately 57% of input N remained in the fir bark substrate at three months of production and may be due to Osmocote CRF that has five to six-month longevity at the mean substrate

temperatures recorded (Pitton et al., 2021). In a study with *V. tinus*, only 5% of applied N remained in the CRF (Narvaez et al., 2012) with the majority of substrate N immobilized in the media. Producing plants using a CRF product with greater longevity than needed to complete the production cycle ensures sufficient fertilizer is available for plant health while waiting for purchase by the end consumer.

5. Conclusion

The N balance indicates that applying N fertilizer that exceeds plant demand results in leaching and runoff losses as well as greenhouse gas emissions. Along with other nursery best management practices, optimizing N application rates to meet plant demand improves nitrogen use efficiency (Chen *et al.*, 2001) and many extension publications exist on implementing best management practices in nursery production. However, outreach efforts should continue to be made to inform growers on how to improve nutrient use efficiency.

6. Acknowledgements

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Tables and Figures

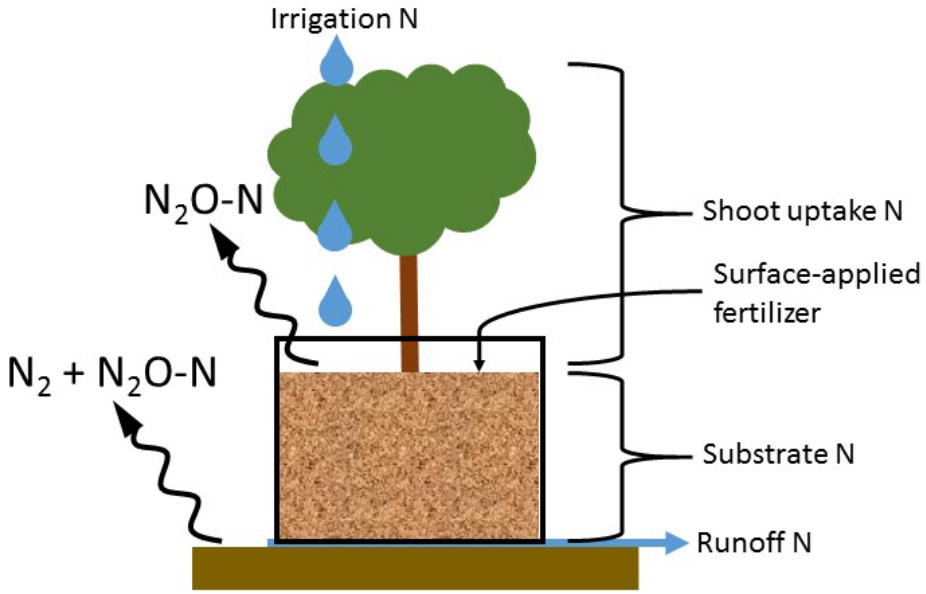

Figure 1.1. Diagram of nitrogen (N) inputs and outputs measured to develop the nursery system N balance. Inputs include substrate N (fertilizer, roots, and media), surface-applied fertilizer, and irrigation water N. Outputs include N in runoff water, shoot uptake, remaining in substrate, and nitrous oxide gas from substrate (N_2O-N) and growing bed soil ($N_2 + N_2O$-N).

Table 1.1. The mean number of plants and nitrogen (N) inputs and outputs from two types of experimental beds at an ornamental production nursery. Values represent mean of four experimental beds per treatment. Percentages of total input N are presented below each input or output. Lowercase letters following values indicate significant differences ($p < 0.05$) between lined and unlined beds.

Bed type	Mean plants bed^{-1}	Mean Nitrogen (kg N bed^{-1})							
		Inputs			Outputs				
		Substrate[a]	Irrigation	20-9-9 fertilizer[b]	Shoot uptake	Substrate[c]	N_2O-N[d]	Runoff	Unaccountable
Lined	153	4.680	0.007	1.071	0.296	3.317	0.089	0.521 a	1.683
		81%	<0.1%	19%	5.1%	58%	1.5%	9%	29%
Unlined	151	4.773	0.007	1.057	0.292	3.274	0.087	0.377 b	1.797
		82%	<0.1%	18%	5.0%	56%	1.5%	6.5%	31%

a. The amount of input N in substrate is based on number of plants per bed and includes fir bark substrate, controlled release fertilizer, and transplant roots.

b. The amount of input N from 35 g pot^{-1} of surface-applied 20-9-9 fertilizer is calculated based on number of plants per bed.

c. The amount of output N in substrate includes fir bark substrate, remaining fertilizer, and plant roots and is based on number of plants per bed.

d. The amount of N_2O-N lost is based on total N_2O-N emitted from substrate during the 81-day production cycle (Pitton et al. 2021) and is based on the number of plants per bed.

* Indicates significant difference at $p < 0.01$.

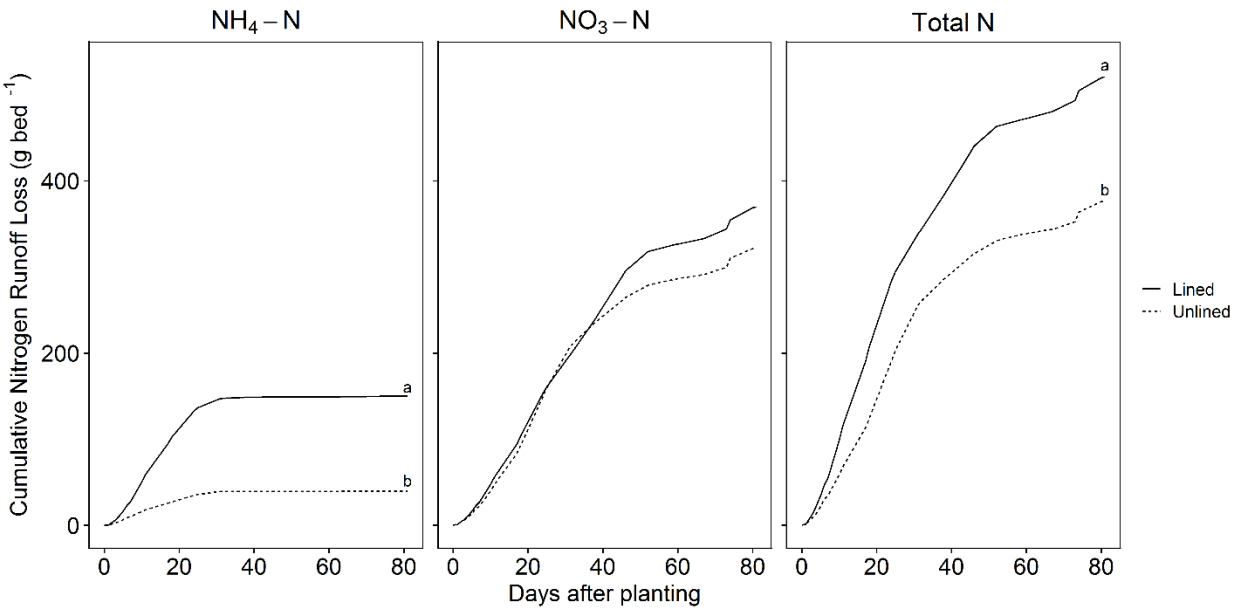

Figure 1.2. Cumulative ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), and total nitrogen (Total N) in runoff water for lined and unlined beds during production of a woody ornamental plant at a container-plant nursery. Significant differences ($p < 0.05$) between lined and unlined beds for each nitrogen species are designated by different lowercase letters next to the lines.

Table 1.2. Total cumulative nitrogen load from experimental beds. Lowercase letters following values indicate significant differences ($p < 0.05$) between lined and unlined beds.

	Total Nitrogen Load (g bed^{-1})		
Bed type	NH_4-N	NO_3-N	Total N
Lined	150 a	370	521 a
Unlined	40 b	322	377 b

Table 1.3. Nitrogen balance for a container plant production system at a nursery. The first row below each column header is the mass of nitrogen per hectare and the second row is the amount of nitrogen as a percentage of the total applied nitrogen.

	Input[a]	**Sold[b]**	**Substrate N_2O-N**	**Bed runoff[c]**	**Bed infiltration[d]**	**Bed (N_2 + N_2O)-N**	**Unaccounted[e]**
Nitrogen (kg ha^{-1})	917.83	568.23	13.93	59.70	22.47	0.33	253.18
Percent of Input N	100.0%	61.9%	1.5%	6.5%	2.4%	<0.01%	27.7%

a. Input N is the sum of N from incorporated CRF, fir bark, and plant roots at transplanting, fertilizer surface-applied on day 3, and irrigation water throughout the experiment.

b. The amount of N sold is the sum of total N in plant product at time plants are ready for sale and includes N remaining in the CRF, fir bark substrate, and plant shoots and roots.

c. Calculated as the total mass of N in the runoff from the unlined beds.

d. The mass of N infiltrating the soil below the growing bed was calculated as the difference of runoff N from the lined and unlined beds.

e. Unaccounted N is the sum of Sold, Substrate N_2O-N, Bed runoff, Bed infiltration, and Bed (N_2 + N_2O)-N subtracted from the total Input N.

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Chapter 2: Greenhouse gas emissions and global warming potential from a woody ornamental production system using soilless growing substrate

ABSTRACT

This research aimed to estimate methane (CH_4) and nitrous oxide (N_2O) fluxes and subsequent global warming potential (GWP) for a Douglas fir (*Pseudotsuga menziesii*) bark-based substrate production system. Fir bark-based substrate had controlled release fertilizer (CRF) incorporated with differing amounts of surface-applied fertilizer. In a nursery study and greenhouse experiment, gas flux samples were regularly collected. Total cumulative N_2O emissions and GWP were greatest from the greenhouse treatment with the most surface-applied fertilizer. A regression model indicated that significant predictors of N_2O flux were pour-through extract ammonium (NH_4-N) and nitrate (NO_3-N) concentration, volumetric water content (VWC), and substrate temperature. Net CH_4 flux was negligible for all treatments during both studies. The N_2O-N seasonal emission factor was 2.58 – 3.08, greater than for soil-grown California horticultural crops. These results indicate that N_2O is the major greenhouse gas from soilless substrate and should be the focus of mitigation efforts.

Keywords: Nitrous oxide, methane, nursery, container plants, growing media

INTRODUCTION

Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are the three largest anthropogenic contributors to the greenhouse effect, in that order.[1] From 2005 to 2013, there was a 10%, 2%, and 6% increase in global CO_2, CH_4, and N_2O emissions, respectively.[1] Agriculture is a significant contributor of GHGs to the global pool.[2] As with most global CO_2

emissions, agricultural CO_2 emissions are mainly attributed to fossil fuel consumption,[2] while soils also release CO_2 after tillage events.[3] Methane from agriculture is produced under anoxic conditions[4] and is linked to animal, rice, and dairy production.[2] Increases in atmospheric N_2O concentration are predominantly from agriculture as a result of synthetic nitrogen fertilizer and manure use.[2]

Global warming potential (GWP) quantifies the greenhouse effect contribution and may be applied to industries, practices, production systems, etc.[1] Global warming potential allows for normalization of gas emissions to a common scale in CO_2 equivalents (CO_2e).[5] Normalization of GHGs to CO_2e is based on radiative forcing over a specific timeframe. Radiative forcing of CH_4 and N_2O over one hundred years is estimated 34 and 298 times more than the same mass of CO_2, respectively.[1] Understanding GWP from agricultural production systems is important because it can be utilized in Life-Cycle Assessments to determine the total GWP of a harvestable product.[6] Quantification of GHG from soilless crop production systems is central to estimating the associated GWP, increasing the accuracy of Life-Cycle Assessments, and assessing the environmental impact of changes in production practices.

Previous research on GHG emissions from soilless growing substrates has focused on those containing pine bark[7-9] or peat.[10,11] Unsurprisingly, larger volumes of pine bark-based substrate resulted in greater GHG emissions than smaller volumes.[7] Incorporating controlled release fertilizer (CRF) into pine bark-based substrate generated greater N_2O emissions than placing CRF into a one or more holes in the substrate ("dibbling"), or surface-applying with CRF.[8] Further research indicated that overhead irrigated pine bark-based substrate generated more N_2O emissions than drip irrigated when both had CRF incorporated.[9] In the presence of nitrate,

peat based-substrates generated greater N_2O emissions at greater VWC, suggesting that N_2O emissions are from denitrification processes.[10,11]

Pine bark is the predominant organic substrate component used by nursery growers in the Eastern United States,[12] and peat-based substrates are common in greenhouse production.[13] Along with peat and pine bark, a number of other organic amendments are used in growing substrates in Western U.S. nursery crop production, including coconut coir, rice hulls, and fir or redwood bark and sawdust.[14] As limited research has been performed on GHG emissions from soilless substrates, it is unclear if all organic substrate amendments produce similar GHG emissions and GWP.

The goal of this research was to identify the magnitude and drivers of GHG fluxes and GWP for a typical woody ornamental plant produced in Douglas fir (*Pseudotsuga menziesii*) bark-based substrate in California, USA. To the authors' knowledge, no previous research has attempted to understand the soilless substrate physical and chemical characteristics that drive GHG emissions from soilless production systems. Additionally, this is the first estimation of N_2O seasonal emission factor from a soilless production system and the first comparison of this emission factor with other California soil-based horticultural crops. Douglas fir bark is commonly used as a soilless substrate amendment in California and the Western U.S. due to its availability as a byproduct from the timber industry. Estimation of GHG emissions and GWP for this production system will provide insight into the magnitude of soilless production's contribution to the greenhouse effect.

MATERIALS AND METHODS

Nursery site and greenhouse conditions

Two studies were conducted to determine the GHG emissions from fir bark-based substrate and the factors that influence emission rates.

