Published August 1, 2019

Soil Fertility & Plant Nutrition Note

Asymbiotic Nitrogen Fixation is Greater in Soils under Long-Term No-Till Versus Conventional Tillage

David W. Franzen*
North Dakota State Univ.
School of Natural Resource Sciences
Dep. of Soil Science
Fargo, ND 58108

Patrick Inglett
Univ. of Florida
Soil and Water Sciences Dep.
Gainesville, FL 32611

Caley K. Gasch
North Dakota State Univ.
School of Natural Resource Sciences
Dep. of Soil Science
Fargo, ND 58108

A series of N-rate experiments previously conducted in spring wheat, corn, and sunflower in North Dakota indicated that less N was required when fields were in six years or more continuous no-till compared to conventional till. The objective of this study was to determine whether part of the reason for the decreased requirement for N was the greater activity of asymbiotic N-fixing organisms. Twelve paired-samplings were conducted in 2018. A surface 0- to 5-cm deep sample was obtained in a long-term no-till field directly across the fence or road from a similar soil in conventional till. Samples were incubated in an acetylene-reduction procedure to estimate N fixation rate. Ten of twelve paired samplings had greater asymbiotic N fixation compared to the conventional till counterpart. This indicates that long-term no-till soils support greater N production from soil microorganisms than conventional till soils, which would result in lower input costs to no-till farmers.

Microbial biomass is usually greater in no-till soils than soils in conventional-till systems (Feng et al., 2003; Helgason et al., 2009, 2010). Analysis of over 100 nitrogen (N)-rate trials in spring wheat and durum wheat, over 120 N-rate trials in corn and over 30 N-rate trials in sunflower conducted in North Dakota shows that long-term no-till sites (greater than 6 yr continuous no-till management) require less N to achieve maximum economic yield than conventionally tilled sites (Franzen, 2016, 2017, 2018).

It is possible that one explanation for the reduced N required in no-till sites is generally greater N use efficiency and protection from leaching/denitrification due to microbial N uptake. Another possible reason for reduced N requirement in long-term no-till is greater N fixation from asymbiotic N-fixing organisms. These are free-living organisms and do not have a symbiotic relationship with plant roots as do Rhizobium, Bradyrhizobium, or other symbiotic bacteria important to legumes. According to Roper and Gupta (2016), most soil nitrogenase enzyme is found in species within the Bacteria and Archaea domains. A limitation to the activity of asymbiotic N-fixing organisms is the food source. Asymbiotic N-fixing bacteria are present in nearly all cultivated soils (Wilson, 1958).

Long-term no-till fields generally have a greater supply of C and more diverse C sources compared to a conventional-tilled soil (Awale et al., 2013; Smith et al., 2016). Also, the greater stable aggregation in a no-till soil would provide a habitat for N-fixers and protect them from biocides that they might otherwise encounter (Gupta and Roper, 2010).

Microbial communities found in stable aggregates under no-till have greater soil organic matter cycling-process rates than similar microbes in conventional systems where aggregates are disturbed or destroyed. No-till management systems are associated with greater number of N-fixing genes compared to soil from conventional-till fields (Smith et al., 2016).

Core Ideas

- **Microbial communities are different between conventional till and no-till managed soils.**
- **Asymbiotic N-fixation was greater in farmer-managed no-till than neighboring conventional till.**
- **N recommendations in long-term continuous no-till should be separate from conventional till N recommendations.**

Soil Sci. Soc. Am. J. 83:1148–1152
doi:10.2136/sssaj2019.03.0062
Received 7 Mar. 2019.
Accepted 4 May 2019.
*Corresponding author (david.franzen@ndsu.edu).

