Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Volume 21 Article 12

Fall 2020

Microdialysis: a method for quantifying in situ nitrogen fluxes in soil microsites

Srusti Maddala
University of Arkansas, Fayetteville, vssrusti@gmail.com

Mary C. Savin
University of Arkansas, Fayetteville, msavin@uark.edu

Julie A. Stenken
University of Arkansas, Fayetteville, jstenken@uark.edu

Lisa S. Wood
University of Arkansas, Fayetteville, lswood@uark.edu

Microdialysis: a method for quantifying in situ nitrogen fluxes in soil microsites

Cover Page Footnote

Srusti Maddala is an honors program graduate with a dual degree in Environmental, Soil, and Water Science and Chemistry (Biochemistry) and a minor in Biology. Mary C. Savin, a faculty mentor, is a professor in the Department of Crop, Soil, and Environmental Sciences. Julie A. Stenken, a faculty mentor, is a professor in the Department of Chemistry and Biochemistry. Lisa S. Wood, a faculty mentor, is a professor in the Department of Crop, Soil, and Environmental Sciences and Assistant Dean of Honors and International Programs for the Dale Bumpers College of Agricultural, Food, and Life Sciences.

Microdialysis: a method for quantifying in situ nitrogen fluxes in soil microsites

Meet the Student-Author

Srusti Maddala

Research at a Glance

- Microdialysis data provide information about nutrient movement in the soil, which may improve understanding of soil processes and reform current fertilizer usage.
- An advantage of using microdialysis is that it can be used to sample nutrients without moving any soil particles from where they are around the plant.
- Through a series of experiments, we found the best flow rate to be 2.0 µL/min. We also discovered that we could measure multiple forms of nitrogen at the same time because we did not have to worry about the measurement of one form of nitrogen interfering with another.

I am from Springdale, Arkansas, and graduated from Har-Ber High School in 2016. In May of 2020, I graduated from the University of Arkansas with a dual degree in Environmental, Soil, and Water Science and Chemistry (with a concentration in Biochemistry) and a minor in Biology. Funding for my undergraduate studies was generously provided by the Bodenhamer Fellowship and the Arkansas Governor's Distinguished Scholar scholarship. Funding to conduct and present research was provided by the Honors College, Bumpers College, and the Arkansas Department of Higher Education through a SURF grant.

Growing up, I had always loved the natural sciences and was exposed to gardening at a young age. At the start of college, I knew I wanted to major in Chemistry, but also decided to major in Environmental, Soil, and Water Science because it seemed interesting. As I took courses in the Crop, Soil, and Environmental Sciences department, I began to see the beauty of the interdisciplinary nature of environmental science and how vital it was to conduct research. Over the course of my four years as an undergraduate at the University of Arkansas, I had the opportunity to conduct an honors thesis project that integrated my interests in both majors and provided me with invaluable research experience. I also was able to participate in study abroad programs in Belgium and Belize. Thank you to Dr. Savin for her guidance, Dr. Stenken for her support, and Dr. Wood for her assistance.

Srusti working on her greenhouse experiment at the University of Arkansas System Division of Agriculture's Altheimer Lab.

Microdialysis: a method for quantifying in situ nitrogen fluxes in soil microsites

Srusti Maddala,[*] *Mary C. Savin,*[†] *Julie A. Stenken,*[§] *and Lisa S. Wood*[‡]

Abstract

Microdialysis, a diffusion-based sampling technique commonly used in biomedical research, has recently been recognized as a candidate for monitoring chemical changes in the rhizosphere. The information it provides about nutrient diffusion may improve nitrogen use efficiency, leading to enhanced management and success of restoration projects. The objective of this study was to determine the efficacy of microdialysis sampling to quantify the relative recoveries (*RR%*) of nitrate-N and ammonium-N, the two inorganic nitrogen compounds typically found in soil. The effects of microdialysis flow rate, sample medium concentration, and the presence of both analytes in solution on the relative recoveries obtained from dialysate samples were investigated. In comparison to 3.75 and 5.0 μL/min, a flow rate of 2.0 μL/min resulted in an increased relative recovery for both nitrate-N and ammonium-N solutions, at 42.7% and 51.0%, respectively, and was determined to be an optimum rate for subsequent experiments using CMA 20 microdialysis probes. The *RR%* for both nitrate-N and ammonium-N did not display a statistically significant dependence on the concentration of analyte present in the sample medium. The analytes also did not exhibit interferences, and the presence of both nitrate-N and ammonium-N in the same solution did not influence the *RR%* of either analyte. The results obtained from this study will assist in validating a novel approach to measuring in situ nitrogen availability in soil with minimal disturbance.

