

# Summary of Regional Studies in Corn on Selected Commercial Asymbiotic N-fixing Organisms and Suggestions for Companies Developing Similar Products

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Evidence for asymbiotic N-fixing organisms finds that these organisms were active 1.5 billion years ago- some of the oldest organisms found in the fossil record.

(Boyd & Peters, 2013, *Frontiers in Microbiology*)

Compared with about 59 million years ago for symbiotic N-fixers (Sprent and James 2007, *Plant Physiology*)

*Point #1- Asymbiotic N-fixing organisms are a natural part of soils*

## Asymbiotic N-fixing organisms

Organisms, usually a species of bacteria, that have the ability to fix atmospheric N ( $N_2$ ), transforming it into  $NH_3$ , which is immediately attached to a 'carbon-skeleton', safeguarding it.

The fixation requires energy, which when conducted in soil comes from organic matter.

N-fixation is an energy-expensive process.

The enzyme that serves as 'fixation facilitator' in bacteria is *nitrogenase*.

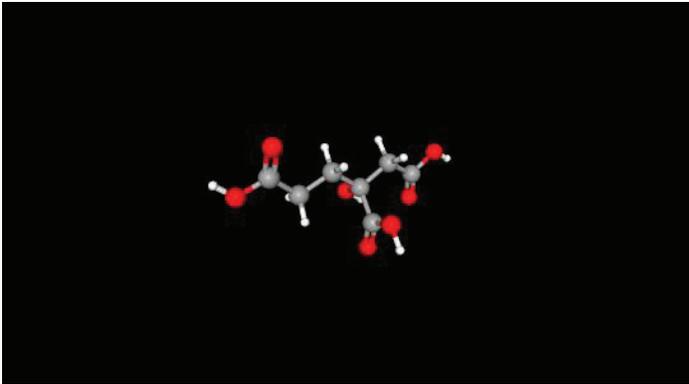
To convert 1  $N_2$  to 1  $NH_3$  requires 16 ATP molecules (produced during photosynthesis) and 8 electrons.

Energy limits N fixation.

(Smercina et al., 2019, *Applied Environmental Microbiology*)

*For comparison, production of 1 peptide bond in protein synthesis requires only 5 ATP (still considered 'high energy requirement')*

## Nitrogenase enzyme

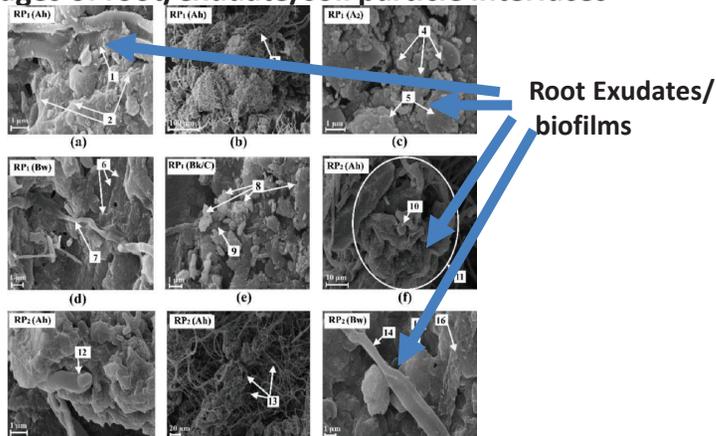


## Substrates to meet energy requirement

Energy sources for asymbiotic N-fixing organisms close to plant roots are the root exudates that surround many roots, and also includes components of soil organic matter and residue decay intermediary compounds in the bulk soil.

N-fixing organisms that exist inside the plant tissue have to receive substantial energy from the plant within which they reside.

## Images of root/exudate/soil particle interfaces



RP 1 images (SEM) of fine root (1), root exudates/biofilms (2), fine roots (3), clay mineral grains (4), clay mineral aggregates (5), root exudates/biofilms (6), organic filament (7), fine clay aggregates (8), fine root (9), and RP 2 images (SEM) of aggregates with abundant root remnants (10), pollen (11), root tap protruding through clay aggregate coated by exudates (12), dominant network of fine roots (13), and organic filament, primary mineral, clay minerals coating organic filament (14, 15, 16).  
From Razzaghi et al., 2017.

