

IMPACTS OF CHANGING PRECIPITATION ON NITROGEN CYCLING IN DIFFERENT  
LANDSCAPE POSITIONS AND CROPPING SYSTEMS

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## ABSTRACT

### IMPACTS OF CHANGING PRECIPITATION ON NITROGEN CYCLING IN DIFFERENT LANDSCAPE POSITIONS AND CROPPING SYSTEMS

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Soil nitrogen (N) influences crop yields and can interact with climate change. Soil N has many transformations and transfers that are hard to quantify and control. These N transformations and transfers are mediated by many factors, including temperature, water, and carbon. Thus, impending climate change may strongly affect N cycling across cropping systems.

To minimize N losses and increase crop production, we must maximize N use efficiency (NUE). Past research shows precipitation and soil moisture act as the primary physical drivers of terrestrial N cycling and losses. To improve NUE with changing precipitation patterns, controls on N cycling in terrestrial systems must be identified. Thus, experiments to elucidate the linkage between hydrological and biogeochemical controls are valuable (Chapter 1). Many aspects of the N cycle are influenced by a changing climate - two are especially important: nitrous oxide fluxes ( $N_2O$ ) and biological nitrogen fixation (BNF).

$N_2O$  is a powerful greenhouse gas with over 250 times the radiative forcing of  $CO_2$ . In Chapter 2, I test the hypothesis that changing rainfall patterns strongly alter  $N_2O$  fluxes in agricultural soils as modulated by cropping system. I use rainfall manipulation shelters to expose soils to the same amount of rainfall delivered at different intervals (3-days, 14-days, and 28-days). Results from the 2016 and 2017 field seasons show cumulative  $N_2O$  fluxes were 1.4 to 2 times higher when rainfall occurred in 28-day than shorter intervals in corn systems. Fluxes were related to changes in denitrifier enzyme activity for both years. In switchgrass systems  $N_2O$  emissions were not significantly affected by rainfall intervals.

In Chapter 3, I test the hypothesis that changing rainfall patterns that alter N<sub>2</sub>O fluxes will be modulated by landscape position as landscape position affects soil texture and carbon. Over two field seasons cumulative N<sub>2</sub>O fluxes were higher in toeslope positions than in summit positions, and longer rainfall intervals had higher fluxes in summits only, consistent with higher soil carbon and finer soil texture in toeslope positions. Knowledge of these landscape patterns deserve inclusion in models of current and future climate change effects in order to better quantify and mitigate agricultural N<sub>2</sub>O fluxes.

In Chapter 4, I test the hypothesis that BNF is particularly vulnerable to changing rainfall patterns in till vs. no-till and in summit vs. toeslope positions due to differences in texture and organic matter. Results reinforce the importance of topographic position for predicting soybean BNF and show that summit positions are more sensitive to additional rainfall. Results also show changes in rainfall intensity affect BNF in tilled differently than in no-till soils. Models that incorporate these interactions will be better able to characterize legume crop performance and N fixation across landscapes and improve global estimates for BNF.

Understanding these interactions in the agricultural US Midwest may help us improve sustainability of N use in cropping systems with a changing climate.

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## Chapter 1: Introduction

### **Background**

Nitrogen (N) is an important element for crop growth and known contributor to environmental pollution. Humans' ability to transform inert N to reactive N ( $N_r$ ) has been a double-edged development with positive and negative effects. Society has increased  $N_r$  inputs to the biosphere to ~5 times those of pre-industrial times, mainly to cropping systems (Houlton et al., 2013). These N inputs, through fertilizer, biological nitrogen fixation (BNF), and deposition, have allowed us to support an ever-increasing need for food, fuel, and fiber for a growing human population. However, the majority of N applied is not taken up by plants but lost to the environment, far exceeding planetary boundaries (Rockström et al., 2009) and even N taken up by plants makes its way back to the environment through animal and human waste.  $N_r$  from crop production is released as different N species either to the atmosphere or to ground and surface waters via leaching and runoff. Negative impacts include climate change, eutrophication, biodiversity declines, and smog formation.

These losses are influenced by complex soil N cycle transformations and transfers - which operate differentially across the environment at different temporal and spatial scales. Together, water and carbon (C) greatly influence soil N cycling, and in the US Midwest water dynamics are changing with climate change. Climate change in the US Midwest is causing more extreme precipitation events with longer dry periods in between (Pryor et al., 2014) and more overall annual precipitation, at least in Michigan (Frankson and Kunkel, 2017). The Midwest is a very important agricultural region and projected to be increasingly impacted by climate change.

In this dissertation, I aim to examine how changes in rainfall patterns influence N loss and N acquisition by crops in different cropping systems and in different topographical positions.

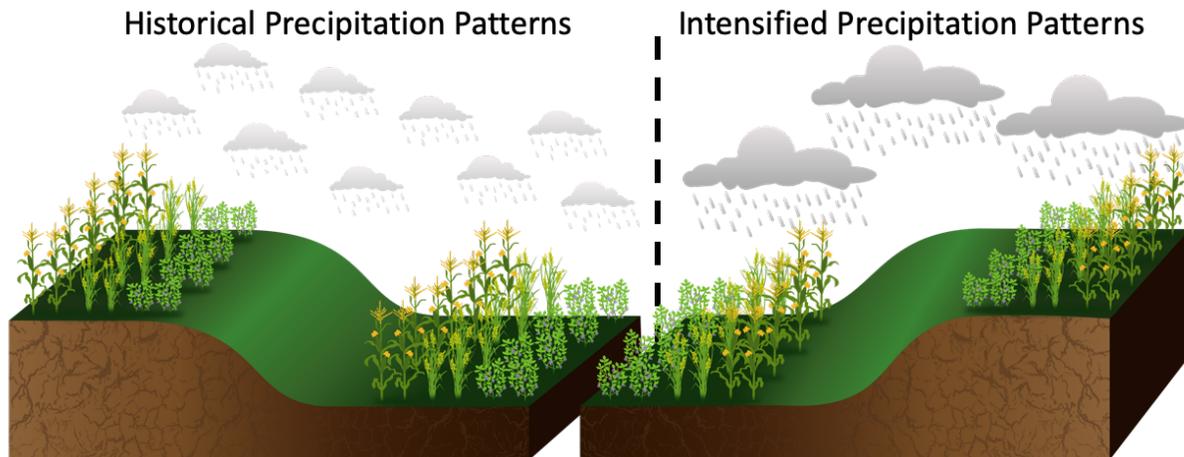
There are several aspects of the N cycle likely to be particularly affected by changing rainfall; two of the most interesting and important are soil nitrous oxide (N<sub>2</sub>O) emissions and biological nitrogen fixation (BNF).

Approximately two-thirds of N<sub>r</sub> created by contemporary human activity is lost to air as opposed to water. This includes nitrogen oxides (NO<sub>s</sub>), ammonia, dinitrogen, and N<sub>2</sub>O. N<sub>2</sub>O is a potent greenhouse gas that contributes greatly to climate change through radiative forcing. A majority of anthropogenic N<sub>2</sub>O emissions are from agricultural soils (IPCC 2014). Additionally, changing rainfall patterns are known for their capacity to affect N<sub>2</sub>O emissions (Bergsma et al., 2002; Gelfand et al., 2015; Kim et al., 2012).

A second important N-cycle process likely to be affected by climate change due to soil N transformations is BNF, such as in soybean (*Glycine max* L.) and other legumes. BNF is the capture of atmospheric N<sub>2</sub> and its transformation to ammonia by symbiotic N<sub>2</sub>-fixing rhizobia. N acquired by crops is removed in harvest, decomposed, or stored in the cropping system in soil organic matter. Legumes have the potential to build soil fertility and provide N to subsequent crops. BNF is affected by many soil factors including texture, pH, and inorganic N pools (Bottomley and Myrold, 2015; Salvagiotti et al., 2008). Leguminous N-fixing crops are important worldwide as BNF also occurs in other important crops, including peas (*Vigna* spp.), clovers (*Trifolium* spp.), vetches (*Vicia* spp.), and perennial legumes such as alfalfa (*Medicago sativa* L.). BNF may be even more relevant in the future with the potential introduction of N<sub>2</sub>-fixing corn (*Zea mays*), wheat (*Triticum aestivum* L.), and rice (*Oryza sativa*) to reduce the use of fertilizer, which may influence N<sub>r</sub> in the environment (Bottomley and Myrold, 2015). Understanding controls on BNF can help farmers make management decisions.

## Chapter Overview

In the remainder of this dissertation I describe three projects to explore how changing rainfall and topography will affect N<sub>2</sub>O emissions, BNF, and farmer vs researcher perspectives in Michigan cropping systems. Figure 1.1 illustrates the factors explored in this dissertation.



**Figure 1.1:** An illustration showing differences in precipitation patterns (historical and intensified), in landscape positions (toeslope and summit), and in cropping systems (corn, switchgrass, and soybeans). This is intended to show some factors explored in the dissertation.

Research on N<sub>2</sub>O emissions appears in two chapters. In Chapter 2, I test the hypothesis that changing rainfall patterns strongly alter N<sub>2</sub>O fluxes in agricultural soils as modulated by cropping system. I used rainfall manipulation shelters to expose soils to the same amount of rainfall delivered at different intervals (3-days, 14-days, and 28-days) and quantified mineral N, nitrous oxide reductase, and labile C.

In Chapter 3, I test the hypothesis that changing rainfall patterns that alter N<sub>2</sub>O fluxes will be modulated by landscape position insofar as landscape position affects soil texture and carbon content. I again used rainfall manipulation shelters to expose soils to the same amounts of rainfall delivered at different intervals (3-days, 14-days, and 28-days) placed at both summit and toeslope positions within conventionally tilled corn fields.

In Chapter 4, I test the hypothesis that BNF is particularly sensitive to changing rainfall patterns in till vs. no-till and in summit vs. toeslope positions due to landscape differences in texture and organic matter. I measured soybean BNF by  $^{15}\text{N}$  natural abundance using nodulating and non-nodulating isolines to test effects of landscape position, and tillage management, and their potential interaction with rainfall.

The results of both the  $\text{N}_2\text{O}$  and BNF experiments elucidate how the N cycle will be influenced by changing rainfall patterns. The findings challenge the assumptions that longer dry periods with less consistent rainfall will lower  $\text{N}_2\text{O}$  emissions and decrease BNF consistently.  $\text{N}_2\text{O}$  results show this may not be the case due to accelerated emissions upon drought cessation, especially for annual systems with high fertilizer inputs. BNF results show that changes in rainfall will differ by landscape position and tillage management. My conclusions point to potential feedbacks and challenges of changing rainfall patterns for farmers and society. The controls identified will be important in designing models, adapting practices, and creating mitigation plans for the intensifying precipitation patterns predicted.

## Chapter 2: Consequences of Changing Rainfall Patterns on Nitrous Oxide (N<sub>2</sub>O) Fluxes in an Annual vs. Perennial Cropping System

### **Abstract**

Nitrous oxide (N<sub>2</sub>O) is the dominant natural ozone-consuming substance in the stratosphere and a strong greenhouse gas with 265-300 times the radiative forcing of CO<sub>2</sub>. N<sub>2</sub>O is produced by soil microbes and fluxes are closely linked to soil moisture. Consequently, documented and future changing rainfall patterns due to climate change will likely influence N<sub>2</sub>O fluxes. Since most anthropogenic N<sub>2</sub>O produced globally is from agricultural soils, where fluxes are controlled by numerous factors including oxygen, nitrate, and carbon availability (all of which are strongly tied to soil moisture status), it seems important to understand the impact of more extreme precipitation patterns on N<sub>2</sub>O emissions. I tested the hypothesis that changing rainfall patterns strongly alter N<sub>2</sub>O fluxes in agricultural soils as modulated by cropping system. I used rainfall manipulation shelters to expose soils to the same amount of rainfall delivered at different intervals (3-days, 14-days, and 28-days). The experiment was conducted for 10-weeks during two growing seasons in replicated no-till continuous-corn and switchgrass systems at a site in the upper US Midwest. Results show cumulative N<sub>2</sub>O fluxes were 1.4 to 2.0 times higher when rainfall occurred in 28-day rather than shorter intervals in corn systems. Response patterns were related to drought-induced changes in denitrifier enzyme activity for both years. In switchgrass systems N<sub>2</sub>O emissions were not significantly affected by different rainfall intervals. Understanding patterns and mechanisms for N<sub>2</sub>O fluxes from managed soils is important for achieving more sustainable agriculture, developing mitigation practices, and parameterizing global biogeochemical models.

