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ORIGINAL ARTICLE

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# Variability of asymbiotic N-fixation organism activity with distance and time in North Dakota transitional no-till soils

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## Abstract

Asymbiotic nitrogen fixation (N-fixation) is a microbial process that may result in the introduction of plant-available N into the soil. Although the process of N mineralization in soils is mediated by many microorganisms and is related mostly to soil moisture, the soil factors which regulate asymbiotic N-fixation are relatively unknown and the number of microorganisms with the ability to fix N is small relative to the whole soil microorganism pool. Soils under long-term no-till management have greater asymbiotic N-fixing organism activity compared to no-till. This study was conducted to determine the variance of N-fixing activity over distance, and the temporal variability of asymbiotic N-fixing organism activity at six sites with transitional no-till soils in eastern North Dakota over three growing seasons. Sites were sampled at the same location each month of the growing season from 2019 to 2021. At one of the sampling dates, each year additional samples were obtained at distances from the central sampling location for use in statistical analysis. The sampling over distance indicated that to characterize a large field area, multiple samples should be taken and analyzed separately or mixed together for single analysis. Monthly sampling in all 3 years indicated that peak N-fixation activity was favored by a moist, warm environment. Dry periods and excessively wet periods resulted in low activity. Heavy rains within 48 h of sampling resulted in extremely low N-fixation activity at the subsequent sampling date. Models were constructed relating the accumulative rainfall from the 30 days prior to sampling, and the mean air temperature from the 30 days prior to sampling. These data indicate that the activity of asymbiotic N-fixing organisms increased with temperature and cumulative rainfall prior to measurement.

# 1 | INTRODUCTION

Microbial communities that live and function in the soil are related to the congregate soil, cropping, and environmental conditions present (Upton et al., 2020). Among the organisms present in many soils are those capable of asymbiotic N-fixation. Asymbiotic N-fixation in soil is also referred to as free-living N-fixation (Smercina et al., 2019) and nonsymbiotic N-fixation (Roper & Gupta, 2016). Asymbiotic N-fixing organisms have a long genetic history, with their presence found as early as 1.5 billion years ago (Boyd & Peters, 2013). Environmental factors that limit N-fixation are theoretically broader with asymbiotic N-fixation compared with symbiotic N-fixation; however, the environmental factors that limit the activity of specific N-fixing organisms are much narrower (Smercina et al., 2019). Asymbiotic organisms are incapable of fixing N without available organic carbon (C) substrates. These substrates could be delivered through root exudates, soluble organic matter, or organic films coating soil particles and aggregates (Smercina et al., 2019). The quality of the organic C source is also important, with some sources shown to inhibit N-fixing activity while others enhance N-fixation (Smercina et al., 2019).

Soil oxygen concentration is also important in asymbiotic N-fixation (Kox et al., 2018), with anaerobic conditions required for some N-fixing organisms to function, while other organisms in non-wetland conditions employ internal strategies to isolate nitrogenase from free oxygen (Rice et al., 1967). Internal oxygen management requires energy, and therefore organisms that fix N in aerobic environments subsequently have lower N-fixing activity compared to organisms adapted to an anaerobic environment (Rice et al., 1967). Asymbiotic N-fixation is also dependent on soil temperature and water content (Reed et al., 2011). Temperature and soil water content affect not only N-fixation rate but the composition of the N-fixing community (Reed et al., 2011).

Asymbiotic N-fixation rate, as estimated by the acetylene reduction method (Weaver & Danso, 1994), varies by location and land use. In a survey of Austrian land uses on asymbiotic N-fixation, peatlands had the greatest N-fixation, ranging from 951.9 to 39.1 nmol $C_2H_4$ $m^{-2}$ $day^{-1}$. Saline soils had the next highest rates, ranging from 642.1 to 207.9 nmol $C_2H_4$ $m^{-2}$ $day^{-1}$. The explanation for the high rates in saline soils was that the timing of sampling coincided with soil drying in late spring and the development of algal crusts, consisting at least in part of blue-green algae (genera *Nostoc*, *Calothrix*, *Tolypothrix*, *Fischerella*, *Anabaena*, and *Aphanothece*) with high N-fixation ability. The land use with the next to lowest N-fixation rate was arable fields, ranging from 2.3 to 13.1 nmol $C_2H_4$ $m^{-2}$ $day^{-1}$, with non-peatland coniferous forests ranging from 1.3 to 3.5 nmol $C_2H_4$ $m^{-2}$ $day^{-1}$ (Zechmeister-Boltenstern & Kinzel, 1990). Fixation rates from arable lands and non-peatland coniferous forests were about less than 1% of the activity of peatlands.

**Core Ideas**

- Asymbiotic N-fixing organism activity is variable over sampling locations.
- Asymbiotic N-fixation was low in early spring after thaw (mid-April to late April).
- Asymbiotic N-fixation activity was favored by moist, warm soil.
- Asymbiotic N-fixation activity was lowest during dry or excessively wet conditions.

Asymbiotic N-fixation has been shown to have a higher rate in soils under long-term no-till management than in conventional tillage (Franzen et al., 2019; Lamb et al., 1987). In a wheat-fallow system in western Nebraska (Lamb et al., 1987), soil sampling for asymbiotic organism activity was conducted on three dates in 1983, and in April, May, June, July, and September, 1984. In 1983, the soil was very dry all season (0.21–0.26 $m^3$ $m^{-3}$), which was probably why there were no differences in activity between dates. After a moist April in 1984 in this study, the soil again became dry and differences between treatments diminished as the soil became drier. Lamb et al. (1987) noted that N-fixation decreased with decreased soil moisture.

The objective of these studies was to determine how asymbiotic N-fixing organism activity varied throughout multiple seasons in North Dakota in response to cumulative rainfall prior to sampling and temperature and the degree of variability in sample fixation value.

# 2 | METHODS

Six experimental sites were identified that were early-transitional no-till fields. The experimental sites were located near Mooreton, Jamestown, New Rockford, Lakota, Logan Center, and Gardner, North Dakota (Figure 1). Soil properties of the sites are provided in Table 1, with crops grown in each site year provided in Table 2. The crops grown at the sites are for informational value only. This study was not designed to indicate if crops grown in the year of sampling had any effect on asymbiotic N-fixing activity.