The first study was performed at an outdoor ornamental plant nursery near Sacramento, California, USA during a normal production cycle from May 4 – July 24, 2018. The nursery grows a large variety of woody and herbaceous perennials for sale to retail garden centers and landscape contractors. The average air temperature and relative humidity during the study period was 22°C and 57%, respectively. Rain occurred twice during the study period: 3 mm on May 16 and 13.4 mm on May 25. Potted plants were grown on 61-cm centers (one plant per center), resulting in 24,062 plants ha^{-1}. Irrigation was applied overhead to the plants with rotary stream sprayers (MP-3000, Hunter Industries, San Marcos, CA) twice per day.

The follow-up experiment was conducted from March 12 – June 4, 2019 in a glass greenhouse at the University of California, Davis. The primary objective was to determine the effect of adding supplemental surface-applied fertilizer on GHG emissions. Typical nursery production practices were followed. The mean greenhouse air temperature was 21°C and relative humidity was 75% during the experiment. Plants were irrigated by hand with deionized water, as needed.

Plants, Substrate, and Fertilizer

The soilless plant growing substrate in both experiments consisted of 7:1 (v:v) Douglas fir (*Pseudotsuga menziesii*) bark:washed concrete sand, incorporated with 3.47 kg Apex 9-2-0 sulfur coated urea (J.R. Simplot Co., Boise, ID), and 6.16 kg Osmocote Plus 15-9-12 CRF (Scotts

MiracleGro, Marysville, OH) per m^3. For the #3 (≈11.4-L) container utilized, approximately 9.0 – 10.6 L of soilless substrate was placed in the pot, resulting in a substrate depth of 23 – 25 cm. Volumetric water content (VWC) and air-filled porosity at container capacity were 39.6% and 44.8%, respectively. Bulk density was 0.45 g cm^{-3}, the organic matter component constituted 45% and mineral component was 55% by weight. Mean temperature of thermocouples placed 12-cm deep in substrate of six containers in the nursery experiment was 24.1°C.

For both experiments, on day zero, *Lagerstroemia indica* 'Whitt II' plants in #1 (3.8-L) containers were individually transplanted into #3 containers filled with the fir bark-based substrate. Among commercially produced ornamental plants, *Lagerstroemia indica* is an average nitrogen user[15]. Prior to transplanting, all experimental plants were pruned to approximately one meter in height. All experimental plants were placed off-center in the pot to allow for installation of a 10-cm diameter static chamber and base for gas flux sampling. For the nursery study, these pots were randomly placed in an area among plants spaced on 61-cm centers that were not used for gas flux measurement. For the greenhouse experiment, 24 plants were placed on 61-cm centers in the greenhouse and randomly separated into three treatments, with eight plants per treatment.

All treatments had the same amount of CRF incorporated into the substrate, but varied in the amount of surface-applied fertilizer. Plants in the nursery experiment had 35 g of surface-applied 20-9-9 fertilizer (Loveland Products, Greeley, CO) applied to substrate surface, resulting in a total of 21.3 g nitrogen $plant^{-1}$. Greenhouse treatments included 0 (0 g), 5 (5 g), or 35 g (35 g) of surface-applied to the substrate surface. This resulted in a total of 13.7, 14.7, and 20.7 g nitrogen fertilizer $plant^{-1}$ for the 0 g, 5 g, and 35 g treatments, respectively. There was a slight

discrepancy in total nitrogen applied to the nursery and 35 g treatments because the nursery containers had a slightly larger volume than the containers used for the greenhouse experiment. However, ANOVA indicated that there was no significant difference ($p > 0.05$) in mean shoot dry biomass at the end of the studies between any of the greenhouse and nursery treatments (data not shown).

Gas flux measurements

Nitrous oxide substrate-to-atmosphere gas fluxes were measured 14 times during the nursery production cycle. The nursery study was sampled between 11:50 and 14:30 on days 1, 4, 5, 7, 11, 18, 32, 39, 46, 53, 60, 67, 74, and 81 after planting. Methane substrate-to-atmosphere gas fluxes were measured on days 18, 32, 39, 46, 53, 60, 67, 74, and 81. For the greenhouse experiment, N_2O and CH_4 substrate-to-atmosphere gas fluxes were measured on days 1, 4, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, 70, 78, and 84 for a total of 15 sampling events. Gas sampling during the greenhouse experiment was initiated between 09:30 and 14:30.

On each sampling day of the nursery study, eight potted plants were randomly selected for gas sampling to measure N_2O flux, and four of the selected plants were also used for the CH_4 flux measurements. Biogenic carbon from the decomposition of bark in the plant container is considered carbon neutral[16] and as such, CO_2 is not a net contributor to GHG in nursery production and was not reported. The same gas samples were used for both N_2O and CH_4 analysis. During the greenhouse experiment, all 24 pots were gas sampled and analyzed for N_2O and CH_4 every sampling day. For the nursery study, at least 24 hours prior to gas sampling, a 10-cm long piece of 10-cm diameter polyvinyl chloride chamber base was pushed 8 cm into the

potted substrate. For the greenhouse experiment, the bases were installed at the same depth as the nursery study on day zero and not removed for the experiment duration.

Insulated, vented, round polyvinyl chloride chambers (10-cm diameter and 15-cm height, with a polyvinyl chloride lid) were used for gas sampling. Each chamber had a stainless-steel vent tube secured into its side. The lid of each chamber had a hole sealed with a rubber septum. Each chamber was sealed onto a chamber base, with a rubber gasket overlapping base and chamber, just prior to gas sampling. Gas samples were collected at each of four time intervals by inserting a needle attached to a 20-ml syringe into septum on top of chamber, withdrawing a 20-ml sample, and injecting the sample into an evacuated 12-ml Exetainer vial fitted with a grey butyl rubber septum (Labco Ltd., Lampeter, UK). The gas samples were analyzed on a Shimadzu gas chromatograph (Model 2014, Shimadzu Corp., Kyoto, Japan) linked to a Shimadzu auto sampler (Model AOC-5000). The GC was equipped with a ^{63}Ni electron capture detector for N_2O, a thermal conductivity detector for CO_2, and a flame ionization detector for CH_4. The GC was calibrated before each operation by using analytical grade CH_4, CO_2, and N_2O gas standards (Airgas Inc., Sacramento, CA) prepared at the same time the samples were collected. The majority of individual CH_4-C flux samples from the nursery and greenhouse studies were not significant ($p > 0.05$), and the null hypothesis (H_0: CH_4-C flux = 0 mg CH_4-C m^{-2} hr^{-1}) could not be rejected. This indicates that there is negligible net negative or positive CH_4-C flux from the fir bark-based substrate.

Gas flux calculation

Chamber gas concentrations were converted to mass per volume using the ideal gas law and chamber air temperature, which was measured by a HOBO temperature datalogger (Onset Corporation, Bourne, MA) at the time of sampling. Gas fluxes were calculated with the 'gasfluxes' package for R using chamber volume, surface area, and rate of change in chamber gas concentration.[17] The 'gasfluxes' package in R used the linear method when chamber gas concentrations had a constant rate of increase over time;[17] the robust linear method when interpolation between points was necessary; and the curvilinear method when chamber gas concentrations changed at a decreasing rate over time[18]. The curvilinear method is applicable for high porosity soils or substrates in which gas can move horizontally, and/or when high flux rates result in achieving equilibrium of chamber gas concentration before the end of the sampling period.[18] Kappa.max was employed to select a flux result from the three flux calculation methods following the guidelines from Hüppi et al.[19] Minimum curvilinear flux detection limit was calculated via a Monte Carlo simulation as described by Parkin et al.[20]. The null hypothesis when estimating gas flux was that flux was equal to zero (H_0: flux = 0 mg GHG m^{-2} hr^{-1}). A p-value was computed by 'gasfluxes' package for each individual gas flux sample to test the null hypothesis.[21] It was determined that curvilinear flux estimates have greater p-value than linear estimates and that it was not prudent to remove any flux estimate based on large p-value. Therefore, kappa.max was the only selection criterion used to determine the final gas flux estimate.

Cumulative gas emissions and emission factor (EF) estimation

Gas fluxes measured each sampling day were used to estimate cumulative pot-scaled gas emissions with trapezoidal integration of daily fluxes, under the assumption that the measured fluxes represented daily mean fluxes and the mean daily fluxes changed linearly between measurements.[22] It was assumed that GHG efflux solely escaped the plastic container at the top of the substrate. Cumulative pot-scaled emissions were based on mean daily GHG flux measurement for the nursery study. For the greenhouse experiment, emissions were based on the individual pot GHG flux measurements. Daily mass-based GHG flux was calculated as the cumulative pot-scaled gas emissions divided by the number of production days (81 days for the nursery study and 84 days for the greenhouse experiment) and either the mean initial substrate dry weight (DW) (for the nursery study) or the initial substrate DW for individual pots (in the greenhouse experiment). Mean initial substrate DW for the nursery study was 4.77 kg. The mean initial substrate DW for the greenhouse experiment was 4.55 kg.

The growing season EF for fir bark-based substrate was calculated as the total N_2O-N emissions divided by the total nitrogen fertilizer applied. Global warming potential was calculated by converting mass of N_2O and CH_4 to CO_2e, using a 100-yr timeframe and climate-carbon feedback (298 and 34 for N_2O and CH_4, respectively).[1]

The total nursery production area in California was estimated by multiplying the number of California Department of Food and Agriculture licensed nursery producers in 2019 (2,473)[23] by the average nursery size (11.45 ha) in California from the 2012 Census of Agriculture.[24] Specialty crop N_2O-N emissions per area per day were estimated by dividing the total N_2O-N emissions by the number of days in the growing season as reported for each cited reference.

The total California specialty crop N_2O-N emissions per day were estimated as the product of N_2O-N emissions per area per day from cited reference and total cropping system production area from the 2017-2018 California Agricultural Statistics Review.[25]

Substrate temperature, moisture, pH, electrical conductivity (EC), and inorganic N analysis

For both studies, substrate temperature was measured before gas flux sampling by inserting a 10-cm long temperature probe (Fisher Scientific, Hampton, NH) into the substrate in the center of the pot. For the greenhouse experiment, the plant, substrate, pot, and chamber base were weighed together after sampling. Volumetric water content was calculated using the plant/substrate/pot/chamber base weight after gas flux sampling and the weight at container capacity. Container capacity weight was measured weekly, approximately 1 hour after irrigation. The substrate volume was determined based on the substrate height in each individual container and dimensions of the container.

In the greenhouse, after the container was weighed, the VWC was returned to container capacity by slowly adding water to the substrate. Once container capacity was reached, a pour-through extract[26] was collected into a 50-ml centrifuge tube by lifting one side of the pot. Aliquots of the pour-through extract were analyzed for EC and pH (Horiba, Irvine, CA). The remaining pour-through extract was frozen until analyses for nitrate (NO_3-N) and ammonium (NH_4-N) concentration via spectrophotometric method.[27]

Statistics

To improve the normality of the residual errors and homoscedasticity, the N_2O-N flux, substrate temperature, and VWC data from the greenhouse experiment were transformed using a Box-

Cox transformation. Optimal lambda (λ) were 0.30, -3.12, and 3.21 for the N_2O-N flux, substrate temperature, and VWC data, respectively. Before Box-Cox transformation, the N_2O-N data was translated by adding 0.01 mg N_2O-N m^{-2} hr^{-1} per value to remove negative values. No transformation was performed on the pH data. Treatment means were determined and Tukey's test was used for means separation ($\alpha = 0.05$) of transformed N_2O-N flux, substrate temperature, VWC, and non-transformed pH data between treatments within the greenhouse experiment on each day. For comparing the weekly N_2O-N flux between the 35 g greenhouse treatment and the nursery study, N_2O-N flux was transformed using a Box-Cox transformation ($\lambda = 0.0496$). Student's t-test was used to indicate significant differences ($\alpha = 0.05$) between the transformed mean of 35 g or nursery treatment for each week. All statistics were performed in R statistical software. Confidence intervals for the total cumulative N_2O-N and GWP for the nursery experiment were computed using trapezoidal integration, with the upper and lower 95% confidence limits of the mean flux and GWP calculated for each sampling day. A regression model was developed to estimate the predictors ($p < 0.05$) of the common logarithm ($\log_{10}(x)$) of N_2O-N flux from bark substrate using the common logarithm of variables (substrate temperature and VWC, pour-through NH_4-N concentration, NO_3-N concentration, pH, and container number) measured at gas flux sampling time.

RESULTS

Temperature

The nursery study mean substrate temperature at time of flux sampling was 23.6 ± 1.6°C (Figure 2.1). The lowest (15.7°C) and highest (28.1°C) mean substrate temperature recorded at

sampling time occurred on day 11 and 46 (Figure 2.1), respectively. The greenhouse experiment mean substrate temperature at time of flux sampling was 23.6 ± 0.1°C (Figure 2.2). The minimum substrate temperature during greenhouse sampling was 20.5°C, and the maximum was 31.3°C.