[*] Srusti Maddala is a May 2020 honors program graduate with a dual degree in Environmental, Soil, and Water Science and Chemistry (Biochemistry) and a minor in Biology.
[†] Mary C. Savin, a faculty mentor, is a professor in the Department of Crop, Soil, and Environmental Sciences.
[§] Julie A. Stenken, a faculty mentor, is a professor in the Department of Chemistry and Biochemistry.
[‡] Lisa S. Wood, a faculty mentor, is a professor in the Department of Crop, Soil, and Environmental Sciences and Assistant Dean of Honors and International Programs for the Dale Bumpers College of Agricultural, Food and Life Sciences.

Introduction

Microdialysis is a diffusion-based sampling method used for nearly three decades in the field of medicine and has been established as a major research tool in studying the effects of drugs and disease on brain tissue (Duo et al., 2006; Kehr, 1993; Stenken, 2006). The central component of the microdialysis device is a semipermeable membrane 500 µm in diameter and 10 mm in length (Fig. 1), which is very similar in size to 0.5-mm mechanical pencil lead. A perfusion fluid is passed through the inlet of the device at flow rates in units of µL/min, which drives diffusion of compounds from the sample medium into the probe according to their concentration gradient. These compounds of interest—in this study nutrients such as nitrate-N and ammonium-N—are then carried to the outlet to undergo chemical analysis (de Lange, 2013; Stenken, 2006).

In the soil, namely the rhizosphere (Fig. 2)—a zone of dynamic microbial activity and interconnecting relationships between microorganisms, plant roots, and the soil—microdialysis has the potential for providing real-time, in situ data with greater temporal and spatial resolution than current standard methods, which are highly destructive (Mulvaney, 1996). Comparatively, salt extractions using potassium chloride (KCl) and potassium sulfate (K_2SO_4) are the conventional methods of sampling nitrogen from the soil, which destroy soil structure and also require significant periods of time between sampling and analysis.

Recent studies have investigated applications of microdialysis to sample inorganic forms of N (nitrate-N and ammonium-N) and measure the chemical changes in the rhizosphere without removing soil or destroying the soil structure (Inselsbacher et al., 2011). The microdialysis technique measures N flux (rate of movement in space) over time, which can enhance the understanding of the mechanisms involved in the availability of nutrients to plants and nutrient diffusion. By discerning the patterns and the trends of fluxes of nitrogen in the rhizosphere, more efficient use of fertilizers is possible and can also improve the management and success of remediation projects.

While the overarching purpose of this research was to implement microdialysis sampling in the rhizosphere soil, initial optimization of the technique was required to ensure 1) acquaintance with the equipment and procedures involved with microdialysis, including equipment set-up, probe handling, and sample analysis using colorimetric assays and 2) acquisition of preliminary data regarding the efficiency of the sampling technique in solutions of nitrate-N and ammonium-N using percent relative recovery (*RR%*). This study focuses on optimizing the microdialysis technique in the laboratory setting for further application to the rhizosphere of plants. The results of this study could aid in identifying the most effective flow rate of perfusate and quantifying the effects of sample medium concentration and the presence of potential interferences the analytes may exhibit on one another.

