Combined Proceedings IPPS 72:79-86. 2022.

# **Container Grown Plants are Gassy**

Forrest J. Brown<sup>1</sup>, James S. Owen Jr.<sup>2</sup> and Alex X. Niemiera<sup>1</sup>

<sup>1</sup>School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA, USA <sup>2</sup>U.S. Department of Agriculture, Agriculture Research Service, Application Technology Research Unit, Wooster, OH, USA

### fjbrown92@vt.edu

*Keywords*: Nitrogen, containerized crops, soilless substrates, nursery production, control-release fertilizer, Fourier Transform Infrared Spectroscopy (FTIR)

#### Summary

Rising mineral nutrient and polymer costs are placing a direct economic burden on nursery crop producers utilizing controlledrelease fertilizer. Nitrogen (N) inputs in the containerized crop production have potential inefficiencies under a broad scope of cultural practices. While N gas emissions have been investigated in other forms of crop production, emission research on container-grown nursery production has been limited due to analytical methodology and the complexity of gaseous fate. The objective was to compare two control-release fertilizers (CRFs), ammonium nitrate (AN) vs urea ammonium nitrate (UAN), to determine the gaseous emissions between fertilizer treatment based on time of day using fourier transform infrared spectroscopy

(FTIR). These data were then used to estimate seasonal flux loss over a 51-day period. Results showed there was a higher degree of variability of gaseous flux [nitrous oxide (N<sub>2</sub>O), nitric oxide (NO), and nitrogen dioxide (NO<sub>2</sub>)] in the beginning of the season when CRFs began releasing, more consistent fluxes were exhibited during the mid to late production season. Gaseous fluxes of N species were similar regardless of CRF and time treatments for all N species; only summation of N species  $(\sum N)$ showed statistical differences. The study of gaseous emissions in nursery production is still in its infancy and more research is necessary to gain a better understanding of gaseous flux and factors influencing flux for container-grown crops.

### IPPS Vol. 72 - 2022

79

Copyright© Brown et al. The use, distribution or reproduction of materials contained in this manuscript is permitted provided the original authors are credited, the citation in the Proceedings of the International Plant Propagators' Society is included and the activity conforms with accepted Academic Free Use policy.

# INTRODUCTION

Daily fertilization is essential in containerized crops and a routine practice in nursery production. Despite modern advancement in mineral nutrient delivery and the science of understating mineral nutrient fate, applied nitrogen (N) remains complex and is poorly understood due to the numerous chemical and biological pathways of the N cycle within the growing media (Creamer et al., 2022). Closing the gap in understanding N fate can help pave the way for a more economically efficient and environmentally friendly nursery industry.

Nitrogen is applied to containerized nursery crops typically as a controlled release fertilizer (CRF) containing a combination of ammonium (NH4), nitrate (NO3), or urea [CO(NH2)2]. Each of these N forms result in different, concurrent microbial and chemical processes. This adds another layer of complexity to the question, "Where is the applied N going?".

Nitrogen budgets quantifying the various pathways of applied N (**Fig. 1**; e.g., plant, substrate, leached) have helped growers and scientists learn and build upon previous estimates of N fate (Navarez et al., 2012; Navarez et al., 2013; Pitton et al., 2022; Warren et al., 1995). However, quantification of N losses vary considerably due to methodology and production practices (Marble et al., 2011; Marble et al., 2012; Pitton et al., 2021).

Nitrogen applied to containerized bark-based substrates undergo several transformations and can ultimately, depending on conditions, form various gaseous species generally termed nitrous oxides (NOx). Specifically, NOx is any form of gaseous N species with an oxide group excluding dinitrogen (N<sub>2</sub>) and ammonia (NH<sub>3</sub>).





One of the NOx species receiving significant scrutiny, nitrous oxide (N<sub>2</sub>O), is a major climate warming gas of concern due to its long-lived nature and global warming potential 298 times greater than carbon dioxide (CO<sub>2</sub>) (Myhre et al., 2013). While gaseous N losses are an environmental concern, there is also an economic concern for growers. The grower's overarching goal is to provide a crop with adequate N throughout the production cycle to produce a saleable product in the shortest time possible. Growers suffer a monetary loss when a large portion of applied N is unused by the plant and specifically lost as a gas from the container.

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical method used for measuring and determining gas emissions and enables individuals to measure NH<sub>3</sub>, N<sub>2</sub>O, NO, and NO<sub>2</sub> simultaneously, in real-time in field settings with a 'rugged' instrument. By measuring the N gaseous species previously listed we can solve and account for  $N_2$  gas lost from complete denitrification (NO<sub>3</sub> microbially ultimately transformed to  $N_2$  gas in a multi-step reaction). Utilizing this technology, our objective for this portion of the research project was to better understand the gaseous N losses from container-grown plants.

