Continuous in situ soil nitrate sensors: The importance of high-resolution measurements across time and a comparison with salt extraction-based methods

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Continuous *in situ* soil nitrate sensors: The importance of high-resolution measurements across time and a comparison with salt extraction-based methods

by

Yunjiao Zhu

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Soil Science

Program of Study Committee:
Michael J. Castellano, Co-major Professor
Sotirios V. Archontoulis, Co-major Professor
Robert Horton

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2021

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ABSTRACT

Soil nitrate (NO$_3^-$) is one of the most important forms of nitrogen in nature. It is an important plant nutrient, but it also easily lost to the environment where it can diminish air and water quality. However, the ability to measure soil NO$_3^-$ is limited by manual sampling and laboratory analyses, and only low-resolution data can be obtained with conventional procedures. In this thesis, a new kind of instantaneous soil NO$_3^-$-N sensor was used to measure soil NO$_3^-$-N concentration both in the laboratory and in a continuous maize fertilizer nitrogen (N) rate trial during the 2019 maize growth season. First, agreement between status-quo salt-extraction based measurements and sensor measurements was assessed using linear regression models, Bland and Altman plots, and intraclass correlation coefficients (ICCs). All three quantitative comparisons demonstrated good agreement, especially at relatively high NO$_3^-$-N concentrations that are relevant for fertilizer N management. Subsequently, using the validated sensors, temporal variability of soil nitrate and the effect of temporal soil sampling resolution were quantified in situ using 60 days of soil NO$_3^-$-N measurements every 10 seconds. A new finding, made possible with the new sensors, was that there was no consistent within-day pattern in soil NO$_3^-$-N concentration. Across-days, when soil solution NO$_3^-$-N was dynamic and sampling frequency was >5 days, estimates of mean daily NO$_3^-$-N concentration were >20% from the actual mean daily concentration. The underlying temporal variability can affect the efficiency of given sampling management, which means the soil NO$_3^-$-N sensor can have a promising application in the future soil fertilizer management. In the future, research will be required to interpret sensor measurements and spatial variability of sensor measurements.
CHAPTER 1. GENERAL INTRODUCTION

Nitrogen (N), which is required for the synthesis of amino acids and proteins, is one of the most fundamental nutrients for plants. Ammonium (NH$_4^+$) and nitrate (NO$_3^-$) are the most important forms of soil inorganic nitrogen because they are the primary source of plant N uptake. Most soils cannot supply sufficient NH$_4^+$ and nitrate NO$_3^-$ to meet plant N uptake requirements in high-intensity cereal crop systems. As a result, most cereal crop systems rely on synthetic N fertilizer in the form of urea, NH$_4$ or NO$_3$. Globally, agricultural synthetic N fertilizer use has tremendously increased in the past 50 years to meet the requirements of a growing population (Cao et al., 2018; Nishina et al., 2017; Lu and Tian, 2017). However, fertilizer use efficiency remains low; in the U.S, less than 50% of the of synthetic N fertilizer is used by crops (Environmental Protection Agency, 2011). Low N fertilizer use efficiency is a major economic loss for farmers and the cause of serious environmental problems such as eutrophication.

Precision management of soil N fertilizer is a growing need to reduce the cost of agricultural inputs for farmers and mitigate the negative environmental effect of agriculture (Fahsi et al., 1998; Ma and Biswas, 2015).

With progress in wireless communications and the miniaturization of electronic devices, coupled with advances in chemical, molecular, and optics signaling platforms, sensor technology has been an important part of precision measurement and management in agriculture. In the past decades, sensors have been used to measure parameters in soil science, for example, soil moisture and soil temperature. Breakthroughs in soil moisture sensor technology and cost led to improvements in soil evaporation models (Ventura et al., 2006) and crop irrigation management (Blonquist et al., 2006). With the development and deployment of advanced materials that can provide soil data at the molecular level, ecosystem and cropping systems can achieve similar
benefits from chemical-biological-optical-electrical signaling NO₃⁻ sensors at a very high frequency and data density (NAS, 2019). Numerous attempts to develop soil sensors have been previously reported (Hummel et al., 1996; Sudduth et al., 1997; Adamchuk et al., 2004; Rogovska et al., 2019). Recently, Ali et al. (2019) developed an all-solid-state miniature potentiometric soil sensor to continuously measure NO₃⁻ concentration with a minimum detection limit of 1 mg NO₃⁻ L⁻¹. This method has the potential to increase N fertilizer use efficiency and improve environment quality. However, this new sensor has not been tested in the field. In addition, it is unknown how its measurement corresponds to conventional, salt-extraction based measurements of soil NO₃⁻ concentration.

Hence, objectives of this thesis were to:

1) Compare sensor (Ali et al. 2019) and salt-based extract (Hart et al. 1994) measurements of soil NO₃⁻-N concentration in well-controlled laboratory conditions and in field conditions.

2) Use the sensors to explore in situ diurnal variation in soil NO₃⁻-N concentration

3) Quantify the effect of sampling frequency on estimations of mean daily in situ NO₃⁻-N concentration both within and across days.

In this thesis, I compared sensor measurements and salt-based method measurements in well-controlled laboratory conditions and in field conditions. In the lab condition, the influence of soil moisture and temperature on measurement accuracy of the sensor was analyzed. After demonstrating the accuracy of sensors in the laboratory, I deployed the sensors in the field to investigate questions about the temporal dynamics of soil nitrate levels that scientists have not been previously able to investigate given the absence of an accurate nearly continuous soil nitrate sensor. In the field, sensors were deployed in 2019 in a continuous maize N fertilizer rate trial. In
Chapter 2, I examine the agreement between the two methods and temporal variation in soil NO$_3^-$-N concentration. Based on these results, I provide recommendations about frequency of soil sampling in different stages of plant growing season. Ultimately, my goal is to advance my capacity to predict, explain and manage ecosystem nitrogen dynamics.