## Introduction

Climate change is causing shifts in U.S. temperatures and precipitation, resulting in increasingly more intense and frequent precipitation events, longer frost-free seasons, and higher night-time temperatures across much of the Midwest U.S. (Pryor et al., 2014). Associated effects on biodiversity, human health, and ecosystem productivity are projected to intensify under all projected future emissions scenarios (IPCC, 2007).

Rainfall intensification in the US Midwest, an area especially important to agriculture, can be expressed as more variable growing season rainfall with longer dry periods between rain events. Rainfall intensification is particularly worrisome for farming, which depends on predictable rainfall for high yields and efficient nitrogen (N) use. Rainfall dramatically affects soil biogeochemical processes, especially for carbon (C) and N flows (Fierer and Schimel, 2002), and rainfall intensification, often quantified as the heaviest 1% of events, is an important attribute of precipitation regimes, especially for agriculture. Future N management decisions will require a thorough understanding of N responses to altered rainfall (Bowles et al., 2018; Robertson et al., 2013), which may lead to even greater losses of N to groundwater, waterways, and the atmosphere.

Nitrogen gas losses from agricultural systems include the nonreactive gas dinitrogen ( $N_2$ ) as well as nitric oxides ( $NO_x$ ) and nitrous oxide ( $N_2O$ ).  $N_2O$  is a main cause of stratospheric ozone depletion and is a powerful greenhouse gas (Ravishankara et al., 2009), with 265 to 300 times the radiative forcing of  $CO_2$  (IPCC, 2014). Agriculture is a major global source of  $N_2O$  and is responsible for most of the rise in atmospheric  $N_2O$  concentrations since the 1950s (Fowler et al., 2009). About 60% of agricultural  $N_2O$  emissions are emitted from soil, where  $N_2O$  is produced by microbes primarily during denitrification and nitrification (Zhu-Barker et al., 2015).

Denitrification appears to be the dominant source of  $N_2O$  in most mesic cropping systems (Aronson and Allison, 2012). Heterotrophic denitrifiers, mainly bacteria but also archaea and fungi, use soil organic C as an energy source and nitrate, which is thermodynamically similar to oxygen, as a terminal electron acceptor when oxygen is unavailable. Denitrification is often enhanced after precipitation (Bergsma et al., 2002; Gelfand et al., 2015) and especially in soil aggregates, rhizospheres, and decomposing litter, where soil microbes have ample access to C but limited access to oxygen (Khalil et al., 2004; Kravchenko et al., 2017; Loecke et al., 2008; Sexstone et al., 1988). The denitrification pathway products nitrite, nitric oxide,  $N_2O$ , and dinitrogen are formed sequentially (Cooper and Smith, 1963), such that  $N_2O$  is released to the soil atmosphere when denitrification is not carried out completely.

Although considered a minor source of  $N_2O$  in comparison to denitrification, nitrifying archaea and bacteria also produce  $N_2O$  from hydroxylamine decomposition following ammonia oxidation (Bremner and Blackmer, 1978). Since nitrifiers require oxygen ( $O_2$ ) to oxidize nitrite to  $NO_3^-$ , nitrification proceeds fastest in aerobic conditions, but can occur in soils with up to 60% water filled pore space (WFPS) (Abbasi and Adams, 2000). Nitrifier denitrification and coupled nitrification-denitrification can also produce  $N_2O$  (Wrage et al., 2001). Most  $N_2O$  produced by nitrifiers appears to be from nitrifier denitrification stimulated by wet-dry cycles (Wrage-Mönnig et al., 2018).

Thus, both nitrification and denitrification are strongly affected by soil moisture, so likely to be influenced by changing rainfall patterns (G P Robertson and Groffman, 2015). The effects of longer dry periods during the growing season on C and N will likely influence nitrous oxide ( $N_2O$ ) emissions in inconsistent ways (Kim et al., 2012). Changing rainfall patterns are an aspect of climate change that may shift controls on biogeochemical mechanisms, leading to spatial

variability and episodic moments that have higher than average influence on N fluxes (Bernhardt et al., 2017).

Plants influence N<sub>2</sub>O fluxes by competing with microbes for available N and this likely differs in annual and perennial cropping systems. Plants in annual cropping systems are not active for the entire growing season – as compared to perennial systems – and as well cannot retranslocate N to roots for use the following year, so might leave more soil N for microbes to transform into N<sub>2</sub>O (Oates et al., 2016). Perennial systems can shift the microbial community composition by improving niches for functional groups (Liang et al., 2012) and change the physiological ability of the soil community towards enzyme production (Hargreaves and Hofmockel, 2014).

How might changing rainfall intensification, with its effects on C and N biogeochemistry, affect N<sub>2</sub>O fluxes? Some research shows higher N<sub>2</sub>O emissions following rewetting of dry soil in the field (Gelfand et al., 2015). Numerous lab experiments have also reported N<sub>2</sub>O emissions stimulated by wetting dry as compared to wetting wet soils (e.g. Bergsma et al., 2002; Christensen and Prieme, 2001). Groffman and Tiedje (1988) suggest that denitrification is stimulated by reduced oxygen following rewetting due to accelerated respiration. Others hypothesize that pulses of N<sub>2</sub>O are likely caused by mineralization of both C and N due to the hysteretic effects of microbial biomass' increasing substrate availability and denitrifier activity (Congreves et al., 2018).

Here I examine the degree to which changing rainfall patterns might accelerate N<sub>2</sub>O fluxes in different cropping systems. I test the hypothesis that longer dry periods between rainfall events will strongly increase N<sub>2</sub>O fluxes in agricultural soils and that these increases will be especially pronounced in annual (versus perennial) cropping systems where more N is more

available to microbes. Understanding these patterns and the mechanisms that underlie them may be crucial for predicting reactive N responses to climate change and for designing mitigation strategies to forestall potential increases in N<sub>2</sub>O production.

## **Methods**

### ***Experimental Site***

I conducted this study in the Great Lakes Bioenergy Research Center's Bioenergy Cropping System Experiment at the Kellogg Biological Station (KBS) Long-Term Ecological Research site, Hickory Corners, Michigan, USA (42° 24'N, 85° 23'W 288 m elevation). Average temperatures at KBS range from -3.8°C (January) to 22.9°C (July) with an annual mean of 10.1°C. Yearly precipitation is 1005 mm and the 30-year average in monthly precipitation ranges from 40 mm in February to 100 mm in May (Peters et al., 2013). About 17% of precipitation occurs during the winter months from January to April, with the rest evenly distributed throughout the growing season. Over the past 30 years, precipitation events from April to December were, on average, 2.5 mm and ~3.6 days apart; around eight events per season were greater than 25 mm (<https://lter.kbs.msu.edu/datatables/7>). Potential evapotranspiration exceeds rainfall for four months out of the year (Crum et al., 1990; Hamilton, 2015).

Soils at KBS are well-drained mesic Typic Hapludalfs developed from glacial till and outwash consisting of co-mingled Kalamazoo (fine-loamy, mixed, semiactive) and Oshtemo (coarse-loamy, mixed, active) series (Crum and Collins, 1995; G Philip Robertson and Groffman, 2015) with intermixed loess (Luehmann et al., 2016). The predominant land use in the region consists of cultivated and successional fields, woodlots, residences, lakes, and wetlands.

Field experiments were conducted in the Great Lakes Bioenergy Research Center's Biofuel Cropping System Experiment experimental design as described in Sanford et al. (2016).

I used two of ten cropping systems established in 2008 in a randomized complete block design with five replicate blocks. I used four of the replicate blocks. Treatment plots were  $27 \times 43$  m (0.12 ha) with 12-m alleyways between adjacent plots and planted to either continuous no-till corn (*Zea mays*) or switchgrass (*Panicum virgatum*, variety “Cave-in-Rock”) to represent annual and perennial crops, respectively. Both crops were managed as per common practice in the region. Corn was planted in 76 cm rows and fertilized at  $150 \text{ kg N ha}^{-1}$  in two applications: 25 kg N at planting and the remainder side-dressed ~6 weeks later when plants were at the V6 stage. Switchgrass was fertilized at  $56 \text{ kg N ha}^{-1}$  in late spring. About 50% of aboveground corn residue was harvested in the fall along with grain (Gelfand et al., 2020); switchgrass was harvested each fall following first frost. Detailed agronomic protocols are available online (<https://lter.kbs.msu.edu/protocols/122>).

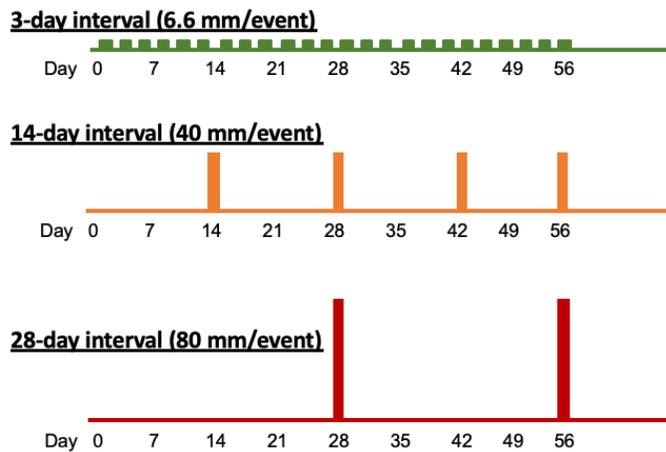
### ***Rainfall Manipulation Shelters***

Small rainfall manipulation shelters were used to create rainfall environments in which soils were exposed to different rainfall intervals but the same amounts of total rainfall, which was added to each shelter at different times. Well-vented shelters were  $61 \times 122 \times 40$  cm high and placed between corn rows and switchgrass crowns; each shelter contained a cylindrical stainless-steel gas sampling frame (28.5 cm diameter  $\times$  30 cm high) and sufficient soil surface area to allow periodic soil sampling. Shelters were constructed of polycarbonate (roofs), plexiglas (sides), and sheet metal (bottom edging). Bottom edging on the sides allowed 3 cm insertion into the soil to eliminate the potential for storm-derived lateral surface flow, which was in any case low due to high infiltration rates in these well-drained soils. Three large holes 6.4 cm diameter drilled in the sides of the shelters allowed free airflow and eliminated humidity buildup. Before implementing the experiment,  $\text{N}_2\text{O}$  flux measurements taken inside and outside small

shelters placed under a larger (10 x 5m) shelter. The small shelters showed no significant shelter effects on fluxes during either low or high flux events (Glanville in prep). Roofs were hinged at the back to allow for periodic soil and gas sampling. Each shelter covered a 0.6 m<sup>2</sup> area.

### ***Rainfall Intervals***

Rainfall additions were based on a 30-year regional average calculated from 1984 to 2014. To each of three shelters per plot, rainfall was applied at either 3-day (long-term average for the region), 14-day, or 28-day intervals (Figure 2.1). The 3-day average was 7.1 mm with a 1.3 mm standard deviation not accounting for interception by the canopy. Interception of the canopy was accounted for, lowering the average of 7.1 mm to 6.6 mm rainfall. All treatments received 80 mm of precipitation per month. Since the soil under each shelter was protected from ambient rainfall, the amount of water added was independent of droughts or unusually wet weather; thus, the experiment was repeatable across field seasons because water was not limiting.