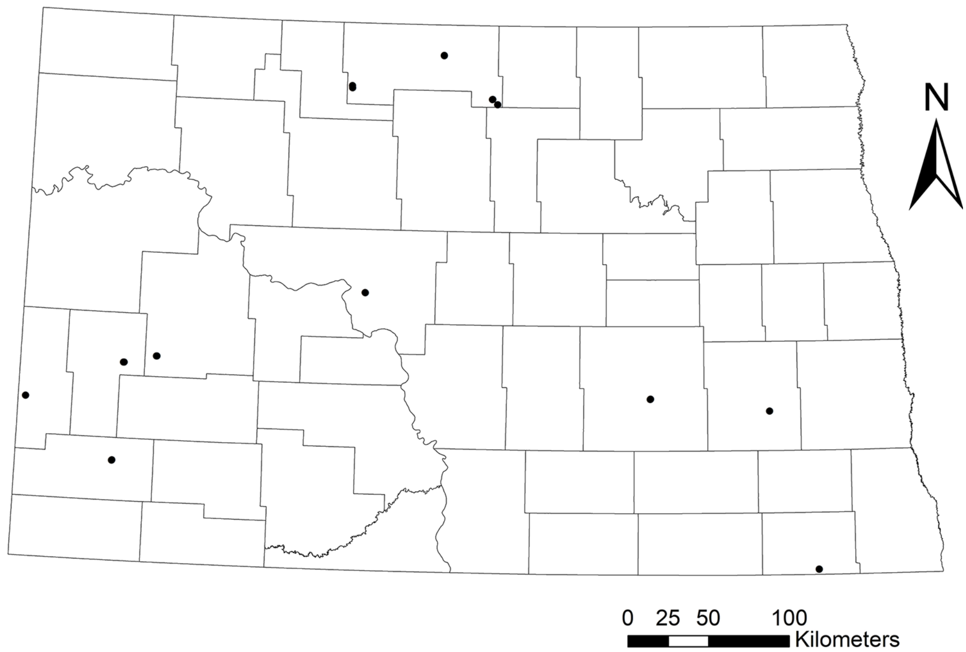

Fig. 1. Locations of twelve paired-sample sites.

There is no literature specifically comparing asymbiotic N-fixing in soils due to tillage differences. The objective of this study is to determine whether asymbiotic N-fixing is different in long-term no-till fields compared to adjacent fields in conventional tillage. A preprint of this work is available online (Franzen et al., 2018).

MATERIALS AND METHODS

Twelve paired sites were identified and sampled in North Dakota between April 26 and May 1 in 2018. Each soil in a pair was within the same soil series, mapped by NRCS (Natural Resource Conservation Service) as its paired comparison. The soil series was confirmed through a field visit. The NRCS Web Soil Survey was used as a guide to which area of the paired fields would represent a similar soil at each paired site (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx). One soil of each pair was located in a no-till field with at least 6 yr continuous no-till. No-till for the purposes of these comparisons included a 'purist' no-till, with only the seed disc cutting through the residue to the soil; strip-till, where a residue managers remove the residue from about 20% of the soil surface and a thin shank penetrates to the soil to about 20 cm in depth, but leaves the rest of the soil undisturbed; and one-pass seeding, which is a shallow tillage using field cultivator shovels no deeper than 5 cm at the time of seeding. The no-till location was directly across the fence, or road, no more than 50 m away from the conventional tillage location. The conventional till location was in a field in which tillage had been performed continuously to a depth greater than 15 cm at least once each year. The conventional till soils were subjected to a tillage pass in the fall greater than 15 cm in depth, and at least one spring tillage pass using an off-set disc or field cultivator at a 10-cm depth.

Locations of the sites are provided in Fig. 1. The locations were widely distributed within North Dakota. Samples were obtained during spring thaw before any spring fieldwork was performed. The frost depth at the four northernmost sites was about 15 cm below the soil surface, and frost depth at the remaining sites was about 30 cm below the surface. One sample was obtained from each site. For each sample, the residue was brushed from the soil surface at a location about 40 m inside the field boundary to avoid end-rows. A clean knife was used to scribe a 10-cm diameter cylinder of soil 5 cm deep. The cylinder was lifted from the soil using a stainless steel spatula and wrapped in aluminum foil to keep the soil intact as much as possible. The wrapped cylinders were then placed in 11.4-cm diameter, 8.3-cm deep Ziploc screw-lid containers and placed in a cooler. The knife and spatula were cleaned of soil between sites. Samples were stored in a refrigerator at −14°C until ready to ship for analysis. Previous crop and soil series for each site are listed in Table 1.