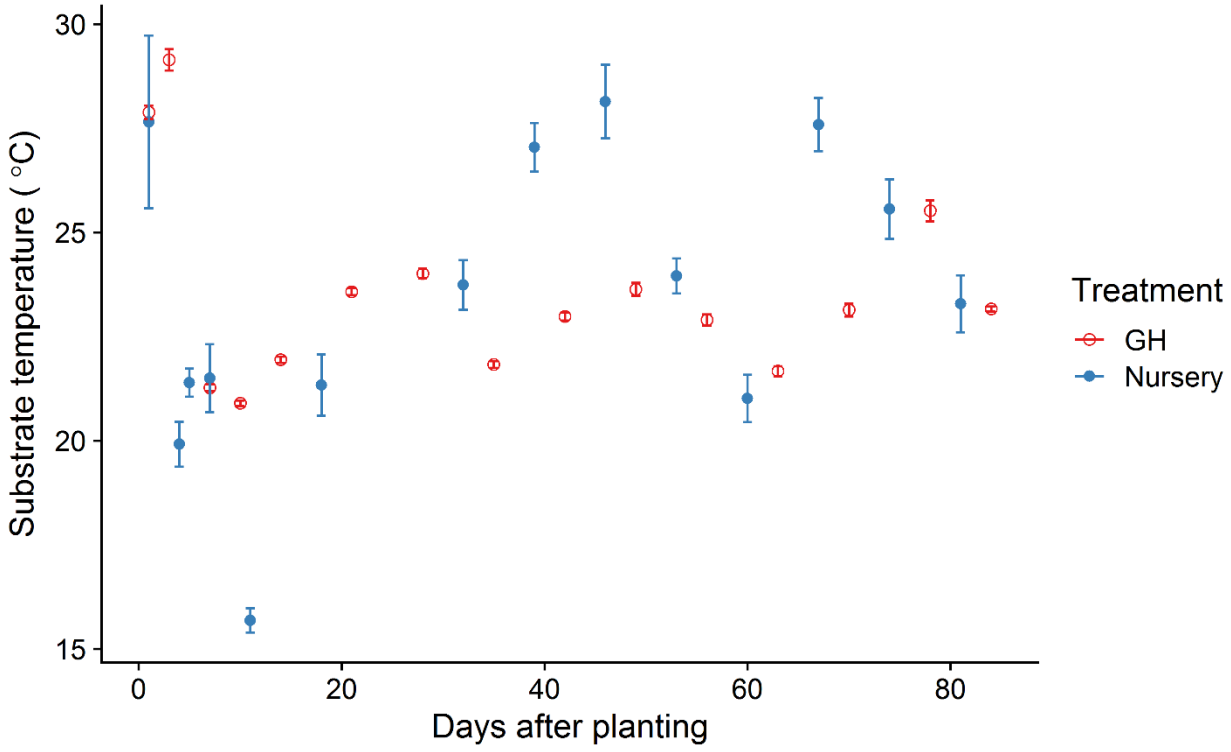

Figure 2.1. Mean fir bark-based soilless substrate temperature at the time of gas flux sampling for the greenhouse (GH) and nursery (Nursery) experiments. The mean for the greenhouse experiment represents all replicates from all treatments. Error bars represent one standard error of the mean (GH, n = 24; Nursery, n = 8).

Volumetric water content

In the greenhouse experiment, the mean VWC of the fir bark-based substrate at the time of gas flux sampling for all three treatments was 31.3 ± 0.3%. Volumetric water content did not differ

among treatments, except on day three, when the VWC in the 35 g treatment substrates were greater than those in the 0 g treatment (Figure 2.2). Mean VWC was near container capacity initially, then began to decrease around day 21 with the lowest mean VWC observed on day 56 (Figure 2.2). After day 56, plants were watered more frequently, which increased the mean VWC at sampling time (Figure 2.2).

Pour-through extract EC and pH

The mean EC of pour-through extracts during the entire experiment were 1.69, 1.84, and 2.32 dS m^{-1} for the 0 g, 5 g, and 35 g treatments, respectively. The mean extract EC was similar among all greenhouse treatments on day one after planting (Figure 2.2). Starting on day four after planting, the extract of the 35 g treatment was always greatest and the extract EC of the 0 g treatments usually lowest (Figure 2.2).

The mean extract pH for all greenhouse treatments was 6.08 during the course of the experiment. There was no significant difference ($p > 0.05$) in pH between any of the treatments on any of the sampling days (Figure 2.2).

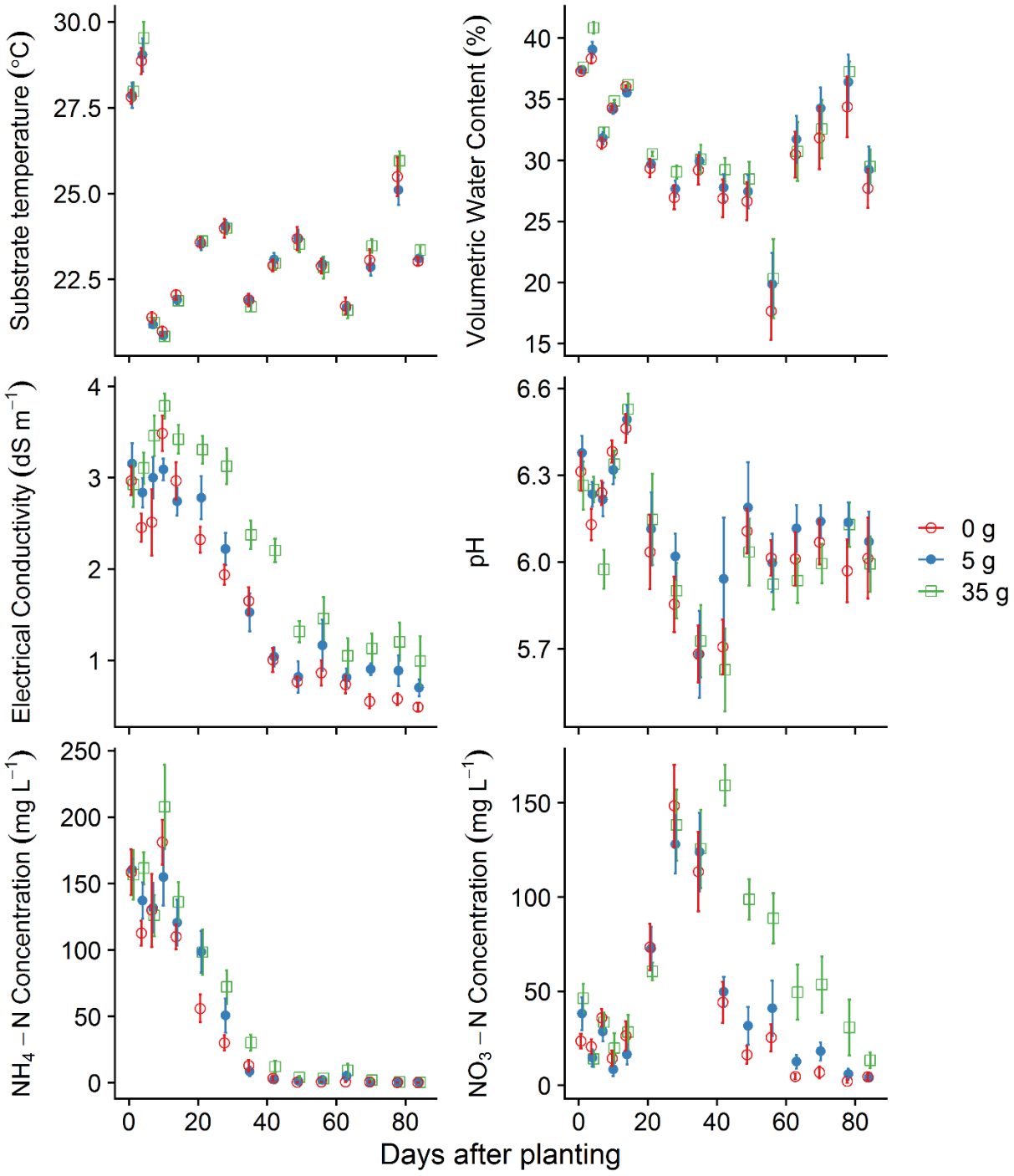

Figure 2.2. Mean substrate temperature, volumetric water content (VWC), electrical conductivity, pH, and NH_4-N and NO_3-N concentrations of fir bark-based soilless substrate at

the time of sampling for the 0 g, 5 g, and 35 g surface-applied fertilizer treatments in the greenhouse experiment. Error bars represent one standard error of the mean (n = 8).

Pour-through extract NH_4-N concentration and NO_3-N concentration

Data on NH_4-N and NO_3-N concentration was collected during the greenhouse experiment only. The mean pour-through extract NH_4-N concentration across all sampling days was 53.1, 58.3, and 67.9 mg L^{-1} for the 0 g, 5 g, and 35 g treatments, respectively. The mean NH_4-N concentration for all treatments was high for the first two weeks, then declined steadily for four weeks, after which it remained low for the rest of the experiment (Figure 2.2). The mean NH_4-N concentrations of all replicates within a treatment were similar on days one and seven after planting (Figure 2.2). On all sampling days except day 84, the 35 g treatment had the greatest mean NH_4-N concentration (Figure 2.2). On days 10, 35, 42, and 70 after planting, the extracts from the 0 g treatment had greater NH_4-N concentration than the extracts from the 5 g treatment (Figure 2.2).

The mean pour-through extract NO_3-N concentration for all replicates across all sampling days was 37.3, 39.6, and 64.0 mg L^{-1} for the 0 g, 5 g, and 35 g treatments, respectively. The mean NO_3-N concentrations of the extracts for all the greenhouse treatments was initially low and increased greatly on day 21, then peaked on day 28 (Figure 2.2). The mean NO_3-N concentration was similar for all treatments until day 35, after which the NO_3-N concentration in the extract from the 35 g treatment was consistently highest and that from the 0 g treatment was consistently lowest (Figure 2.2).

Nitrous oxide fluxes

The mean N_2O-N flux during the entire nursery study was 5.14 ± 0.683 mg N_2O-N m^{-2} hr^{-1}, with fluxes from individual samples ranging from -0.59 to 57.2 mg N_2O-N m^{-2} hr^{-1}. The mean N_2O-N flux from the potting substrate during the first week was 4.29 mg N_2O-N m^{-2} hr^{-1}. The daily mean N_2O-N flux decreased markedly a few days after planting, until day seven when flux was 1.24 mg N_2O-N m^{-2} hr^{-1} (Figure 2.3). The mean N_2O-N flux increased sharply after day seven, peaked at 14.3 mg N_2O-N m^{-2} hr^{-1} on day 18, and then decreased steadily until day 60 (1.86 mg N_2O-N m^{-2} hr^{-1}) (Figure 2.3).

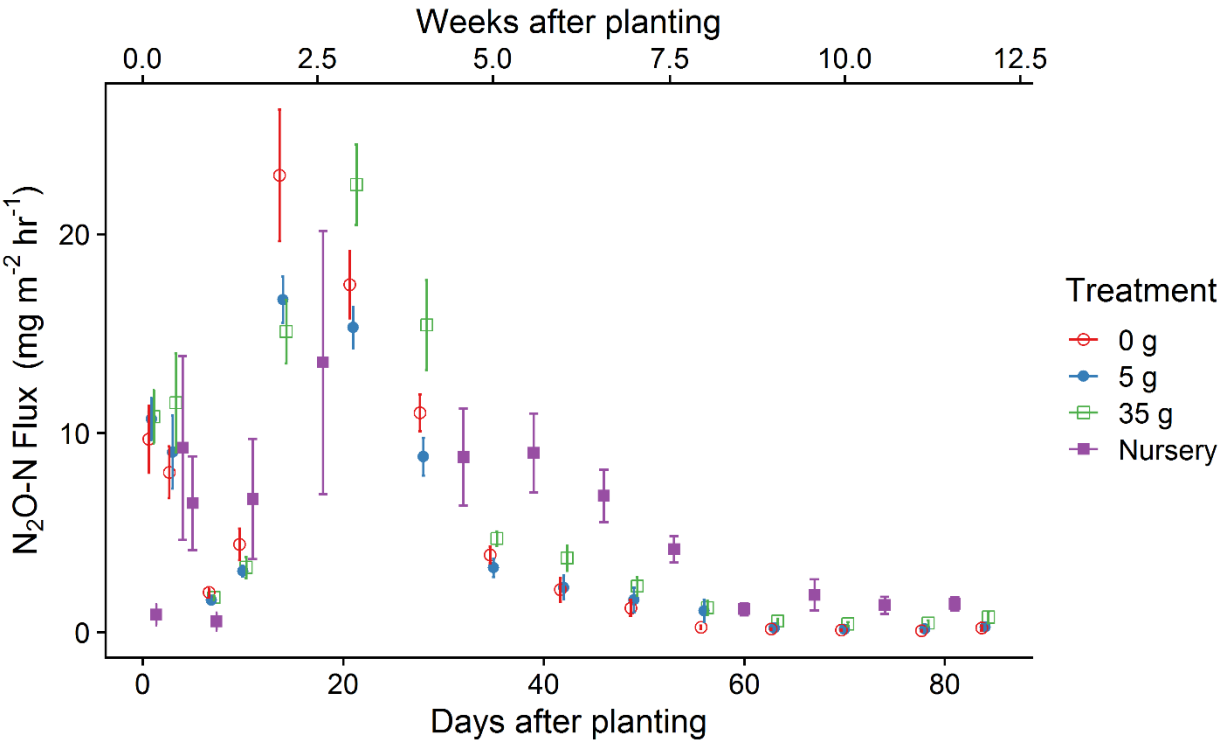

Figure 2.3. Mean nitrous oxide (N_2O-N) flux from fir bark-based soilless growing substrate in the greenhouse experiment treated with 0, 5, or 35 g of surface-applied fertilizer and at a production nursery with 35 g of surface-applied fertilizer (Nursery). Error bars represent one standard error of the mean (n = 8).

For the greenhouse experiment, no significant differences in mean transformed N_2O-N fluxes were observed between treatments until day 14 after planting. Flux in the 35 g treatment was significantly higher ($p < 0.05$) than in the 0 g treatment on days 14, 42-56, and 78, and significantly higher ($p = 0.022$) than in the 5 g treatment on day 28 (Figure 2.3).