A sampling location was found at each of the six experimental sites and these locations were georeferenced. The initial sampling was performed at the exact georeferenced location, and subsequent sampling in 2019, and again in 2020 and 2021 sampling was performed within a meter of this initial point. Only one sample jar was used to represent the location at each sampling time. The locations were sampled at the 0–5 cm depth for asymbiotic N-fixing organism activity at the sampling dates indicated in Table 3. The 0–5 cm depth was

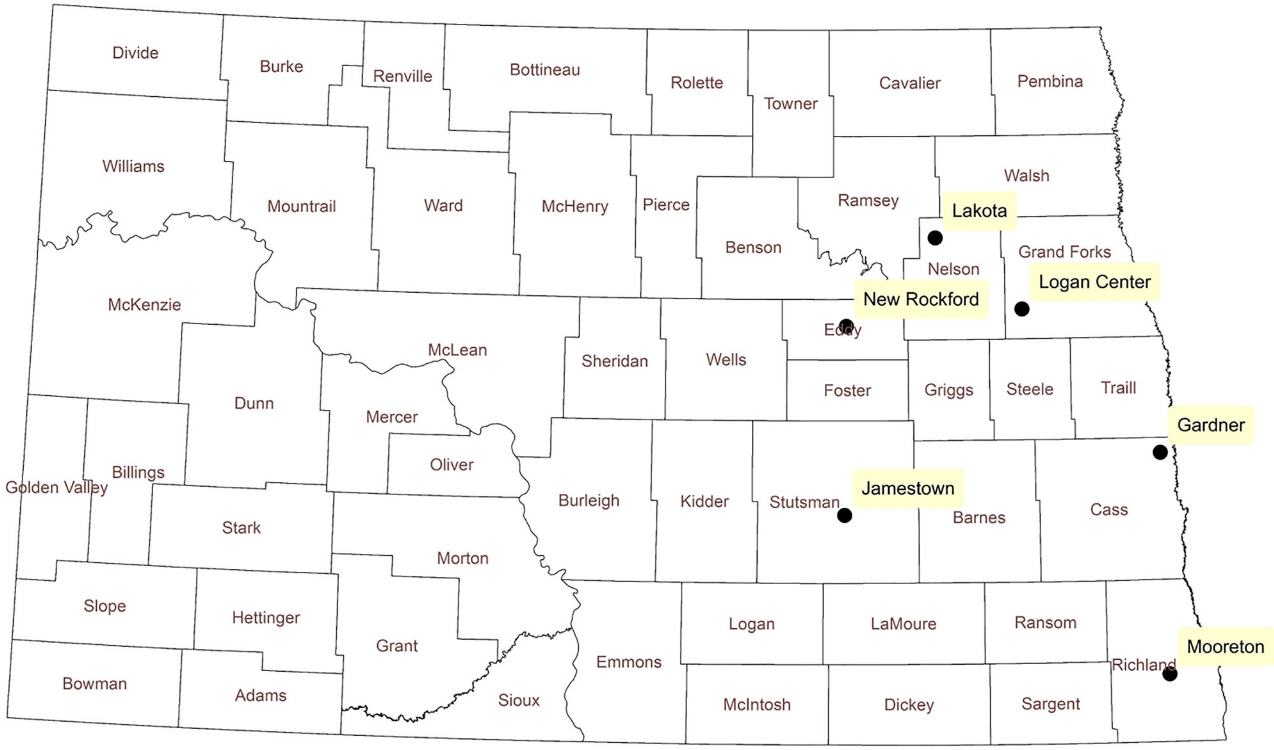


**FIGURE 1** Experimental sites subjected to soil sampling to determine asymbiotic N-fixing activity, 2019–2021.

**TABLE 1** Experimental site characteristics.

| Site | No-till status in year 1 | Soil texture | Soil series | Soil taxonomy |
|---|---|---|---|---|
| Mooreton | Third year no-till | Silty clay | Fargo | Fine, smectitic, frigid Typic Epiaquerts |
| Jamestown | First year no-till | Loam | Barnes | Fine-loamy, mixed, superactive, frigid Calcic Hapludolls |
| New Rockford | First year no-till | Sandy loam | Walum | Sandy, mixed, frigid Oxyaquic Hapludolls |
| Lakota | First year no-till | Sandy loam | Arvilla | Sandy, mixed, frigid Calcic Hapludolls |
| Logan Center | First year no-till | Loam | Barnes | Fine-loamy, mixed, superactive, frigid Calcic Hapludolls |
| Gardner | Third year no-till | Clay | Fargo | Fine, smectitic, frigid Typic Epiaquerts |

**TABLE 2** Crop grown each year at each site 2019–2021.

| | Crop | | |
|---|---|---|---|
| Site | 2019 | 2020 | 2021 |
| Mooreton | Corn[a] | Soybean | Spring wheat |
| Jamestown | Soybean | Flax | Spring wheat |
| New Rockford | Soybean | Sunflower | Spring wheat |
| Lakota | Barley | Dry bean | Corn |
| Logan Center | Dry bean | Corn | Soybean |
| Gardner | Corn | Soybean | Spring wheat |

[a]Corn (Zea mays L.); soybean (*Glycine max* L. Merrill); spring wheat (*Triticum aestivum* L.); flax (*Linum usitatissimum* L.); sunflower (*Helianthus annuus* L.); barley (*Hordeum vulgaris* L.); dry bean (*Phaseolus vulgaris* L.).

chosen because in many North Dakota soils, past wind/water erosion has reduced the "topsoil" to a shallow depth. Also, in a dry year, once deeper depths are dried due to evapotranspiration, any rainfall wets the surface and may not be great enough to wet deeper depths. Also, the closer the soil is to the surface, the greater the oxygen in the soil atmosphere to support N-fixation (Topp et al., 2000). A 10 cm diameter hard-plastic screw-top jar was pushed into the soil to a 5 cm depth, and the soil was kept in the jar when removed by inserting a 12-cm wide drywall joint knife under the inverted jar top and lifting the jar with the knife from the soil. Although initially the aim was to be able to ship intact soil so as not to disturb soil structure and natural organism living spaces, the samples always fell apart. So essentially the samples were bulk soils. The sample jars were transported during the sampling expeditions in a Styrofoam cooler with ice packs and placed in a refrigerator for up to 3 days at 3°C before shipment. The samples were shipped overnight with ice packs to the University of Florida Wetland Biogeochemical Laboratory, Gainesville, FL, where an incubation acetylene reduction procedure was

**TABLE 3** Sampling dates, 2019–2021, all sites sampled on the same dates.

| Years | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2019 | | | | | 2020 | | | | 2021 | | | | | |
| Sampling dates | | | | | | | | | | | | | | |
| 4/25 | 5/29 | 6/27 | 7/29 | 8/27 | 6/15 | 7/15 | 8/17 | 9/15 | 4/14 | 5/17 | 6/14 | 7/19 | 8/23 | 9/27 |