Samples were shipped to and analyzed by the Wetland Biogeochemistry Laboratory, University of Florida, Gainesville, FL. Despite careful sampling, wrapping and shipping procedures, the samples did not arrive in a state that would allow an intact incubation. The soils were too dry at the time of sampling for them to remain as an undisturbed unit. The soils were therefore incubated separately as a bulk sample. Estimated N fixed per day utilized a 3:1 acetylene to N reduction ratio suitable for arable soils (Seitzinger and Garber, 1987).

Nitrogen fixation potential of soil was assessed with the acetylene reduction assay using the procedures of Inglett (2013) and Matson et al. (2015). Briefly, soil samples were stored at 4°C until analysis (no more than 3 d). Soil samples were homogenized by hand mixing and added to 125 mL glass jars with metal, sealing lids which were ported to receive a luer connection, stopcock and syringe needle port. Two samples of field moist soil

Table 1. Selected site characteristics.

Sample location	Tillage	Previous crop	Soil series
Amidon	Conventional	Spring wheat†	Rhodes sil‡, fine, smectitic, frigid, leptic Vertic Natrustolls
	No-till	Spring wheat	
Barton	Conventional	Corn	Embden fsl, coarse loamy, mixed, superactive, frigid, Typic Argiustolls
	No-till	Corn	
Beach	Conventional	Spring wheat	Chama sil, fine-silty, mixed, superactive, frigid, Typic Calciustolls
	No-till	Corn	
Belfield	Conventional	Spring wheat	Chama sil, fine-silty, mixed, superactive, frigid, Typic Calciustolls
	No-till	Winter wheat	
Bottineau	Conventional	Spring wheat	Overly sicl, fine-silty, mixed, superactive, frigid Pachic Hapludolls
	No-till	Spring wheat	
Dickinson	Conventional	Summer fallow	Morton sil, fine-silty, mixed, superactive, frigid Typic Argiustolls
	No-till	Spring wheat	
Jamestown	Conventional	Field pea/pea cover crop	Barnes l, fine-loamy, mixed, superactive, frigid Calcic Hapludolls
	No-till	Soybean	
Lansford	Conventional	Spring wheat	Hamlet l, fine-loamy, mixed, superactive, frigid Typic Calciaquolls
	No-till	Spring wheat	
Riverton	Conventional	Soybean	Williams l, fine-loamy, mixed, superactive, frigid, Typic Argiustolls
	No-till	Corn	
Rutland	Conventional	Soybean	Colvin sicl, fine-silty, mixed, superactive, frigid Pachic Hapludolls
	No-till	Corn	
Valley City	Conventional	Spring wheat	Hamerly l, fine-loamy, mixed, superactive, frigid Aeric Calciaquolls
	No-till	Soybean	
Willow City	Conventional	Corn	Hecla lfs, sandy, mixed, frigid Oxyaquic Hapludolls
	No-till	Corn	

† Spring wheat/winter wheat (*Triticum aestivum* L.); Field pea (*Pisum sativum* L.); Soybean (*Glycine max* L. Merr.); Corn (*Zea mays* L.).
‡ l, loam; sil, silt loam; sicl, silty clay loam; lfs, loamy fine sand; fsl, fine sandy loam.

were prepared for each site with an additional sample taken for separate dry weight determination at 105°C.