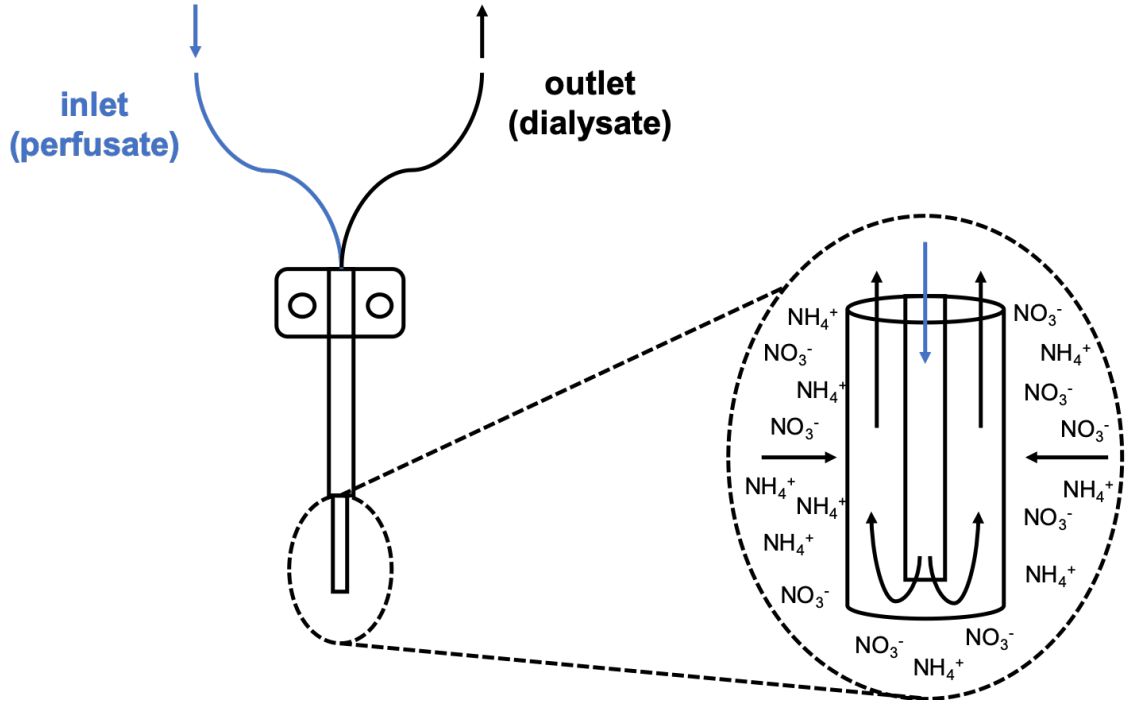

Fig. 1. An overview of the microdialysis device showing the potential for diffusion of nitrate and ammonium ions from soil solution through the probe membrane as an example in the expanded inset.

Materials and Methods

Microdialysis Set-Up

Four syringe pumps (MD-1001, BASi, Lafayette, Indiana) were equipped with a total of 12 gas-tight syringes (MDN-0250, 2.5 mL, BASi) that delivered the perfusate high performance liquid chromatography (HPLC)-grade water (VWR, Radnor, Pennsylvania) at the specified flow rates using a four-syringe drive pump controller (MD-1020, BASi). The HPLC-grade water was used ubiquitously as the perfusate for all microdialysis experiments in this study. Each syringe was connected to extra tubing (MF-5164, 1 meter, FEP (fluorinated ethylene propylene), 0.65 mm OD × 0.12 mm ID, BASi), which was connected to the inlet of a CMA 20 Elite probe (CMA8010436, 10-mm membrane length, PAES (polyarylethysulfone) membrane, and 20-kDa molecular weight cut-off, Harvard Apparatus, Holliston, Massachusetts). The equilibration time used at the beginning of every sampling was 15 minutes.

Determination of Optimum Flow Rate

A 10-µg/mL nitrate-N solution was prepared in HPLC-grade water using sodium nitrate (Mallinckrodt, St. Louis, Missouri), and three microdialysis probes were placed into the solution. Dialysates (180 µL) were collected in pre-weighed 0.5-mL microcentrifuge tubes at flow rates of 2.0, 3.75, and 5.0 µL/min (0.8, 1.5, and 2.0 µL/min on the pump controller). The relative recovery percent (*RR%*) was calculated at each flow rate using Equation 1:

$$RR\% = \frac{C_d}{C_{SM}} \times 100\% \qquad \text{Eq. (1)}$$

where C_d is the concentration of nitrate-N in the dialysate, and C_{SM} is the concentration of nitrate-N in the sample medium. An equilibration time of 15 minutes was used between changing flow rates. The same procedure was repeated with a 10-µg/mL solution of ammonium-N prepared using ammonium chloride (Mallinckrodt, St. Louis, Missouri).