The N_2O-N flux patterns in the nursery study were similar to those of the greenhouse experiment. As the 35 g treatment in the greenhouse and nursery study had nearly the same amount of fertilizer applied, weekly N_2O-N flux was compared between these two. Significant differences ($p < 0.05$) in transformed N_2O-N fluxes were observed between the 35 g and nursery treatment during weeks 1, 2, 3, 7, and 8, with the 35 g treatment having greater N_2O-N flux during weeks 1, 2, and 3, while the nursery study had greater N_2O-N flux during weeks 7 and 8 (Figure 2.3).

The total cumulative pot-scaled and substrate DW based daily N_2O-N emissions for the 35 g treatment were significantly greater than the emissions from the 5 g treatment (Table 2.1). However, significant differences were not observed in total cumulative pot-scaled nor substrate DW based daily N_2O-N emissions between the nursery experiment and any of the greenhouse treatments (Table 2.1).

Table 2.1. Total cumulative N_2O-N emissions and global warming potential (CO_2e) per pot or Mg of dry fir bark-based substrate. Significant differences among treatments are indicated by different letters and were based on trapezoidal integration of 95% confidence intervals. Confidence intervals for the Nursery experiment were calculated by trapezoidal integration of

the upper and lower confidence limits for the mean flux of each sampling day. Letters after value indicate significant differences between values in each column.

Treatment	Experiment Duration (days)	mg N_2O-N pot^{-1}	mg N_2O-N day^{-1} Mg DW $Substrate^{-1}$	g CO_2e pot^{-1}	g CO_2e day^{-1} Mg DW $Substrate^{-1}$
0 g	84	494.5 ab	1,342 ab	232 ab	628 ab
5 g	84	431.5 b	1,195 b	202 b	559 b
35 g	84	574 a	1,566 a	269 a	733 a
Nursery	81	579.4 ab	1,500 ab	271.3 ab	702 ab

The uncorrected growing season emission factor, the percent of applied N fertilizer lost as N_2O-N, for the greenhouse experiment and nursery study were all greater than 2% (Table 2.2). Emission factor was greatest for the 0 g treatment and lowest for the 35 g treatment (Table 2.2).

Table 2.2. California specialty cropping systems and their respective production area, production time, nitrogen input, nitrous oxide (N_2O-N) emissions per area per day, total estimated California N_2O-N emissions, and growing season emission factor.

Cropping System	Production Area (ha)[a]	Production Time (days)[b]	N Input (kg N ha^{-1})[b]	Emissions (g N_2O-N ha^{-1} day^{-1})[c]	Total California Emissions (kg N_2O-N day^{-1})[d]	Emission Factor (%)[e]	Reference
Almonds	538,232	244	336	2.09	1,127	0.15	[28]
		244	336	3.20	1,723	0.23	
		244	336	4.25	2,285	0.31	
Grapes	335,485	182	52	0.72	241	0.27	[29]
		195	52	0.77	258	0.27	[30]
		182	52	1.03	344	0.38	[29]
		195	5.4	1.65	553	5.56	[30]
		182	275	2.03	682	0.13	[31]
		182	129	4.18	1,401	0.59	[31]
Lettuce	80,411	54	0	2.96	238	na	[32]
		54	56	6.48	521	0.63	
		42	190	13.1	1,053	0.30	
		54	112	14.6	1,176	0.71	
		54	168	18.5	1,489	0.60	
		54	225	21.3	1,713	0.51	
Processing Tomatoes	89,840	177	111	3.28	294	0.28	[33]
		177	73	11.4	1,020	0.85	
Nursery[f]	28,322[g]	84	288	124	3,501	2.84	5 g[h]
		84	264	142	4,012	3.08	0 g[h]
		84	431	164	4,657	2.58	35 g[h]
		81	431	172	4,874	2.72	Nursery[h]

a. Cropping system area is total harvested production area from the 2017 California Agriculture Statistics Review.[25]

b. Production time and nitrogen input are from cited references.

c. Emissions of nitrous oxide-N (N_2O-N) per area per day are estimated by dividing reported production cycle N_2O-N emissions by total production time from cited reference.

d. Total California N_2O-N emissions are estimated by multiplying N_2O-N emissions per area by total production area.

e. Growing season emission factor (EF) is percentage of applied N lost as N_2O-N. The values presented for non-nursery cropping systems were uncorrected EF values reported.[34]

f. The N_2O-N emissions per area for the nursery cropping system are based on 24,062 14-L pots per hectare.

g. Total nursery production area in California is estimated by multiplying the number of California Department of Food and Agriculture licensed nursery producers (2,473)[23] by the mean nursery area according to the 2012 Census of Agriculture.[24]

h. The values in each row are based on a greenhouse experiment with 0 (0 g), 5 (5 g), or 35 g (35 g) and at a production nursery (Nursery) with 35 g of surface-applied fertilizer.

Relationship between nitrous oxide fluxes and substrate characteristics

The final regression model indicated that the common logarithm of pour-through extract NH_4-N concentration ($p < 2.2 \times 10^{-16}$), NO_3-N concentration ($p < 2.2 \times 10^{-16}$), substrate temperature ($p = 1.0 \times 10^{-3}$), and VWC ($p = 6.0 \times 10^{-12}$) at time of gas flux sampling were predictors of common logarithm of N_2O-N flux. (CH_4-C flux was not modeled because it was negligible). The model shows a strong positive correlation between predictor variables and response ($R^2 = 0.76$). The final regression model is:

$$log_{10}(N_2O - N) = 0.42 \times log_{10}(NH_4 - N) + 0.42 \times log_{10}(NO_3 - N) + 1.91 \times log_{10}(temp) + 1.69 \times log_{10}(VWC) - 10.53 + \varepsilon$$

where *N_2O-N* is the N_2O-N flux, *NH_4-N* is NH_4-N concentration in pour-through extract, *NO_3-N* is NO_3-N concentration in pour-through extract, *temp* is substrate temperature, *VWC* is VWC at time of gas flux sampling, and ε is residual error of the model. When significant variables were

individually removed from the model, mean square error increased by 210%, 141%, 115%, and 103% for common logarithm of NH_4-N, NO_3-N, VWC, and temperature, respectively.

DISCUSSION

Nitrous oxide emissions

Based on our studies, we estimated that the daily N_2O-N emissions per production area were nearly two orders of magnitude greater than for four major soil-based horticultural crops in California (Table 2.2). Even though nursery production occupies a small fraction of the area used for production of all horticultural crops in California, the total daily estimated N_2O-N emission from nursery production is far greater than from annual or perennial horticultural crops, whether woody or herbaceous. The uncorrected growing season emission factor, the percent of applied N fertilizer lost as N_2O-N, for the fir bark-based substrate production system is 3 to 24 times greater than for four horticultural crops in California (Table 2.2).[34] A single reported high emission factor for grapes was 5.56%, which may have been due to the abnormally low nitrogen input of 5.4 kg N ha^{-1}.[30] The ornamental plant harvestable product is the entire plant plus the substrate, which contains unexpended CRF, making it possible that N_2O-N emissions could continue after shipping the product from the nursery. However, the majority of N_2O-N emissions occurred in the first six weeks of production and additional emissions after this period are minor (Figure 2.3). High inorganic nitrogen concentration (Figure 2.2) of pour-through extract during the first six weeks of the GH experiment explain why large N_2O-N emissions occurred during this time. The high nitrogen concentrations and EC (Figure 2.2) during this time may be due to CRF prills that were damaged during mechanical

incorporation into the fir bark-based substrate. *L. indica* 'Petite Orchid' only requires 65 mg nitrogen L^{-1} for sufficient plant growth[15] but the *Lagerstroemia* plants in these studies regularly had >150 mg nitrogen L^{-1} available in substrate solution during the first six weeks. Reducing damage to CRF prills or applying soluble nitrogen in irrigation water could provide more consistent nitrogen application rate while still meeting plant nutrient needs throughout the production process.

The lack of significant difference in total cumulative N_2O-N emissions between the 0 g and 5 g treatments (Table 2.1) indicated that the 10% greater nitrogen in the 5 g treatment did not affect total cumulative N_2O-N emissions. There was not a significant difference in total cumulative N_2O-N emissions between the 0 g and 35 g treatments (Table 2.1), even though 70% more nitrogen was used for the 35 g treatment. The 35 g treatment emitted the greatest total cumulative N_2O-N, but plant dry biomass and relative growth index after 84 days was not significantly different between any of the treatments (data not shown). This suggests that CRF provides sufficient nitrogen for plant growth, and reducing or removing the surface-applied fertilizer could mitigate N_2O-N emissions without compromising *L. indica* plant growth or quality.

Aside from peat, many soilless substrate amendments are waste products from another industry: coir from coconut processing,[14] pine bark from the paper and pulp industry,[35] rice hulls from rice production, and fir bark from timber harvesting. As the fir bark is a waste product of the timber industry, if not used for growing substrate it would decompose, either at the lumber mill, landfill, or possibly a commercial composting facility. Similar to other organic growing substrates, the fir bark-based substrate is decomposing while growing plants,[36] so it is

reasonable to compare N_2O-N emissions to those for composted plant material. Zhu-Barker et al.[22] found total N_2O-N emissions from composted green waste in California between 152 and 511 mg N_2O-N day^{-1} Mg^{-1} of initial compost DW. The higher N_2O-N emissions (511 mg N_2O-N day^{-1} Mg^{-1} DW) were from a composting cycle that lasted from May 22 to July 19, 2012, a 57-day cycle at the same time of year as the plant production cycle in this experiment. Although daily mean substrate DW-based N_2O-N emissions are higher from fir bark-based substrate (Table 2.1) than green waste compost, the compost emissions indicate that a portion of the N_2O-N emissions observed in this study may have been released if the fir bark was sent to a commercial composting facility. This suggests that using bark as a soilless substrate may be more sustainable than composting or disposing of it in a landfill because the bark is repurposed for soilless plant production and some N_2O-N would be emitted during decomposition in either scenario.

Two studies that evaluated N_2O-N emissions from pine bark-based substrate in containers planted with a woody ornamental presented interesting comparisons to our results. One estimated cumulative N_2O-N emissions to be 228.8 mg N_2O-N pot^{-1} over a 12-week period for #3 (11.4 L) pot.[7] The other estimated cumulative emission of 104.8 mg N_2O-N pot^{-1} in 84 days for a #3 pot.[9] Although our study estimated greater total cumulative N_2O-N emissions from Douglas fir bark-based substrate over a shorter period of time, most of the N_2O-N emissions in the cited studies occurred during the first few months of the experiments. The total cumulative N_2O-N emission from any treatment with fir bark-based substrate was much greater than those reported for the pine bark-based substrate which implies that other factors were driving the greater N_2O-N emissions from the fir bark-based substrate.

A regression model indicated that N_2O-N flux increased in proportion to pour-through extract NH_4-N and NO_3-N concentrations, temperature, and VWC. Temperature affects N_2O-N flux because microbial activities that drive gas production increase with temperature. Removal of the common logarithm of NH_4-N pour-through extract concentration from the regression model resulted in the largest increase in mean square error, showing that NH_4-N was the greatest driver of N_2O-N emission from the fir bark-based substrate. This suggests that ammonia-oxidation was probably the predominant pathway of N_2O-N production in the fir bark-based substrate. Zhu et al.[37] found that ammonia oxidation was a significant pathway of N_2O-N production when oxygen concentration was ≥0.5% for soil in microcosms. The fir bark-based substrate used in these experiments had a high air-filled porosity, even at container capacity, indicating that oxygen was available for oxidative chemical reactions. Furthermore, a large amount of the fertilizer in the fir bark-based substrate was urea-N or NH_4-N, between 70 and 80% depending on amount of surface-applied fertilizer added, resulting in more nitrification than if NO_3-N was the sole N source. The abundance of NH_4-N could explain the difference in total cumulative N_2O-N emissions from pine[7,9] and fir bark-based substrates. The pine bark-based substrate had Polyon 17-5-11 incorporated[7,9] which is approximately equal parts of NH_4-N and NO_3-N, with no urea-N. The Osmocote CRF contributed 5.2 to 5.5 g NH_4-N and 4.1 to 4.3 g NO_3-N to each container of the fir bark-based substrate. Additionally, 4.3 to 11.3 g urea-N was applied per pot to the fir bark-based substrate depending on the amount of surface-applied fertilizer. In soils and soilless substrates, urea is converted to ammonia through hydrolysis, which in acidic conditions (e.g., as in fir bark-based substrate, Figure 2.2) increases the concentration of ammonium in substrate solution. The results of the regression model and

greater use of NH_4-N and urea-N in fir bark-based substrate indicate that increased emissions from the fir bark-based substrate compared to the pine bark-based substrate could be attributed to fertilizer formulation. This suggests that reducing NH_4-N and urea-N and replacing with NO_3-N fertilizer sources may reduce N_2O-N emissions from soilless substrates.

Global Warming Potential (GWP)

Global warming potential was calculated as CO_2e, based on the radiative forcing of N_2O compared to CO_2.[1] Methane was not included in the estimate of GWP because net flux was at or near zero, and CO_2 was not included because it derives from the decomposition of plant material and was considered neutral when evaluating GWP.[16] The greatest GWP per DW substrate was from those with 35 g of surface-applied fertilizer (Table 2.1), due to their high N_2O-N emissions.

The cumulative GWP from the fir bark-based growing substrate (Table 2.1) was lower than that for a commercial compost pile (1,240 g CO_2e Mg DW^{-1} day^{-1}) during the same time of year as the nursery experiment.[22] Methane was the major contributor to compost GWP, and N_2O contributed only 19% of the total GWP for composting emissions.[22] This contrasts with the fir bark in a growing substrate, which is probably more oxygenated than windrow composting, which would result in no net CH_4 production. Using Douglas fir bark as a growing substrate may be more sustainable than composting it because less CH_4 and a lower total GWP per dry plant material mass are produced.