To one set of prepared jars, acetylene (generated by adding water to CaC_2) was added to each jar to enrich the headspace to approximately 10% by volume. Gas samples were taken at 4, 12, and 48 h of incubation for determination of ethylene production from both acetylated and control (non-acetylated) soils. Ethylene values of acetylated jars were then corrected for ethylene in both the added acetylene (gas blanks) and that produced by the soil in the unacetylated controls. Gas samples were analyzed for ethylene using a Shimadzu GC-8A gas chromatograph equipped with a flame ionization detector (110°C) and a Poropak-N column (80°C). Two standard gases (1 ppm and 10 ppm; Scott Specialty Gases, Inc., Plumsteadville, PA) were used to calibrate the measurement, which was expressed per gram of dry weight soil per day (nmol C_2H_4 g DW soil^{-1} d^{-1}). The data were subjected to statistical analysis in SAS 9.2 (SAS Institute, Cary, NC) as a paired *t* test, using a 95% confidence interval.

An estimate of N reduced using the acetylene reduction values was made. This estimate is based on several assumptions: the incubation procedure is similar to in situ processes and N fixation only occurs in the surface 5 cm of soil. One estimate of the N contribution from conventionally tilled soil to a wheat crop is about 10 kg N ha^{-1} per season (Kennedy and Islam, 2001).

RESULTS

Of the twelve paired sites, there were statistical differences ($P < 0.05$) at ten sites (Table 2; Fig. 2), where there was greater acetylene reduction in the no-till sample compared to the conventional-till sample. At three sites, there was no detectable acetylene reduction activity in the conventional-till site. The Lansford and Bottineau sites were not different in acetylene reduction compared to the conventional-till site. It is possible that what the no-till farmers considered to be 'conventional tillage' at these sites was actually a one-pass seeding. The higher rates of acetylene reduction in the conventional-till soils at these sites compared to reduction rates at other conventional till sites over the study suggest that the true tillage management was mischaracterized. Further conversation with the no-till farmer at Bottineau confirmed that he had confused conventional tillage (tillage deeper than 5 cm depth) with the shallow tillage one-pass seeding used by the neighbor in our paired sampling.

The previous crop is noted in Table 1 for the record, but it is unlikely that a previous crop had any influence on acetylene reduction results. For example, three sites had spring wheat as previous crop before no-till and conventional-till soils (Amidon, Lansford and Bottineau). Although the acetylene reduction rates for conventional and no-till soils at Lansford and Bottineau soils were not different, the acetylene reduction rates for soils at Amidon were greatly different.

An estimate of g N fixed ha^{-1} d^{-1} is provided (Table 2). If we use the mean difference in these comparisons of 0.48 nmol

Table 2. Soil nitrogenase activity of twelve paired samplings, no-till and conventional till.

Sample location	Tillage	C_2H_4 reduced†‡	Estimated N fixed§
		nmol g DW soil^{-1} d^{-1}	g N ha^{-1} d^{-1}
Amidon	Conventional	Undetectable	0
	No-till	1.10*	7.2*
Barton	Conventional	0.36	2.4
	No-till	1.12*	7.3*
Beach	Conventional	Undetectable	0
	No-till	1.07*	7.0*
Belfield	Conventional	0.80	5.2
	No-till	0.93*	6.1*
Bottineau	Conventional	0.77	5.0
	No-till	0.75	4.9
Dickinson	Conventional	0.72	4.7
	No-till	1.66*	10.8*
Jamestown	Conventional	0.71	4.6
	No-till	1.15*	7.5*
Lansford	Conventional	0.48	3.1
	No-till	0.49	3.2
Riverton	Conventional	Undetectable	0
	No-till	1.28*	8.4*
Rutland	Conventional	0.83	5.4
	No-till	1.38*	9.0*
Valley City	Conventional	0.72	4.6
	No-till	2.02*	13.2*
Willow City	Conventional	0.35	2.3
	No-till	0.59*	3.9*

* Significant difference at $P < 0.05$ between No-till and Conventional-till reduction.

† Units are nanomoles of C_2H_4 reduced per gram of dry weight (DW) soil per day.

‡ The LSD for reduction is 0.1 nmol C_2H_4 reduced g DW soil^{-1} d^{-1}.