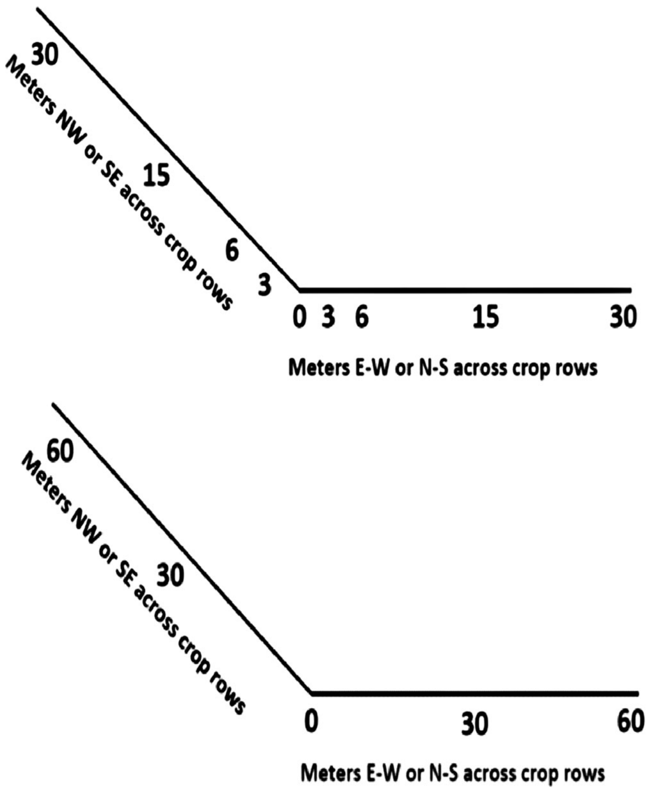


**FIGURE 2** Sampling for N-fixing organism activity over distance, upper figure, 2019 and 2020; lower figure 2021.

performed, as detailed in Franzen et al. (2019). At the second sampling, samples were obtained from the initial Global Positioning System (GPS) sampling location, and 3, 6, 15, and 30 m away from center in two directions from the GPS sampling location in 2019 and 2020, and from a 30 and 61 m distance in two directions in 2021 (Figure 2). The samples taken at distance from the center were subjected to the acetylene reduction procedure and the results were subjected to basic statistical analysis. To provide a proper semivariogram, it is necessary to have at least sampling locations (Kerry & Oliver, 2007). Since these experiments consisted of only nine distance locations in 2019–2020, and only five in 2021, a full geostatistical analysis was not possible.

In addition, at the start of each year of experiments, soil samples were also obtained within a meter of the primary sampling for soil pH, Phosphorus(P), and Potasium (K) at the 0–5 cm and 5–15 cm depth. These background soil fertility samples were obtained using a 2.5 cm soil probe to obtain 5 cores, which were mixed to produce the soil sample submitted to the laboratory. The results are shown in Table 4.

Models were developed using all site, all year data to characterize the relationships between temperature and asymbiotic N-fixing activity and rainfall before sampling and activity. For each site-year trial, the accumulated daily total rainfall of 30 days before the first sampling was extracted from North Dakota Agricultural Weather Network (NDAWN, 2022). For samplings other than the first sampling, the total rainfall of the days in between the current sampling date and the previous sampling date was obtained from NDAWN. Note that the period length between two sampling dates was also ∼30 days (Table 3). The same data extraction operation was applied to temperature, except that the mean temperature instead of summation of temperature was calculated for each site-year sampling. All of the 96 sampling records from 3-year and six-location trials were pooled together for preprocessing and modeling. Each record contains five fields: location, sampling date, total rainfall, mean temperature, and activity. All records were first sorted and grouped into six categories by the value of total rainfall, and then the mean total rainfall (specifically, mean 30-day total rainfall), mean temperature, and the mean activity for each category was calculated and listed in Table 5. To relate the temperature to the activity, several simple statistical regression models including simple linear regression, exponential regression, and secondorder polynomial regression were tested, and the exponential regression model is found to best fit the data in terms of $R^2$ value. However, for the relationship between activity and rainfall, the simple linear regression model presents the highest and close-to-one $R^2$ value. From both the very high $R^2$ values and visible reasonable trends shown in the graph, we found these simple statistical regression models were best to characterize the relationships of interest in our study; so, there was no need to explore more complicated models. All statistical data preprocessing, modeling, and graphing were conducted using Microsoft Excel Professional Plus 2019 with the Analysis ToolPak for Excel.

## 3 | RESULTS AND DISCUSSION

The experimental sites are transitional no-till, with no site more than 5 years continuous no-till in 2021; yet, some stratification of pH, P, and K are seen in years where the 0–5 cm

**TABLE 4** Soil test pH, P, and K with depth.

| Site | Depth (cm) | pH 2019 | pH 2020 | pH 2021 | P ($mg\ kg^{-1}$) 2019 | P 2020 | P 2021[a] | K ($mg\ kg^{-1}$) 2019 | K 2020 | K 2021 |
|---|---|---|---|---|---|---|---|---|---|---|
| Mooreton | 0–5 | 7.6 | 7.3 | 7.3 | 11 | 22 | | | 420 | |
| | 0–15 | 7.5 | 7.3 | 7.3 | 20 | 11 | 18 | 340 | 276 | 415 |
| Jamestown | 0–5 | 7.7 | 7.6 | 7.6 | 14 | 12 | | | 270 | |
| | 0–15 | 7.7 | 7.1 | 7.0 | 13 | 3 | 10 | 222 | 185 | 240 |
| New Rockford | 0–5 | 5.9 | 4.9 | 5.3 | 41 | 38 | | | 219 | |
| | 0–15 | 8.0 | 7.8 | 7.6 | 27 | 12 | 19 | 182 | 205 | 295 |
| Lakota | 0–5 | 7.9 | 7.4 | 7.4 | 10 | 11 | | | 304 | |
| | 0–15 | 8.0 | 7.8 | 7.6 | 8 | 6 | 10 | 263 | 205 | 225 |
| Logan Center | 0–5 | 7.7 | 6.7 | 6.8 | 14 | 23 | | | 372 | |
| | 0–15 | 7.8 | 7.7 | 7.4 | 11 | 20 | 11 | 259 | 251 | 310 |
| Gardner | 0–5 | 7.7 | 7.7 | 7.7 | 25 | 18 | | | 485 | |
| | 0–15 | 7.5 | 7.3 | 7.3 | 13 | 7 | 7 | 410 | 276 | 370 |

[a]2021 sampling for P and K 0–15 cm depth only, 2019 K 0–15 cm only.

**TABLE 5** Categories and mean values of total 30 days rainfall, air temperature within 30 days of sampling, and N-fixing activity used in building models for the relationships between temperature and activity, and rainfall and activity.

| Mean 30-days total rainfall (cm) | Mean total rainfall (cm) | Mean temperature (˚C) | Mean activity (nmol $C_2H_2$/gDW/h) |
|---|---|---|---|
| [0, 2.54) | 1.14 | 7.65 | 0.09 |
| [2.54, 5.08) | 3.96 | 16.57 | 0.15 |
| [5.08, 7.62) | 6.12 | 15.68 | 0.18 |
| [7.62, 10.16) | 8.66 | 18.51 | 0.27 |
| [10.16, 12.70) | 11.28 | 19.77 | 0.31 |
| [12.70, +∞) | 16.50 | 19.51 | 0.51 |

depth results were compared with the 0–15 cm depth. Stratification was evident even after 2 years into no-till. Stratification of soil P and K is generally not restrictive to crop productivity (Grove et al., 2007); however, acidification of soils with low buffering capacity (resistance to pH change) is an issue in many areas of long-term no-till production in North Dakota, Montana, and South Dakota (Buetow, 2022). The main source for this acidification is the nitrification of ammonium-based fertilizers used by the growers (urea or anhydrous ammonia) (Kissel et al., 1988). Conventionally tilled soils with low buffering capacity are also susceptible to acidification; however, the change happens slowly due to dilution of surface acidity development with tillage. The acidification develops at the depth of ammonium-N application with urea, anhydrous ammonia, and ammonium nitrate solutions. None of the pH values observed in this study are known to inhibit N-fixing bacteria activity.