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CHAPTER 2. CONTINUOUS IN SITU SOIL NITRATE SENSORS: THE IMPORTANCE OF HIGH-RESOLUTION MEASUREMENTS ACROSS TIME AND A COMPARISON WITH SALT EXTRACTION-BASED METHODS

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Abstract

Soil nitrate (NO₃⁻) affects microbial processes, plant productivity and environmental nitrogen losses. However, the ability to measure soil NO₃⁻ is limited by manual sampling and laboratory analyses. Hence, temporal variation in soil solution NO₃⁻ concentration – both within and across days – is poorly understood. Here, we evaluated a potentiometric sensor that continuously measures soil solution NO₃⁻ concentration with unprecedented specificity due to a novel membrane that serves as a barrier to interfering anions (Ali et al. 2019). First, we compared sensor and salt extraction-based measurements of soil NO₃⁻ in well-controlled laboratory conditions. Second, using 60 days of in situ soil NO₃⁻ measurements every 10 seconds, we quantified temporal variation and the effect of sampling frequency on field estimations of mean daily NO₃⁻ concentration within and across days. In the laboratory, sensors measured soil NO₃⁻ concentration without significant difference from theoretical adjusted soil NO₃⁻ concentration or conventional salt extraction-based methods. In the field, the sensors demonstrated no within-day pattern in soil NO₃⁻ concentration. However, measurements of soil NO₃⁻ within-days varied by >10%. Across-days, when soil solution NO₃⁻ was dynamic and
sampling frequency was >5 days, estimates of mean daily NO$_3^-$ concentration were >20% from the actual mean daily concentration. *In situ* soil sensors are becoming more common and offer potential to improve fundamental and applied sciences. However, in most situations, sensors will measure soil properties in a different manner than conventional salt-extract soil sampling-based approaches. Hence, research will be required to interpret sensor measurements and optimize sensor deployment.

**Introduction**

Accurate measurement of soil nitrate (NO$_3^-$) is a critical challenge for agriculture and the environment. In experiments and models, soil NO$_3^-$ concentration has profound effects on primary productivity and environmental N losses. Whether measured or simulated, accurate soil NO$_3^-$ data are important to predict and explain critical ecosystem processes such as plant growth, nitrous oxide emissions and NO$_3^-$ leaching (Del Grosso et al. 2008; Loecke et al. 2012; Iqbal et al. 2018). Collection of high-quality soil NO$_3^-$ data is challenging because soil NO$_3^-$ concentration is extremely variable in time and space (Archontoulis et al. 2020; Cambardella et al. 1994; Robertson et al. 1988). At a seasonal time scale, temporal variability in soil NO$_3^-$ concentration is largely affected by the balance of annual weather-driven patterns of nitrification, environmental N losses, and plant N uptake (Lovett et al. 2000; Martinez-Feria et al. 2018; Archontoulis et al. 2020). However, temporal variability in soil NO$_3^-$ concentrations within a single growing season is not well understood because the costs of manual soil sampling and laboratory processing limit the number of samples across time. In addition, the spatial variability in soil NO$_3^-$ concentrations is extremely high. For example, at a scale of 10-1000 m$^2$, there may be no spatial autocorrelation between individual soil NO$_3^-$-N concentration measurements (Cambardella et al. 1994).
This spatiotemporal variability creates enormous challenges. From basic understanding of the soil N cycle to the optimization of N fertilizer inputs, a lack of high-resolution soil NO$_3^-$ data limit science. Current approaches to soil NO$_3^-$-N measurement require enormous amounts of time. On an individual sampling date, soil must be collected from field, returned to the laboratory, homogenized, and extracted in a strong salt solution (typically 5:1 ratio of soil:2 M KCl; Hart et al. 1994). Subsequently, NO$_3^-$ concentration is measured in the salt extract and scaled to mg NO$_3^-$-N kg soil, but only after water content of the soil is determined by oven-drying to a constant mass. Nevertheless, soil NO$_3^-$ data is critical to my understanding of ecosystem N dynamics and my ability to manage agricultural systems. For example, the late spring soil NO$_3^-$ test (LSNT) can optimize springtime N fertilizer applications to maize, reduce environmental N losses and improve profitability (Jaynes et al. 2004). However, farmer adoption of the LSNT is limited due to the high costs of sample analysis (private laboratories charge $\sim$10 USD per sample for analysis) and collection.

Recent innovations in electrical engineering promise new opportunities to measure soil NO$_3^-$ concentration at low cost and high resolution. Recently, Ali et al. (2019) developed an all-solid-state miniature potentiometric soil sensor to continuously measure NO$_3^-$ concentration with a minimum detection limit of 1 mg NO$_3^-$-N L$^{-1}$. This method has the potential to improve N use efficiency and environment quality. However, the approach has not been tested in the field. In addition, it is unknown how the new sensor measurement corresponds to conventional salt-extract based measurements of soil NO$_3^-$ concentration. In contrast to salt extractions of soil NO$_3^-$, which are reported as mg NO$_3^-$-N kg$^{-1}$ dry soil (Hart et al. 1994), the sensors report mg NO$_3^-$-N L$^{-1}$ soil solution. Importantly, all methods of soil NO$_3^-$ measurement are simply indicators of the NO$_3^-$ pool size at a particular time and space. Available methods (e.g.,
lysimetry, water-based extractions, and salt-based extractions) all produce different measurements of the soil NO$_3^-$-N pool (e.g., Darrouzet-Nardi & Intraub 2014). Hence, no approach to measuring soil NO$_3^-$-N is ‘correct’.

Instead, the goal is to produce an indicator of the NO$_3^-$-N pool size that predicts and explains processes of interest such as plant growth or environmental N losses. All methods have strengths and weaknesses that vary with application. For example, salt- and water-based extractions allow the user to account for spatial variability by subsampling soil at many locations, pooling those subsamples, and making one extraction from a homogenized sample that represents an average soil NO$_3^-$-N concentration in the sampled space (e.g., Mueller et al. 2018). However, the approach is laborious in the field and laboratory; as a result, temporal resolution is poor. In contrast, lysimetry and sensors measure the soil solution NO$_3^-$ concentration at a specific location. Although lysimetry remains laborious and limited in temporal resolution, the sensors can record data in real time at frequencies <1s. Moreover, when sensor manufacturing is industrialized, it is likely that sensors will cost < $20 USD per unit (L. Dong, personal communication).