# MATERIALS AND METHODS

The experiment took place from 15 July 2020 to 4 September 2020 at the United States Department of Agriculture, Agriculture Research Service (USDA-ARS) Application Technology Research Unit in Wooster, OH.

This experiment was a single factor experiment comparing two N source formulations in a completely randomized block design. Sampling and harvest of three experimental unit replicates were completed on: 1, 2, 3, 6, 9, 12, 15, 19, 23, 27, 31, 35, 39, 43, 47, 51 days after initiation (DAI). At each date, substrate solution extract was collected analyzed for N species using an ion chromograph, fertilizer was collected and digested to determine N content remaining, plant (root and shoot) and substrate were harvested and analyzed for total N content, and N gas flux was measured using FTIR at 1000 hrs. [morning(10:00am)] and 1500 hrs. [afternoon (3:00pm)].

*Forsythia* ×*intermedia* Showoff<sup>®</sup> liners (Baileys Nursery, St. Paul, MN) were transplanted in #2 trade containers (7.33L, C900, Nursery Supplies Inc., Chambersburg, PA) with a pine bark substrate (T.H. Blue Inc., Eagle Springs, NC) amended with 1.51 kg·m<sup>-3</sup> of ground dolomite lime (95.0% CaCO3 equivalent, 21.6% Ca, 10.0% Mg; Soil Doctor, Atlanta, GA) and 0.89  $kg \cdot m^{-3}$  granular micronutrient fertilizer (Micromax, Everris, Dublin, OH) on July 10, 2020.

The two N source treatments were month) control-release fertilizers (3-4 (CRFs): 42.0 g per container of 15.0N:3.9P:9.9K ammonium-nitrate based CRF (AN) (15-9-12; 8% NH4-N, 7% NO3-N; Osmocote, ICL, Charleston, SC with a water-soluble pre-charge of 0.23 % applied N): and 35.0 g per container of 18.0N:2.2P:6.6K urea ammonium nitrate CRF (UAN) (18-5-8; 6.3% NH4-N, 5.4% NO3-N, 6.3% Urea-N) (Osmocote, ICL, Charleston, SC) Each CRF was top-dressed on July 14, 2020 and surface incorporated into the substrate surface (2-3 cm below the surface by hand). This resulted in 6.3 g of N being supplied per container for CRF treatments based on the fertilizer label.

Containers were then placed in an open-air nursery setting and received 18 mm daily over-head irrigation application (05:00 HR) via upright mini-Wobbler sprinklers (#4 nozzle, 1.6 mm orifice; Senninger, Clermont, FL, U.S.) on 91-cm risers.

Gas analysis occurred on all sampling days using FTIR (Gasmet Terra GT5000, Vantaa, Finland). To measure gas flux from a container, a chamber was made using a plastic 22.7 (L) bucket with Polyurethane Tubing (McMaster-Carr, Cleveland, OH) to make a closed loop with the FTIR (Fig. 2). One experimental unit (planted and fertilized container) was placed inside the chamber of the closed loop system, and the gas flux was measured over five minutes. The Gasmet apparatus would then be brought back to ambient gas concentrations; sampling atmospheric air between each experimental sample (3 min) prior to sampling the next replication. Data were

used to calculate gas flux by using Equation [1] (Gyawali et al., 2019).

$$f_{NOx} = \frac{P_0 V_c}{RToA} \times \frac{\Delta C}{\Delta t} [1]$$

The formula is defined by  $P_o$  the pressure within the chamber [M  $L^{-1} t^{-2}$ ]. This is assumed to be equivalent to the atmospheric pressure. V<sub>c</sub> is the cumulative

volume of the chamber, apparatus internal volume, and tubing volume  $[L^3]$ , R is the ideal gas law constant  $[M L^2 N^{-1} K^{-1} t^{-2}]$ , T<sub>0</sub> is the air temperature [K], A is the exposed surface area of an individual container or experimental unit  $[L^2]$ ,  $\Delta C$  is change in concentration of a given gas on a molar basis  $[N N^{-1}]$  which is then compared to change in time in seconds  $\Delta t$  [s]. Three samples per treatment were measured (n = 3). (Gyawali Et al., 2019).



Figure 2. Depiction of gas sampling setup which includes (from left to right) Fourier transform infrared gas analyzer FTIR), 1/4" (6.35 mm) tubing creating a closed loop with a 6-gal (22.7-L) sealed chamber containing #2 containerized *Forsythia* placed upon a 1" (25.4 mm) PVC ring. A desiccant and water filter were added to the chamber return or FTIR intake to control humidity and protect internal mirrors.