Effects of Sample Medium Concentration on *RR%*

Standard solutions of 1, 3, 5, 8, and 10 µg/mL nitrate-N and ammonium-N were prepared in HPLC-grade water. Microdialysis sampling was performed in each solution using a 2.0-µL/min flow rate to collect 180 µL of dialysate. Equilibration times of 15 minutes were used between changing sample medium concentrations. The *RR%* was calculated for each sample medium concentration using Equation 1.

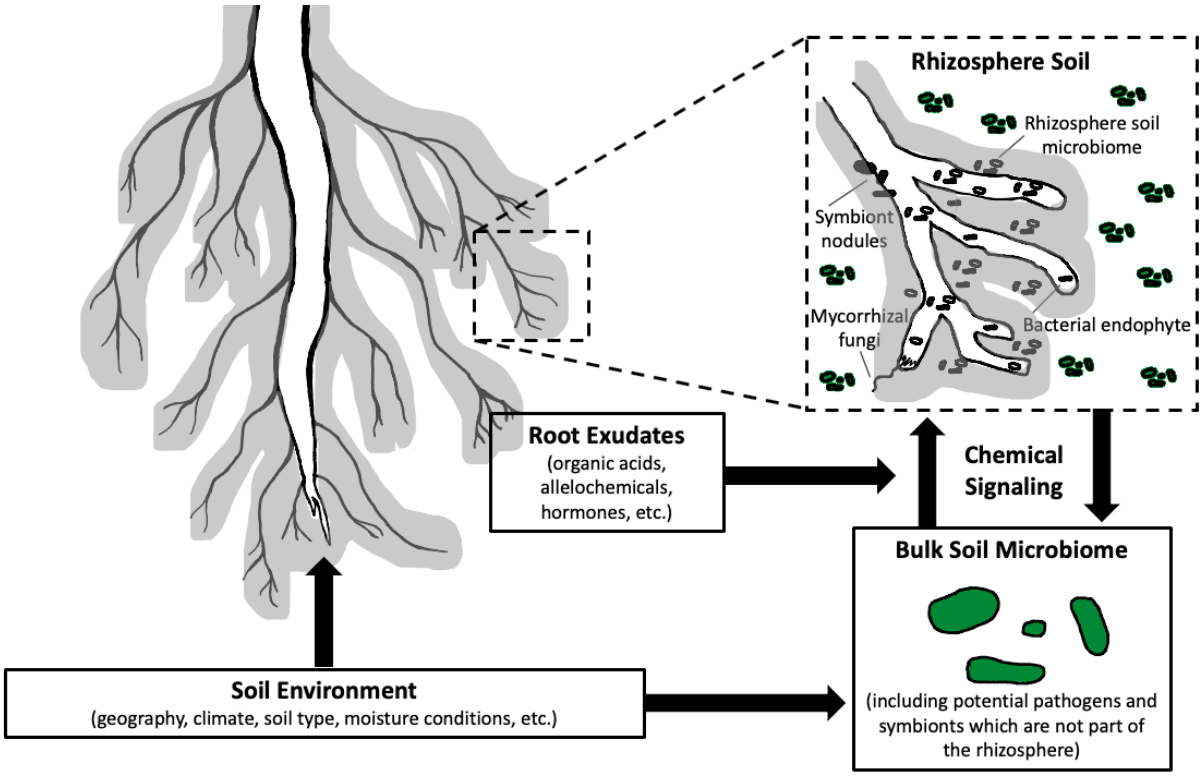

Fig. 2. The rhizosphere, a zone of dynamic symbiotic relationships at the plant root-soil interface.

Comparison of *RR%* in a Combined Solution of Nitrate-N and Ammonium-N

Standard solutions of 5 µg/mL nitrate-N and 5 µg/mL ammonium-N were prepared in HPLC water. Three microdialysis probes were placed into the 5-µg/mL nitrate-N solution and sampled for 30-minute intervals for a total of 90 minutes at 2.0 µL/min. Aliquots from the sample medium were collected before the initial collection and at the end of each 30-minute interval. Microdialysis was performed in the 5-µg/mL ammonium-N solution using the same sampling procedure. After dialysates were collected from each ion solution, 10-µg/mL nitrate-N and 10-µg/mL ammonium-N solutions were added to a 5-mL centrifuge tube in a 1:1 (v/v) ratio. The solution was vortex-mixed to ensure homogeneity, and microdialysis was performed at 2.0 µL/min for 60 minutes. The *RR%* for the individual ion solutions, as well as the combined solution, was calculated using Equation 1.