Regardless of method, it appears that future soil NO$_3^-$ measurements will incorporate the use of in situ soil sensors that record a spatially explicit concentration of NO$_3^-$-N in soil solution at high temporal resolution. Hence, my objectives were to:

1) Compare sensor (Ali et al. 2019) and salt-based extract (Hart et al. 1994) measurements of soil NO$_3^-$-N concentration in well-controlled laboratory conditions and in field conditions.

2) Use the sensors to explore in situ diurnal variation in soil NO$_3^-$-N concentration
3) Quantify the effect of sampling frequency on estimations of mean daily *in situ* NO$_3^-$-N concentration both within and across days.

**Materials and Methods**

**Laboratory Experiments**

We conducted two laboratory experiments to test the agreement between sensors and salt extract measurements. In contrast to field experiments, these laboratory experiments aimed to measure a similar pool of NO$_3^-$ with each method. In the first laboratory experiment, the influence of soil moisture on measurement accuracy of the sensor was analyzed. Soils were sampled (0-30 cm) from the Iowa State University Agronomy and Agricultural Engineering Research Farm (41°55′N, 93°45′W), air-dried and sieved (2 mm). The sampling area contained USDA soil series Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). After air-drying and sieving, soils were combusted in a muffle furnace at 400 °C to reduce the effects of microbial processes on soil solution NO$_3^-$ concentration: our goal was to adjust the NO$_3^-$ concentration to a constant level and thus we had to minimize the effects of microbial N mineralization-immobilization dynamics. Next, the combusted soil was adjusted to a bulk density of 1.25 g cm$^{-3}$ and prepared with two gravimetric moisture contents (20% and 30%) and three NO$_3^-$-N soil solution concentration levels (10 mg L$^{-1}$, 100 mg L$^{-1}$, and 200 mg L$^{-1}$) in 118 mL polyethylene cups. We selected these gravimetric water contents and NO$_3^-$ concentrations because they encompass the typical ranges observed during the growing season in the rainfed Corn Belt (e.g., Archontoulis et al. 2020). One sensor was installed in the center of each cup. Sensor measurements were recorded after readings stabilized (~60 minutes) and, at the same
time, soil samples (three per cup) were immediately mixed with 2 M KCl (5:1 solution/soil ratio) by reciprocal shaking for 1 h at 180 rpm. The soil slurry was then filtered through preleached Whatman 1 filter paper. The NO$_3^-$-N concentrations of the filtrate (i.e., extraction) were measured with colorimetry in microplates using the Griess–Ilosvay reaction with vanadium(III) chloride as a reducing agent and the Berthelot reaction, respectively (Hood-Nowotny et al., 2010). The NO$_3^-$-N concentrations of the filtrate are scaled to mass of dry soil that is mixed with the 2M KCl such that the reported unit is mg NO$_3^-$-N kg$^{-1}$ dry soil.

In the second laboratory experiment, the effect of temperature on the sensor performance was analyzed by measuring the standard NO$_3^-$-N solutions across four temperatures (0 °C, 10 °C, 20 °C, and 30 °C). The stability of each sensor across the four temperatures was obtained by calculating the error rate of the measurement result in each concentration of the NO$_3^-$-N solution. This experiment was performed in solution rather than soil to adjust the temperature more accurately.

**Field Experiments**

Sensors were deployed in 2019 in a continuous maize N fertilizer rate trial at the Iowa State University Agricultural Engineering and Agronomy Research Farm in Boone county, IA (42.02 N, 93.77W). Long-term (35-year) average annual precipitation and temperature were 87.2 cm and 9.4°C. Soil series include Webster (fine-loamy, mixed, superactive, mesic Typic Hapludolls) and Nicollet (fine-loamy, mixed, superactive, mesic Aquic Hapludolls).

The trial was established in 2017. The experimental design was a randomized complete block design with three N fertilizer rate treatments (0, 168, and 336 kg N ha$^{-1}$; onward N0, N168, and N336). Each treatment had 3 replicate plots and was blocked to account for soil series. Corn was planted in all years in all plots and fertilized with hand-broadcast urea prior to
planting. The soil was chisel-plowed in the fall after harvest and cultivated in the spring prior to corn planting. In 2019, N fertilizer was applied on April 24th and maize (111 day relative maturity) was planted on May 16th at 80,000 plants ha\(^{-1}\) in 76 cm rows.

In each plot, soil temperature and soil volumetric water content were measured at 15 and 45 cm depth with commercial soil sensors (METER 5TM soil moisture and temperature sensors). These sensors were placed near the center of each plot between maize rows. The new soil NO\(_3^-\) sensors were installed in the field on June 8th when corn was at the 2\(^{nd}\) leaf growth stage (crop planting in 2019 was delayed across the Corn Belt due to record precipitation). During installation, the sensors were inserted in slurry of soil and water to ensure good sensor-soil contact (a procedure similar to the installation of lysimeters). The installation depth was 25 cm in the middle of two maize rows nearby within 25 cm of the soil moisture and temperature sensors. After field deployment, sensors were retrieved and recalibrated.

Salt extract-based soil NO\(_3^-\) measurements during the sensor measurement period were made on four dates: June 13th, June 27th, July 9th and July 15th. On each date, three random 0-30 x 2.54 cm soil cores were collected from each plot within 20 cm of the corresponding sensor. The soil cores were immediately returned to the laboratory, homogenized, and soil NO\(_3^-\) concentration was measured using the salt extraction procedure described above. Although the sensors made point-based measurements at ~25 cm depth, we sampled from 0-30 cm because my objective was to compare the sensors to the conventional salt-extraction approach for soil NO\(_3^-\) measurement in Midwest US maize fields (e.g., Jaynes et al. 2004).