Gas measurements occurred during both the morning and the afternoon of sampling dates; this was conducted to see if there was a difference between gaseous flux based on time of the day. Preliminary analysis of N gas measurements was made by graphing ~180s to determine change in concentration over change in time (Equation 1). These data were then graphed and checked by the expected linear increase of  $CO_2$  gas concentration ( $R^2 < 0.90$ ).

Data analysis occurred using JMP Pro (JMP® Pro ver. 14, SAS Institute, Cary, NC). Data was analyzed by morning or afternoon measures (AM or PM); pooling across approximately 14-day segments to represent early, mid, late production period based on observed CRF release and subsequent gas emissions. Additionally, gas emissions occurring in the AM, PM or both were pooled across the entire 51 production period to compare means. All data were not normally distributed, therefore a non-parametric analysis, Wilcoxon / Kruskal-Wallis Test, was used to determine statistically significant differences between means, comparing differences between morning and afternoon or fertilizer formulation.

## **RESULTS AND DISCUSSION**

There was no difference in gaseous species losses during three periods of the growing season: (early, mid, and late production) for fertilizer treatments, however, the sums of all reactive N gaseous species ( $\sum RN$ ) were different between CRF treatment (Fig. 3). For both CRF treatments,  $\sum RN$  was highest in the first period (early season) and stabilized thereafter, and gaseous flux was more consistent in the mid to late season periods. Over the entire season time of day data were the same (**Fig. 3**).



**Figure 3**. N species gaseous losses based on CRF treatment shown as box plots (AN vs. UAN) during morning (AM) and afternoon (PM) at three stages of the production cycle (early (1-17 DAI), mid (18-34 DAI), late season (35-51 DAI)). Gaseous flux ( $f_{NO}$ -N(Eq. 1) is defined as (ug cont-1 sec-1) is shown on a logarithm scale (b=10).  $f \sum NnXn$  is the sum of all N species measured.

There were no differences in N gaseous species throughout the entire growing sea-

son; the only differences are the  $\sum RN$  values; PM values were higher than AM values for both fertilizer treatments (**Table 1**).

**Table 1**. N species gaseous flux of two CRF fertilizer based on time of day (AM/PM) and over entire season. Flux of N species (f) and sum of N gaseous species ( $\sum$ N) Statistically significant (P-value <0.05) values denoted by red text and asterisk (\*). Average values and  $\pm$  standard deviation are shown for all gaseous species.

Fertilizer for- mulation	Time of Day	N	fN2O-N	fNH3-N	fNO-N	fNO2-N	$f \sum N$
					(µg cont <sup>-1</sup> min <sup>-1</sup> )		
Ammonium- nitrate	AM	46	0.26±0.04	0.0±0.0	0.00±0.00	0.01±0.01	0.28±0.04
	PM	36	0.49±0.07	0.0±0.0	0.11±0.11	2.30±1.32	2.90±1.29
	p- value		0.0658		0.2583	0.1888	0.0018
	Mean	82	0.36±0.04	0.0±0.0	0.05±0.05	1.02±0.59	1.43±0.58
Urea-ammo- nium-nitrate	AM	46	0.45±0.08	0.0±0.0	0.00±0.00	0.29±0.15	0.74±0.15
	PM	34	0.15	0.0±0.0	$0.08 \pm 0.07$	1.75±0.88	2.71±0.85
	p- value		0.1015		0.0979	0.7630	0.0227
	Mean	80	0.63±0.08	0.0±0.0	0.03±0.03	0.91±0.39	1.58±0.38
Fertilizer for- mulation	p- value		0.2420		0.5564	0.1550	0.0203

Using all the collected RN gaseous and liquid pathways of this study, over half of the applied N was lost via gaseous pathways. We hypothesized that the two major processes influencing gaseous losses are denitrification and nitrification. Denitrification and nitrification are two microbial processes in which there are several gaseous N intermediates or potential fates (NO, N<sub>2</sub>O, NO<sub>2</sub>, NH<sub>3</sub>, N<sub>2</sub>). Based on this research, we estimate gasses lost from highest to lowest concentration are: N<sub>2</sub> > NO > N<sub>2</sub>O. Since N<sub>2</sub> was not measured, we inferred that N<sub>2</sub> evolved via the microbial oxidation of N<sub>2</sub>O (Havlin et al., 2014). Any N not accounted for in plant tissue, fertilizer harvested, substrate, aqueous samples, or RN gas samples was lost as N<sub>2</sub> gas. Unfortunately, there is not currently a preferential methodology to determine  $N_2$  concentrations in gaseous samples (Takaya et al., 2003; Wang et al., 2011).