Nitrate-N Chemical Assay (Griess Reaction)

Nitrate-N in microdialysis samples was analyzed using vanadium chloride (VCl_3) and the Griess reaction based on the technique described by Miranda et al. (2001) and adapted to microplate analysis. A 0.05-M solution of VCl_3 (Strem Chemicals, Newburyport, Massachusetts) was prepared in 1M-HCl and filtered using a 0.2-µm Whatman syringe filter (GE Healthcare, Chicago, Illinois). Griess reagent 1 consisted of 1% (w/v) sulfanilamide (Tokyo Chemical Industry, Tokyo, Japan) prepared in 5% (v/v) phosphoric acid. Griess reagent 2 consisted of 0.1% (w/v) N-naphthylethylenediamine (Sigma-Aldrich, St. Louis, Missouri) in HPLC water. The assay for nitrate in a 96-well plate (3590, Corning Inc., Corning, New York) was as follows: 50 µL of sample or standard, 50 µL of VCl_3, 50 µL of Griess reagent 1, and 50 µL of Griess reagent 2. The plate was covered in aluminum foil and placed on a plate shaker at 200 RPM for 2.5 hours at room temperature.

Ammonium-N Chemical Assay (Indophenol Berthelot Reaction)

Ammonium-N in the dialysate samples was analyzed using the microplate adaptation of the Indophenol Berthelot reaction based on the technique as described by Baethgen and Alley (1989) and Willis et al. (1996). The plate was covered in aluminum foil and placed on a plate shaker at 200 RPM for 1.5 hours at room temperature.

UV-Vis Spectrophotometry

Absorbance values were measured using a Tecan infinite m200 plate reader (Tecan, Männedorf, Switzerland) at 540 nm and 650 nm for the Griess and Indophenol Berthelot reactions, respectively.

Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's hon-

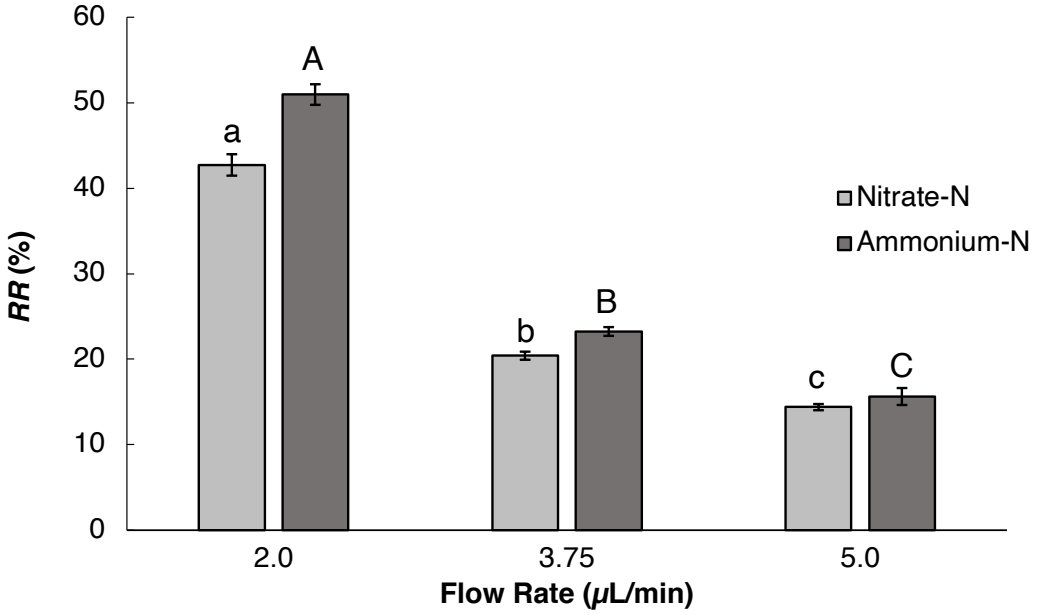

Fig. 3. The relative recovery percent (*RR%*) of nitrate-N and ammonium-N obtained from microdialysis sampling in solutions of 10 µg/mL at flow rates of 2.0, 3.75, and 5.0 µL/min. Nitrate-N and ammonium-N *RR%* were analyzed separately. Bars represent means ± SE (n = 3). Different lowercase letters indicate differences between *RR%* of nitrate-N ($P < 0.001$), and different uppercase letters indicate differences between *RR%* of ammonium-N ($P < 0.001$).

estly significant difference as a post-hoc test and Student's *t*-test using SigmaPlot 14.0 (Systat Software, Inc). All statistical analyses were performed using a 95% confidence interval; therefore, differences were considered statistically different at $P \leq 0.05$.