**Figure 2.1:** Simulated rainfall in experimental treatments by day of experiment. Bar heights are relative to the amounts of water applied (shown in parentheses). All treatments received the same total amount of water (80 mm per four weeks).

### ***Gas sampling***

N<sub>2</sub>O, CH<sub>4</sub>, and CO<sub>2</sub> fluxes were measured three times per week on average to capture responses to rainfall events, using a static chamber method in which airtight lids were clamped to the chamber top and headspace N<sub>2</sub>O and CH<sub>4</sub> concentrations measured periodically (Holland et. al 1999) using one of two techniques. First, for the "GC method" I took four chamber headspace gas samples (20 mL) every 15 minutes for 60 minutes. The samples were stored over-pressurized in glass vials and analyzed for N<sub>2</sub>O, CO<sub>2</sub>, and CH<sub>4</sub> concentrations in the lab using a gas chromatograph (7890A Agilent Technologies Inc., DE, USA) equipped with an ECD for N<sub>2</sub>O, an FID for CH<sub>4</sub>, and an infrared gas absorption analyzer (LI-820, LI-COR, NE, USA) for CO<sub>2</sub> (<http://lter.kbs.msu.edu/protocols/113>). The second technique was the "MICOS (Mobile Integrated Cavity Output Spectroscopy) method" that used a quantum cascade laser (QCL) analyzer (Los Gatos Research Inc., CA, USA) in the field for continuous chamber headspace measurements of N<sub>2</sub>O and CH<sub>4</sub> concentrations and a closed-path infrared gas analyzer (LI-7000, LI-COR, NE, USA) for CO<sub>2</sub> concentrations. During the measurement period, the headspace air inside each chamber was circulated through the QCL analyzer for 5 mins and gas concentrations were analyzed at 1s intervals.

### ***Soil Sampling***

Soil moisture and temperature were measured in every shelter every 10 minutes with sensors (5TM, Decagon, WA, USA) installed vertically at the surface and stored on data loggers (EM50, Decagon, WA, USA). Soil was sampled around co-occurring rewetting events to 20 cm depth for several assays: denitrification enzyme activity (DEA), gravimetric soil moisture, inorganic N, and labile and dissolved organic C. Soil texture to 20 cm depth was also measured once from each shelter area in both 2016 and 2017.

The DEA assay is a short-term slurry incubation wherein C as glucose and  $\text{NO}_3^-$  was provided in excess (Groffman et al. 1999). For each assay 10 g of fresh soil were added to 150 mL airtight jars. I evacuated the headspace of each jar then added either  $\text{N}_2$  or  $\text{N}_2$  plus 10% acetylene. Acetylene inhibits the last step of denitrification wherein  $\text{N}_2\text{O}$  is converted to dinitrogen. While jars were gently shaken on shaker tables, headspace  $\text{N}_2\text{O}$  concentrations were measured as described earlier over a 90 minute period and production rates were compared with and without the acetylene block to provide a measure of nitrous oxide reductase (NOS) activity (<https://lter.kbs.msu.edu/protocols/193>). For gravimetric soil moisture 10 g of fresh soil were oven-dried at  $60^\circ\text{C}$  for 48 hours (<https://lter.kbs.msu.edu/protocols/24>)

Inorganic N was measured via a 1 M KCl 100 mL extraction of 10g of fresh soil performed in duplicate. Soils were shaken on a shaker table overnight and allowed to settle before filtering. Extracts were frozen until analysis. A flow injector analyzer (QuickChem 8500 Series 2, Lachat Instruments, CO, USA) was used to determine nitrate and ammonium concentrations in extracts (<https://lter.kbs.msu.edu/protocols/33>).

Labile C was measured with a carbon mineralization assay wherein 10 g of dry soil were rewetted to 55% WFPS in a 150 mL jar (Franzluebbers et al., 2000):  $\text{CO}_2$  concentrations were measured every hour for four hours on an infrared gas analyzer (LI-7000, LI-COR, NE, USA). Dissolved organic C was measured via a water extraction with a 1:3 soil water ratio: The soil water combination was shaken overnight then centrifuged and the supernatant then filtered through a  $0.2\ \mu\text{m}$  membrane. The extracts were then analyzed for C on a total organic carbon analyzer (TOC-VWS and ASI-V autosampler, Shimadzu; Columbia, Maryland).

Soil texture was measured in the shelter locations within each plot using the hydrometer method (<https://lter.kbs.msu.edu/protocols/108>). Here, 40 g of soil were shaken for 24 hours in

200 mL jars using hexametaphosphate as a dispersant. The slurry was then put in 1000 mL cylinders and supplemented with water. Hydrometer and temperature readings were taken over eight hours. Since sand falls out of solution too quickly to accurately record density changes, to supplement hydrometer readings sand was sieved out separately with a 53  $\mu\text{m}$  sieve.

### ***Statistical Analysis***

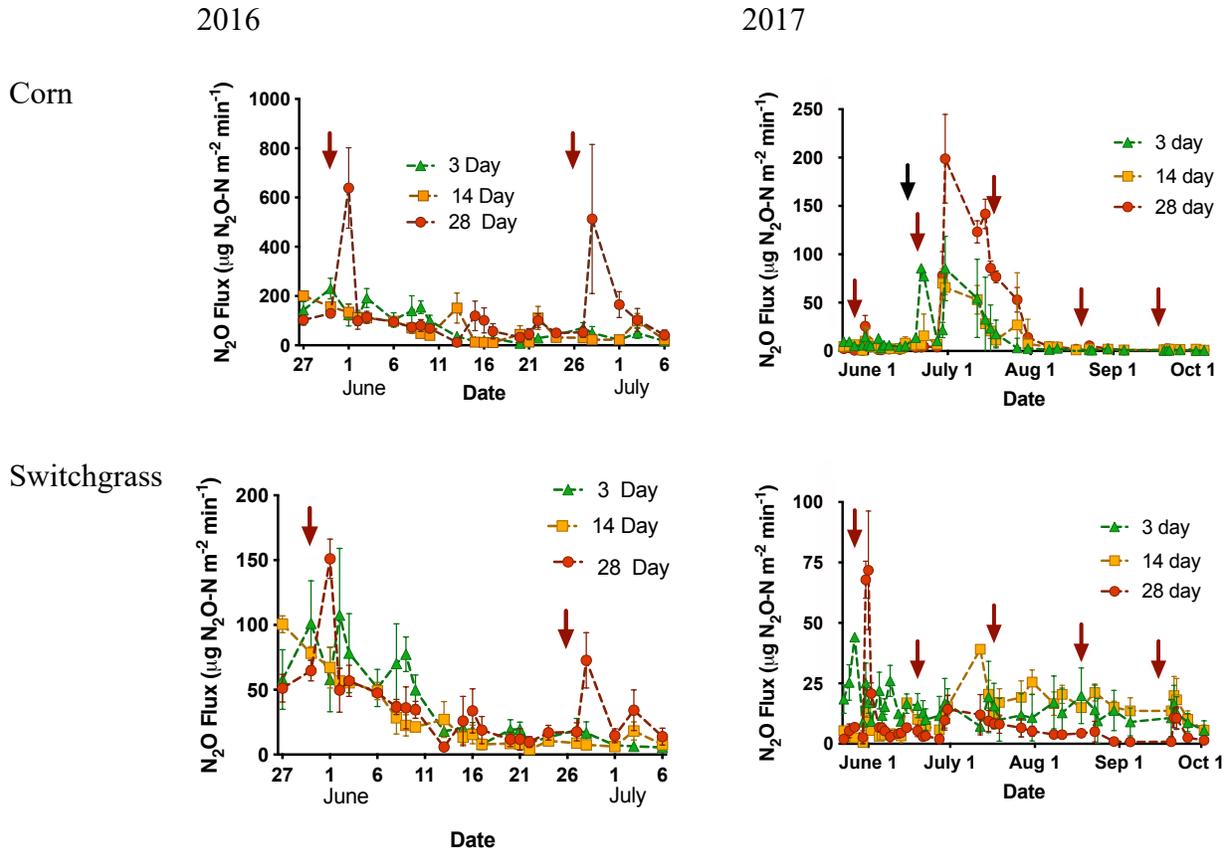
All statistical analyses were performed using R software version 3.6.1 (R Development Core Team 2019) and with a significance value of  $P < 0.05$ . The statistical model included two cropping systems, two years, and three rainfall-interval treatments with the interactions among them considered fixed factors. Blocks, crops nested within blocks, and rainfall-interval treatments nested within blocks and crops were considered random factors. Analysis of variance was used by considering cropping system as a whole plot factor and rainfall-interval treatments and years as subplot and sub-subplot factors. Normality of residuals was visually observed, and no violations of assumptions were found. Homogeneity of variance assumptions were checked and no heterogeneous variance was detected by Levene's test. DEA means were compared for significance using t-tests. Regression analysis was used to determine the relationship between  $\text{N}_2\text{O}$  flux and temperature, moisture, and soil texture.

## **Results**

### ***$\text{N}_2\text{O}$ emissions***

Daily  $\text{N}_2\text{O}$  emissions in corn and switchgrass for 2016 and 2017 are shown in Figure 2.2. In corn, fluxes ranged from 3.1 to 1000  $\mu\text{g N}_2\text{O-N m}^{-2} \text{ min}^{-1}$  in 2016 and from 2.1 to 258  $\mu\text{g N}_2\text{O-N m}^{-2} \text{ min}^{-1}$  in 2017. In corn, higher fluxes occurred during the summer months of June and July before declining to below  $\sim 20 \mu\text{g N}_2\text{O-N m}^{-2} \text{ min}^{-1}$  for August through December of both years.

In switchgrass, N<sub>2</sub>O fluxes ranged from 1.7 to 183 in 2016 and 0.4 to 105 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup> in 2017, with fluxes highest in May, June, and July, and lower in August and September. Flux variability was greatest in April and May. Flux measurements were not taken past September in either year.