**Soil Sensors**

The sensors in this study measured NO\(_3^-\) concentration in the soil solution (mg NO\(_3^-\)-N L\(^{-1}\)) using a solid-state miniature potentiometric sensor that works in direct contact with soil to measure NO\(_3^-\)-N concentration in soil solution with parts-per-million (ppm) resolution using a
working electrode and a reference electrode. Although the sensors are field-deployable for long-term measurement, they are currently hand-manufactured by the inventors in an academic laboratory setting; the sensors for use in practical applications herein go through strict quality check and systematic calibrations under different conditions in the research lab (Ali et al. 2019). The major innovation in these sensors is the integration of a NO₃⁻-selective membrane and a solid-state ion-to-electron transducing layer that minimize interference from other anions.

The working electrode is formed from a thin layer of silver (Ag) deposited on a patterned gold (Au) electrode and covered with the ion-to-electron transducing layer and the NO₃⁻-selective membrane. The reference electrode comprises a screen-printed silver/silver chloride (Ag/AgCl) electrode covered by a protonated Nafion layer to prevent chloride (Cl⁻) leaching in long-term measurements. A waterproof epoxy covers the entire surface of the sensor and allows only the center area of the membrane to be exposed to the soil solution.

The sensors provide long-term, continuous measurement of soil solution NO₃⁻ concentration at a specific point in space where they are deployed (e.g., < 1 cm³); they sense only the solution with which they contact. In the field study reported herein, the sensors were programmed to record one NO₃⁻-N concentration per ten seconds.

Pre- and post-deployment, the soil NO₃⁻ sensors were calibrated with standard NO₃⁻-N solutions, using a range that includes soil solution NO₃⁻-N concentrations that will be encountered in the sensing environment (for Iowa maize systems, 1−5000 mg NO₃⁻-N L⁻¹ soil solution). Note: most of this range (500-5000 mg L⁻¹) is due to the widespread use of concentrated N fertilizer applications (i.e., ‘banding’). The sensors were calibrated by recording their voltage responses (in mV) after being immersed in standard NO₃⁻-N solutions (10 mg L⁻¹, 100 mg L⁻¹, 200 mg L⁻¹, 1000 mg L⁻¹ and 5000 mg L⁻¹) for 3 minutes. The standard NO₃⁻-N
solutions were prepared by dissolving NaNO₃ in deionized water. The sensors were calibrated in NO₃⁻-N solutions from low to high concentrations, repeated for three times. The average voltage response for each solution were used for representing the corresponding NO₃⁻-N concentration. Sensors were considered functional when the difference between the pre- and post-deployment calibration curves is < 20%. Although this difference may appear to be large, it should be interpreted in the context of exceptionally high within-field spatial variation and minimal spatial autocorrelation in soil NO₃⁻-N concentrations (Cambardella et al. 1994).

**Salt Extraction-based Soil Nitrate Measurements**

In contrast to the soil sensors, which measure the NO₃⁻-N concentration in soil solution (mg NO₃⁻-N L⁻¹ soil solution), salt extract-based soil NO₃⁻-N measurements are reported in units of mg NO₃⁻-N kg⁻¹ dry soil. Hence, the transformation of sensor data from mg NO₃⁻-N L⁻¹ to mg NO₃⁻-N kg⁻¹ dry soil was required for comparison of the sensor method with the conventional salt-extract method and requires two coefficients: soil water content and soil bulk density. We tested the sensitivity of the data transformation to uncertainty across relevant ranges of these coefficients. We used Eq. 1 to estimate soil gravimetric moisture content (θₘ) from volumetric soil moisture content (θᵥ) sensor data and estimates of soil bulk density (ρₜ). In the calculation, we assumed bulk density was 1.25 g cm⁻³. Then, we used Eq 2. to convert mg NO₃⁻-N L⁻¹ soil solution to mg NO₃⁻-N kg⁻¹ soil. According to the equations, the NO₃⁻-N concentration change linearly with bulk density and volumetric water content. For this analysis, we used ranges of uncertainty in bulk density of 1.15 g cm⁻³ ~ 1.3 g cm⁻³ and volumetric water content of ±10 percent difference of the measurement (e.g., 0.30 +/- 0.03 cm⁻³ H₂O cm⁻³ soil).

\[
\text{Eq 1. } \theta_m = \theta_v \times \rho_w / \rho_b
\]

Where, \( \rho_w \) is water density, which is assumed as 1g/cm³. Thus, Eq 1. can be written as: \( \theta_m = \theta_v / \rho_b \)
\[ Eq. \ 2. \ mg \ NO_3^-\text{-N kg}^{-1}\text{ soil} = mg \ NO_3^-\text{N L}^{-1}\text{ soil solution} * \theta_m \]

**Comparisons of Salt-extract and Sensor Measurements**

During the 2019 growing season, 32 pairs of salt extract and sensor-based measurements from 14 sensors in all three N rate treatments and nine plots were made for the four sampling dates listed above. Some comparisons from particular plots are missing due to sensor failures. These data were used to compare the two methods using converted soil sensor NO$_3^-$-N data from mg NO$_3^-$-N L$^{-1}$ soil solution to mg NO$_3^-$-N kg$^{-1}$ dry soil.

We used three quantitative methods to compare the salt extraction-based and sensor-based NO$_3^-$ measurements. First, we used a linear regression model ($Y = aX + b$) to describe the relationship between two methods. The coefficient of determination ($r^2$) was used to quantify the variance in the dependent variable (converted sensor data) that was explained by the independent variable (salt-extract data). We determined whether the slope significantly differed from 1:1.

Second, we used Bland-Altman (B&A) plots (Bland & Altman, 1986 and 1999), which are a more informative graphical method to describe the agreement between a new and established measurement technique. The B&A plot analysis is a simple way to evaluate bias between the mean differences of two methods, and to estimate an agreement interval within which 95% limit of agreement of the second method. In this graphical method, the differences between the two methods are plotted against the averages of the two methods or, when there is an increase in variability of the absolute differences as the magnitude of the measurement increases, the percent differences are plotted against the average of the two methods (Giavarina, 2015). In these plots, the X-axis is the mean value of the paired conventional and sensor-based measurements and the Y-axis is the percentage difference of the two paired measurements. Horizontal lines are drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference
(d) plus and minus 1.96 times the standard deviation (sd) of the differences. If the differences are normally distributed, 95% of the limits of agreement will be between d-1.96sd and d +1.96sd.