Results and Discussion

Optimum Flow Rate Determination

The effect of differing flow rates on the recovery of nitrate-N and ammonium-N was studied in order to determine the optimal flow rate for microdialysis sampling. The *RR%* of nitrate-N (denoted as mean ± SE) were 42.7 ± 1.3 %, 20.4 ± 0.5 %, and 14.4 ± 0.4 % for flow rates of 2.0, 3.75, and 5.0 µL/min, respectively (Fig. 3; n = 3). The recoveries yielded by the flow rates were all statistically different from each other, with 2.0 µL/min yielding the greatest *RR%* ($P < 0.001$).

The *RR%* of ammonium-N were 51.0 ± 1.2 %, 23.2 ± 0.5 %, and 15.6 ± 1.0 % for flow rates of 2.0, 3.75, and 5.0 µL/min, respectively (Fig. 3; n = 3). The recoveries yielded by the three different flow rates were all statistically different from each other, with 2.0 µL/min yielding the greatest *RR%* ($P < 0.001$).

Flow rate affects both recovery and sampling times: slower flow rates yield greater relative recoveries but also result in longer sampling times, which could be problematic to the microdialysis equipment such as the syringe pumps as they are exposed to heat, moisture, and a non-sterile environment for longer periods of time. Prior studies employ a flow rate of 5.0 µL/min and report low recoveries of target molecules from the soil (Buckley et al., 2017; Inselsbacher et al., 2014; Inselsbacher et al., 2011; Shaw et al., 2014).

A flow rate of 2.0 µL/min was determined to be an optimum rate for subsequent experiments using CMA 20 microdialysis probes (Fig. 3). Relative recoveries at 2.0 µL/min were significantly greater than the other two flow rates—twice greater than a flow rate of 3.75 µL/min—and would result in sampling times of 60 minutes in order to sample the needed volumes for nitrate-N and ammonium-N chemical analysis.

Effect of Differing Sample Medium Concentrations

The effect of compound concentration on the relative recoveries was studied in order to determine if the concentrations of analytes obtained in the dialysate samples were still proportional to the concentrations in the sample medium regardless of the magnitude of the actual concentration of the sample medium. Microdialysis was performed in solutions of 1, 3, 5, 8, and 10 µg/mL nitrate-N and ammonium-N, respectively, and the relative recoveries of the analytes from each sample medium were calculated. The relative recoveries of nitrate-N (denoted as mean ± SE) were 37.0 ± 3.1%, 31.0 ± 1.1%, 29.3 ± 1.7%, 38.2 ± 3.7, and 29.9 ± 0.3% in 1, 3, 5, 8, and 10 µg/mL solutions, respectively (Fig. 4; n = 3). Varying the concentration of the sample medium did not yield statistically different relative recovery percentages for nitrate-N ($P = 0.093$).