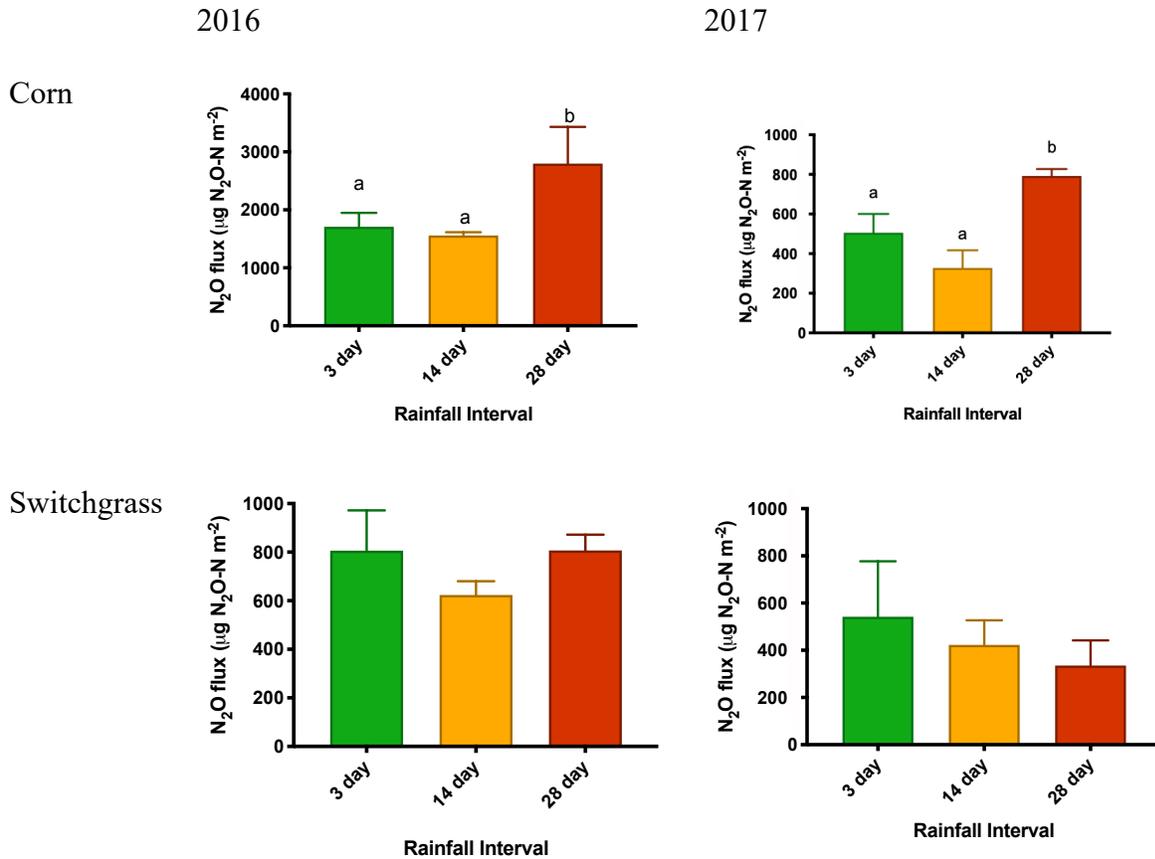


**Figure 2.2:** Daily N<sub>2</sub>O response to different rainfall patterns in the no-till corn and switchgrass systems in 2016 (left) and 2017 (right). Error bars represent standard errors of the mean, n=4 replicate plots (each with three rainfall intervals). Red arrows denote 28-day watering events. Black arrow denotes fertilizer application.

Cumulative N<sub>2</sub>O emissions are sums of measured fluxes; values are not integrated.

Cumulative N<sub>2</sub>O emissions in corn and switchgrass yielded similar trends for 2016 and 2017 (Figure 2.3), however, the difference between the 28-day interval and the other intervals in corn was smaller in 2017 than in 2016. For corn, cumulative N<sub>2</sub>O in 2016 was highest under the 28-day rainfall interval (2798 ± 633 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-2</sup>); fluxes under the 3-day and 14-day

intervals were not significantly different from one another ( $1709 \pm 240$  and  $1559 \pm 55 \mu\text{g N}_2\text{O-N m}^{-2} \text{ min}^{-1}$ , respectively). In 2016 and 2017 switchgrass  $\text{N}_2\text{O}$  fluxes under the 3-day, 14-day, and 28-day intervals were not significantly different from one another (in 2016:  $807 \pm 166$ ,  $624 \pm 57$ , and  $807 \pm 65 \mu\text{g N}_2\text{O-N m}^{-2} \text{ min}^{-1}$ , respectively).

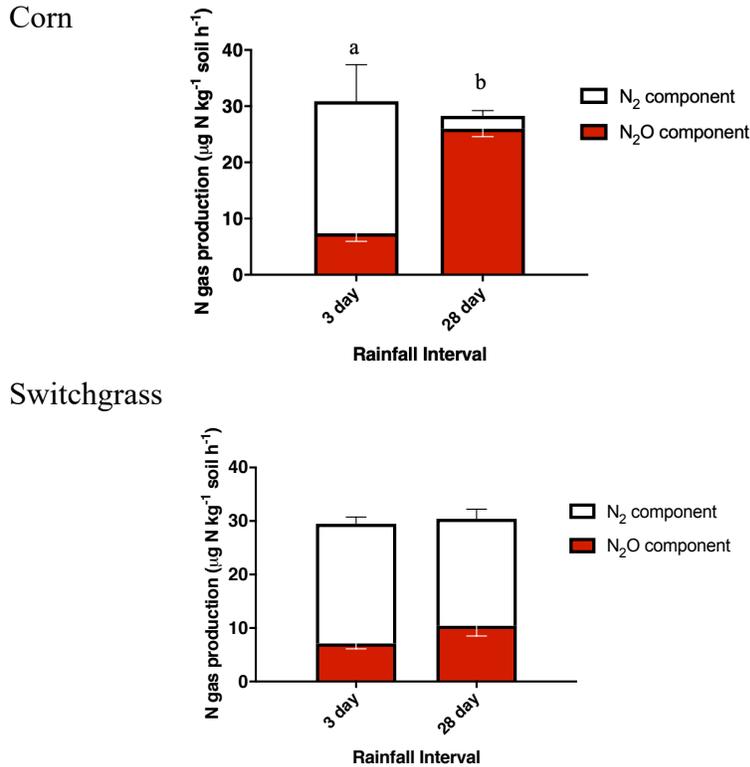


**Figure 2.3:** Cumulative  $\text{N}_2\text{O}$  responses to different rainfall patterns in the no-till corn and switchgrass systems in 2016 and 2017. Error bars represent standard errors of the mean,  $n=4$  replicate plots (each with three rainfall intervals).

### *DEA Assays*

Total DEA rates were similar for both cropping systems and did not differ between 3-day and 28-day rainfall intervals for the June 2017 rewetting interval (Figure 2.4). Denitrification rates in corn were similar ( $30.9 \pm 7.9$  and  $28.0 \pm 2.3 \mu\text{g N gas kg}^{-1} \text{ soil h}^{-1}$  for the different

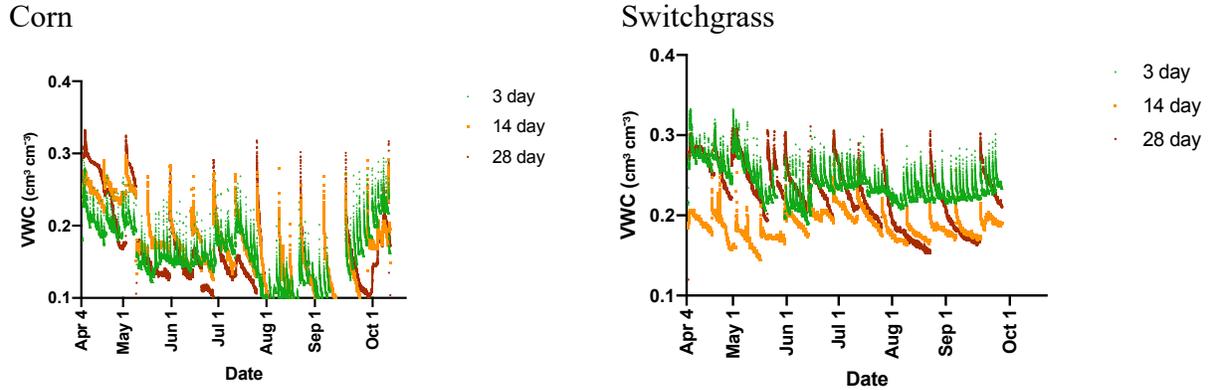
intervals, respectively). However, the proportion of N<sub>2</sub>O converted to N<sub>2</sub> differed between the 3 day and 28-day intervals in corn with NOS converting N<sub>2</sub>O into N<sub>2</sub> at 76% and 8% efficiencies, respectively. There was no difference in the proportion of N<sub>2</sub>O converted to N<sub>2</sub> in switchgrass.



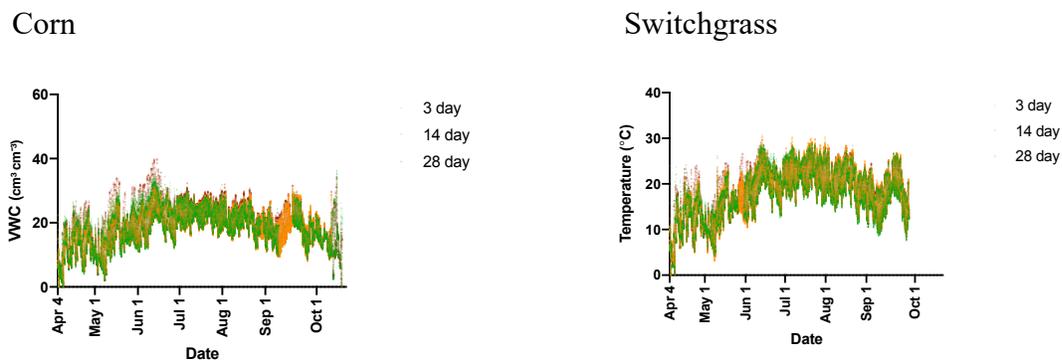
**Figure 2.4:** Composite NOS enzyme activity for before and after rewetting in June 2017. Error bars represent standard errors of the mean, n=4 replicate blocks.

### *Soil Moisture and Temperature*

Soil moisture in corn and switchgrass for 2017 are shown in figure 2.5. In corn, soil moisture VWC ranged from 0 to 0.332 cm<sup>3</sup> cm<sup>-3</sup> in 2017. In corn, higher water content was observed during the months of April and October, congruent when the crop is not using water. In switchgrass, water content was highest in the 3-day rainfall interval, but lower in both the 14-day interval and 28-day interval. In switchgrass, soil moisture VWC ranged from 0 to 0.333 cm<sup>3</sup> cm<sup>-3</sup>. Soil temperature shown in figure 2.6 was slightly cooler in switchgrass than corn ( $18.12 \pm 0.09$  and  $17.49 \pm 0.03$  µg N gas kg<sup>-1</sup> soil h<sup>-1</sup> for the different intervals, respectively).



**Figure 2.5:** Soil moisture in corn and switchgrass for 2017.



**Figure 2.6:** Soil temperature for corn and switchgrass for 2017.

## Discussion

With longer dry periods between rainfall events one might predict lower  $N_2O$  fluxes due to less consistent anoxic conditions. I found the converse: with longer dry periods during the portion of the year with normally high  $N_2O$  fluxes, a 28-day drought increased rather than decreased cumulative  $N_2O$  fluxes. Overall results, then, support the hypothesis that longer dry periods between rainfall events increase  $N_2O$  fluxes, but only for the annual (continuous corn) cropping system. In this system cumulative fluxes after 28 d of rainfall exclusion during the peak growing season were 1.4 times greater than cumulative fluxes from the control (3 d) rainfall interval in 2016, and 2.0 times greater in 2017. In the perennial (switchgrass) system rainfall interval changes had no detectable effects on  $N_2O$  fluxes in either year. Responses were more muted in other parts of the growing season. Response differences can best be explained by

differences in NOS enzyme dynamics; my hypothesis that differences are due to soil C and N pools was not supported.

### ***N<sub>2</sub>O dynamics by cropping system***

Overall, the perennial system had lower N<sub>2</sub>O emissions than the annual system, though in 2017, the cumulative emissions were more similar. Across both cropping systems, fluxes were comparable to those measured for the unsheltered portions of plots in prior years (Oates et al., 2016). N<sub>2</sub>O trends differed each year even though the rainfall pattern was the same. In corn systems, fluxes peaked in mid-June following fertilization in both years. Variations in N<sub>2</sub>O production between the two years did not appear related to inorganic N or temperature patterns over the period; presumably some factors other than temperature, soil N, and rainfall interacted to affect fluxes.

Though increases in N input accelerate N<sub>2</sub>O emissions (Shcherbak et al., 2014), microbial community differences between corn and switchgrass cropping systems may also impact the N<sub>2</sub>O produced, independent of N inputs. Plant species can affect N cycling by shaping the microbial community composition (Hawkes et al., 2005) including composition of the denitrifier community (Cavigelli and Robertson, 2001), and perennial systems can increase enzyme production and nitrogen mining (Hargreaves and Hofmockel, 2014). This experiment did not address the direct effects of microbial community composition, but it is known to be effected by drying/ wetting treatments (Peralta et al., 2013).