§ Assumes 1.4 g soil cm^{-3}, ratio of C_2H_4 to N_2 reduced is 3:1. Reduction under incubation conditions.

C_2H_4 reduced g DW soil^{-1} d^{-1} in conventional-till compared with 1.13 nmol C_2H_4 reduced g DW soil^{-1} d^{-1} in no-till, and a factor of 2.35 for C_2H_4 to N, we can calculate an estimate of the growing season N contribution in no-till. That value would be 23.5 kg N ha^{-1} per a 100-d growing season; an increase of 13.5 kg N ha^{-1} per season over a conventional till soil. The 13.5 kg N ha^{-1} increase available in no-till from conventional till is approximately one-quarter to one-third of the N of the N reduction indicated from N-rate studies on no-till compared to conventional till in North Dakota.

DISCUSSION

Although several studies have indicated that greater asymbiotic N fixation is possible under no-till (Gupta and Roper, 2010; Smith et al., 2016) this is the first study to indicate that greater asymbiotic N is produced in soils under long-term no-till management. This study will hopefully lead to more careful examination of paired fields, or carefully constructed tillage experiments. It takes time to establish credible no-till experimental sites. Establishing credible tillage experiments is difficult for most researchers. Experimental farms often have space allotments that reduce the area that could be devoted to tillage research. Sufficient space must be allocated so that no-till treatments are not driven on to reach a tillage treatment. It is essential that timing of planting be conducted when the soil is fit for the tillage practice. However, in most tillage studies, a choice has to be made whether to plant when the conventional-till plots are fit, or the no-till plots are fit. A farmer would choose the ideal planting date for their tillage system. A researcher usually gives up something because they cannot go back and forth multiple times to plant on the ideal date for each tillage treatment. We therefore have chosen the paired-sample method between closely neighboring fields with similar soils to take advantage of the farmer ability to conduct their operations in the timeliest manner, whether the sites were no-till or conventional till. Our study found that asymbiotic N-fixation activity was greater in long-term no-till compared to neighboring conventional till soils.

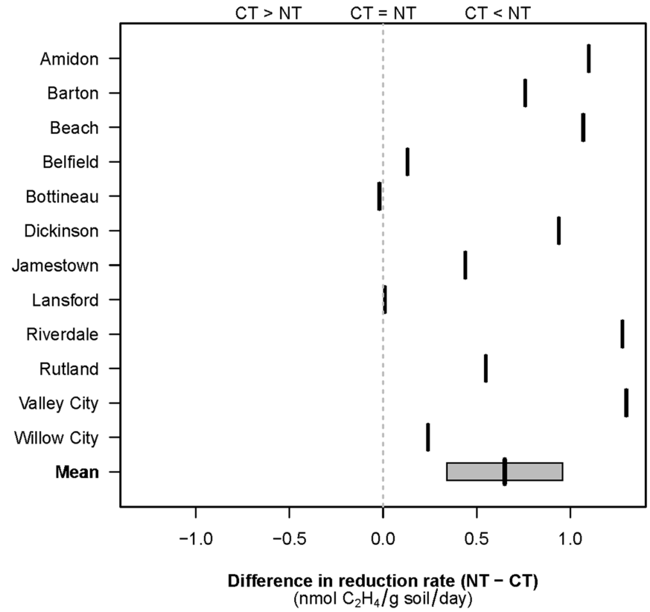

Fig. 2. Difference in acetylene reduction rate between conventional till (CT) and long-term no-till (NT) soil samples at each location.