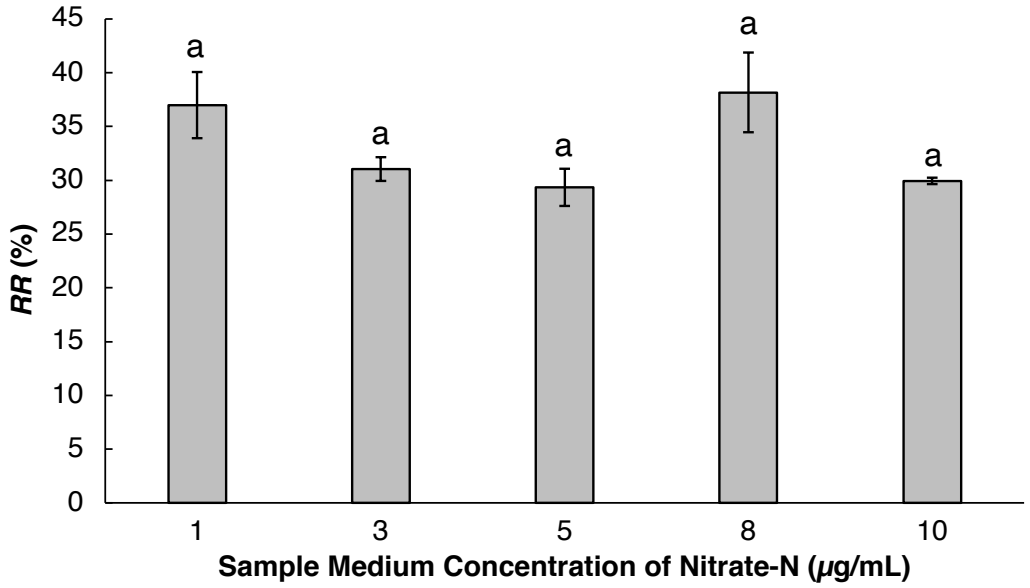

Fig. 4. The relative recovery percent (RR%) of nitrate-N obtained from microdialysis sampling in 1, 3, 5, 8, and 10-µg/mL solutions at 2.0 µL/min. Bars represent means ± SE (n = 3).

The relative recoveries of ammonium-N were 46.9 ± 1.3 %, 40.5 ± 3.3 %, 37.2 ± 1.9 %, 43.3 ± 2.8 %, and 45.1 ± 2.8 % in 1-, 3-, 5-, 8-, and 10-µg/mL solutions, respectively (Fig. 5; n = 3). Varying the concentration of the sample medium did not yield statistically different relative recovery percentages for ammonium-N ($P = 0.130$).

It was important to determine that the concentrations of nitrate-N and ammonium-N do not impact the precision of quantification of either compound. The concentration of N in the soil is a dynamic property and is constantly changing depending on microbial activity, precipitation, temperature, and other abiotic and biotic factors. The results of this study indicate that the recoveries of neither compound depended on concentration.

Comparison of *RR%* of Individual Analytes with *RR%* in Combined Solution

The presence of nitrate-N and ammonium-N in the same solution was studied in order to study the differences in microdialysis performance in the laboratory setting. Since the analytes are of opposite charges and different molecular weights, this study was performed to determine if the presence of both analytes imposed interferences or confounded measurement. Both forms of N are present in the soil environment and were sampled simultaneously during microdialysis sampling. In the 5-µg/mL nitrate-N solution, the average *RR%* (denoted as mean ± SE) was 39.9 ± 0.7 % (n = 3); while in the nitrate-N and ammonium-N combined solution, the average recovery was 41.2 ± 0.6 % (n = 3). The *RR%* obtained from the individual nitrate-N solution and *RR%* obtained from the combined solution were not statistically different ($P = 0.199$).

The average *RR%* in the 5-µg/mL ammonium-N solution and the combined nitrate-N and ammonium-N solution were 43.7 ± 0.8 % (n = 3) and 43.9 ± 1.1 %, respectively (n = 3). The recoveries yielded by the individual ammonium-N solution and the combined solution were not statistically different ($P = 0.863$).

Conclusions

This study revealed that a flow rate of 2.0 µL/min was optimum for subsequent rhizosphere studies, as it resulted in significantly greater recoveries of both nitrate-N and ammonium-N and would result in a sampling time of 60 minutes for collecting the volumes of dialysate required for colorimetric analysis. The concentrations of the analytes in the surrounding solution, as well as the presence of both analytes in the same solution, did not have a significant effect on the recovery of either analyte. The results of this study indicated that the nature of the analytes did not exert any significant effects on the recoveries; therefore, subsequent differences of recoveries observed in a soil-based sample medium can be attributed to analyte-soil-plant interactions.