### ***N<sub>2</sub>O emissions as affected by rainfall pattern***

The 28-day rainfall interval in corn expressed the highest emissions and there were no cumulative differences for other rainfall treatments. In 2017 the magnitude of N<sub>2</sub>O fluxes for

switchgrass was higher than in 2016. There are responses to individual water additions at all rainfall intervals in both cropping systems.

### ***Seasonal patterns of N<sub>2</sub>O in response to changing rainfall regimes***

Effects of rainfall patterns were most pronounced in the summer, when soil N levels and soil temperatures were highest. In April and May, intensified rainfall patterns had little if any effect on N<sub>2</sub>O fluxes. In June and July, in contrast, the same rainfall patterns had very discernable effects. By early fall, however, effects were again small and in early winter effects were again not discernable. This is likely due to a different hierarchy of controlling factors by season in each cropping system.

N<sub>2</sub>O production mostly comes from nitrification and denitrification, which are largely influenced by soil mineral N (Davidson and Swank 1986), so it makes sense that I see the highest N<sub>2</sub>O production in the summer months after fertilizer is applied and when temperature is highest. During the summer the lack of response, when seen, may be due to plant N uptake's depressing soil N pools, as that is when plant N demand is the highest. In the spring and fall, when soil temperature is lower and soil moisture is greater (due to higher water reserves in the spring and less ET in the winter), microbial activity is lower, and the NOS enzyme may not be depleted as it was in summer months.

### ***Denitrification rates and NOS enzyme persistence***

NOS enzyme activity might explain different rainfall responses. Denitrification enzymes do not persist indefinitely and can disappear when not used for long periods of time such as during droughts when soil moisture is not amenable to denitrification and denitrifiers do not allocate resource to enzyme maintenance (G P Robertson and Groffman, 2015). The induction of NOS, which transforms N<sub>2</sub>O to N<sub>2</sub>, lags NIR induction, such that N<sub>2</sub>O could be produced before

NOS is able to further reduce  $N_2O$  to  $N_2$  (Cooper and Smith, 1963). This would result in high  $N_2O$  emissions in treatments with depressed NOS when conditions next become amenable to denitrification. I saw this in corn with the 28-day rainfall interval. Presumably, the 14-day interval was not sufficient to make NOS disappear in corn systems (I did not assay NOS activity for 14-day intervals). In contrast, in switchgrass systems NOS was persistent even through a 28-day interval, shown by the lack of  $N_2O$  emitted after rewetting, despite high NIR activity as indicated by total denitrification in DEA results (Figure 2.4). Many of the other soil variables I measured were not strongly correlated with  $N_2O$  production rates and this contrasts with many other studies (Burger et al., 2005; Liang et al., 2016). NOS enzyme was best related to  $N_2O$  emissions being greater in 2017 in the co-occurring June rewetting intervals.

While both nitrification and denitrification can produce  $N_2O$ , denitrification is accelerated when there are anaerobic conditions that I expect to see after rewetting soils. For example, in a nearby experiment, I found that 80-90% of  $N_2O$  is produced through denitrification after rewetting from isotopomer results not included in this dissertation. I saw the same total denitrification rates in switchgrass and corn treatments using the acetylene block method (Groffman et al. 1999) though for many reasons (Groffman et al., 2006) this method may underestimate actual denitrification and perhaps differentially by treatment.

Pulses of  $CO_2$  after rewetting is termed the "Birch effect", after its discoverer (Birch 1958). These pulses of  $CO_2$  are thought to be caused by both physical and biological factors, namely the changes in diffusion-limited substrates under drought and changes in microbial biomass (Evans et al. 2016). The same may be true for these pulses of  $N_2O$  following rewetting.

### *Implications for predicting future effects of changing rainfall patterns on N<sub>2</sub>O emissions*

The mechanisms that control N<sub>2</sub>O emissions in the future may shift with intensified precipitation patterns. Although N<sub>2</sub>O emissions are small compared to other nitrogen fluxes, they have a profound effect on atmospheric chemistry because N<sub>2</sub>O is the main stratospheric ozone depleting agent and a powerful greenhouse gas. Thus, intensified precipitation patterns could change the global warming impacts of future cropping systems.

This work challenges the assumption that with longer and more drier conditions, one might expect lower cumulative N<sub>2</sub>O emissions due to less consistent anoxic conditions. Results here show this may not be the case for long droughts in annual systems with high fertilizer inputs due to accelerated emissions upon drought cessation. Finding the controls on specific processes leading to N<sub>2</sub>O emissions in cropping systems will be critical to improving predictions and modeling in the response to more intensified precipitation regimes.

## Chapter 3: Consequences of Changing Rainfall Patterns on Nitrous Oxide Fluxes at Different Landscape Positions

### **Abstract**

Fluxes of nitrous oxide ( $\text{N}_2\text{O}$ ), a strong greenhouse gas produced by microbes in agricultural soils, are closely linked with soil moisture as moisture affects oxygen, nitrate, and carbon availability. Changing rainfall patterns due to climate change are thus likely to influence  $\text{N}_2\text{O}$  fluxes. I tested the hypothesis that changing rainfall patterns will alter  $\text{N}_2\text{O}$  fluxes in agricultural soils but with responses modulated by landscape position as positions affects soil texture and carbon contents. I used rainfall manipulation shelters to expose soils to the same amounts of rainfall delivered at different intervals (3-days, 14-days, and 28-days). Shelters were placed at both summit and toeslope positions within conventionally tilled corn fields in southwest Michigan, USA and  $\text{N}_2\text{O}$  fluxes were measured three times per week during the summer for two years.

Results show cumulative  $\text{N}_2\text{O}$  fluxes under normal conditions were 2.3x higher in toeslope positions than in summit positions, consistent with higher soil carbon and finer soil texture in toeslope positions. Large rewetting events (longer dry intervals) increased cumulative  $\text{N}_2\text{O}$  fluxes 32% in summit positions but did not change fluxes in toeslope positions. Changes in fluxes were associated with soil texture, inorganic N, and total C, but not C: N ratios. Knowledge of landscape patterns deserve inclusion in models of current and future climate change effects in order to better quantify and mitigate agricultural  $\text{N}_2\text{O}$  fluxes.

## Introduction

Climate change influences biogeochemical cycles through alterations in temperature and precipitation, at times unpredictably (Greaver et al., 2016). Throughout the Midwest, rising temperatures and more extreme rainfall patterns have been observed and are expected to continue (Pryor et al., 2014), impacting potentially important biogeochemical fluxes. Though all greenhouse gas emission scenarios project intensified temperature and precipitation trends (IPCC, 2014), climate model projections for temperature are more certain than projections for precipitation (Pryor et al., 2014). Likewise, models predicting the biogeochemical responses to the effects of temperature are more certain than the models predicting responses to precipitation change (Robertson et al., 2013). Thus, it seems especially critical to better understand biogeochemical impacts of changing rainfall regimes.

Agricultural nitrogen (N) cycles are likely to be particularly affected by changing rainfall patterns. Farmers manage soil nitrogen (N) from fertilizer and biological sources against losses from leaching, microbial processes, and plant uptake (Robertson and Vitousek, 2009), and N pollution to groundwater, surface waters, and the atmosphere represent major perturbations to the biosphere (Rockström et al., 2009). Dry periods and precipitation influence all soil biogeochemical processes, especially those that involve N (Greaver et al., 2016). Understanding N responses to changes in precipitation is important for designing future management strategies for mitigation and adaptation (Bowles et al., 2018; Robertson et al., 2013).

Nitrous oxide (N<sub>2</sub>O) as a greenhouse gas is an important source of contemporary radiative forcing (IPCC 2014), and agricultural soils represent the largest anthropogenic source. Soil N<sub>2</sub>O fluxes exhibit extremely high spatial and temporal variability, related to different

combinations of soil attributes including carbon (C), nitrogen, and oxygen availabilities, microbial community composition, and plant-microbe-soil interactions.

While we know much about the factors that control denitrification in the laboratory (primarily oxygen, nitrate, and available C), the complex regulation of these factors in situ creates variability that is difficult to quantify and model at field scales. High variability creates particular problems when scaling chamber-based measurements to the field or regional scale, which limits our ability to determine treatments that might best mitigate future emissions (Kravchenko and Robertson, 2015; McDaniel et al., 2014). In many ecosystems, these soil factors vary predictably by landscape position, with higher positions characterized by coarser soils with lower C contents and sometimes plant productivity, as compared to lower positions that are typically higher in clay, carbon, and associated properties (Beehler et al., 2017; Ladoni et al., 2016; Wickings et al., 2016).

Izaurre et al. (2004), for example, found along agricultural toposequences in Alberta and Saskatchewan greater N<sub>2</sub>O emissions in fine-textured depressions than in coarse-textured summit positions. Along a catena in Puerto Rico, McSwiney et al. (2001) found that N<sub>2</sub>O fluxes increased downslope as a function of redox status.

Other factors known to affect N<sub>2</sub>O production do not necessarily differ by landscape position. In Iowa, heterogeneous soil landscapes had higher microbial biomass nitrogen at depressions and toe slope positions, but not nitrate pools (Kaleita et al., 2017). And Groffman (1993) found across a tall grass prairie landscape in Kansas that denitrification was not affected by slope position, although N<sub>2</sub>O-only production was not evaluated. And a study in southwest Michigan showed higher N<sub>2</sub>O fluxes in depressions, with no additional effect of cover crop presence (Negassa et al., 2015).

Soil texture may be particularly important as it has also been found to strongly influence N<sub>2</sub>O fluxes in laboratory settings (Zhu et al., 2013). After flooding soil columns representing three landscape positions from Pennsylvania, matric potential was shown to be the strongest predictor of the timing of N<sub>2</sub>O fluxes across soils that differed in texture, structure, and bulk density (Castellano et al., 2010). Also in the laboratory, Krichels et al. (2019) showed that the ability of a soil to drain or pond water influenced N<sub>2</sub>O fluxes. In an Illinois laboratory study of N<sub>2</sub>O emissions, iron redox reactions were found to strongly influence N<sub>2</sub>O fluxes along micro-topographical gradients (Krichels et al., 2019).

Denitrification appears to be the dominant source of N<sub>2</sub>O in most mesic cropping systems (Aronson and Allison, 2012). Heterotrophic denitrifiers, mainly bacteria but also archaea and fungi, use soil organic C as an energy source and nitrate, which is thermodynamically similar to oxygen, as a terminal electron acceptor when oxygen is unavailable. Denitrification is often enhanced after precipitation (Bergsma et al., 2002; Gelfand et al., 2015) and especially in soil aggregates, rhizospheres, and decomposing litter, where soil microbes have ample access to C but limited access to oxygen (Khalil et al., 2004; A N Kravchenko et al., 2017; Loecke et al., 2008; Sexstone et al., 1988). The denitrification pathway products nitrite, nitric oxide, N<sub>2</sub>O, and dinitrogen are formed sequentially (Cooper and Smith, 1963), such that N<sub>2</sub>O is released to the soil atmosphere when denitrification is not carried out completely.

Although considered a minor source of N<sub>2</sub>O in comparison to denitrification, nitrifying archaea and bacteria also produce N<sub>2</sub>O, from either nitrifier denitrification or hydroxylamine decomposition following ammonia oxidation (Bremner and Blackmer, 1978). Since nitrifiers require oxygen (O<sub>2</sub>) to oxidize nitrite to NO<sub>3</sub><sup>-</sup>, nitrification proceeds fastest in aerobic conditions but can occur in soils with up to 60% water filled pore space (WFPS) (Abbasi and Adams,

2000). Nitrifier denitrification and coupled nitrification-denitrification can also produce  $N_2O$ , most of which appears to be from nitrifier denitrification stimulated by wet-dry cycles (Wrage-Mönnig et al., 2018; Wrage et al., 2001).