In contrast, a meta-analysis comparison of no-till and conventional-till sites (Lundy et al., 2015) found that no-till yield was reduced compared to that of conventional-till yield. Also, N rate had to be greater in no-till, particularly in the first 2 yr of no-till, than conventional till. The conflict between our results and those of this study is due to the difference in many researchers' perception of no-till and ours. In our experience, a cessation of tillage is not no-till, it is the beginning of no-till. A better term for the first 4 to 6 yr of no-till management might be 'transitional' no-till. The observation of Lundy et al. (2015) that greater N needed to be applied to the early years of no-till production is common in the literature (Pittelkow et al., 2015; Habbib et al., 2016). However, our study compared only long-term no-till sites, with at least 10 yr continuous no-till management, where the microbiology had most likely transitioned from that of the conventional-till system. The problem with a meta-analysis that considers all 'no-till' references to be no-till are that many of these studies are short-term (2–4 yr) experiments that probably have not transitioned to a no-till microbiological system.

Grandy et al. (2006) provides evidence in a long-term no-till and conventional-till comparison that soil nitrates levels are reduced under no-till while no-till and conventional till yields are similar. In this comparison, what is missing is a series of N rates that would show whether a similar rate of N is required for optimum yield of no-till and conventional tillage. In North Dakota experiments, long-term no-till soils required less N for maximum yield compared with sites under conventional tillage (Franzen, 2017, 2018; Schultz et al., 2018).

This study examined only one 0- to 5-cm deep sample within a no-till location. This study may lead to future research to investigate the spatial variability of asymbiotic N-fixing organism activity that might be present within 1 m, 5 m, 100 m, etc. Additional studies are required to provide a range of N fixation values for consideration if an N credit toward N rate reduction is to be constructed or modified in the future regarding long-term no-till soil management.

CONCLUSIONS

Long-term no-till soils tended to have greater asymbiotic N fixation than neighboring soils under conventional tillage. This indicates that reduced fertilizer N requirements for corn, wheat and sunflower in North Dakota long-term no-till fertilizer recommendations have at least one explanation. Future studies should include the spatial nature of asymbiotic N fixation in no-till fields and whether differences are present in no-till variations, including penetration of soil with the seeding disc only, or strip-till, or shallow (<5 cm deep) tillage in one-pass seeding systems, which are all generally categorized as 'no-till'. This study also provides evidence that greater farm sustainability is possible from transitioning to no-till, due to reduced N inputs required to produce a crop.

REFERENCES

Awale, R., A. Chatterjee, and D. Franzen. 2013. Tillage and N-fertilizer influences on selected organic carbon fractions. Soil Tillage Res. 134:213–222. doi:10.1016/j.still.2013.08.006

Feng, Y., A.C. Motta, D.W. Reeves, C.H. Burmester, E. van Santen, and J.A. Osborne. 2003. Soil microbial communities under conventional-till and no-till continuous cotton systems. Soil Biol. Biochem. 35:1693–1703.

Franzen, D.W. 2016. Fertilizing sunflower. North Dakota Ext. Circ. SF-713. North Dakota State Univ., Fargo. https://www.ndsu.edu/fileadmin/soils/pdfs/SF713__Fertilizing_Sunflower.pdf.

Franzen, D.W. 2017. Soil fertility recommendations for corn. North Dakota Ext. Circ. SF-722. North Dakota State Univ., Fargo. https://www.ag.ndsu.edu/publications/crops/soil-fertility-recommendations-for-corn/sf722.pdf.

Franzen, D.W. 2018. Fertilizing hard red spring wheat and durum. North Dakota Ext. Circ. SF-712. North Dakota State Univ., Fargo. https://www.ag.ndsu.edu/publications/crops/fertilizing-hard-red-spring-wheat-and-durum-1/sf712.pdf.

Franzen, D.W., P.W. Inglett, and C.K. Gasch. 2018. Asymbiotic nitrogen fixation is greater in soils under long-term no-till versus conventional tillage. bioRxiv. doi:10.1101/502757

Grandy, A.S., T.D. Loecke, S. Parr, and G.P. Robertson. 2006. Long-term trends in nitrous oxide emissions, soil nitrogen and crop yields of till and no-till cropping systems. J. Environ. Qual. 35:1487–1495. doi:10.2134/jeq2005.0166