Third, we used intraclass correlation coefficients (ICCs), which assess agreement of quantitative measurements in the sense of consistency and conformity between two or more measurements (Fisher, 1954). Consistency is defined as the agreement of two quantitative measurements where neither is assumed “correct” or “standard”. Hence, consistency handles questions of intra- as well as inter-observer repeatability of measurement scales. In contrast, the concept of conformity is defined as the agreement of a first measurement with a reference that is established as the “standard” (Müller and Böttner, 1994). Modern ICCs are calculated by mean squares obtained through ANOVA. According to Koo and Li (2016), ICC values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability.

The laboratory experiments allowed us to better control soil NO$_3^-$ concentrations and evaluate sensor response to soil temperature and water content, thus justifying the field evaluation of the sensors. The in situ field experiments allowed us to compare the similarity of the two methods in the context of environmental variability (i.e., spatiotemporal variation) and methodological differences that necessarily result in different NO$_3^-$ measurements with each method. Moreover, the field sensor data also allowed us to characterize intra- and inter-daily fluctuations of soil solution NO$_3^-$ -N concentration with unprecedented resolution.

**Time Series Analyses of Sensor Data**

We used time series data analysis to test for temporal patterns of NO$_3^-$ concentration and soil moisture content. The seasonality of a time series is defined as a pattern that repeats itself over fixed intervals of time (Makridakis et al., 1998). The full (Ha) model for the analysis is: $y_t = T_t + S_t + R_t$, where $y_t$ is the original data, $T_t$ is trend component, $S_t$ is seasonality component and
R_t is residual component. The null (H0) model: \( x_t = T_t + R_t \), is the time series after seasonality adjusting. We used a type I error p-value > 0.05 to indicate that there is not enough evidence to reject H0, which means there is a recurrent temporal pattern. In contrast, if the p-value was < 0.05, we rejected H0 indicating the absence of a recurrent temporal pattern. When rejecting H0, the quantitative measure of seasonality is: \( F_s = 1 - \frac{\text{Var}(R_t)}{\text{Var}(R_t + S_t)} \). Ultimately the temporal pattern is normalized to a value from 0-1 to indicate the degree of presence of the seasonality. A measure near 0 for a certain time series indicates an absence of seasonality, while a measure near 1 indicates a strong presence of the seasonality (Wang et al., 2006).

In the early spring, soil NO\textsubscript{3}\textsuperscript{-}-N concentrations are generally stable and high. In the late spring and early summer, soil NO\textsubscript{3}\textsuperscript{-}-N concentrations are generally dynamic and high. In mid-to-late summer, soil NO\textsubscript{3}\textsuperscript{-}-N concentrations are generally stable and low due high rates of maize N uptake. This pattern is widespread and well-known in maize-based agroecosystems (Archontoulis et al. 2020). Hence, we used the three periods (June 8-22, July 1-15 and August 1-15) to test the effects of sampling frequency on estimates of soil NO\textsubscript{3}\textsuperscript{-}-N concentration. Within days, we investigated the effect of intra-daily sampling frequency (i.e., every 1 to 12 h corresponding to 12-24 samples per day) on actual mean daily soil NO\textsubscript{3}\textsuperscript{-}-N concentration calculated from the 10-second sensor measurements. Across days, we investigated the effect of daily sampling frequency (every 1 to 7 days or 2-15 samples per 15 days) on estimates of mean daily NO\textsubscript{3}\textsuperscript{-}-N concentration across the 15-day period calculated from the 10-second measurements. To quantify the effects of sampling frequency on measured soil NO\textsubscript{3}\textsuperscript{-}-N concentration, we used a jackknife subsampling procedure (Parkin, 2008). Using the continuous measurements of NO\textsubscript{3}\textsuperscript{-}-N, we constructed subsets of hourly or daily concentration data from measured values every 10-seconds throughout the sample period (Efron, 1979; Efron and Gong,
1983). The influence of sampling frequency on the accuracy of NO$_3^-$-N estimates was obtained by computing the percent difference of each jackknife subset from the average of the 10-second data.

**Results**

**Laboratory Experiments**

In laboratory comparisons of soil NO$_3^-$-N concentration after organic matter removal, the salt extract and sensor measurements were similar to the adjusted theoretical soil solution NO$_3^-$-N concentration (i.e., the targeted NO$_3^-$-N concentration). Salt extract and sensor measurements did not significantly differ from each other (P=0.95) or the theoretical adjusted NO$_3^-$-N concentration (Fig. 1, P=0.99). Percent differences between the salt extract measurements and adjusted theoretical NO$_3^-$-N concentrations ranged from -6.5% to 31% whereas the percent differences between the sensor measurements and adjusted theoretical concentrations ranged from 0.9% to 22%. (Fig. 1).

The differences between the sensor measurements and the adjusted theoretical NO$_3^-$-N concentrations ranged from -24% to 27%. The differences between sensor measurements and prepared solution concentration were not significant (Fig. 2, P=0.51).

**Field Experiments**

Nitrogen fertilizer rate had a significant effect on mean daily soil NO$_3^-$ concentration as measured with sensor and salt extract methods (Fig. 3 and 4). The sensors measured NO$_3^-$-N concentrations every ten seconds from June 8th to August 20th with some periods of data loss due to battery or electronic failures. Across all sensors, data loss accounted for 32.7% of the total deployment time. Nitrate concentrations reached a maximum in late June and then decreased until all measurements were lower than 1 mg NO$_3^-$-N kg$^{-1}$ dry soil with both sensor and salt-
extract methods (Figs. 3 and 4). In the zero N fertilizer treatments, the mean concentration was ~3 mg NO$_3^-$-N kg$^{-1}$ dry soil and the highest concentration was ~20 mg NO$_3^-$-N kg$^{-1}$ dry soil. In the 168 kg N fertilizer ha$^{-1}$ treatments, the mean concentration was ~5 mg NO$_3^-$-N kg$^{-1}$ dry soil and the highest concentration was ~35 mg NO$_3^-$-N kg$^{-1}$ dry soil. In the 336 kg N fertilizer ha$^{-1}$ treatments, the mean concentration was ~9 mg NO$_3^-$-N kg$^{-1}$ dry soil and the highest concentration was ~65 mg NO$_3^-$-N kg$^{-1}$ dry soil. Figure 4 displays the comparison between salt-extract and sensor measurements of soil NO$_3^-$-N concentration in two example experimental units in field experiments.