Regardless of its source, since both denitrification and nitrification are strongly affected by soil moisture (G P Robertson and Groffman, 2015),  $N_2O$  fluxes are likely to be influenced by changing rainfall patterns. In particular, the effects of longer dry periods during the growing season on C and N will likely influence  $N_2O$  emissions in inconsistent ways (Kim et al., 2012). Changing rainfall patterns are an aspect of climate change that may shift controls on biogeochemical mechanisms, leading to spatial variability and episodic events with higher than average influence on N fluxes (Bernhardt et al., 2017).

Understanding interactions of changing rainfall patterns and landscape positions may be useful for better predicting the effects of future climate changes on greenhouse gas fluxes from agricultural soils, especially if different landscape positions respond uniquely to changing rainfall patterns. In general, experiments exploring topographical positions have shown greater emissions in fine-textured depressions than in coarse-textured summit positions, as noted above. But little data is available to elucidate the effects of changing rainfall patterns on soil  $N_2O$  emissions across differing topographic positions.

In this study, I hypothesize that under more intensified rainfall patterns, the greater organic matter, moisture, nitrate, and proportion of smaller pore spaces in toeslope positions will support higher rates of  $N_2O$  production. Three specific questions follow:

1. How will  $N_2O$  fluxes differ between toeslope and summit positions that differ in OM and soil texture;
2. Will rainfall intensification magnify fluxes; and, if so

3. Will landscape position influence magnification differently in toeslope and summit positions?

## **Methods**

### ***Experimental Site***

I conducted this study at the Kellogg Biological Station (KBS) Long-Term Ecological Research site in Hickory Corners, Michigan, USA (42° 24'N, 85° 23'W 288 m elevation). Average temperatures at KBS range from -3.8°C (January) to 22.9°C (July) with an annual mean of 10.1°C. Yearly precipitation is 1005 mm and the 30-year average (from 1981) for monthly precipitation ranges from 40 mm in February to 100 mm in May (Robertson and Hamilton, 2015). About 17% of precipitation occurs during the winter months from January to April, with the rest evenly distributed throughout the growing season. Over the past 30 years, precipitation events from April to December were, on average, 2.5 mm and ~3.6 days apart; around eight events per season were greater than 25 mm (<https://lter.kbs.msu.edu/datatables/7>). Potential evapotranspiration exceeds rainfall for four months out of the year (Crum et al. 1990).

Soils at the site are well-drained mesic Typic Hapludalfs developed from glacial till and outwash (Collins and Crum 1995) with intermixed loess (Luehmann et al. 2016), and consist of co-mingled Kalamazoo (fine-loamy, mixed, semiactive) and Oshtemo (coarse-loamy, mixed, active) series soils (Robertson and Hamilton 2015). The predominant land use in the region is cultivated and successional fields, woodlots, residences, lakes, and wetlands.

Experiments were conducted in fields of at least 2 hectares growing corn, soybean, wheat, and alfalfa in various rotations. I used fields in the corn phase of these rotations in both 2016 (three replicate fields) and 2017 (four replicate fields). Corn was managed as per common practice in the region, planted in 76 cm rows and fertilized at 150 kg N ha<sup>-1</sup> in two applications:

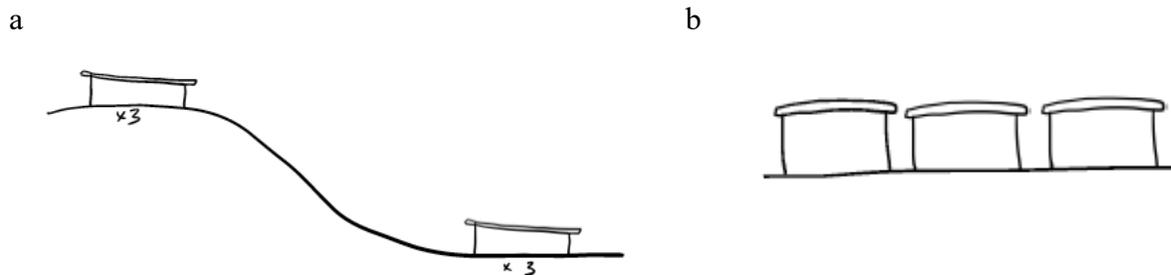
25 kg N at planting and the remainder side-dressed ~6 weeks later when plants were at the V6 stage. Experimental treatments were established at two topographical positions in each field: summit and toeslope, as determined using a LiDAR-based digital elevation model and using terrain parameters of slope, profile curvature, and relative elevation (Table 3.1).

Field	Highest Elevation (m)	Lowest elevation (m)	Length of slope (horizontal distance from highest to lowest elevation) (m)	Slope (°)
F80	280.6	274.0	202.5	3.3
F83	283.6	280.7	123.5	2.4
F85	287.7	283.3	126.6	3.5
F91	284.8	272.5	152.1	8.0
A0S	285.0	282.5	70.0	3.6
F98-04-01	285.2	281.8	80.6	4.2

**Table 3.1:** Field identification, summit elevation, toeslope elevation, length of slope, and slope degree of toposequences.

### *Rainfall Manipulation Shelters*

Small rainfall manipulation shelters were used to create rainfall environments whereby soils were exposed to different rainfall intervals, such that the same amounts of water were added to each shelter but at different times. There were three shelters at each landscape position in each field. The distance between each shelter was ~10 cm and rainfall treatments were assigned at random (Figure 3.1).



**Figure 3.1:** Sketch of experimental design a) from a profile view of two landscape positions in a field and b) outline of three shelters side by side.

Shelters were well-vented and  $61 \times 122 \times 40$  cm high and placed between corn rows; each shelter contained a cylindrical gas sampling frame (28.5 cm diameter  $\times$  30 cm high; 19.1 L) and sufficient soil surface area to allow periodic soil sampling. Shelters were constructed of polycarbonate (roofs), plexiglas (sides), and sheet metal (bottom edging). Bottom edging allowed 3 cm insertion into the soil to eliminate the potential for storm-derived lateral surface flow, which was in any case low to absent due to high infiltration rates in these well-drained soils. Three large holes 6.4 cm diameter drilled in the front and back sides of the shelters allowed airflow and eliminated humidity buildup.  $\text{N}_2\text{O}$  flux measurements taken inside and outside small shelters placed under a larger (10 x 5m) shelter showed no significant small shelter effects on fluxes during either low or high flux events (Glanville, in prep). Roofs were hinged at the back to allow for periodic soil and gas sampling. Each shelter covered a  $0.6 \text{ m}^2$  area.

### ***Rainfall Intervals***

Rainfall additions were based on a 30-year regional average calculated from 1984 to 2014. For each transect, to each of three shelters, per landscape position, rainfall was applied at either 3-day (long-term average for the region), 14-day, or 28-day intervals. The 3-day average was  $7.1 \pm 1.3$  mm (standard deviation) not accounting for interception by the canopy. Since the soil under each shelter was protected from ambient rainfall, the amount of water added was independent of unusually dry or wet weather; thus, the experiment was repeatable across two field seasons.

### ***Gas sampling***

$\text{N}_2\text{O}$ ,  $\text{CH}_4$ , and  $\text{CO}_2$  gas fluxes were measured an average of three times per week to capture responses to rainfall events using the static chamber method (Holland et al. 1999).  $\text{N}_2\text{O}$  and  $\text{CH}_4$  were measured using two techniques. First, for the "GC method" I took four chamber

headspace gas samples (20 mL) every 15 minutes for 60 minutes. The samples were stored over-pressurized in glass vials and analyzed for N<sub>2</sub>O, CO<sub>2</sub>, and CH<sub>4</sub> concentrations in the lab using a gas chromatograph (7890A Agilent Technologies Inc., DE, USA) equipped with an ECD for N<sub>2</sub>O and an infrared gas absorption analyzer (LI-820, LI-COR, NE, USA) for CO<sub>2</sub> (<http://lter.kbs.msu.edu/protocols/113>). Fluxes were calculated as the linear increase in N<sub>2</sub>O concentrations over the 60-minute closure period. The second technique was the "MICOS (Mobile Integrated Cavity Output Spectroscopy) method" that used a portable quantum cascade laser (QCL) analyzer (Los Gatos Research Inc., CA, USA) in the field for continuous chamber headspace measurements of N<sub>2</sub>O and CH<sub>4</sub> concentrations, and a closed-path infrared gas analyzer (IRGA; LI-7000, LI-COR, NE, USA) for CO<sub>2</sub> concentrations. During the measurement period, the headspace air inside each chamber was circulated through the QCL and IRGA analyzers for 5 minutes and gas concentrations were analyzed at 1 second intervals.

### ***Soil Sampling***

Soil moisture and temperature were measured every 10 minutes with sensors (Model 5TM, Decagon, WA, USA) installed vertically at the surface with readings stored on data loggers (EM50, Decagon, WA, USA). Soil was sampled to 20 cm depth for inorganic N, labile C, and dissolved organic C around co-occurring rewetting events, as described below. Soil texture was also measured in the shelter locations within each plot using the hydrometer method (<https://lter.kbs.msu.edu/protocols/108>). Here, 40 g of soil were shaken with 100 mL water for 24 hours using hexametaphosphate as a dispersant. The slurry was then put into cylinders, brought to 1000 mL with supplemental water, and shaken to suspend soil particles. Hydrometer and temperature readings were then taken over 8 hours. Since sand falls out of solution too

quickly to accurately record density changes, sand was determined separately with a 53  $\mu\text{m}$  sieve.

Once during the season, inorganic N was measured via 1 M KCl 100 mL extractions of 10g of fresh soil performed in duplicate. Soils were shaken on a shaker table overnight and allowed to settle before filtering. Extracts were frozen until analysis. A flow injector analyzer (QuickChem 8500 Series 2, Lachat Instruments, CO, USA) was used to determine nitrate and ammonium concentrations in extracts (<https://lter.kbs.msu.edu/protocols/33>). For gravimetric soil moisture 10 g of fresh soil were oven-dried at 60°C for 48 hours (<https://lter.kbs.msu.edu/protocols/24>).

For total C and N analyses soil samples were finely ground and analyzed in triplicate by combustion with an elemental combustion system (ECS 4010, Costech Analytical Technologies, Valencia CA, USA) using acetanilide as a standard (<https://lter.kbs.msu.edu/protocols/148>).

### ***Statistical Analysis***

All statistical analyses were performed using R software version 3.6.1 (R Development Core Team 2019) and with a significance value of  $P < 0.05$ . The statistical model included 2 landscape positions  $\times$  3 rainfall-interval treatments  $\times$  2 years and the interactions among them were considered fixed factors. Fields were considered a random factor. Landscape position is specified as the whole plot factor, the interaction between fields and landscape position is considered a random factor, and this interaction is used to test landscape position effects. The interaction among field, landscape position, and rainfall-interval treatment is specified as a random factor, in order to test for rainfall-interval effects. Rainfall-interval is the subplot factor. Analysis of variance was performed with the “lmer” function in the “lme4” package by considering landscape positions as a whole plot factor, and rainfall-interval treatments and years

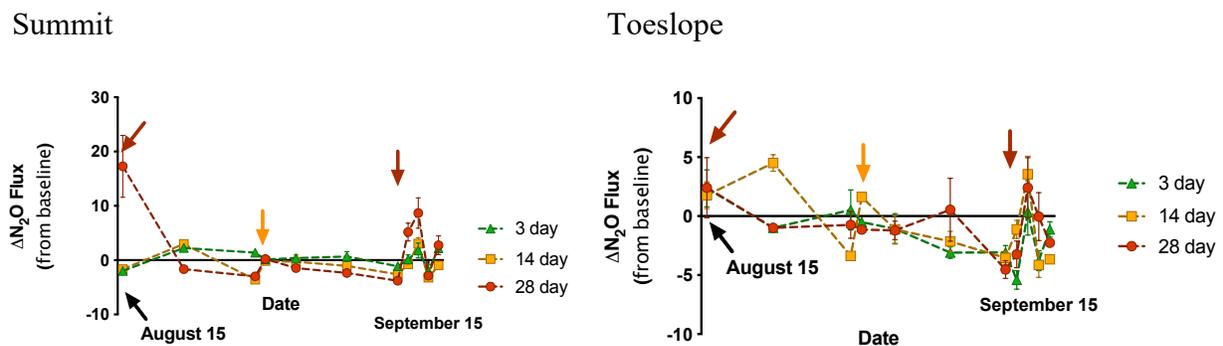
as subplot and sub-subplot factors. Normality of residuals was checked by plotting residuals against the fitted values, and no violations of assumptions were found. Homogeneity of variance assumptions were examined by the “leveneTest” function in the “car” package and no heterogeneous variance was detected. Regression analysis was used to explore the relationships between N<sub>2</sub>O flux and inorganic N, total C, and soil texture.