Summary statistics for N-fixation rates for the 2019 distance sampling are provided in Table 6. The results of 2019,

**TABLE 6** Standard statistics from distance from GPS location 2019, all sites.

| Site | Mean | Standard error | CV% | Variance |
|---|---|---|---|---|
| Mooreton | 0.206 | 0.01879 | 27.4 | 0.00318 |
| Jamestown | 0.196 | 0.01100 | 16.8 | 0.00110 |
| New Rockford | 0.180 | 0.00986 | 16.4 | 0.00088 |
| Lakota | 0.190 | 0.04280 | 20.2 | 0.00148 |
| Logan Center | 0.194 | 0.00990 | 15.3 | 0.00089 |
| Gardner | 0.532 | 0.22800 | 128.0 | 0.46800 |

*Note*: Number of samples was 9 at all locations. Units of N-fixation rates are nanomoles acetylene $C_2H_2$ reduced per gram dry soil per day.
Abbreviation: CV, coefficient of variation.

**TABLE 7** Summary statistics from GPS location 2020, all sites.

| Site | Mean | Standard error | CV% | Variance |
|---|---|---|---|---|
| Mooreton | 0.079 | 0.00426 | 14.3 | 0.000127 |
| Jamestown | 0.056 | 0.00451 | 23.0 | 0.000163 |
| New Rockford | 0.076 | 0.00206 | 7.1 | 0.000030 |
| Lakota | 0.073 | 0.00603 | 24.7 | 0.000327 |
| Logan Center | 0.066 | 0.00391 | 17.8 | 0.000137 |
| Gardner | 0.087 | 0.00767 | 24.9 | 0.000470 |

*Note*: Number of samples was 9 at all locations. Units of N-fixation rates are nanomoles acetylene reduced per gram dry soil per day.
Abbreviaton: CV, coefficient of variation.

2020 (Table 7), and 2021 (Table 8) distance from center samplings indicate that when studying asymbiotic activity, multiple soil cores might be necessary to characterize an area or field, since small-scale to larger-scale variability, based on

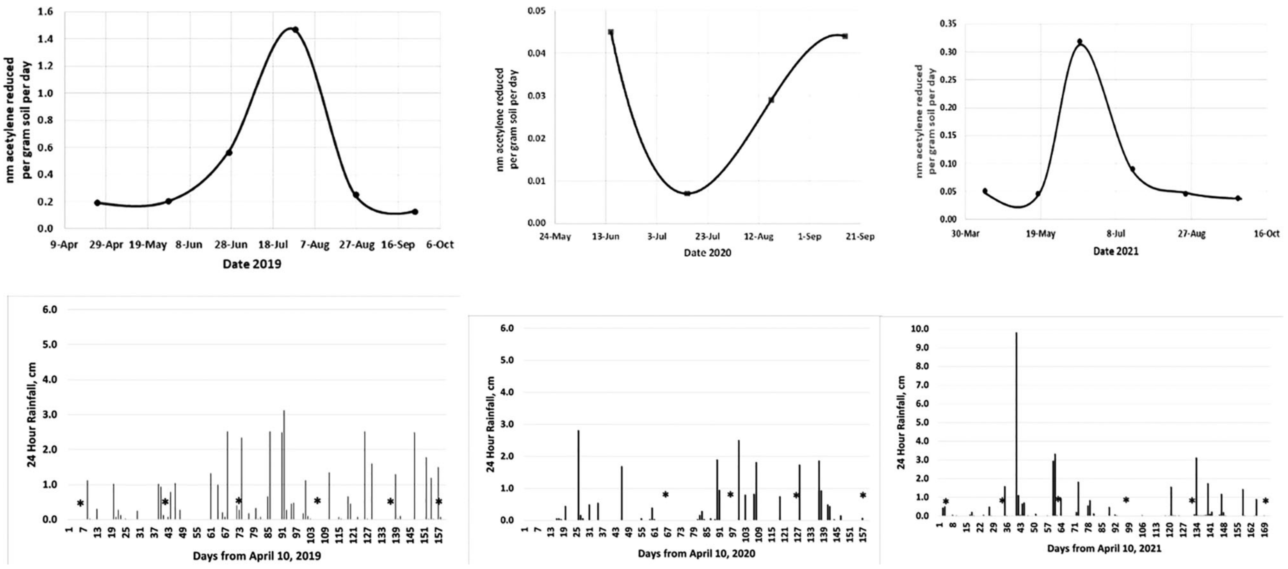


**FIGURE 3** Jamestown top row: Asymbiotic N-fixing activity in nmol $C_2H_2$ reduced $g^{-1}$ dry soil $day^{-1}$ 2019 (left), 2020 (middle), and 2021 (right) over season. Bottom row: Rainfall (cm) 2019 (left), 2020 (middle), and 2021 (right). *Sampling event.

**TABLE 8** Asymbiotic activity of distance from Global Positioning System (GPS) location, 2021, all sites.

| Site | Mean | Standard error | CV% | Variance |
|---|---|---|---|---|
| Mooreton | 0.073 | 0.01300 | 39.9 | 0.0008550 |
| Jamestown | 0.046 | 0.00116 | 2.5 | 0.0000013 |
| New Rockford | 0.088 | 0.01870 | 47.5 | 0.0017400 |
| Lakota | 0.085 | 0.00992 | 26.1 | 0.0004920 |
| Logan Center | 0.073 | 0.01310 | 39.8 | 0.0008550 |
| Gardner | 0.094 | 0.01170 | 27.8 | 0.0006870 |

*Note*: Sample number was 5 at all sites. Units are nanomoles acetylene reduced per gram dry soil per day.
Abbreviation: CV, coefficient of variation.

the coefficient of variation of the sites was large for most comparisons.

The distance from center sampling results, therefore, indicates that multiple cores should be obtained and analyzed separately, or mixed as a composite to provide more consistent values, unless the sample locations are GPS benchmarked and visited at the same locations at each sampling date, as the monthly and annually obtained samples were in these experiments.

At Jamestown, in all 3 years activity peaked during a moist but not excessively wet environment as indicated by rainfall patterns (Figure 3). In 2019, the peak activity in late July (Day 110) was three times the activity of late June (Day 78) during a period of frequent rainfall. In 2020, the peak was in mid-June (Day 66) during a moist period, with the least activity during a period of frequent rainfall (Day 97) resulting in saturated soil conditions. In 2021, the late May sampling (Day 37) before the site experienced a large rainfall event was a period of low activity, and a month later when the soil was still moist from the large rainfall event activity was greatest (Day 65).

At Logan Center (Figure 4) peak activity in 2019 was in a moist, but not wet environment in late July (Day 99). In 2020, peak activity was early (Day 66) and later season (Day 129), with the lowest activity during a period of high rainfall (Day 97). In 2021, peak activity was early in the season (Days 37 and 65), with much less activity during later season higher rainfall.

At Lakota (Figure 5) in 2019, peak activity was in late July (Day 110) during a period of moist soils from earlier rainfall that was not excessive. Activity decreased with frequent rains from early August through the rest of the season. In 2020, peak activity was in the first sampling (Day 66) in mid-June and was much less during periods of frequent and high rainfall in July (Days 97 and 129). In 2021, peak activity was in mid-June in a moist condition due to snow melt, with much less activity in late July (Day 99) when the site experienced high rainfall events.