The effects of uncertainty in bulk density and volumetric soil water content were less than the variations in NO$_3^-$ concentrations within and across plots (Fig. 5). The effects of uncertainty in bulk density and volumetric soil water content changed in proportion to bulk density and volumetric soil water content. The effects of uncertainty in bulk density were limited in -4% to 8%. The effects of uncertainty in volumetric water content were limited to ±10%.

We examined 32 pairs of salt extract and sensor-based measurements from all 14 sensors. The relationship between salt extract and sensor measurements did not differ from 1 (Fig. 6a). The mean difference between paired salt-extract and sensor measurements was -22% (Fig. 6b). Throughout all measurements, the variability in NO$_3^-$-N concentrations across days within plots was ± 99%. As a percent of the NO$_3^-$-N concentration, differences between the sensor and salt extract measurements were relatively high when the NO$_3^-$-N concentration was low. However, the percent difference was relatively low when the mean concentration of the paired measurements was management-relevant (i.e., >10 mg NO$_3^-$-N kg$^{-1}$). According to published guidelines (Koo and Li, 2016), the intra-class correlation (ICC) that was used to quantify the reliability of sensor measurements indicated ‘good’ correlation: $\rho = 0.87$. 
Temporal Analyses of Sensor Data

Time series analysis showed there was no intra-daily (i.e., diurnal) pattern in the NO$_3^-$-N concentration (P>0.99). Despite times when soil moisture content showed a strong intra-daily pattern (which is well-known to occur; e.g., Jackson et al, 1997) there was no intra-daily pattern in the corresponding NO$_3^-$-N concentration (e.g., Fig. 7). For example, from June 8th to June 13th, soil moisture content exhibited a strong diurnal temporal pattern (Fs = 0.7157), but there was no pattern in the NO$_3^-$-N concentration (Fs = 0.0978).

As a percent of the daily mean, the diurnal range in soil moisture content is extremely small relative to the diurnal range of NO$_3^-$-N concentration. Figure 8 displays the daily range of soil NO$_3^-$-N concentration from ten-second measurements across nine days. During this time, the diurnal range of soil NO$_3^-$-N concentration spanned ±0.85% to ±7.8% of the daily mean whereas the diurnal range of soil water content spanned ±0.25% to ±0.52% of the daily mean.

Within days, there was an effect of intra-daily sampling time on estimated mean NO$_3^-$-N concentration calculated from the 10-second measurements. Although the effect was random due to the lack of diurnal pattern in NO$_3^-$-N concentration, it was large during times of the year when soil NO$_3^-$-N concentration was high and dynamic (e.g., Figs 9 and 10). In the early spring when soil NO$_3^-$-N concentration was high but stable (June 8-22), the number of measurements per day (2 to 24) had little effect on the estimate of actual mean NO$_3^-$-N concentration for that day; the mean of two measurements, separated by 12 h, were within 5% of the actual mean value for the day calculated from the 10 s measurements. In contrast, when NO$_3^-$-N concentration was high and dynamic (July 1-15), sampling intervals <4 hours (i.e., 6 measurements per day) were required to provide an estimate that was within 5% of the actual daily mean of the 10-second measurements. When NO$_3^-$ concentration was low and stable (August 1-15), percent differences were large but absolute differences were small (Fig. 9).
Across days, as the number of days between measurements increased, the inter-daily differences in mean daily NO$_3^-$-N concentration became increasingly large (Fig. 10). However, the effect of sampling interval (i.e., days between measurements) on the estimated mean daily NO$_3^-$-N concentration differed with the growth stage of maize and seasonal progression from late spring to summer. In the early spring when soil NO$_3^-$-N concentration was high but stable (June 8-22), sampling interval had a small effect on estimated mean daily NO$_3^-$-N concentration; linear interpolation of mean daily NO$_3^-$-N concentration sampled on days 1 and 8 was within 5% of the actual daily mean calculated as the mean of all eight days. However, when NO$_3^-$-N concentration was high and dynamic (July 1-15), sampling frequencies with greater than 3 d intervals were >20% of the actual daily mean. When NO$_3^-$-N concentration was low and stable (August 1-15), sampling interval had an intermediate effect on estimation of the daily mean.

**Discussion**

The instantaneous, continuous soil NO$_3^-$ sensors were accurate and measured soil NO$_3^-$ similar to conventional salt-extract methods based on manual sampling (Figs 1-2). The sensors enabled unprecedented temporal resolution of sampling, which demonstrated that diurnal and inter-daily variation in soil NO$_3^-$-N pool size is important sometimes, but not others (Figs 6-7). My results indicate that the sensors can be powerful tools to better understand soil nitrogen cycling processes and better predict ecosystem processes such as crop production, nitrous oxide emissions, and NO$_3^-$ leaching.

Due to the difficulty of measuring soil NO$_3^-$ concentration, current ecosystem models and experiments rely on extremely few empirical data in both time and space. Although sub-daily in situ measurements of nitrous oxide (N$_2$O) emissions and NO$_3^-$ leaching are available (e.g., Jarecki et al. 2008; Daigh et al. 2015), sub-daily measurement of soil NO$_3^-$-N concentration – a
critical control on these processes – are unavailable. For example, Jarecki et al. (2008) measured N$_2$O emissions every 6 h for more than 200 consecutive days (>800 times); yet, during this time, they measured soil NO$_3^-$-N concentration only six times. Using these data, the authors demonstrated that the ecosystem process model DAYCENT predicted N$_2$O emissions within 25% of actual emissions. Because NO$_3^-$ is the substrate for N$_2$O production, it is possible that high-resolution NO$_3^-$-N concentration measurements could have improved the model performance.