## Results

### *N<sub>2</sub>O emissions*

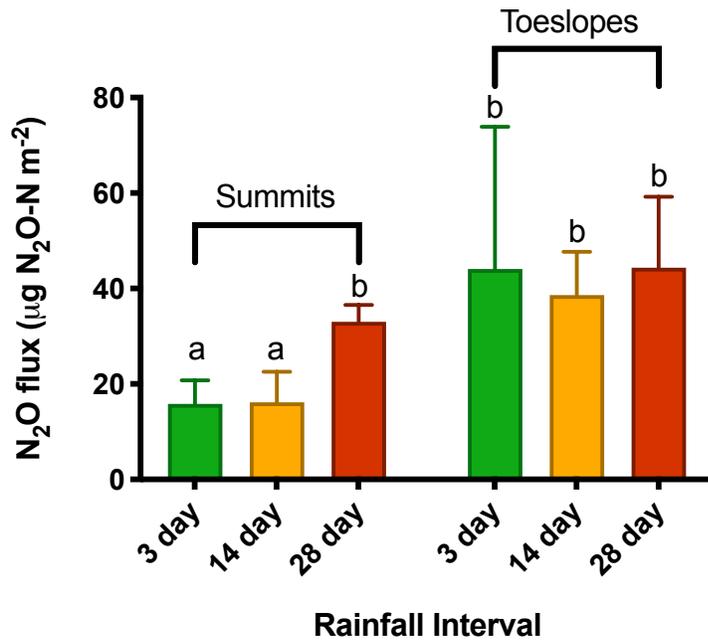
Daily N<sub>2</sub>O emissions in summits and toeslopes for 2016 and 2017 are shown in Figure 3.1. In summits, fluxes ranged from 0 to 245 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup> in 2016 and 0 to 251 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup> in 2017. In summits, higher fluxes occurred during the summer months of May, June, and July before declining to below ~1 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup> for August.

The toeslope position fluxes ranged from 5.1 to 678 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup> in 2016 and 0 to 718 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup> in 2017, with fluxes highest in May, June, and July, and lower in August. Flux measurements were not taken past August in either year since earlier experiments (Chapter 2) had shown no rainfall N<sub>2</sub>O responses outside of the main growing season.



**Figure 3.2:** Daily N<sub>2</sub>O response to different rainfall patterns in the summit and toeslope landscape positions in 2016 and 2017. Error bars represent standard errors of the mean, n=4 replicate plots (each with three rainfall intervals). Red arrows denote 28-day watering events. Months omitted lack temporal resolution.

Cumulative N<sub>2</sub>O emissions are sums of measured fluxes. Cumulative N<sub>2</sub>O emissions in summit and toeslope positions yielded similar trends for 2016 and 2017 (Figure 3.2). For summits, cumulative N<sub>2</sub>O in 2016 was highest under the 28-day rainfall interval (5420 ± 2351 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-2</sup>); fluxes under the 3-day and 14-day intervals were not significantly different from one another (3351 ± 1073 and 4229 ± 1040 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup>, respectively). In 2016 and 2017 toeslopes N<sub>2</sub>O fluxes under the 3-day, 14-day, and 28-day intervals were not significantly different from one another (in 2016: 8053.3 ± 954.9, 8281.2 ± 890.0, and 7924.4 ± 949.2 μg N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup>, respectively).



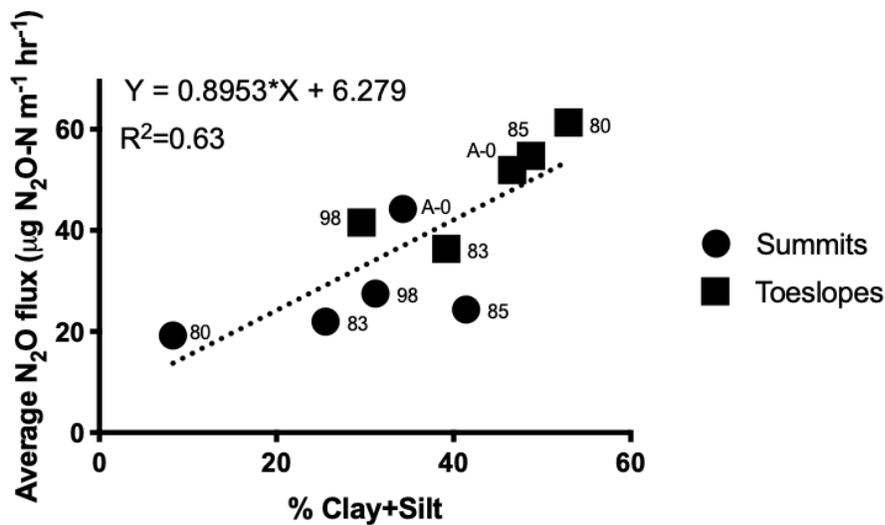
**Figure 3.3:** Cumulative N<sub>2</sub>O responses to different rainfall patterns in the summit and toeslope positions in 2016. Error bars represent standard errors of the mean, n=4 replicate plots (each with three rainfall intervals).

***Soil Properties: Soil texture, C:N, moisture, and temperature***

N<sub>2</sub>O fluxes increased linearly with % clay + silt content; in general, there was a higher percent sand in summit positions and lower percent sand in toeslope positions, resulting in a positive linear regression ( $r^2 = 0.63$ ,  $P < 0.05$ ; Figure 3.3). C: N ratios ranged from 8.6 to 13.6 (Table 3.2)

with no detectable relationship to N<sub>2</sub>O flux. Soil temperature ranged from 9.7 to 29.4 and 8.7 and 28.4 °C, in summits and toeslopes respectively. (Figure 3.4).

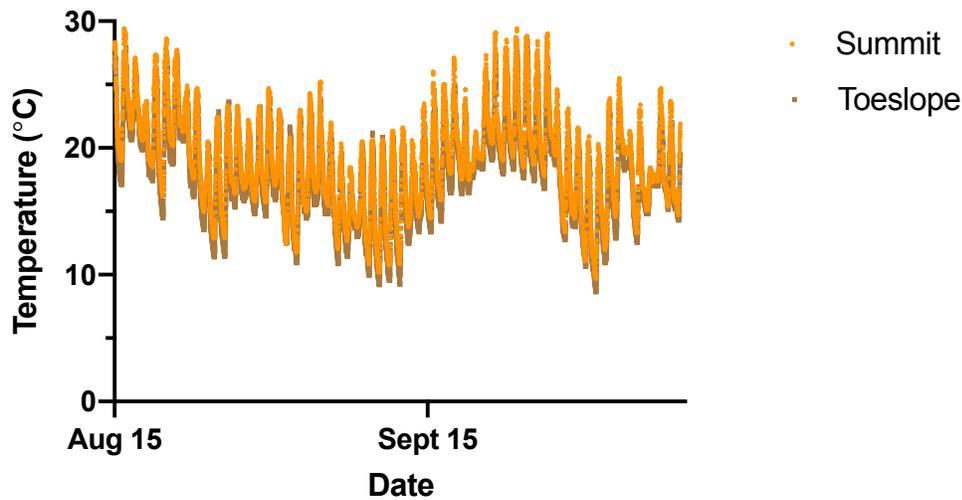
For soil moisture (Figure 3.5) in summit positions, the 14-day interval treatment had both the maximum and minimum values of 0.266 and 0.062 cm<sup>3</sup> cm<sup>-3</sup>, respectively. The 3-day interval had the highest mean VWC at 0.129 cm<sup>3</sup> cm<sup>-3</sup> and the 28-day interval had the lowest mean at 0.107 cm<sup>3</sup> cm<sup>-3</sup>. In toeslope positions, the lowest VWC was 0.003 cm<sup>3</sup> cm<sup>-3</sup> in the 28-day interval while the maximum was 0.289 cm<sup>3</sup> cm<sup>-3</sup>, also in the 28-day interval. The 3-day and 14-day intervals had the highest mean VWC at 0.122 and 0.125 cm<sup>3</sup> cm<sup>-3</sup> respectively, while the 28-day interval in the toeslope had the lowest mean VWC at 0.077 cm<sup>3</sup> cm<sup>-3</sup>.



**Figure 3.4:** Percent clay + silt by average N<sub>2</sub>O flux for the 3-day rainfall interval. Error bars are omitted for clarity. The dashed line represents a linear regression had significant effects (P<0.05).

	Total N (%)	Total C (%)	C:N	Inorganic N (mg kg <sup>-1</sup> )	
				NO <sub>3</sub> -N	NH <sub>4</sub> -N
Toeslopes	0.09±0.01	0.90±0.14	10.0±0.13	6.1± 4.5	14.5 ± 2.1
Summits	0.07±0.01	0.73±0.09	10.7±0.29	1.9 ± 0.5	3.1 ± 1.6

**Table 3.2:** Mean values for five fields with standard error for total C, total N, C:N, and inorganic N.



**Figure 3.5:** Soil temperature fluctuations in summit and toeslope positions.

## Discussion

### *N<sub>2</sub>O dynamics by landscape position (Question 1)*

How do N<sub>2</sub>O fluxes differ between toeslope and summit positions as influenced by OM and soil texture? Average N<sub>2</sub>O fluxes at toeslope positions were higher than for summits, by a factor of 2.2 for normal rainfall intervals and 1.7 for 28-day intervals. Across both positions, fluxes were comparable to those measured for the unsheltered portions of neighboring fields planted to corn in prior years (Gelfand et al., 2016; Oates et al., 2016). Higher fluxes in toeslope positions appears associated with higher total C (Table 3.2) and higher % clay and silt (Figure 3.3).

These results agree with other studies that found higher OM and N<sub>2</sub>O emissions from toeslopes than from summits at the same site (Negassa et al., 2015) and as well over larger regions (Florinsky et al., 2009). That soil texture is highly correlated with average N<sub>2</sub>O flux in the 3-day rainfall interval is consistent with laboratory incubation studies (Castellano et al., 2010; Zhu et al., 2013). This is likely because of textural effects on WFPS, which in turn controls aeration (Schjønning et al., 2003).

### ***N<sub>2</sub>O emissions as affected by rainfall pattern (Questions 2 and 3)***

Will rainfall intensification magnify fluxes and, if so, will landscape position influence this magnification differently in toeslope positions? The 28-day rainfall interval in corn accelerated N<sub>2</sub>O emissions – cumulative fluxes were 2x higher than cumulative fluxes for the normal rainfall interval, but only for summit positions. The higher N<sub>2</sub>O fluxes in toeslope positions were not affected by rainfall intervals. Figure 3.1 shows responses to individual water additions at all rainfall intervals in both landscape positions.