## Diversity of Asymbiotic bacteria types-

A Chinese/Tibetan paper, using genetic screening, found Asym-bacteria from 6 Phyla, 13 Classes and 43 genera (Li et al, 2021, Front. Ecol. Evol. 13 August. Vol 9)

Genera include species from

*Azotobacter*, *Ochrubacterum*, *Sphingomonas*, *Opitutus*, *Clostridium*, *Pseudomonas* and 'a host of others'.

There are many species, some more efficient than others, Their activity is linked to substrate and soil condition.

## Asymbiotic N-fixing bacteria are in most soils.

Their activity increases when tillage decreases. (*Food & Housing*)

### Lamb, Doran and Peterson, 1987

Nonsymbiotic dinitrogen fixation in no-till and conventional tillage SSSAJ 51:356-361

Recorded greater activity with no-till, but concluded that it was not great enough to contribute to any N credit. They considered the values from incubation to be values that might be experienced in the field. But the disturbance of soil probably killed billions of N-fixing critters, so values are index.

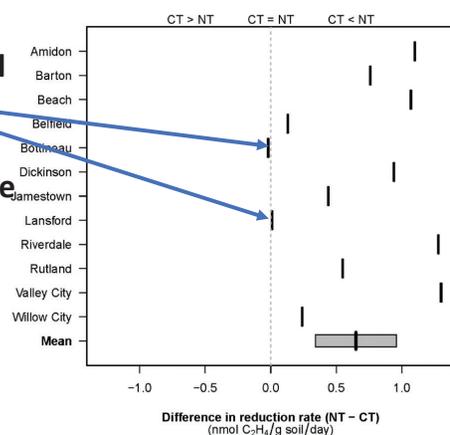
In North Dakota, there is an N credit for 6 or more years of continuous no-till, one-pass shallow tillage, shank strip-till, amounting to 40-50 pounds N per acre.

Part of this credit probably comes from the increased microbial biomass under no-till that protects N from loss. But a part of the credit, perhaps 25-33% may come from greater asymbiotic activity in long-term no-till.

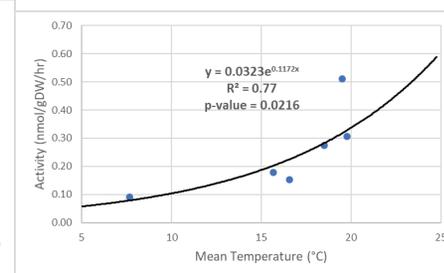
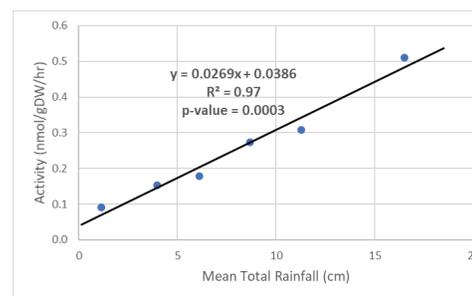
Franzen et al. 2019, SSSAJ

Took paired no-till/conventional till across ND.

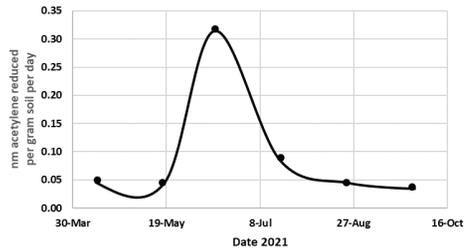
Turns out the 'conventional till' site across the fence was one-pass shallow tillage, so the same tillage category



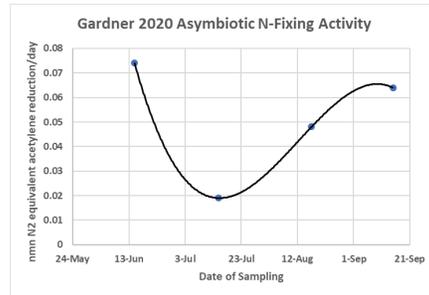
From 2019 thru 2021, 6 sites in eastern North Dakota were sampled each month for asymbiotic N fixing activity. Change in activity was related to rainfall within 30 days before sampling and mean air temperature. Franzen et al., SSSAJ, 2023



The relationship of N-fixing activity to soil condition made a large impact on the trend of activity over a season



Jamestown, 2021. Period from late May to early July moist, then very dry.