The substrate in this study had CRF incorporated and was overhead-irrigated, a combination that produced the highest GWP for pine bark-based substrate.[9] Murphy et al.[9] included CO_2 in

calculating GWP, but since CO_2 is from a biogenic source[16] and pine bark is a waste product of the paper and pulp industry,[35] CO_2 does not contribute to GWP from these substrates. Also, Murphy et al.[9] reduced the GWP from the pine bark-based substrate due to insignificant net CH_4 consumption. Significant net CH_4 flux did not occur in fir bark-based substrate and it is plausible that similar conditions exist in pine bark-based substrate, resulting in negligible net CH_4 flux. Therefore, an equitable comparison of GWP between substrates containing fir bark and pine bark requires evaluating GWP solely from N_2O. Nitrous oxide-generated GWP from the fir bark-based substrate was 202 to 271 g CO_2e pot^{-1} (Table 2.1). The comparable 84-day pine bark-based substrate treatment of Murphy et al. and Marble et al. produced 49.1 and 107.1 g CO_2e pot^{-1}, respectively.[7,9] These are equivalent to 2.40 to 3.35 and 0.59 to 1.28 g CO_2e pot^{-1} day^{-1} for fir bark- and pine bark-based substrates, respectively. The pine bark-based substrate had lower daily GWP than the fir bark-based substrate indicating that bark-based soilless substrates produce different amounts of N_2O emissions which may be influenced by N fertilizer formulation, water content, or temperature.

Abbreviations: CH_4-C, Methane-Carbon; CO_2, Carbon Dioxide; CO_2e, Carbon Dioxide Equivalents; CRF, Controlled Release Fertilizer; DW, Dry Weight; EC, Electrical Conductivity; GHG, Greenhouse Gas; GWP, Global Warming Potential; NH_4-N, Ammonium-Nitrogen; NO_3-N, Nitrate-Nitrogen; N_2O, Nitrous oxide; VWC, Volumetric Water Content.

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Chapter 3: Contribution of denitrification and nitrification to nitrous oxide emissions from three organic soilless substrates

Abstract

Nitrous oxide (N_2O) emissions from soilless growing substrates are significant and it is believed that heterotrophic denitrification accounts for the bulk of these emissions. However, soilless substrate has physical and chemical properties that could promote nitrification- and denitrification-derived N_2O simultaneously. Fir bark, peat, and peat:fir bark substrates were fertilized with $^{15}NH_4NO_3$, $NH_4{}^{15}NO_3$, unenriched NH_4NO_3, or unfertilized to determine primary N_2O production pathway. Soilless substrate volumetric water content was maintained near container capacity and gas flux samples were collected every other day for 21 days and analyzed for $^{15}N_2O$ content. Fir bark and peat:fir bark substrate had significantly greater total N_2O emitted than the peat substrate when fertilized with NH_4NO_3. Heterotrophic denitrification accounted for almost all the N_2O emitted from all three substrates but played a larger role in the fir bark and peat:fir bark substrates. Nitrification-derived N_2O emissions began on day 11 in the peat substrate and continued to increase until the experiment ended, contributing to 6% of total N_2O emission from this substrate. Strategies to reduce denitrification-derived N_2O emissions from soilless substrates should be evaluated and implemented to reduce the global warming potential of container-plant production.

Introduction

Atmospheric nitrous oxide (N_2O) is a potent greenhouse gas, with a 100-year radiative forcing estimated to be 298 times that of carbon dioxide (Myhre *et al.*, 2013). Additionally, N_2O is the most significant anthropogenic compound involved in the depletion of stratospheric ozone (Ravishankara *et al.*, 2009). Global atmospheric N_2O concentration has increased by 0.84 ppb yr^{-1} since 1977 (NOAA, 2021), and agricultural use of synthetic nitrogen fertilizer and manure is the primary anthropogenic cause of this increase (Fowler *et al.*, 2009).

Denitrification and nitrification in agriculturally-managed and natural soils generate the majority of N_2O in Earth's atmosphere (Zhu-Barker and Steenwerth, 2018). Heterotrophic denitrification is performed by bacteria consuming organic carbon while using nitrate or nitrite, instead of oxygen, as an electron acceptor (Knowles, 1982). Multiple biotic pathways can produce N_2O from ammonia oxidation (Firestone and Davidson, 1989), the first step in nitrification. The ammonia oxidation pathways are nitrifier nitrification, nitrifier denitrification, and nitrification-coupled denitrification (Zhu *et al.*, 2013).

Heterotrophic denitrification is an anaerobic process known to occur in anoxic conditions (Knowles, 1982). When soil moisture increases, oxygen content decreases until anoxic conditions occur which increases heterotrophic denitrification potential. Many authors use soil moisture content as an indicator of oxygen content in soils (Linn and Doran, 1984). A common belief among researchers is that N_2O from denitrification is produced in significant amounts at >70% water-filled pore space (Bateman and Baggs, 2005; Dobbie *et al.*, 1999). Ammonia oxidation requires oxygen to convert ammonium to hydroxylamine, and researchers typically

attribute N_2O from soils at <70% water-filled pore space to ammonia oxidation (Dobbie et al., 1999; Linn and Doran, 1984; Venterea *et al.*, 2010). However, along with oxygen diffusion rate, soil moisture content also controls substrate availability and microbial activity (Zhu et al., 2013). Zhu et al. (2013) determined that soils with oxygen concentration >0.5% generate significant N_2O from ammonia oxidation pathways and N_2O emissions increased as oxygen concentration decreased further.

Soilless growing substrates are used in place of mineral soils to provide adequate physical properties for plant production in containers (Passioura, 2006). To ensure sufficient oxygen for plant roots, soilless substrates are recommended to have a "high air volume" (de Boodt and Verdonck, 1972), with a consensus that >10% air-filled porosity by volume is satisfactory for most plant taxa (Bunt, 1976; Evans *et al.*, 2009; Nkongolo and Caron, 2006; Paul and Lee, 1976). There is an exponential relationship between air-filled porosity of soilless substrates and oxygen diffusion rate (Bunt, 1991; Schmitz *et al.*, 2013). Greater air-filled porosity resulted in greater oxygen diffusion rates among a variety of substrates (Bunt, 1991; Paul and Lee, 1976; Schmitz et al., 2013). To provide sufficient moisture for plant growth, soilless substrates should have a "high capacity of available water" (de Boodt and Verdonck, 1972) with 40-65% water holding capacity by volume (Evans, 2014b; Yeager *et al.*, 2013). At container capacity, soilless substrates have a perched water table, i.e. saturated zone at the bottom of the container (Evans, 2014b; Passioura, 2006; Yeager et al., 2013). The height of this saturated zone depends on average pore size of the soilless substrate and is consistent among different container heights (Evans, 2014b; Yeager et al., 2013).

Soilless substrates have unique chemical properties that are different from mineral soil. Organic amendments, the primary component of soilless substrates (Bilderback *et al.*, 2005), typically have high dissolved organic carbon (DOC) content related to humic acids (Pigoli *et al.*, 2019), which can act as an energy source and electron donor for denitrifiers (Knowles, 1982). Multiple studies have shown increased N_2O emissions from denitrification when organic carbon was added to mineral soils in laboratory conditions (Gillam *et al.*, 2008; Myrold and Tiedje, 1985; Weier *et al.*, 1993). The regular addition of nitrogen fertilizers to crops grown in soilless substrates, either in irrigation water or through controlled release fertilizers (Evans, 2014a; Yeager et al., 2013), provides a readily available substrate source for nitrification and/or denitrification. Many controlled release fertilizers contain substrate for nitrification and denitrification in each granule by providing nitrogen as ammonium, nitrate, and/or urea (Agro and Zheng, 2014; Cabrera, 1997).

Previous research has attributed N_2O emissions from soilless substrates to denitrification without acknowledging the possibility of nitrification-produced N_2O (Agner and Schenk, 2005a, b, 2006a, b; Marble *et al.*, 2012a; Marble *et al.*, 2012b; Murphy *et al.*, 2018). Agner and Schenk (Agner and Schenk, 2005a, b, 2006a, b) were among the first to study N_2O emissions from soilless substrate and grew plants with nutrient solution containing 105 mg NH_4-N and 45 mg NO_3-N L^{-1}. Decreased redox potential during N_2O flux measurement, associated with decreased oxygen and denitrification, corresponded with increased N_2O emissions (Agner and Schenk, 2006a). However, two hours prior to every N_2O flux measurement, plants were flood irrigated with 150 mg NO_3-N L^{-1} nutrient solution (Agner and Schenk, 2006a), effectively leaching any NH_4-N from substrate solution and eliminating the possibility of significant N_2O emissions from

nitrification. Recent research showed that pour-through extract NH_4-N and NO_3-N concentrations are significant predictors of N_2O emissions from a soilless substrate incorporated with controlled release fertilizer composed of roughly equal parts of NH_4-N and NO_3-N (Pitton *et al.*, 2021).

High air-filled porosity, oxygen diffusion rates, water holding capacity, DOC concentration, NH_4-N and NO_3-N concentrations in substrate solution, and presence of a saturated zone suggest that nitrification and denitrification pathways may be simultaneously producing N_2O in soilless substrate. To investigate the origin of N_2O from soilless substrates, nitrogen-15, provided either as $^{15}NH_4$-N or $^{15}NO_3$-N, was used with three soilless substrates to identify the contribution of nitrification and denitrification to N_2O emissions from soilless substrates. This, to the authors' knowledge, is the first report of the contribution of nitrification and denitrification to N_2O emissions from soilless substrates, as well as the first to compare N_2O emissions from soilless substrates composed of different organic amendments at similar physical properties.

Materials and Methods

Substrate

Three soilless growing substrates were used to identify the predominant pathway for N_2O production. Douglas Fir (*Pseudotsuga menziesii*) bark substrate was prepared from 7:1 (v:v) Douglas fir bark and washed sand incorporated with 6.79 g dolomite lime m^{-3} substrate. Peat substrate was prepared with 7:1 (v:v) sphagnum peat moss (Professional Sphagnum Peat Moss, Berger, QC, Canada) and washed sand incorporated with 29.94 g dolomite lime m^{-3} substrate. A combination of Douglas Fir bark and peat substrate was prepared with 3.5:3.5:1 (v:v) fir bark,

peat, and sand, respectively, incorporated with 18.31 g dolomite lime m^{-3} substrate. Fir bark and peat were leached with DI water to remove excess salts, then dried for four days in an oven at 60°C before mixing with sand. After removal from the oven, DI water was added to the fir bark and peat to increase water content to 55% and 360% gravimetric water content, respectively, and left to incubate for five days. One week before adding ammonium nitrate, the substrates were mixed to final amendment ratio for the three substrates and DI water was added to adjust gravimetric water content to 27%, 78%, and 42% for the fir bark, peat, and peat:fir bark substrate, respectively. The substrates were incubated at room temperature throughout the experiment.

Characterization of the physical properties of substrate included measurement of bulk density, mineral and organic content, volumetric water content, and calculation of total and air-filled porosity at container capacity. Bulk density of the two substrates was determined using samples that had been placed in cylinders (25-cm height x 10-cm diameter) with holes at the bottom for drainage. The substrate was packed to approximate the anticipated bulk density during plant growth. The total porosity was calculated using the equation,

$$\Phi\ (\%) = \left(1 - \frac{1}{V_b}\left(\frac{g_m}{\rho_m} + \frac{g_o}{\rho_o}\right)\right) \times\ 100 \quad (1)$$

where Φ is total porosity, V_b bulk volume in cm^{-3}, g is the soil dry weight in grams, ρ is the particle density in g cm^{-3} and the subscripts *m* and *o* signify the substrate mineral and organic

fractions, respectively. The particle densities for silica (2.65 g cm^{-3}) and cellulose (1.60 g cm^{-3}) were used for ρ_m and ρ_o, respectively.

The substrate in the cylinders was saturated with water, then drained for two hours, and reweighed. Volumetric water content (VWC) at container capacity was calculated using the ratio of water volume in the substrate to the bulk volume of the substrate, expressed as a percentage. The air-filled porosity at container capacity was calculated by subtracting VWC at container capacity from the total porosity.

Samples of each substrate were placed in tin cans and oven-dried for two days. The organic and mineral contents of the substrates were determined by the loss on ignition method (Davies, 1974). Physical and chemical properties of the three substrates prior to addition of ammonium nitrate solutions are presented in Table 3.1.

Saturated media extracts (Warncke, 1986) were prepared with DI water and set for 1.5 hours until measurement of pH and electrical conductivity with a portable probe (Oakton PCTSTestr™ 50 Waterproof Pocket pH/Cond/TDS/Salinity Tester, IL, USA). Dissolved organic carbon concentration was analyzed as non-purgeable organic carbon determined by the combustion catalytic oxidation method on a Shimadzu TOC-L/TN Analyzer from saturated media extract prepared with DI water and filtered through a 0.45 µm PVDF filter (Durapore, Millipore Sigma, MA, USA) after 1.5 hours. Total C of substrate was determined via the loss on ignition method. Total N of substrate was determined via ISO method 13878.

To standardize relative gas diffusion rate, the three substrates were maintained at similar air-filled porosity throughout the experiment. This resulted in 51.1%, 56.2%, and 53.0% VWC for

the fir bark, peat, and peat:fir bark substrates, respectively. Three ammonium nitrate fertilizer treatments and one treatment without nitrogen fertilizer (Unfertilized) were applied to each substrate. The treatments were NH_4NO_3 at natural abundance, ^{15}N-enriched $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ (at 10 atom%), and background treatment which had no added NH_4NO_3. Ammonium nitrate was added to each substrate to achieve 200 mg L^{-1} N in substrate solution. The NH_4NO_3 fertilizers were applied to substrates in solution with DI water and mixed by gloved hands until substrate was uniformly wetted.