At New Rockford (Figure 6), peak activity in 2019 was in late July (Day 110) during a period of moist, but not wet soils. In 2020, the peak activity was during a moist mid-June sampling (Day 66) with lower activity during periods of high rainfall in mid-July to late July. In 2021, peak activity was in late June (Day 65), with less activity during late July high rainfall.

At Mooreton (Figure 7), in all three seasons peak activity was in moist periods, and not in the mid of major rainfall events. In 2019, the major rainfall events were early and late in the season, resulting in peak activity in late July (Day 99). In 2020, major rainfall events were in the middle of the

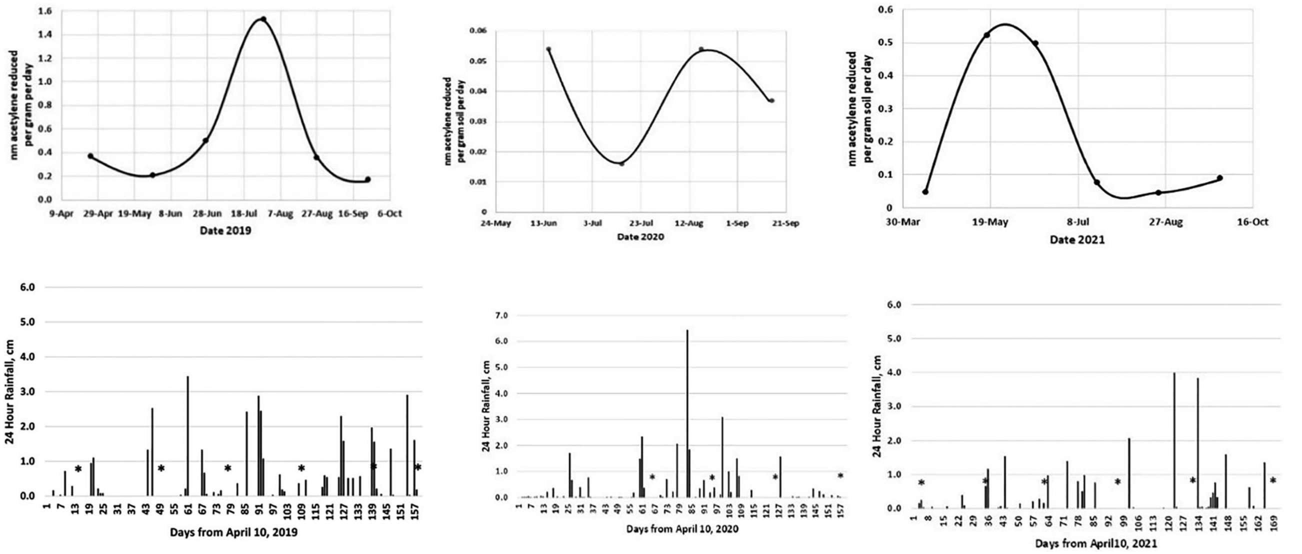


**FIGURE 4** Logan Center top row: Asymbiotic N-fixing activity in nmol $C_2H_2$ reduced $g^{-1}$ dry soil $day^{-1}$ 2019 (left), 2020 (middle), and 2021 (right) over season. Bottom row: Rainfall (cm) Kempton station, 2019 (left) because Logan Center weather station was not online until late July. Logan Center, 2020 (middle) and 2021 (right). *Sampling event.

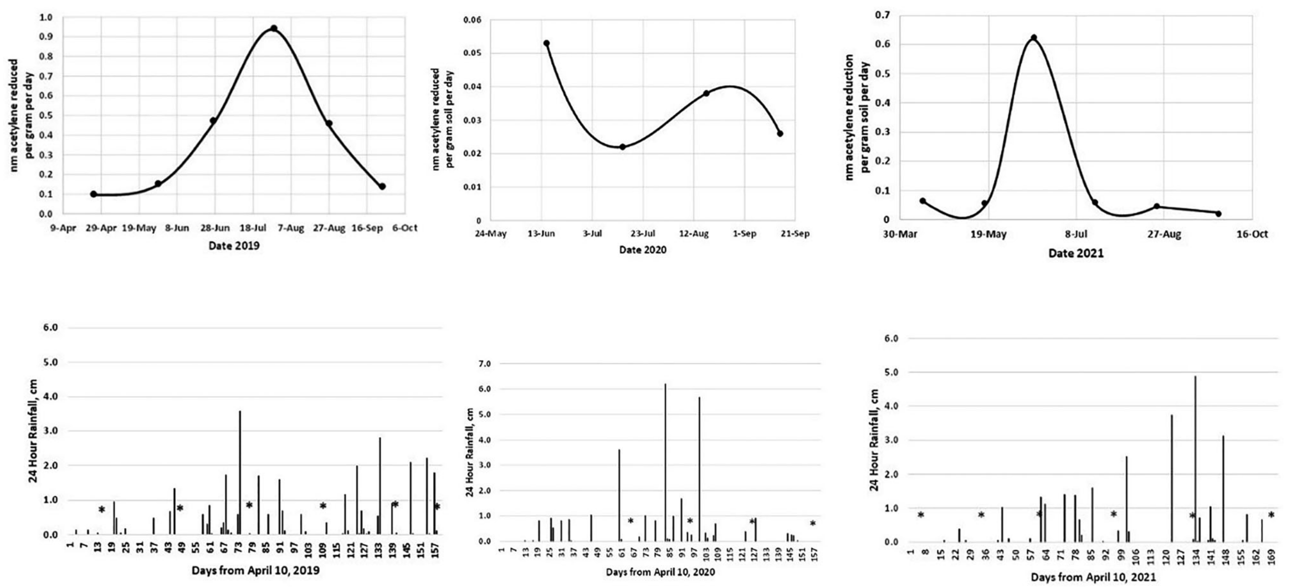


**FIGURE 5** Lakota top row: Asymbiotic N-fixing activity in nmol $C_2H_2$ reduced $g^{-1}$ dry soil $day^{-1}$ 2019 (left), 2020 (middle), and 2021 (right) over season. Bottom row: Rainfall (cm) near Lakota, 2019 (left), 2020 (middle), and 2021 (right). *Sampling event.

season, resulting in peak activity in mid-June (Day 66). In 2021, mid-season was moist and activity higher (Days 65 and 99) with major rainfall events late in the season and very cold soils inhibiting activity at the earliest sampling.

At Gardner (Figure 8), peak activity was in moist periods, but not in periods of highor frequent rainfall. In 2019, the unusual lack of activity at the May and June sampling (Days 49 and 78) compared to the other five locations was due to frequent and high rainfall mid-season, resulting in peak activity before and after these events. In 2020, again high rainfall events occurred mid-season (Day 97), so peak activity was again in mid-June (Day 66), August (Day 129), and September (Day 158). In 2021, the peak activity was after the soils warmed and the soil was still moist before drying during the drought (Day 65). The high rainfall in August (Day 134) did not result in greater activity, probably due to saturated surface soil conditions in its high clay soil.