We maintain that using linear increase of CO<sub>2</sub> gas concentration ( $\mathbb{R}^2 < 0.90$ ) to determine gaseous flux of N or other gaseous species is not an appropriate method of analysis. This method would have simplified analysis by using a single gas to determine trends of gaseous species of interest. Each gaseous species exhibits a unique pattern potentially influenced by analytical detection limits and head space saturation that differ on an experimental unit basis. Therefore, analysis of individual RN gaseous species provides more insight into emissions rather than the method examined in this analysis.

Pour-through extractions of the pine bark substrate were conducted prior to gas sampling during this experiment on the

### LITERATURE CITED

Creamer, R.E., Barel, J.M., Bongiorno, G., and Zwetsloot, M.J. (2022). The life of soils: Integrating the who and how of multifunctionality. Soil Biol. Biochem. *166*:1-15.

Gyawali, A.J., Lester, B.J., and Stewart R.D. (2019). Talking SMAAC: A new tool to measure soil respiration and microbial activity. Front. Earth Sci. 7:1-7.

Havlin, J.L., Tisdale, S.L., Nelson, W.L., and Beaton J.D. (2014). Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 8<sup>th</sup> Edition. Pearson, N.Y., N.Y. same experimental units. This likely led to some of the N cycle products to have been flushed from the system that may have resulted in reduced N gas losses.

#### CONCLUSION

In conclusion, we found that more than half of applied N exits as a gas or is unaccounted presumably due to denitrification and nitrification. Hence, there is a need to further investigate and understand the various factors affecting gaseous N losses from a container system. Further investigation into the influence of the zone of saturation within the container, irrigation regime, fertilizer type and placement, and substrate physical properties are factors that may influence gaseous losses in nursery production. A comprehensive understanding of N gaseous emissions in nursery production can serve to close the gap on N application inefficiencies presented by gaseous loss of N from the container.

Marble, S.C., Prior, S.A., Runion, G.B., Torbert, H.A., Gilliam, C.H., Fain, G.B., Sibley, J.L., and Knight, P.R. (2011). Determining trace gas flux from containergrown woody ornamentals<sup>©</sup>. Comb. Procd. Internat. Plant Propagators' Soc. *61*:469-475.

Marble, S.C., Prior, S.A., Runion, G.B., Torbert, H.A., Gilliam, C.H., Fain, G.B., Sibley, J.L., and Knight, P.R. (2012). Effects of fertilizer placement on trace gas emissions from nursery container production. HortScience 47:1056-1062. Myhre, G., Shindell, D., Breon, F.M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.F., Lee, D., Mendoza, B., Nakajima, A., Roback, A., Stephens, G., Takemura, T., and Zhang, H. (2013). Anthropogenic and natural radiative forcing, p. 659-740. Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.). Climate Change 2013: The Physical Science Basis. Contribution of working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Narváez, L., Cáceres, and R., Marfá, O. (2012). Effects of climate and fertilization strategy on nitrogen balance in an outdoor potted crop of *Viburnum tinus* L. Span. J. Agric. Res. *10*:471-481.

Narváez, L., Cáceres, and R., Marfá, O. (2013). Effect of different fertilization strategies on nitrogen balance in an outdoor potted crop of *Osteospermum ecklonis* (DC.) Norl. 'Purple Red' under Mediterranean climate condition. Span. J. Agric. Res. *11*:833-841.

Pitton, B.J.L., Evans, R.Y., Zhu-Barker, X., and Oki, L.R. (2021). Greenhouse gas emissions and global warming potential from a woody ornamental production system using a soilless growing substrate. ACS Agric. Sci. *1*:35-43

Pitton, B.J.L., Oki, L.R., Sisneroz, and J. Evans, R.Y. (2022). A nursery system nitrogen balance for production of a containerized woody ornamental plant. Sci. Hortic. 291:1-7.

Takaya, N., Catalan-Sakairi, M.A., Sakaguchi, Y., Kato, I., Zhou, Z., and Shoun, H., (2003). Aerobic denitrifying bacteria that produce low levels of nitrous oxide. Appl. Environ. Microbiol. *69*:3152-3157.

Wang, R., Willibald, G., Feng, Q., Zheng, X., Liao, T., Brüggemann, N., and Butterbach-Bahl, K. (2011). Measurement of N<sub>2</sub>, N<sub>2</sub>O, NO, and CO<sub>2</sub> Emissions from soil with the gas-flow-soil-core technique. Environ. Sci. Technol. *45*:6066-6072.

Warren, S. L., Bilderback, and T.E., Tyler, H.H. (1995). Efficacy of three nitrogen and phosphorus sources in container-grown azalea production. J. Environ. Hort. *13*:147-151.