Acknowledgments

Funding was provided by the Dale Bumpers College of Agricultural, Food and Life Sciences Creative and Research Grant, a Student Undergraduate Research Fellow-

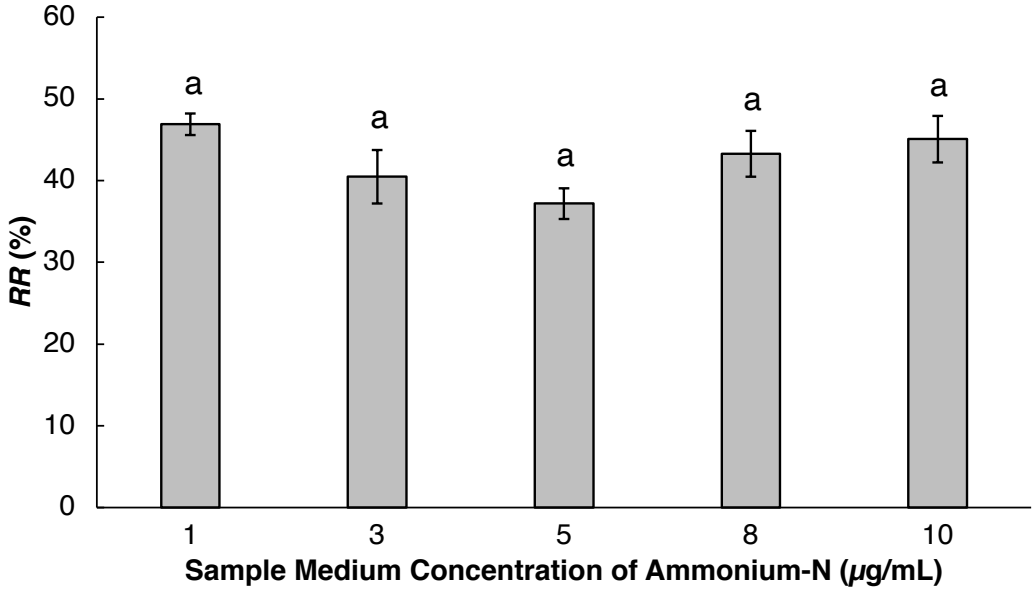

Fig. 5. The relative recovery percent (RR%) of ammonium-N obtained from microdialysis sampling in 1, 3, 5, 8, and 10-µg/mL solutions at 2.0 µL/min. Bars represent means ± SE (n = 3).

ship (SURF) grant, and a University of Arkansas Honors College grant for travel to the ASA-CSSA-SSSA Tri-Societies meeting to present this research.

Literature Cited

Baethgen, W.E. and M.M. Alley. 1989. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. Commun. Soil Sci. Plan. 20(9&10):961-969.

Buckley, S., R. Brackin, T. Näsholm, S. Schmidt, and S. Jämtgård. 2017. Improving in situ recovery of soil nitrogen using the microdialysis technique. Soil Biol. Biochem. 114:93-103.

de Lange, E.C.M. 2013. Microdialysis in drug development. Springer New York, New York, NY. Chapter 2, Recovery and calibration techniques: Toward quantitative microdialysis; p. 13-33.

Duo J., H. Fletcher, and J.A. Stenken. 2006. Natural and synthetic affinity agents as microdialysis sampling mass transport enhancers: Current progress and future perspectives. Biosens. Bioelectron. 22(3):449-457.

Inselsbacher E., O.A. Oyewole, and T. Näsholm. 2014. Early season dynamics of soil nitrogen fluxes in fertilized and unfertilized boreal forests. Soil Biol. Biochem. 74:167-176.

Inselsbacher E., J. Öhlund, S. Jämtgård, K. Huss-Danell, and T. Näsholm. 2011. The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. Soil Biol. Biochem. 43(6):1321-1332.

Kehr, J. 1993. A survey on quantitative microdialysis: Theoretical models and practical implications. J. Neurosci. Methods. 48(3):251-261.

Miranda, K.M., M.G. Espey, and D.A.Wink. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 5(1):62-71.

Mulvaney, R.L. 1996. Nitrogen—Inorganic Forms. Soil Science Society of America & American Society of Agronomy, Madison, Wis.

Shaw R., A.P. Williams, and D.L. Jones. 2014. Assessing soil nitrogen availability using microdialysis-derived diffusive flux measurements. Soil Sci. Soc. Am. J. 78(5):1797-1803.

Stenken, J.A. 2006. Microdialysis sampling. In: Encyclopedia of medical devices and instrumentation. 2nd ed. John Wiley and Sons, Inc., Hoboken, N.J. p. 400-420.

Willis R.B., M.E. Montgomery, and P. R. Allen. 1996. Improved method for manual, colorimetric determination of total kjeldahl nitrogen using salicylate. J. Agric. Food Chem. 44(7):1804-1807.