The trend of asymbiotic N-fixing organism activity is different from that published regarding the activity of N-mineralization organisms. The greatest mineralization found by Contosta et al. (2011) was in early summer in Massachusetts after soils warmed. Temperature was the single

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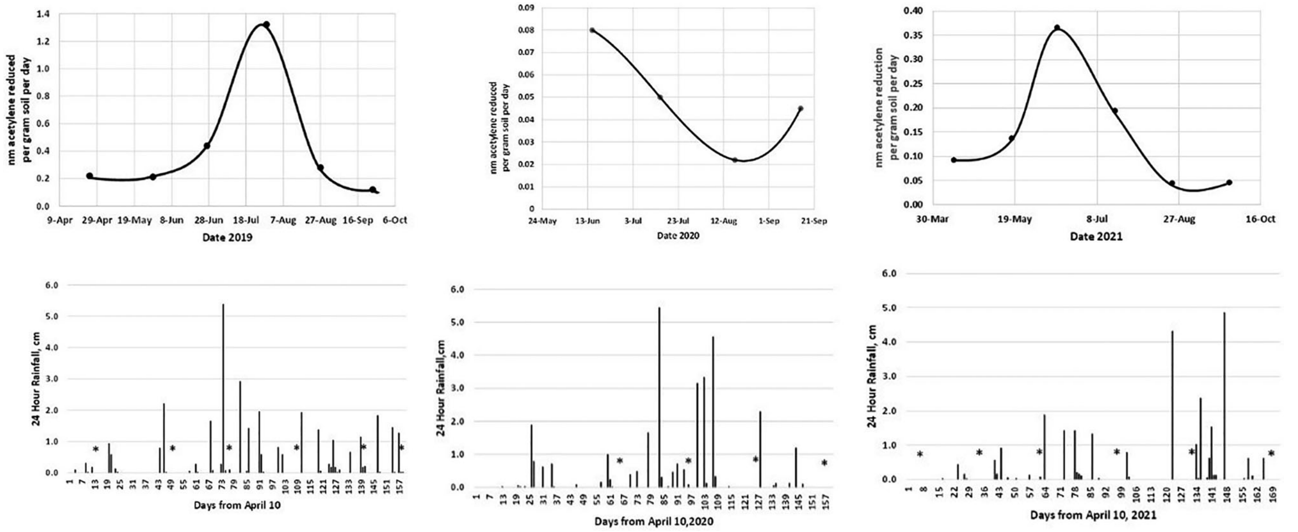


**FIGURE 6** New Rockford top row: Asymbiotic N-fixing activity, nmol $C_2H_2$ reduced $g^{-1}$ dry soil $day^{-1}$ 2019 (left), 2020 (middle), and 2021 (right) over season. Bottom row: Rainfall (cm) near New Rockford, 2019 (left), 2020 (middle), and 2021 (right). *Sampling event.

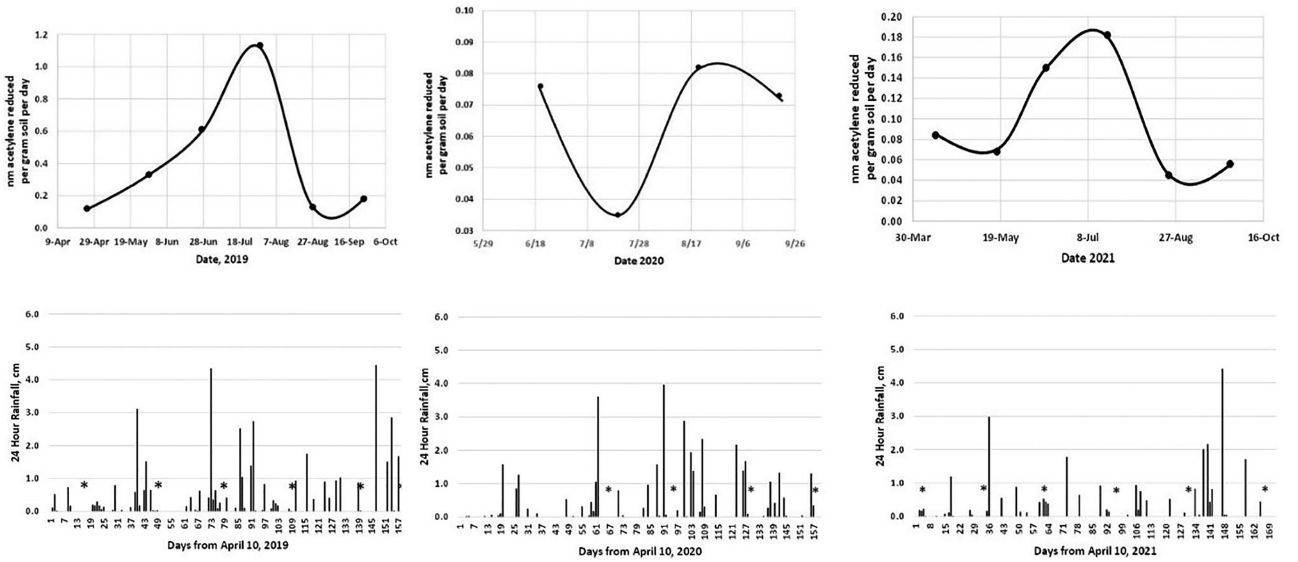


**FIGURE 7** Mooreton top row: Asymbiotic N-fixing activity in nmol $C_2H_2$ reduced $g^{-1}$ dry soil $day^{-1}$ 2019 (left), 2020 (middle), and 2021 (right) asymbiotic N-fixing activity over season. Bottom row: Rainfall (cm) 2019 (left), 2020 (middle), and 2021 (right). *Sampling event.

most important governing factor in the study. The magnitude of the mineralization was governed by soil moisture, but not the seasonal variation. The N-fixing activity in our study was usually least in early spring, then increased as soils warmed and the soil moisture as indicated by rainfall patterns resulted in not too wet and not too dry soil conditions. Nitrogen mineralization in Contosta et al.'s (2011) study was usually lowest in late summer into fall, with some sites experiencing a slight increase with rainfall in the fall that was not excessive. Nitrogen fixation in these North Dakota experiments tended to be elevated at some locations in September if field conditions are still warm and moist, as at Mooreton in 2020.