Our results point towards significant potential for high-resolution NO$_3^-$-N data to advance understanding of soil N dynamics. During times of the year when soil NO$_3^-$-N concentration was high and dynamic, low sampling frequency both within and across days resulted in substantial errors in linear interpolation-based estimates of mean soil NO$_3^-$-N concentration (Figs 8-10). However, during times of the year when soil NO$_3^-$-N concentration is stable, measurement frequency had relatively little effect on NO$_3^-$-N concentration. These results suggest that sensors may add significant value to improving soil tests and model predictions of environmental N losses and plant growth.

Although the soil NO$_3^-$-N concentration varied within days (Fig. 9), we rejected the hypothesis that this variation is due to the well-known intra-daily fluctuation in soil water content (e.g., Fig 8). Volumetric soil water content is well known to follow a cyclical pattern within days due to the daily pattern of plant water uptake. As a result, the intra-daily variability in NO$_3^-$ is likely the result of many factors including differing water potentials and sink strengths (e.g., microbes, crops and ion exchange sites).

We did not investigate the effect of spatial resolution on measurements of soil NO$_3^-$ concentration, however, low-N systems (e.g., pastures and forests) may require more sensors in
The absolute difference between sensors and salt extract measurements was similar at relatively low and high NO$_3^-$-N concentrations (Fig. 6a). Hence, the percent difference was lower at high NO$_3^-$-N concentrations (Fig. 6b).

Given the general similarity between sensor and salt extract measurements in laboratory soils (Figs. 1-2), it is most likely that methodological and spatial variations rather than sensor accuracy led to the differences in soil NO$_3^-$-N measurements with the sensor and salt extract methods (Fig. 4). We compared point-based sensor measurements at 25 cm depth with salt extract measurements based on extraction of several 2.5 x 30 cm homogenized soil cores. At low NO$_3^-$-N concentrations, the salt-extract method tended to produce higher NO$_3^-$-N concentrations (Fig. 4); this difference is consistent with the widespread pattern of a decrease in soil NO$_3^-$ concentration with depth (Wiseler & Horst 1993; Toosi et al. 2014) and the fact that the salt-extract method includes a large fraction surface soils in the sample. As soil NO$_3^-$ moves downward through the soil profile, the optimum depth comparison between the two methods may change.

In the context of differences between the methods, it is important to note that all soil NO$_3^-$-N measurements are indexes – there is no true value of soil NO$_3^-$ concentration because the soil NO$_3^-$ pool varies by size in space and strength of adhesion to soil particles (Darrouzet-Nardi & Intraub 2014). Spatial variation in soil NO$_3^-$ pool size is enormous; there is often no spatial dependence of soil NO$_3^-$-N concentration at scales > 2 m$^2$ (Robertson et al. 1988; Cambardella et al. 1994). Moreover, some soil NO$_3^-$ chemically adheres to anion exchange sites or physically adheres in soil solutions that are bound to soil particles at extremely low pressure potentials. The strength of adhesion varies at a microscale and these factors differently affect the measured concentration of NO$_3^-$-N in any given sample with any given method (e.g., Darrouzet-
Nardi & Tintraub 2014). Hence, the goal of all soil NO$_3^-$-N measurements is to use the measurements to predict and explain processes of interest such as microbial metabolism, plant productivity and environmental N losses. The sensors tested herein show potential to improve prediction and understanding of these processes.

As soil NO$_3^-$ sensors become low-cost and ruggedized for long-term deployment, widespread implementation will advance my capacity to predict, explain and manage ecosystem nitrogen dynamics. Much more detailed salt-extract, lysimeter and sensor comparisons will become increasing possible as sensor manufacturing is industrialized. An increase in manufacturing yield will require an industrial setting for manufacturing, quality check, and maintenance.

The recent National Academies of Sciences, Engineering, and Medicine Report “Science breakthroughs to advance food and agricultural research by 2030” (NAS, 2019) highlighted the need for agricultural sensors to deliver research breakthroughs that are required for the long-term sustainability of global agriculture. Such sensors have already been realized for soil moisture and temperature. Breakthroughs in soil moisture sensor technology and cost led to improvements in soil evaporation models (Ventura et al., 2006) and distributed sensor networks that improved large-scale ecosystem models (Robison et al., 2008). In addition, farmers use the same sensors and similar models to better manage irrigation systems, significantly decreasing water use while improving profitability (Blonquist et al., 2006). Ecosystem and cropping systems can achieve similar improvements from NO$_3^-$ sensors. Future work must to determine optimum spatial deployment of sensors at depth and area, use sensors to improve model algorithms, and determine how sensor data streams can be coupled with models to enhance predictions.
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**Figures**

Figure 1. Laboratory-based measurements of NO$_3^-$ in soils that were adjusted to specific gravimetric water contents and soil solution NO$_3^-$ concentrations after combustion to remove organic matter and reduce microbial mineralization-immobilization. a) Sensor measurements of soil solution NO$_3^-$ concentration. b) Comparison between salt extract and sensor measurements after adjusting to common units (mg NO$_3^-$-N kg$^{-1}$ dry soil). Theoretical data represent target NO$_3^-$-N concentration that soils were adjusted to after combustion. Standard errors (only salt extraction-based measurements) indicate standard error of 3 sub-samples from the same
experimental unit. c) Salt extract vs. sensor, salt extract vs. theoretical, and sensor vs. theoretical did not significantly differ from 1.0. The solid line of equality is shown for reference. The dashed lines show linear regression of each comparison. The linear regression for the sensor vs theoretical is: \( y = 0.9827x + 0.2295, r^2 = 0.99 \); the linear regression for the salt-extract vs Theoretical is: \( y = 1.0196x - 1.6779, r^2 = 0.98 \).