Gupta, V.V.S.R., and M.M. Roper. 2010. Protection of free-living nitrogen-fixing bacteria within the soil matrix. Soil Tillage Res. 109:50–54. doi:10.1016/j.still.2010.04.002

Habbib, H., J. Verzeaux, E. Nivelle, D. Roger, J. Lacoux, M. Catterou, B. Hirel, F. Dubois, and T. Tetu. 2016. Conversion to no-till improves maize nitrogen use efficiency in a continuous cover cropping system. PLoS One 11:e0164234. doi:10.1371/journal.pone.0164234

Helgason, B.L., F.L. Walley, and J.J. Germida. 2009. Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. Soil Sci. Soc. Am. J. 73:120–127. doi:10.2136/sssaj2007.0392

Helgason, B.L., F.L. Walley, and J.J. Germida. 2010. No-till soil management increases microbial biomass and alters community profiles in soil aggregates. Appl. Soil Ecol. 46:390–397. doi:10.1016/j.apsoil.2010.10.002

Inglett, P.W. 2013. Biological dinitrogen fixation. Chapter 31. In: R.D. DeLaune, K.R. Reddy, C.J. Richardson, and J.P. Megonigal, editors, Methods in biogeochemistry of wetlands. SSSA, Madison, WI. p. 593-602. doi:10.2136/sssabookser10.c31

Kennedy, I.R., and N. Islam. 2001. The current and potential contribution of asymbiotic nitrogen fixation to nitrogen requirements on farms: A review. Aust. J. Exp. Agric. 41:447–457. doi:10.1071/EA00081

Lundy, M.E., C.M. Pittlekow, B.A. Linquist, X. Liang, K.J. van Groenigen, J. Lee, J. Six, R.T. Venterea, and C. van Kessel. 2015. Nitrogen fertilization reduces yield declines following no-till adoption. Field Crops Res. 183:204–210. doi:10.1016/j.fcr.2015.07.023

Matson, A.L., M.D. Corre, J.I. Burneo, and E. Veldkamp. 2015. Free-living nitrogen fixation responds to elevated nutrient inputs in tropical montane forest floor and canopy soils of southern Ecuador. Biogeochemistry 122:281–294. doi:10.1007/s10533-014-0041-8

Pittelkow, C.M., B.A. Linquist, M.E. Lundy, X. Liang, K.J. van Groenigen, J. Lee, N. van Gestel, J. Six, R.T. Venterea, and C. van Kessel. 2015. When does no-till yield more? A global meta-analysis. Field Crops Res. 183:156–168. doi:10.1016/j.fcr.2015.07.020

Roper, M.M., and V.V.S.R. Gupta. 2016. Enhancing non-symbiotic N_2 fixation in agriculture. Open Agric. J. 10:7-27. doi:10.2174/1874331501610010007

Seitzinger, S.P., and J.H. Garber. 1987. Nitrogen fixation and $^{15}N_2$ calibration of the acetylene reduction assay in coastal marine sediments. Mar. Ecol. Prog. Ser. 37:65–73. doi:10.3354/meps037065

Schultz, E., T. DeSutter, L. Sharma, G. Endres, R. Ashley, H. Bu, S. Markell, A. Kraklau, and D. Franzen. 2018. Response of sunflower to nitrogen and phosphorus in North Dakota. Agron. J. 110:1–11. doi:10.2134/agronj2017.04.0222

Smith, C.R., P.L. Blair, C. Boyd, B. Cody, A. Hazel, A. Hedrick, H. Kathuria, P. Khurana, B. Kramer, K. Muterspaw, C. Peck, E. Sells, J. Skinner, C. Tegeler, and Z. Wolfe. 2016. Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. Ecol. Evol. 6:8075–8084. doi:10.1002/ece3.2553

Wilson, P.W. 1958. Asymbiotic nitrogen fixation. In: E.K. Allen, et al., editors, Nitrogen metabolism. Encyclopedia of Plant Physiology. (In German.) Vol. 8. Springer, Berlin. doi:10.1007/978-3-642-94733-9_3