Models were developed for N-fixing activity and mean total rainfall 30 days before sampling and N-fixing activity with mean air temperature 30 days before sampling. The categories used to develop the model appear in Table 7. A statistically significant simple linear regression model, $y = 0.0269x + 0.0386$, where $y$ represents the mean activity and $x$ represents the mean total rainfall, best characterizes the relationship between mean activity and mean total rainfall, as illustrated in Figure 9, in that the model reaches a $p$-value of 0.0003 and a $R^2$ value of 0.97. Meanwhile, a strong exponential relationship between mean activity ($y$, dependent variable) and mean temperature ($x$, independent variable) was found and illustrated in

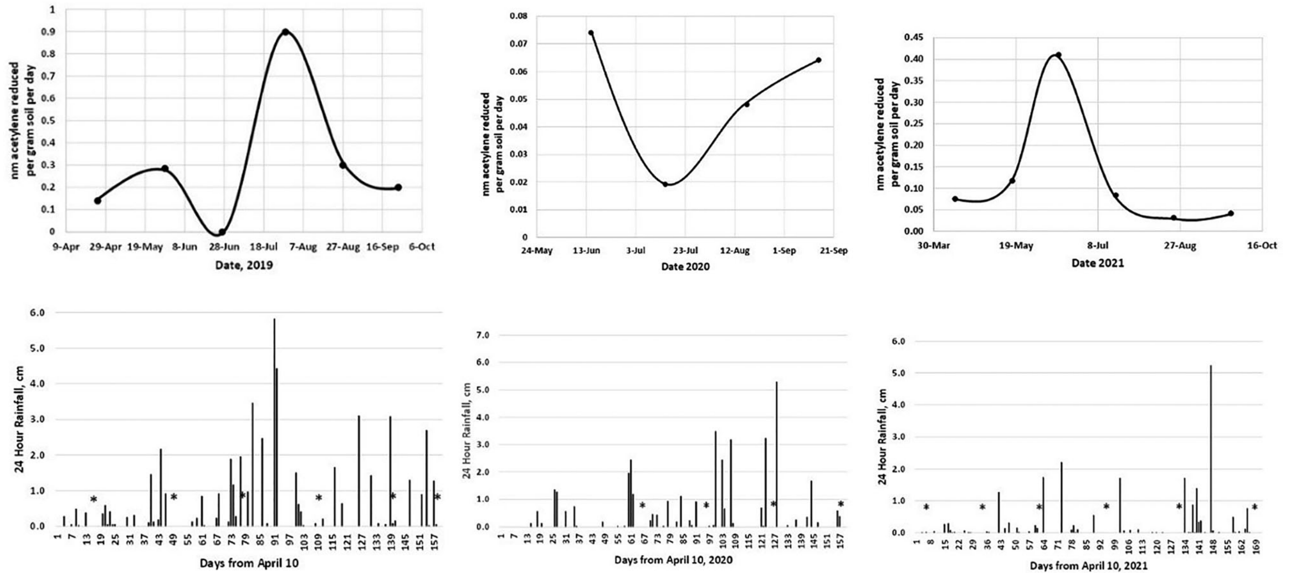


**FIGURE 8** Gardner top row: Asymbiotic N-fixing activity in nmol $C_2H_2$ reduced $g^{-1}$ dry soil $day^{-1}$ 2019 (left), 2020 (middle), and 2021 (right) over season. Bottom row: Rainfall near Gardner (cm) 2019 (left), 2020 (middle), and 2021 (right). *Sampling event.

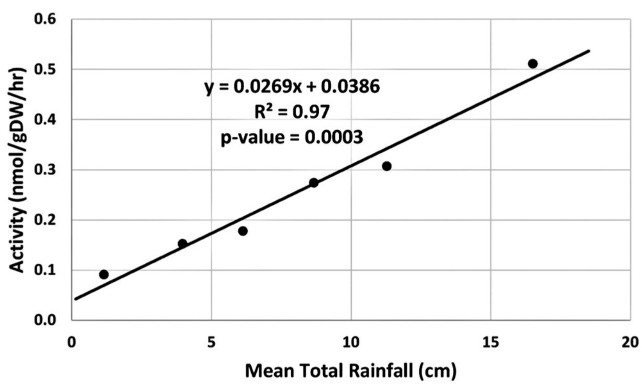


**FIGURE 9** Simple linear relationship between activity and mean total rainfall within 30 days preceding sampling.

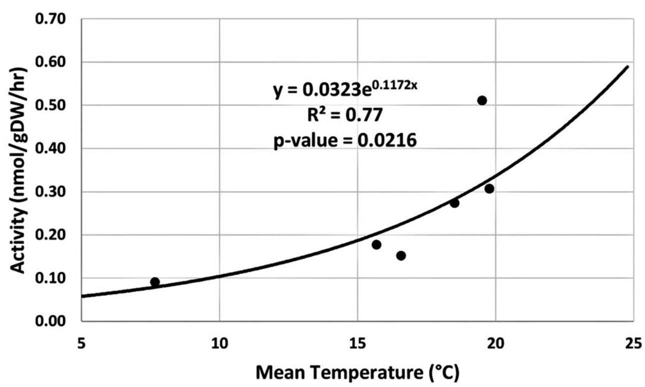


**FIGURE 10** Exponential relationship between N-fixing activity and mean air temperature, 30 days preceding sampling.

Figure 10. This exponential model, $y = 0.0323e^{0.1172x}$, is also highly statistically significant with a $p$-value of 0.0216 and a $R^2$ value of 0.77.

The overriding factor in sustaining greater asymbiotic organism activity was soil moisture. Over all sites, the least activity was experienced during periods of high rainfall (greater than 2.5 cm in a 24-h period) and especially when several high rainfall events were spaced only days apart. Dry soil conditions also inhibited activity, as evidenced at Jamestown, 2021.

One of the reasons why transitional no-till sites were chosen for this study was that a trend to greater N-fixing activity was expected over time. However, each season was unique due to soil moisture and temperature regime, and although previous work indicated that long-term no-till supports greater activity compared to conventional tillage (Franzen et al., 2019), the trend toward greater N-fixation is likely to be observed at a longer time-span than this 3-year sampling study was capable of showing. One might speculate that it would take 10 years to produce enough data to discern a trend because of year-to-year variation in rainfall patterns and spring warm-up conditions.

The total seasonal $N_2$ conversion estimate should be considered qualitative (Table 9). As Mills (2003) points out, activity of microorganisms is concentrated in organic films, particularly in soil aggregates. This is probably why long-term no-till soils have greater asymbiotic N-fixing activity compared to conventionally tilled soils (Franzen et al., 2019; Lamb et al., 1987), since the aggregates are not disturbed under no-till conditions. However, in this study it was not possible to take a soil sample without disturbing its structure and aggregation. Increasing the disturbance was the necessity of shipping the samples to the University of Florida laboratory from North Dakota. With the aggregates disturbed, the

**TABLE 9** Comparative N fixation between 2019, 2020, and 2021 seasons.

| Location | 2019 | | 2020 | | 2021 | |
|---|---|---|---|---|---|---|
| | Area under curve nmol $C_2H_4$ | $N_2$ equivalent conversion | Area under curve nmol $C_2H_4$ | $N_2$ equivalent conversion | Area under curve nmol $C_2H_4$ | $N_2$ equivalent conversion |
| Gardner | 28.8 | 35.8 | 4.1 | 5.1 | 20.5 | 25.5 |
| Jamestown | 77.7 | 96.4 | 2.4 | 3.0 | 16.0 | 19.9 |
| New Rockford | 72.4 | 89.8 | 4.1 | 5.1 | 24.0 | 29.9 |
| Mooreton | 68.4 | 84.8 | 5.9 | 7.3 | 15.7 | 19.5 |
| Logan Center | 83.2 | 103.2 | 3.5 | 4.3 | 35.2 | 43.8 |
| Lakota | 64.1 | 79.5 | 3.0 | 3.7 | 24.1 | 30.0 |

*Note*: In nmol $C_2H_2$ reduced $g^{-1}$ soil.

resulting N-fixation measurements were likely far less than they would have been in their natural state.