Gardner, 2020, Period for July sampling, soil was saturated in a high clay soil.

**Summary of what we know about native activity-**

**Greater in long-term no-till.** Their activity increases when their 'homes' are not destroyed and there is sufficient food to support their N-fixation.

**Moist soil and warm conditions favor N-fixation.** Dry soil conditions, saturated soil conditions, and cold soil temperatures inhibit their activity.

**Regional studies on commercial asymbiotic N-fixation products**

**Products tested-**

**Envita, Azotic North America** *Gluconacetobacter diazotrophicus*

**Utrisha, Corteva Agriscience** *Methylobacterium symbioticum*

**ProveN, PivotBio** *Klebsiella variicola*

**ProveN 40, PivotBio** *Kosakonia sacchari & Klebsiella variicola*

**Summary of results from 10 states.**

No means no difference between same N rate with or without additive  
Yes means a yield increase present at least 1 N rate

State	Envita IF†	Utrisha	ProveN	ProveN 40 IF	ProveN 40 ST
Number of site years included in evaluations					
ND	4 No	4 No	-----	-----	-----
MN	1 No	-----	3 No/1 Yes	-----	-----
IL	2 No	-----	4 No	5 No	2 No
IN	1 No	-----	-----	-----	-----
MO	2 No / 1 Yes	3 No	2 No	1 No	-----
KS	-----	-----	1 No	-----	-----
MI	1 No	1 No	-----	1 No	-----
KY	-----	2 No	-----	-----	-----
NE	-----	-----	5 No	6 No	-----
OH	-----	1 No	-----	-----	-----
<b>Total</b>	<b>11 No/1 Yes</b>	<b>11 No</b>	<b>15 No/1 Yes</b>	<b>13 No</b>	<b>2 No</b>

Total corn experiments 54.  
 52 no benefit to yield over N rate alone.  
 2 benefits with N rate benefits 12-20 lbs N/a

*Growers should be skeptical about new products*

**Point # 2-**

Try them on replicated strips on the farm.

Refer to L. Thompson, 2022 from *Proceedings of the North Central Extension-Industry Soil Fertility Conference* for ideas regarding on-farm testing and data analysis.

**PROMOTING ADOPTION OF PRECISION NITROGEN MANAGEMENT TECHNOLOGIES THROUGH ON-FARM RESEARCH**  
 L.J. Thompson, L.A. Puntel, T. Mieno, J. Iqbal, B. Maharjan, J. Luck, S. Norquest, J. G. C. P. Pinto, C. Uwineza  
 University of Nebraska – Lincoln, Lincoln, NE  
[laura.lthompson@unl.edu](mailto:laura.lthompson@unl.edu) 402-245-2224