Substrate at experiment VWC were packed to their respective bulk densities into 28-cm tall, 10-cm diameter polyvinyl chloride gas cylinders that were closed at the bottom. Substrate column height was approximately 25-cm, which is similar to substrate height in a #3 (14-L) container. There were four replicates of each substrate:fertilizer treatment in the gas cylinders. The experimental design was a completely randomized design with gas cylinders placed on a lab bench and sampled in a randomly selected order each sampling day. There was no blocking effect observed. All cylinders were incubated for 21 days at 23°C after fertilizer and water application. Substrate moisture was maintained by weighing substrate cylinders every other day and adding deionized water to compensate for evaporative loss.

Substrates were also packed to their respective bulk densities into 28-cm tall, 2.54-cm diameter polyvinyl chloride cylinders for use with saturated media extracts during the experiment. One saturated media extract cylinder per substrate:fertilizer treatment, excluding the No N treatment, was selected on day 1, two on days 5 and 11, and three on day 16. Substrate from all gas cylinders was collected on day 21 for saturated media extract. Substrate from each cylinder was placed into a foil tray and oven-dried at 60°C for at least 48 hours or until

completely dry. Inorganic N concentration of dried substrate was determined by the spectrophotometric method (Doane and Horwáth, 2003) from a saturated media extract prepared with 1-M potassium chloride solution and filtered through 0.22 µm cellulose filter paper (Whatman, Cytiva, MA, USA) after 1.5 hours. Isotope analysis of inorganic N was performed on aliquots of the extract using a sequential diffusion method (Sørensen and Jensen, 1991). The ^{15}N isotopic analyses were performed at UC Davis Stable Isotope Facility on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20 – 20 isotope ratio mass spectrometer.

Nitrous oxide substrate-to-atmosphere gas fluxes were measured on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21 after fertilizer application. Insulated, vented, round polyvinyl chloride chambers (10-cm diameter and 15-cm height, with a polyvinyl chloride lid) were used for gas sampling. Each chamber had a stainless-steel vent tube secured into its side. The lid of each chamber had a hole sealed with a rubber septum. Each chamber was sealed onto a gas cylinder, with a rubber gasket overlapping cylinder and chamber, just prior to gas sampling. Gas samples were collected at each of four time intervals (0, 8, 16, 24 min) by inserting a needle attached to a 20-ml syringe through the septum on the chamber, withdrawing a 20-ml sample, and injecting the sample into an evacuated 12-ml Exetainer vial fitted with a grey butyl rubber septum (Labco Ltd., Lampeter, UK). The gas samples were analyzed on a Shimadzu gas chromatograph (Model 2014, Shimadzu Corp., Kyoto, Japan) linked to a Shimadzu auto sampler (Model AOC-5000). The GC was equipped with a ^{63}Ni electron capture detector for N_2O, a thermal conductivity detector for CO_2, and a flame ionization detector for CH_4. The GC was calibrated before each operation by using analytical grade CO_2 and N_2O gas standards (Airgas Inc., Sacramento, CA) prepared at

the same time the samples were collected. Stable N isotope analysis of N_2O from samples collected at 24 min was performed at the UC Davis Stable Isotope Facility, on a ThermoFinnigan GasBench + PreCon trace gas concentration system connected to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (Bremen, Germany).

Gas flux calculation

Chamber gas concentrations were converted to mass per volume using the ideal gas law and chamber air temperature, which was measured by a HOBO temperature datalogger (Onset Corporation, Bourne, MA) at the time of sampling. Gas fluxes were calculated with the 'gasfluxes' package for R using chamber volume, surface area, and rate of change in chamber gas concentration (Fuss, 2019; Hutchinson and Mosier, 1981). The 'gasfluxes' package in R used the linear method when chamber gas concentrations had a constant rate of increase over time (Hutchinson and Mosier, 1981); the robust linear method when interpolation between points was necessary; and the curvilinear method when chamber gas concentrations changed at a decreasing rate over time (Pedersen *et al.*, 2010). The curvilinear method is applicable for high porosity soils or substrates in which gas can move horizontally, and/or when high flux rates result in achieving equilibrium of chamber gas concentration before the end of the sampling period (Pedersen et al., 2010). Kappa.max was employed to select a flux result from the three flux calculation methods following the guidelines from Hüppi et al. (2018). Minimum curvilinear flux detection limit was calculated via a Monte Carlo simulation as described by Parkin et al. (2012).

Contributions of ammonia oxidation or heterotrophic denitrification to N_2O production were calculated based on the results from the ^{15}N-labeled NH_4^+ or NO_3^-, respectively. Nitrous oxide production derived from ammonia oxidation was assumed to be equal to the ^{15}N isotopic enrichment of NH_4^+. Ascribing N_2O production nitrifier-nitrification, nitrifier-denitrification, or coupled nitrification-denitrification was not possible in this experiment. The contribution of N_2O derived from ammonia oxidation was calculated from equation 2 using results from the $^{15}NH_4^+$ labeled treatment; heterotrophic denitrification was calculated from equation 3:

(2)

(3)

$$n = (a_m - a_d)/(a_n - a_d)$$

$$d = 1 - n$$

where n is the contribution of N_2O-N from ammonia oxidation, a_m is the fraction of ^{15}N in mixed N_2O produced by both processes, a_d and a_n are the ^{15}N atom fractions of substrate NO_3^- and NH_4^+ at time of gas sampling, and d is the contribution of N_2O-N from heterotrophic denitrification. The ^{15}N fraction of mixed N_2O (a_m) was taken directly from the gas fluxes measured at each sampling day. For days when the mineral N species was extracted (days 1, 5, 11, 21), a_d and a_n were based on the mean value of ^{15}N fraction for that day. For days when the mineral N species was not extracted (days 3, 7, 9, 13, 15, 17, 19), linear interpolation was used for ^{15}N-enrichment of mineral N species.

Gas fluxes measured each sampling day were used to estimate cumulative cylinder-scaled gas emissions with trapezoidal integration of daily fluxes, under the assumption that measured fluxes represented daily mean fluxes and the mean daily fluxes changed linearly between

measurements (Zhu-Barker *et al.*, 2017). For days when a gas flux measurement was collected, the contribution of ammonia oxidation or heterotrophic denitrification was calculated using equations 1 and 2, respectively. For those days between gas flux measurements, linear interpolation was used to estimate the contribution of ammonia oxidation or heterotrophic denitrification and this value was multiplied by the estimated N_2O flux value for that day. Emission factor was calculated as the total N_2O-N emissions divided by the total nitrogen fertilizer applied. Mean total N_2O emitted per substrate was estimated as the total cumulative N_2O emitted per gas cylinder.

Statistics

The common logarithm [$\log_{10}(x)$] of N_2O and CO_2 gas concentrations of gas flux samples was utilized to improve normality of residual errors of N_2O and CO_2 flux. A constant (1.54) was added to the product of the common logarithm transformation before N_2O flux was calculated to accommodate the inability of 'gasfluxes' (Fuss, 2019) to use negative gas concentration values. A weighted model was fitted to account for heteroscedasticity in N_2O and CO_2 flux response from the different substrates on different days. Heteroscedasticity was linked to daily flux variance and a weighted model was fitted using lmer() from 'lme4' package in R. Kenward-Roger method was used for approximating degrees of freedom for weighted models of log-transformed N_2O and CO_2 flux. Model assumptions were confirmed by Levine test and Shapiro-Wilks test. Gas cylinder was given a random intercept to account for possible dependence among N_2O or CO_2 flux over sampling days. The N_2O flux results using the common logarithm of N_2O gas concentrations were analyzed with the inclusion of the Unfertilized treatment and then the three substrates were analyzed without the Unfertilized treatment to elucidate subtler

differences. The CO_2 flux results were analyzed with all the fertilizer treatments together to elucidate differences between NH_4NO_3 added treatments and the unfertilized treatment. Tukey's test was used for separation of means ($\alpha = 0.05$) for N_2O flux on each sampling day between the NH_4NO_3 and unfertilized treatments within each substrate, total N_2O emitted from NH_4NO_3 treatments per substrate and CO_2 flux on each sampling day between the NH_4NO_3 and unfertilized treatments within each substrate.

Results

Extractable NH_4-N and NO_3-N concentrations were comparable among the three different substrates at experiment initiation and decreased over the course of the experiment (Figure 3.1).

In the peat:fir substrate fertilized with $^{15}NH_4NO_3$, ^{15}N-enrichment of N_2O did not occur until the last three days of the experiment (Figure 3.2). In peat substrate fertilized with $^{15}NH_4NO_3$, ^{15}N-enrichment of N_2O occurred near the middle of the experiment and the proportion of ^{15}N-enrichment of N_2O increased on each subsequent day thereafter. Nitrogen-15 enrichment of NH_4-N and NO_3-N extracted from all substrates fertilized with $^{15}NH_4NO_3$ exhibited a downward and upward trend, respectively (Figure 3.2).

In fir bark and peat:fir bark substrates fertilized with $NH_4{}^{15}NO_3$, increased ^{15}N-enrichment of N_2O (Figure 3.2) coincided with increasing N_2O flux (Figure 3.3). Enrichment of N_2O from peat substrate fertilized with $NH_4{}^{15}NO_3$ followed a similar trajectory until day 11, when ^{15}N-enrichment of N_2O began to decline (Figure 3.2). Peat substrate fertilized with $NH_4{}^{15}NO_3$ had a constant ^{15}N-enrichment of NH_4-N throughout the experiment. Fir bark substrate fertilized with

$NH_4{}^{15}NO_3$ showed an increase ^{15}N-enrichment of NH_4-N throughout the experiment. The ^{15}N-enrichment of NH_4-N in peat:fir bark substrate fertilized with $NH_4{}^{15}NO_3$ increased on day 16 before decreasing on day 21.

Significant differences in N_2O-N flux were occasionally observed among different ^{15}N-enriched NH_4NO_3 treatments on individual sampling days within the different substrates (Figure 3.3). Aside from differences between with- and without-NH_4NO_3 treatments, there was not a clear pattern in differences in N_2O-N flux observed between the ^{15}N-enriched NH_4NO_3 treatments. Nitrous oxide-N flux was low among the three different substrates fertilized with NH_4NO_3 at experiment initiation, and began to increase on day three (Figure 3.3). The fir bark substrate had a rapid increase in N_2O flux, with large emissions observed from day five until almost the end of the experiment (Figure 3.3). Nitrous oxide-N flux from peat:fir bark substrate increased until day 13, after which it fluctuated until the end of the experiment (Figure 3.3). Nitrous oxide-N flux from peat substrate increased slightly until day seven, then decreased until day 17 before increasing again on the last days of the experiment (Figure 3.3). Total N_2O-N emitted was greatest from the fir bark substrate and lowest from the peat substrate (Table 3.2). The peat:fir bark substrate had a mean total N_2O-N emitted that was close to the value observed from the fir bark substrate. The majority of N_2O-N emitted from each substrate was from denitrification, with peat substrate emitting the most N_2O-N from nitrification (Table 3.2, Figure 3.4).

Emission factor was significantly greater for the fir bark and peat:fir bark substrates than the peat substrate (Table 3.2).

There was no significant difference in CO_2-C flux from the peat substrate between the NH_4NO_3 and Unfertilized treatments for any of the gas flux sampling days (Figure 3.5). Starting on day three all the NH_4NO_3 treatments from the fir bark and peat:fir bark substrates had significantly greater CO_2-C flux than the Unfertilized treatment. The only exception was days 17 and 19 from the peat:fir bark substrate, when the $^{15}NH_4NO_3$ and Unfertilized treatments were not significantly different.

Discussion

Heterotrophic denitrification was the major pathway of N_2O-N emissions from all three substrates but played a larger role in the fir bark and peat:fir bark substrates. Denitrification-derived N_2O-N emissions from soils are typically attributed to >70% water-filled pore space to denitrification-derived N_2O-N emissions (Bateman and Baggs, 2005; Dobbie et al., 1999). The three substrates were maintained at between 61-63% water-filled pore space (Table 3.1), and heterotrophic denitrification accounted for almost all N_2O-N emissions from the substrates (Table 3.2). This deviation from the prevailing thought about water content effect on N_2O-N emissions from soils may be due to the sharp vertical stratification of the volumetric water content in soilless substrates (Evans, 2014b; Passioura, 2006; Yeager et al., 2013). Water-filled pore space >70% in the bottom portion of the cylinder generated most heterotrophic denitrification-derived N_2O-N emissions, with a smaller portion associated with anoxic microsites (Flessa and Beese, 1995; Tiedje *et al.*, 1984) in the upper substrate. A decrease in redox potential at the bottom of 8-cm tall containers following flood irrigation with nutrient solution indicated that an anoxic zone and denitrification was the source of N_2O-N emissions from a peat:coir substrate planted with *Pelargonium zonale* plants (Agner and Schenk, 2006a).

Six-percent of total N_2O-N emissions from peat substrate were from ammonia oxidation-derived N_2O-N (Figure 3.4, Table 3.2). This contribution could increase if the experiment were longer, because contribution from ammonia oxidation started on day 11 and increased to day 21. Fir bark substrate had very little ammonia oxidation-derived N_2O-N during the 21-day experiment (Table 3.2, Figure 3.4). The low rate of nitrification as indicated by ammonia oxidation-derived N_2O-N emissions in the three substrates agrees with studies evaluating nitrification in other substrates (Niemiera and Wright, 1987; Ogden and Mills, 1988). Niemiera and Wright (1987) observed little nitrification before day 21 in pine bark substrate. A greater contribution of ammonia oxidation-derived N_2O-N may have been realized from all substrates if nitrifier populations had more than 21 days to grow to levels capable of significant nitrification.