## 4 | CONCLUSIONS

Sampling at six sites was completed monthly at the same GPS location at each site from spring thaw until late September in 2019 and 2021, and from mid-June to late September in 2020 due to COVID-19 restrictions at the University of Florida which prevented earlier sampling. Sample analysis of distance from center asymbiotic N-fixing organism activity showed large variation in activity at small distances (<33 m apart) and at larger distances (>33 m distant) predominated the data indicating the need for multiple samples to characterize activity if repeated temporal sampling is intended for field assessment.

Asymbiotic N-fixing activity was low in late April in 2019 and 2021 (2020 not sampled due to COVID-19), indicating that the organisms require warm temperatures for activity. Soil moisture as inferred from rainfall patterns at the sites was very important. Saturated soil conditions resulted, especially at the high clay sites, in very low activity. Dry soil conditions also slowed activity. A moist, warm soil was optimal for N-fixing organism activity. The construction of mathematical models related to temperature and previous rainfall with soil N-fixing activity support these observations.

This work indicates how asymbiotic N-fixing organisms perform in variable environments over time; however, it may also serve to anticipate possible challenges to the effectiveness of commercial N-fixing inoculants for corn and other crops. If commercial N-fixing inoculants are as sensitive to environmental conditions as natural asymbiotic N-fixing organism activity, then it may not be prudent to consider the N-fixation bacteria amendment as being able to provide a specific quantity of fertilizer N replacement. Only future research will reveal if natural and commercial N-fixing organisms behave similarly or whether they have different environmental requirements that govern their activity.

## AUTHOR CONTRIBUTIONS

**David Franzen**: Conceptualization; funding acquisition; investigation; methodology; supervision; validation; writing—original draft; writing—review and editing. **Abbey Wick**: Conceptualization; resources. **Honggang Bu**: Formal analysis. **Caley Gasch**: Conceptualization; methodology. **Patrick Inglett**: Formal analysis; methodology; validation.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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## REFERENCES

Boyd, E. S., & Peters, J. W. (2013). New insights into the evolutionary history of biological nitrogen fixation. *Frontiers in Microbilogy*, *4*, 1–12. https://doi.org/10.3389/fmicb.2013.00201

Buetow, R. (2022, March 8–9). *Acid soil adaptation management in western North Dakota with hard red spring wheat* [Paper presentation]. 2022 Great Plains Soil Fertility Conference, virtual. https://greatplainssoilfertility.org/proceedings/?action=abstract&id=8654&title=Acid+Soil+Adaptation+Management+in+Western+North+Dakota+with+Hard+Red+Spring+Wheat&search=topics

Contosta, A. R., Frey, S. D., & Cooper, A. B. (2011). Seasonal dynamics of soil respiration and N mineralization in chronically warmed and fertilized soils. *Ecosphere*, *2*, 1–21. https://doi.org/10.1890/es10-00133.1

Franzen, D. W., Inglett, P., & Gasch, C. K. (2019). Asymbiotic nitrogen fixation is greater in soils under long-term no-till versus conventional tillage. *Soil Science Society of America Journal*, *83*, 1148–1152. https://doi.org/10.2136/sssaj2019.03.0062

Grove, J. H., Ward, R. C., & Weil, R. R. (2007). Nutrient stratification in no-till soils. *Leading Edge Journal of No-till Agriculture*, *6*(3), 374–381. https://www.notill.org/sites/default/files/le_6-3_web.pdf

Kerry, R., & Oliver, M. (2007). Comparing sampling needs for variograms of soil properties computed by the method of moments and residual maximum likelihood. *Geoderma*, *140*, 383–396. https://doi.org/10.1016/j.geoderma.2007.04.019

Kissel, D. E., Cabrera, M. L., & Ferguson, R. B. (1988). Reactions of ammonia and urea hydrolysis products. *Soil Science Society of*

America Journal, 52, 1793–1796. https://doi.org/10.2136/sssaj1988.03615995005200060050x

Kox, A. R. M., Aalto, S. l., Penttila, T., Ettwig, K. F., Jetten, M. S. M., & van Kessel, M. A. H. J. (2018). The influence of oxygen and methane on nitrogen fixation in subarctic *Sphagnum* mosses. *AMB Express*, *8*(76), 1–9. https://doi.org/10.1186/s13568-018-0607-2

Lamb, J. A., Doran, J. W., & Peterson, G. A. (1987). Nonsymbiotic dinitrogen fixation in no-till and conventional wheat-fallow systems. *Soil Science Society of America Journal*, *51*, 356–361. https://doi.org/10.2136/sssaj1987.03615995005100020018x

Mills, A. L. (2003). Keeping in touch: Microbial life on soil particle surfaces. In D. L. Sparks (Ed.), *Advances in agronomy* (Vol. 78, pp. 2–43). Academic Press.

NDAWN. (2022). *North Dakota agricultural weather network*. North Dakota State University. https://ndawn.ndsu.nodak.edu

Reed, S. C., Cleveland, C. C., & Townsend, A. R. (2011). Functional ecology of free-living nitrogen fixation: A contemporary perspective. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 489–51. https://doi.org/10.1146/annurev-ecolsys-102710-145034

Rice, W. A., Paul, E. A., & Wetter, L. R. (1967). The role of anaerobiosis in asymbiotic nitrogen fixation. *Canadian Journal of Microbiology*, *13*, 829–836. https://doi.org/10.1139/m67-109

Roper, M. M., & Gupta, V. V. S. R. (2016). Enhancing non-symbiotic $N_2$ fixation in agriculture. *The Open Agriculture Journal*, *10*, 7–27. https://doi.org/10.2174/1874331501610010007

Smercina, D. N., Evans, S. E., Friesen, M. L., & Tiemann, L. K. (2019). To fix or not to fix: Controls on free-living nitrogen fixation in the rhizosphere. *Applied and Environmental Microbiology*, *85*(6), 1–14. https://doi.org/10.1128/aem.02546-18

Topp, G. C., Dow, B., Edwards, M., Gregorich, E. G., Curnoe, W. E., & Cook, F. J. (2000). Oxygen measurements in the root zone facilitated by TDR. *Canadian Journal of Soil Science*, *80*, 33–41. https://doi.org/10.4141/s99-037

Upton, R. N., Selaff, A. C., Hopmocke, K. S., Xu, X., Polley, H. W., & Wilsey, B. J. (2020). Soil depth and grassland origin cooperatively shape microbial community co-occurrence and function. *Ecosphere*, *11*, 1–20. https://doi.org/10.1002/ecs2.2973

Weaver, R. W., & Danso, S. K. A. (1994). Dinitrogen fixation. In R. W. Weaver, J. S. Angle, & P. S. Bottomley (Eds.), *Methods of soil analysis part 2* (pp. 1019–1045). American Society of Agronomy.

Zechmeister-Boltenstern, S., & Kinzel, H. (1990). Non-symbiotic nitrogen fixation associated with temperate soils in relation to soil properties and vegetation. *Soil Biology & Biochemistry*, *22*, 1075–1084. https://doi.org/10.1016/0038-0717(90)90032-u

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