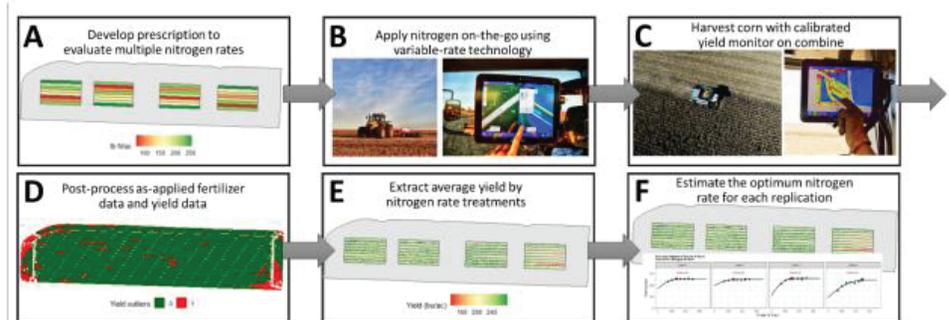
**ABSTRACT**

The Nebraska On-Farm Research Network helps farmers evaluate products and practices that impact the productivity, profitability, and sustainability of their operations. There are many technologies that have potential to increase nitrogen use efficiency (NUE) on corn and winter wheat but typically these technologies have low adoption. Concurrently, farmers have technologies such as GPS, yield monitors, and variable-rate application equipment on their farms that enables them to easily conduct on-farm research to evaluate new technologies and products. Participating farmers evaluated commercially available nitrogen (N) management technologies across Nebraska and their impact on yield, profit, and NUE. We enabled farmer's hands-on experience with technologies that are relevant for their operation and promoted technology adoption. We also collected field data to validate and improve the technology tested. 40 trials are established each year in the three-year project. We utilized an innovative experimental design combining traditional strip trials with small N plots where all treatments are established with variable-rate fertilizer equipment on-the-go. An automated data processing tool was developed for data processing, analysis, and reporting. 98% of the experiments were successfully established in the first year of the study and 90% were analyzed using the automatic process. To measure impact, grower incremental changes in N management strategy and technology adoption were documented.

**INTRODUCTION**

Nitrogen (N) is critical for attaining higher crop yields; however, risks of environmental losses necessitate more precise fertilizer management. Predicting the economic optimum N rate (EONR) remains challenging due to spatial and temporal

**Example from Thompson paper:**



<https://cropwatch.unl.edu/farmstat>

**N** Institute of Agriculture and Natural Resources  
**CROPWATCH**

Home Weather (GDD & ET) Info & Resources Crops Management Related Topics Archives

Nebraska | I&M | Nebraska Extension | CropWatch | On-Farm Research | FarmStat

## FarmStat

FarmStat is a statistical analysis tool that provides quick, accurate, and straightforward analysis for data from agricultural experiments. While FarmStat is provided at no cost, we do require you to register with your name and email address.

First Name Required  
David

Last Name Required  
Franzen

E-Mail Required  
david.franzen@ndsu.edu

**Farmers have GPS**

**Farmers have GPS yield monitors**

**Farmers have everything they need to replicate treatments and test product effectiveness.**

## FarmStat Beta Welcome

Thank You

The screenshot shows a video player titled "FarmStat Complete Guide". The video content displays an Anova table with the following data:

Source	D.F.	F-Value	P-Value
Treatments	4		<0.001
Blocks	3		0.983
Error	12		
Total	19		

Below the table, there are statistics: F-Critical: 2.48, Correction Factor: 1.94729370494+6, Standard Deviation: 18.065, Coefficient of Variation: 0.079. A Mean value of 284.91 is also shown.

[Download FarmStat User Guide](#)

[Download CRD Excel Template](#)

[Download RCBD Excel Template](#)

**Point 3-**

**There should be a quick method of analysis developed to determine whether the organism is alive and functioning in the container, the field or the plant.**

***Point 4-***

**Organisms need to be kept alive through transportation and storage intervals between manufacturer, shipper, warehouses, distributor, dealer and on the farm awaiting application.**

**Storage and Disposal**

**Storage Conditions:** Keep the product in a well-ventilated place. Store at room temperature, do not subject to temperatures below 39 °F (4 °C). Keep the product hermetically closed.

**STORAGE AND HANDLING:** DO NOT FREEZE. This product contains live non-pathogenic organisms. Store between 39°F to 46°F. Store in well ventilated buildings, away from foodstuffs and animal feed. Keep out of reach of children. Keep out of direct sunlight. DO NOT open product container until ready for use.

***Point 6-***

**Organisms should be adapted to variable moisture, variable soil pH and variable soil salts in order to perform its function.**

***Point 5-***

**Organisms should be able to compete and ‘win the war’ with native microorganisms in order to survive and perform its function.**

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