Previous research has shown that microbial community composition differs among substrates (Montagne *et al.*, 2017). Although we do not know the taxa that make up this composition, it is possible that peat has a larger population of nitrifiers than fir bark. *Nitrosomas* and *Nitrobacter* species were both detected in peat soils at pH 5.3-5.9 (Herlihy, 1971), and it would be reasonable to assume these microbes were present in harvested peat as well. Ammonia oxidation-derived N_2O-N from the peat:fir bark substrate (21.5 mg N_2O-N) was approximately the mean value of ammonia oxidation-derived N_2O-N from the separate peat and fir bark substrates (Table 3.2), suggesting that nitrification in the peat:fir bark substrate was closely tied to the peat amendment and possibly the nitrifying organisms associated with it. In soils, nitrifiers are more closely associated with clay than sand particles as a result of the greater cation-exchange capacity of clays, which protects nitrifiers from H^+ ions formed during ammonia oxidation (Powell and Prosser, 1991). Nitrifiers in peat may be afforded similar

protection because it is generally reported that peat has greater CEC than fir or pine bark (Bollen, 1969; Bunt, 1976; Goh and Haynes, 1977; Lemaire, 1995), possibly due to more decomposition and humification of peat (Goh and Haynes, 1977).

Greater heterotrophic denitrification-derived N_2O-N emissions (Figure 3.4) and mean total N_2O-N emissions were recorded from fir bark and peat:fir bark substrate than peat substrate (Table 3.2). Organic carbon is an energy source and electron donor for denitrifers (Knowles, 1982), and denitrification potential is limited by low organic carbon content of soils (Myrold and Tiedje, 1985; Weier et al., 1993). Burford and Bremner (1975) reported that denitrification is more closely associated with water soluble carbon than total organic carbon. There is significantly more dissolved organic carbon content in the fir bark and peat:fir bark substrates than in peat (Table 3.1). Peat is primarily composed of lignin (Carlile and Wilson, 1991), with the majority of easily decomposable carbon consumed before peat was harvested from bogs. Thus, fir bark and peat:fir bark are likely to have more easily decomposable carbon, like sugars, for denitrification. When peat substrate was treated with glucose, N_2O-N, N_2 (Amha and Bohne, 2011) and CO_2-C (Turner and Carlile, 1984) evolution increased, suggesting that peat substrate lacks sufficient easily decomposable carbon to support substantial denitrification.

Carbon dioxide flux from unplanted soils and soilless substrate is a measure of microbial respiration, i.e. microbial activity (Jenkinson and Powlson, 1976; Turner and Carlile, 1984). The increase in CO_2-C flux on day three from fir bark and peat:fir bark with NH_4NO_3 added (Figure 3.5) indicates greater microbial activity in these substrates when nitrogen is added. Lack of significant difference in CO_2-C flux from peat substrate with or without NH_4NO_3 throughout the

experiment is in agreement with previous studies (Jackson *et al.*, 2009) and is likely due to the low DOC content of peat.

It is not clear why ^{15}N content of KCl-extracted NO_3-N in the $NH_4{}^{15}NO_3$ treatment was near 3 atom% for the duration of the experiment. It could be that not all NH_4-N was removed in the first step of the sequential diffusion trap procedure, because unenriched NH_4-N would be captured with the trap intended to capture enriched NO_3-N, thereby diluting the ^{15}N content of the trap. This logic is supported by 10 atom% ^{15}N content of N_2O-N from the $NH_4{}^{15}NO_3$ treatment in fir bark media starting on day five. However, if NH_4-N remained in solution at the end of the first step of sequential ammonia diffusion trapping, the NO_3-N content from the $^{15}NH_4NO_3$ treatment would be enriched, and it was at or near natural abundance on each sampling day. Additionally, to verify the sequential diffusion trap procedure, $^{15}NH_4NO_3$ was dissolved in 1M KCl solution, and there was no ^{15}N enrichment of recovered N from the second step, signifying that the protocol was robust.

It is not clear why the initial concentrations of NH_4-N and NO_3-N extracted with KCl differed, or why they exceeded 200 mg N L^{-1} in the three substrates (Figure 3.1). One possibility is the occurrence of immobile porosity composed of dead end and closed pores. Numerous studies have identified immobile pores in peat substrate (Caron *et al.*, 2015; Hoag and Price, 1997; Ours *et al.*, 1997), and it is reasonable to expect these in all types of organic amendments. Immobile pores delay desorption of salts when testing breakthrough curves (Caron et al., 2015). It is possible that DI water occupied immobile pores before soluble NH_4NO_3 was added to substrates and diffusion of ions into closed pores was delayed or prevented. All three substrates were incubated with DI water for one week before adding 200 mg N L^{-1} as NH_4NO_3 in

solution; the intended N concentration was based on the total volume of water present in all pores, including immobile pores, but NH_4NO_3 was only dissolved in the water that was added at beginning of experiment.

Previous research has shown that N_2O-N emission from fir bark substrate used in California nursery production is significant (Pitton et al., 2021). The results of this study indicate that almost all N_2O-N emissions from this substrate are the product of heterotrophic denitrification. Therefore, identifying practices to decrease heterotrophic denitrification-derived N_2O-N emissions in growing substrates could significantly reduce N_2O-N emissions from container plant production.

One strategy to reduce N_2O-N emissions from growing substrate could be to use organic amendments with less DOC content. Peat, almond shells, and coir typically have lower DOC than wood fiber, composts, and compost-based growing substrates (Pigoli et al., 2019). Fir and pine barks, which are common in US nursery production, have not been extensively tested. Composting fresh organic amendments before use as growing substrate could partially decrease DOC concentration (Giuliana and Fabrizio, 2007), but this is likely to generate greenhouse gas emissions during the composting process (Beck-Friis *et al.*, 2000; Hellmann *et al.*, 1997; Zhu-Barker et al., 2017). Producing plants in container substrates requires leaching to reduce salt buildup (Evans, 2014a), and this leaching may also reduce DOC concentration in substrate solution over time (Shreckhise *et al.*, 2019). Leaching DOC from a fertilized fir bark substrate may have contributed to lowering N_2O-N emissions over time (Pitton et al., 2021), but DOC in pour-through extracts was not quantified. However, increased leaching fraction reduces water (Bilderback *et al.*, 2013) and nutrient efficiency (Niemiera and Leda, 1993; Tyler *et al.*,

1996), thereby trading the benefit of reduced N_2O-N emissions from soilless substrate with other potentially negative environmental effects.

Nitrification-derived N_2O-N emission from all substrates was a small proportion of total emissions, which indicates that a greater proportion of NH_4-N fertilizer, possibly with nitrification inhibitors to maintain N as NH_4-N, could be provided for plant growth. Nitrification inhibitors could reduce the amount of NO_3-N in substrate solution and decrease heterotrophic denitrification (Slangen and Kerkhoff, 1984). However, plants typically have greater preference for NO_3-N and vary in susceptibility to NH_4^+ toxicity (Hawkesford *et al.*, 2012), so care in using excessive NH_4-N fertilizer and nitrification inhibitors should be taken to avoid phytotoxicity.

Although substrate microbial populations are responsible for N_2O-N emissions, plants exert some influence over this system during production. Plants significantly decrease volumetric water content through transpiration, resulting in drier substrate conditions being reached more rapidly than in substrate without plants. Transpiring plants decreased N_2O-N emissions from peat substrate by drying the substrate (Agner and Schenk, 2006b) and possibly taking up NO_3^- that would otherwise be denitrified. Volumetric water content was found to be a significant factor affecting magnitude of N_2O-N emissions from fir bark substrate (Pitton et al., 2021). During this experiment, volumetric water content was maintained at or near container capacity to isolate organic amendment effects which may have increased N_2O-N emissions. Additionally, irrigation application and drainage facilitate oxygen diffusion into the growing substrate. The increased oxygen content may reduce anoxic microsites responsible for a portion of denitrification-derived N_2O-N emissions (Flessa and Beese, 1995; Tiedje et al., 1984) in the upper portion of the substrate.

Further research into organic substrate properties effects on heterotrophic denitrification and ammonia oxidation-derived N_2O emissions could potentially identify additional opportunities to mitigate global warming potential from soilless substrates.

Literature Cited

Tables and Figures

Table 3.1. Physical and chemical properties of each substrate prior to experiment initiation.

Media	Bulk Density (g cm^{-3})	Total Porosity (%)	Water Content (%)[a]	Air-filled porosity (%)[a]	pH[b]	EC (µs cm^{-1})[b]	NH_4-N (mg L^{-1})[c]	NO_3-N (mg L^{-1})[c]	DOC (mg L^{-1})[c]	C:N ratio	WFPS[d]
Peat	0.278	89	57.8	31.1	6.35	371.3	16.71	0.36	123.6 a	143:1	63%
Fir bark	0.436	83.9	51.1	32.9	6.21	449.7	3.05	0.20	453.4 b	162:1	61%
Peat:Fir bark	0.369	85.8	51.8	34	6.39	422.3	5.28	0.03	361.1 b	157:1	62%

a. Measured at container capacity.

b. After addition of 29.94, 6.79, and 18.31 g dolomite lime m^{-3} substrate for the peat, fir bark, and peat:fir bark substrates, respectively.

c. Reported as concentration at experimental volumetric water content.

d. WFPS = Water-filled pore space.

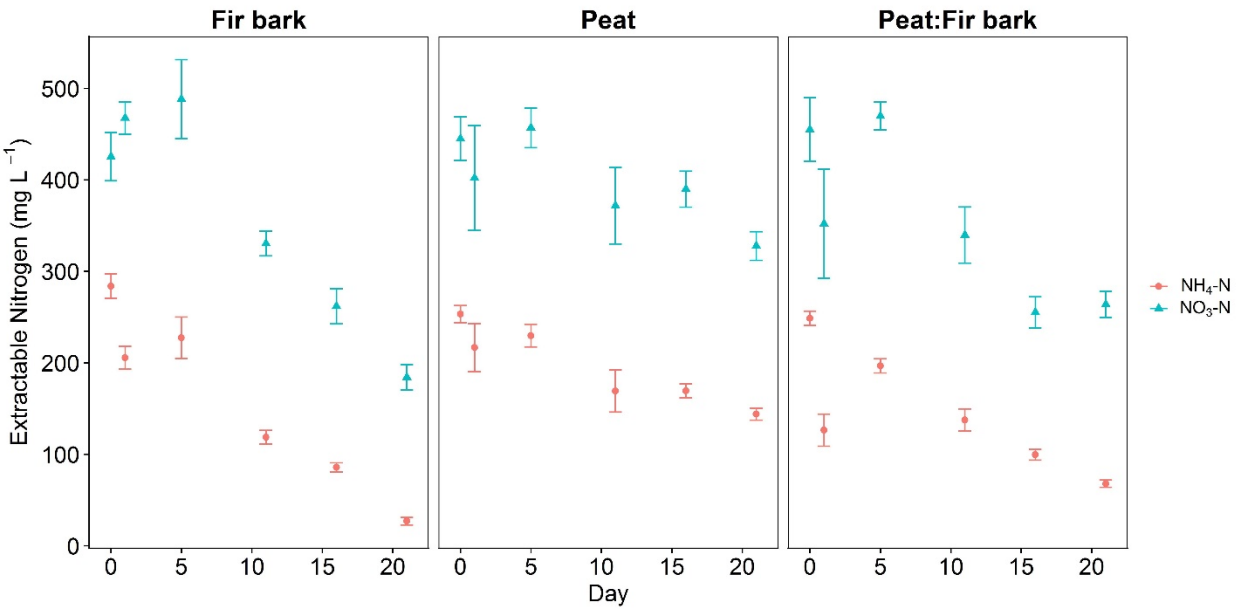

Figure 3.1. Mean extract concentration from substrates fertilized with ammonium nitrate. Error bars represent one standard error of the mean.