Figure 2. a-c) Comparison of NO\textsubscript{3}^-N concentration in laboratory experiments across four different temperatures (0°C, 10°C, 20°C, 30°C) and 3 prepared NO\textsubscript{3}^-N concentration solution (10 mg/L, 100 mg/L, 1000 mg/L). d) The relationship between prepared NO\textsubscript{3}^-N concentration (x axis) and sensor measured NO\textsubscript{3}^-N concentration (y axis). The solid line of room temperature is shown for reference; points on this line are from sensor calibration procedure under room temperature. The dashed lines show linear regression of each temperature. The linear regression for 0 °C is: \( y = 1.04572x - 0.10963, r^2 = 1 \); the linear regression for 10 °C is: \( y = 1.04873x - 0.11823, r^2 = 0.98597 \); the linear regression for 20 °C is: \( y=0.95468x+0.14644, r^2 = 0.99984 \); the linear regression for 30 °C is: \( y=1.05184x - 0.06813, r^2 = 0.99476 \).
Figure 3. Soil solution NO₃⁻ concentration measured every ten seconds in a nitrogen (N) fertilizer rate experiment with 14 sensors in three N fertilizer treatments: 0 kg N ha⁻¹, 168 kg N ha⁻¹ and 336 kg N ha⁻¹.
Figure 4. Example comparison between salt extract and sensor measurements of soil NO$_3^-$ concentration in two field experiment units receiving 0 kg N ha$^{-1}$ and 168 kg N ha$^{-1}$. There were two sensors in each experimental unit (solid and dashed lines). Error bars for the salt extract method indicate standard error of three sub-replicate soil samples in each experimental unit sampled within 30 cm of the sensor.
Figure 5.  a-b) Mean soil NO$_3^-$-N concentration in the 2019 growing season from 14 sensors. Panel a error bars represent the effect of bulk density assumptions (1.15 g cm$^{-3}$ to 1.3 g cm$^{-3}$) on soil NO$_3^-$-N concentration. Panel b error bars represent the effect of soil water content assumptions (volumetric water content limit from -0.1 to +0.1 proportional difference) on soil NO$_3^-$-N concentration.

Figure 6. Soil NO$_3^-$ concentration measured with salt extract and sensor methods in a field experiment. Each point represents one replicate plot in the field experiment on each sampling date. Sensor data represent mean daily soil NO$_3^-$ concentration on the day of soil sampling and salt extraction. a) The solid line of equality is shown for reference. The slope of the linear regression model did not significantly differ from 1.0. The equation for the linear equation is: $y=0.7081x - 0.1766$, $r^2=0.76$; $R=0.87$. b) Bland-Altman plot showing percent differences between salt extract and sensor measurements (y axis) against the mean of the two measurements (x axis). The solid red line indicates the mean difference between methods (-22%), and the blue dashed lines indicate 95% confidence intervals (blue dotted line).
Figure 7. Example comparison of soil volumetric content and soil $\text{NO}_3^-$ concentration in a plot receiving 168 kg N ha$^{-1}$.

Figure 8. Example daily range of soil $\text{NO}_3^-$ concentration from ten-second measurements across nine days (June 17th to June 25th).
Figure 9. The effect of intra-daily sampling resolution on accuracy of estimated mean daily soil NO$_3^-$ concentrations for a single day in the field experiment. The actual single day mean concentration is calculated as the mean of intra-daily field sensor measurements with 10-second sampling resolution. The estimated mean single day concentrations are based on linear interpolation of hourly sampling intervals from 1 to 12 hours (i.e., 24 to 2 intra-daily samples). Panels represent three time periods that have different NO$_3^-$ concentrations. Circles represent 0 kg N ha$^{-1}$; squares represent 168 kg N ha$^{-1}$; triangles represent 336 kg N ha$^{-1}$. Data are offset from hourly sampling intervals to allow visualization of the error bars.
Figure 10. The effect of inter-daily sampling resolution on accuracy of mean daily soil NO$_3^-$ concentration across days in a field experiment. The actual single-day mean concentrations are calculated as the mean of intra-daily field sensor measurements with 10-second sampling resolution. The estimated mean daily concentration across days is calculated by linear interpolation of mean daily concentrations with sampling intervals from one to eight days (i.e., 15 to 2 samples). Panels represent three time periods that have different NO$_3^-$ concentrations. Symbols represent different N fertilizer rates. Circles represent 0 kg N ha$^{-1}$; squares represent 168 kg N ha$^{-1}$; triangles represent 336 kg N ha$^{-1}$. Data are offset from hourly sampling intervals to allow visualization of the error bars.
CHAPTER 3. GENERAL CONCLUSION

The goal of this research was to test the agreement between sensor (Ali et al. 2019) and salt-based extract (Hart et al. 1994) measurements of soil NO$_3^-$-N concentration in both laboratory conditions and in field conditions and examine the temporal pattern of soil NO$_3^-$-N concentration. Field analyses were conducted at the research experiment scale at one long-term continuous maize site in Iowa.

In Chapter 2, my results give compelling evidence that the measurements of this sensor have good agreement with the salt extraction-based measurements in both laboratory and field experiments. Differences between the sensor and salt extract measurements were relatively high when the NO$_3^-$-N concentration was low. However, the percent difference was relatively low when the mean concentration of the paired measurements was $>10$ mg NO$_3^-$-N kg$^{-1}$, which would be around the lower bound of management-relevant nitrate levels in a maize production system. In general, the reliability of sensor measurements is good according to Bland & Altman plot and Intra-class Correlation (ICC).

According to my results, high-resolution NO$_3^-$-N data has significant potential to aid future understanding of soil N dynamics. For the temporal analyses of sensor measurements, we rejected the hypothesis that there was intra-daily pattern in the corresponding NO$_3^-$-N concentration, despite times when soil moisture content showed a strong intra-daily pattern.

Lastly, during times of the year when soil NO$_3^-$-N concentration was high and dynamic, low sampling frequency both within and across days resulted in substantial errors in linear interpolation-based estimates of mean soil NO$_3^-$-N concentration. However, during times of the year when soil NO$_3^-$-N concentration is stable, measurement frequency had relatively little effect
on NO$_3^-$-N concentration. These results suggest that sensors may add significant value to improving soil tests and model predictions of environmental N losses and plant growth.

References

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