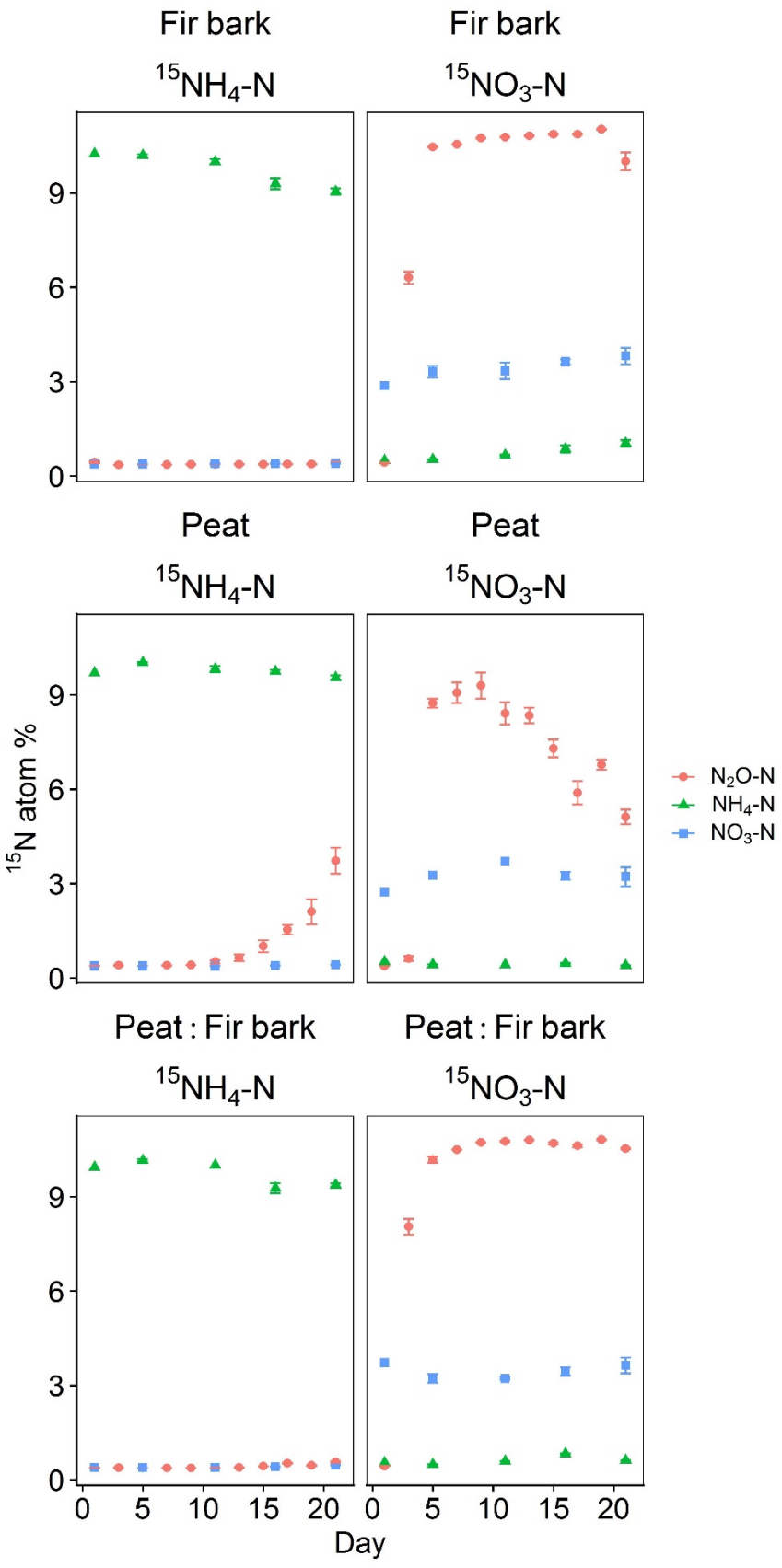
Fir bark
$^{15}NH_4$-N
Fir bark
$^{15}NO_3$-N
Peat
$^{15}NH_4$-N
Peat
$^{15}NO_3$-N
Peat : Fir bark
$^{15}NH_4$-N
Peat : Fir bark
$^{15}NO_3$-N
^{15}N atom %
0
3
6
9
0
5
10
15
20
Day
N_2O-N
NH_4-N
NO_3-N

Figure 3.2. Content of ^{15}N recovered in N_2O-N flux samples, and NH_4-N and NO_3-N in substrate solution from substrates. Error bars represent one standard error of the mean.

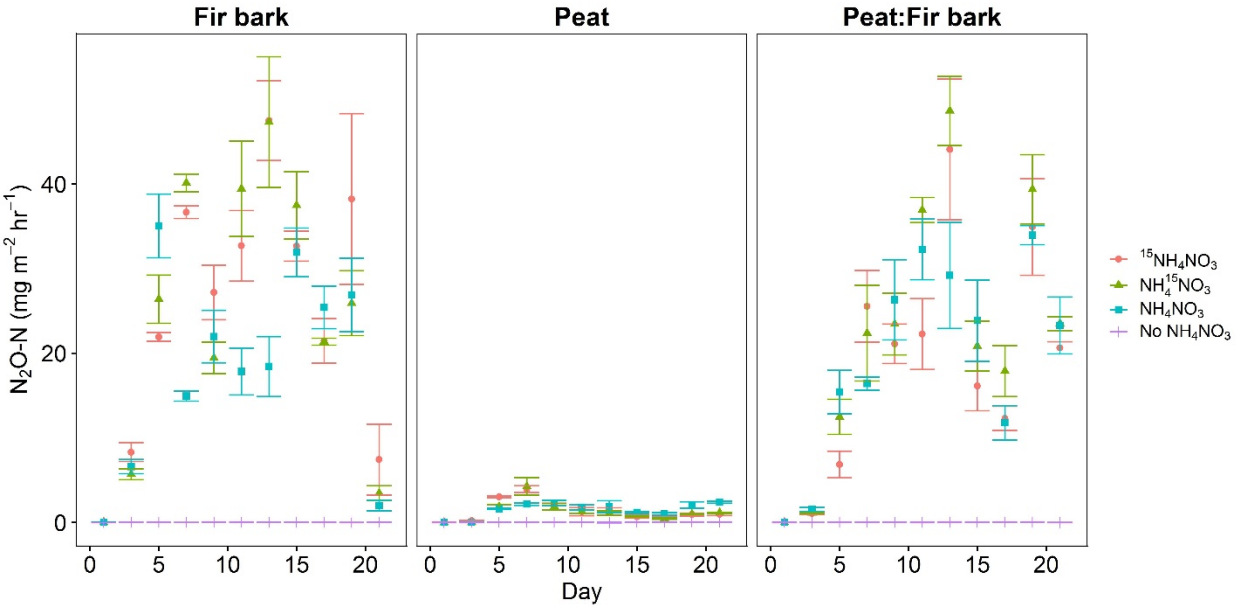

Figure 3.3. Mean nitrous oxide flux from three different substrates fertilized with ammonium nitrate or unfertilized. Error bars represent one standard error of the mean.

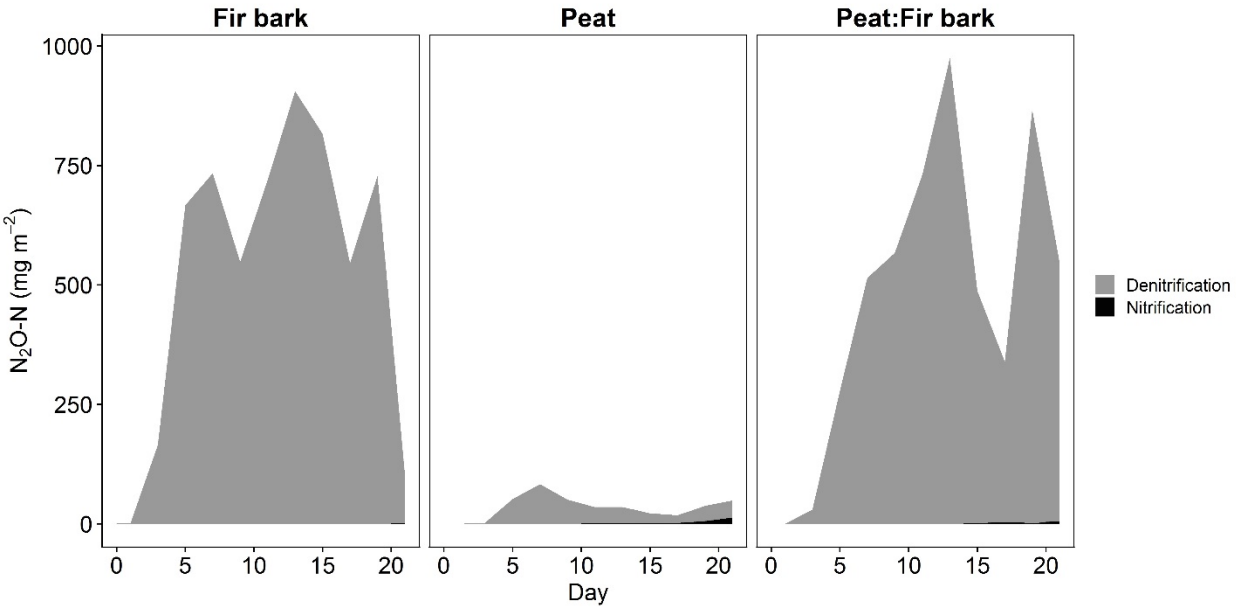

Figure 3.4. Contribution of heterotrophic denitrification or nitrification to nitrous oxide-nitrogen emitted from three substrates. Be aware that different y-axes are present in each facet to show the N_2O-N contribution from nitrification.

Table 3.2. Total N_2O-N emitted, contribution from denitrification (Denitri.) and nitrification (Nitrifi.), and total combined emission factor (EF) for three substrates with NH_4NO_3 applied over 21 days. Lower case letters after values indicate significant differences ($p < 0.05$) in total N_2O-N emitted.

Media	Total N_2O-N emitted (g m^{-2})	Denitri.	Nitrifi.	Combined EF
Fir bark	11.81 a	99.991%	0.009%	42.05%
Peat	0.70 b	94.102%	5.898%	2.75%
Peat:Fir bark	10.37 c	99.793%	0.207%	39.17%

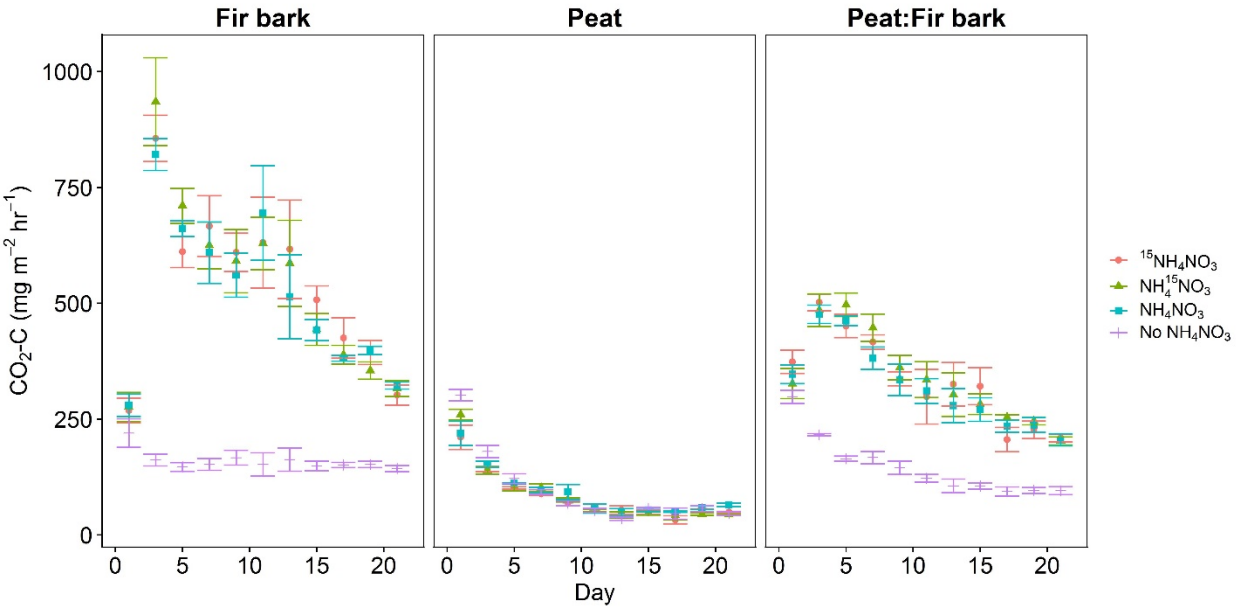

Figure 3.5. Mean carbon dioxide flux from three substrates fertilized with ammonium nitrate or or unfertilized. Error bars represent one standard error of the mean.

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Summary

Complete quantification of N removed at harvest for the large number of plant taxa in California's diverse production container-plant systems is not likely to occur. Quantifying inputs and outputs for estimating a system N balance for nursery production is time-consuming, costly, and exceeds the level of expertise of employees at most nurseries. Facilitating implementation of best management practices may be more effective at reducing N leaching into groundwater than requiring plant nurseries to complete the INMP. The causes of surface- and groundwater contamination by nurseries are N leaching and runoff from soilless substrates, which could be addressed by best management practices to improve water and fertilizer management. For example, avoiding application of soluble fertilizer through overhead irrigation systems reduces N leaching and runoff, thereby supporting the Central Valley Regional Water Quality Control Board's goal to reduce nitrate contamination of groundwater. Multiple publications (Bilderback *et al.*, 2013; Newman, 2014) provide best management practices that summarize the literature on reducing N leaching from container-plant production (Andiru *et al.*, 2015; Broschat, 1995; Cabrera *et al.*, 1993; Chen and Wei, 2018; Conover *et al.*, 1994; Cox, 1993; Fare *et al.*, 1994; Hershey and Paul, 1982; Hoskins *et al.*, 2014; Huett and Morris, 1999; Juntunen *et al.*, 2003; Merhaut *et al.*, 2006; Newman *et al.*, 2006; Ristvey *et al.*, 2004; Tyler *et al.*, 1996; Yelanich and Biernbaum, 1994). Accelerating extension efforts to communicate best management practices to reduce N leaching and runoff may have a greater impact on reducing nitrate leaching to groundwater than implementation of INMPs.

However, best management practice guidelines do not exist for mitigating N_2O-N emissions from soilless substrates because very little research has been done on the topic and factors

contributing to N_2O-N emissions from soilless substrates are not clearly understood. Compared to N leaching losses, few studies have described N_2O-N emissions from soilless growing substrate (Marble *et al.*, 2012a; Marble *et al.*, 2012b; Murphy *et al.*, 2019; Murphy *et al.*, 2018), and even fewer have tried to address the cause of N_2O-N emissions from soilless substrates (Agner and Schenk, 2005, 2006a, b). Best management practices to reduce N_2O-N emissions could have a significant impact on reduction of the global warming contribution from California nursery crop production. Coupling cultural practices of container-plant producers and physical and chemical characteristics of soilless substrates with knowledge of factors contributing to N_2O-N emissions could lead to improved best management practices. Implementation of best management practices that reduce N_2O-N emissions and global warming potential from soilless substrates will help California to meet Executive Order B-55-18 and achieve carbon neutrality goals.

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