Though my work did not elucidate why these soils responded differently by landscape position upon rewetting, other studies show the response is largely dependent on drainage ability and matric potential (Castellano et al., 2010; A. Krichels et al., 2019; Syväsalö et al., 2004). However, these studies did not vary rewetting treatments by amount or interval, so more can be done to understand the impact of changing rainfall patterns.

### ***Factors associated with N<sub>2</sub>O emissions***

Variations in N<sub>2</sub>O production between the two years and positions did not appear related to temperature or C:N patterns over the period examined. A similar absence of relationship to C:N and N<sub>2</sub>O fluxes have been found in other studies (Castellano et al., 2010; Syväsalö et al., 2004). However, I found higher inorganic soil N in toeslope positions (Table 3.2), where N<sub>2</sub>O emissions were high, than in summit positions. Greater resource for nitrification, which produces N<sub>2</sub>O through the oxidation of ammonia (NH<sub>3</sub>), and for denitrification, which produces N<sub>2</sub>O through the reduction of nitrate (NO<sub>3</sub><sup>-</sup>), could both produce more N<sub>2</sub>O (Saha et al., 2017). However, since N<sub>2</sub>O fluxes increase after rewetting when anaerobic microsites proliferate, I infer that higher toeslope position fluxes are from denitrification. Li et al. (2018) found topography to explain the greatest amount of variation in denitrification potential in the Delmarva Peninsula.

### ***Implications for predicting future effects of changing rainfall***

In corn systems, N<sub>2</sub>O emissions will be influenced by predicted intensified precipitation regimes but differently across a field. In corn systems, N<sub>2</sub>O fluxes may be small in comparison to other N losses like total denitrification or nitrate leaching, but N<sub>2</sub>O fluxes have a disproportionately large impact on the radiative forcing of the atmosphere since N<sub>2</sub>O is a powerful greenhouse gas. Results also suggest that including information on landscape position and rainfall intensification in calculations of field-scale N<sub>2</sub>O fluxes would lead to more accurate landscape and regional contributions of N<sub>2</sub>O emissions in corn systems. Additionally, the greatest opportunity for N<sub>2</sub>O mitigation from fields with varying topography is in toeslope positions, where N<sub>2</sub>O emissions are high.

Chapter 4: Impacts of Changing Precipitation Patterns on Soybean (*Glycine Max* L.)  
Biological Nitrogen Fixation as Mediated by Landscape Position and Tillage

**Abstract**

Cropping systems receive N additions primarily from organic amendments, synthetic fertilizers, and biological N<sub>2</sub> fixation (BNF). These processes, most notably the use of fertilizer, have accounted for dramatic anthropogenic increases in reactive nitrogen (N<sub>r</sub>) on Earth over the past 100 years; currently ~25% of terrestrial BNF is from soybean cultivation. BNF differs across cropped fields, which may be related to soil properties that covary with soil texture and water availability.

Changes in rainfall patterns associated with climate change will create changes in the timing of N-mineralization and other N transformations, potentially promoting or suppressing BNF. Given these changes and the importance of BNF to legume crop productivity, soil fertility, the productivity of subsequent non-leguminous crops, and N<sub>r</sub> in the environment, it seems prudent to understand potential effects of future changes in rainfall on BNF. Since BNF may be affected by soil organic matter (OM) and texture, two primary determinants of soil moisture, one might expect the strength of BNF responses to changes in rainfall to vary by tillage and topographic position as affected by OM and texture. In particular, there may be larger impacts of rainfall changes at summits and in tilled systems where there is less OM and less water holding capacity relative to toeslopes and no-till systems, respectively, and therefore greater potential impacts on BNF.

Here I test the hypotheses that BNF is sensitive to changing rainfall patterns in till vs. no-till and in summit vs. toeslope positions due to associative relationships in texture and OM. I measured soybean BNF by <sup>15</sup>N natural abundance using nodulating and non-nodulating isolines

to test effects of landscape position, its potential interaction with rainfall, and tillage management. Soybean BNF was 41% higher at summit than toeslope positions, consistent with soil OM and texture patterns. Percent BNF (%BNF) is the fraction of N from BNF divided by the total N in the plant. When precipitation was increased by 20%, BNF decreased from 75.7 to 51.7 %BNF at summit positions, though was unaffected at toeslope positions. BNF response to 3-week rainfall intervals also differed by tillage. In no-till plots, %BNF decreased 15% with less intense rainfall patterns in conventionally tilled fields, whereas BNF increased 14% with less intense rainfall in no-till plots. Results reinforce the importance of topographic position for predicting soybean BNF responses to changing rainfall patterns and show that summit positions are more sensitive to additional rainfall. Results also show changes in rainfall intensity affect BNF in tilled differently than in no-till soils, perhaps because greater retention of organic matter in no-till systems results in less available plant N. Models that incorporate these interactions will be better able to characterize legume crop performance and N use across landscapes and improve global estimates for BNF.

## Introduction

Cropping systems receive nitrogen (N) additions primarily from organic amendments, manufactured fertilizers, and biological N<sub>2</sub> fixation (BNF). These inputs, most notably the use of fertilizer, account for dramatic increases in reactive N (N<sub>r</sub>) on Earth over the last 100 years (Vitousek et al., 2013). When not taken up by plants or soil microorganisms, N<sub>r</sub> can be lost from cropping systems and become pollutants in water and the atmosphere. BNF contributes ~25% to N<sub>r</sub> inputs to the biosphere (Vitousek et al., 2013); soybeans (*Glycine max* L.) in particular contribute 10.4 Tg N<sub>r</sub> yr<sup>-1</sup>, representing ~18% of total global BNF inputs (Gelfand and Robertson, 2015). Soybean BNF can substitute for N fertilizer application and has a lower environmental cost since systems with BNF as a major source of N<sub>r</sub> require less N fertilizer and tend to have lower hydrologic N<sub>r</sub> losses (Blesh and Drinkwater, 2013; Syswerda et al., 2012). Thus, the sustainability of food production systems can be aided by BNF management and associated feedbacks (Pearson, 2007).

BNF transforms N<sub>2</sub> gas from the atmosphere into a form that can be incorporated into the tissue of certain plants, and global rates of BNF have increased due to agricultural intensification (Herridge et al., 2008). BNF is an energetically expensive process for plants since it requires breaking the N<sub>2</sub> molecule's triple bond when transforming it to NH<sub>3</sub> and then to amino acids. In legumes such as soybeans, BNF occurs through a symbiotic association between plants and bacteria such as *Bradyrhizobium japonicum* (Bottomley and Myrold, 2015) and thus depends on interacting factors that affect crop growth and associated N demand (Salvagiotti et al., 2008). Long-term studies of farms and fields planted to soybeans show a wide range of soybean BNF rates across management and fertilization gradients, with greater N availability's generally

suppressing BNF (Gelfand and Robertson, 2015; Grossman et al., 2011; Oberson et al., 2007; Tamagno et al., 2018).

Soil physical characteristics influencing BNF include redox state and water availability. Chemical controls include the availability of inorganic N and N fixation cofactors such as iron (Fe), molybdenum (Mo), and vanadium (V). Biological factors include plant genotype and growth rates; rhizobia numbers, diversity, and effectiveness; and pests and pathogens (Unkovich et al., 2008). Thus, BNF is influenced by many of the same agronomic and ecological factors that affect plant nutrition, water availability, herbivory, and disease (Valentine et al., 2010).

BNF can supplement N availability to future crops but can also result in decreased N fertility (Bottomley and Myrold, 2015; Salvagiotti et al., 2008) when N exported through legume harvest is greater than N fixed through BNF (Ciampitti and Salvagiotti, 2018).

Global calculations of BNF assume that BNF rates are homogenous over individual fields, but soybean BNF has been shown to vary with soil properties. In southwestern Victoria, Australia, for example, in a survey of 71 dairy pasture sites rates of BNF in white clover (*Trifolium repens*) ranged from 0 to 100% across different soil textures, though were 7% higher on light-textured soils (Riffkin et al., 1999). In a Denmark pea (*Pisum sativum* L.) field sampled at 56 points, BNF ranged from 26% to 81% of total plant N (Hauggaard-Nielsen et al., 2010). At sites in Central New York, USA, soybean BNF at various farms differed was affected controls in different soil types (Schipanski et al., 2010).

Climate change, particularly changes in rainfall intensity and amount, may also affect BNF. The US Midwest, an intensive agricultural region responsible for >80% of US soybean production (USDA NASS 2018), has experienced increasingly more intense and frequent heavy rainfall over the past century (Pryor et al., 2014). Furthermore, global circulation models predict

that both the increasing length of dry intervals between precipitation events and the amount of precipitation falling in single events will further increase (Pryor et al., 2014). In Michigan, both the number of extreme precipitation events and observed annual precipitation amounts are increasing (Frankson and Kunkel, 2017). Changes in rainfall will likely be accompanied by changes in the timing of N-mineralization and other N transformations (Bowles et al., 2018; Robertson et al., 2013), potentially promoting or suppressing BNF.

Given these changes and the importance of BNF to legume crop productivity and as well to soil fertility and subsequent non-leguminous crops, it seems prudent to examine potential effects of future climates on BNF. Since BNF appears related to soil organic matter (OM) and texture, two primary determinants of soil moisture, one might expect the strength of BNF responses to changing rainfall patterns to vary across a field as OM and texture vary, particularly along toposequences. Impacts of rainfall changes at summits may be greater than at toeslopes, for example, where there is usually more OM, clay, and water holding capacity and thus more buffering against rainfall variability.

In general, BNF rates are lower with drought conditions due to nodule sensitivity to decreased phloem flow (Serraj et al., 1999). Studies investigating the interactive effects of N fixation and drought have mostly been performed in controlled greenhouse settings, where plants are not exposed to normal environmental weather and soil conditions and the rhizobia-plant symbiosis does not reflect the impact of rainfall changes. Furthermore, growth in artificial environments alters nodules, as well as nodule depth and distribution (Pueppke, 1986). Field experiments are needed to more accurately quantify fixation responses to changes in topography, rainfall, and tillage. This will aid models for predicting BNF under the varying crop, soil, and

meteorological conditions that may account for the spatial variation of BNF changes with rainfall.

Here I test the hypotheses that BNF is vulnerable to changing rainfall regimes in summit vs. toeslope topographic positions and, separately, in till vs. no-till management as associated with differences in texture and OM. I address three specific questions:

1. How does soybean BNF vary by topographic position as affected by OM and its influence on N-mineralization and water availability;
2. Does BNF in summit and toeslope positions differ in response to added precipitation; and
3. Do changes in precipitation intensity, in particular the length of time between rainfall events, influence soybean BNF in tilled plots differently from no-tilled plots?

### **Experimental Design and Site Description**

The study was conducted over four field seasons (2015-2018) at the Kellogg Biological Station (KBS) Long-Term Ecological Research site, Hickory Corners, Michigan, USA (42° 24'N, 85° 23'W 288 m elevation). Average temperatures at KBS range from -3.8°C (January) to 22.9°C (July) with an annual mean of 10.1°C. Yearly precipitation is 1005 mm on average (30-year mean) and the 30-year monthly precipitation rate ranges from 40 mm month<sup>-1</sup> in February to 100 mm month<sup>-1</sup> in May (Peters et al., 2013). About 17% of precipitation occurs during the winter months from January to April, with the rest evenly distributed throughout the growing season. Over the past 30 years, precipitation events from April to December were, on average, 2.5 mm and ~3.6 days apart; around eight events per season were greater than 25 mm (<https://lter.kbs.msu.edu/datatables/12>). Potential evapotranspiration exceeds rainfall for four months out of the year (Crum et al., 1990).

Soils at KBS are well-drained mesic Typic Hapludalfs developed from glacial till and outwash (Collins and Crum 1995) with intermixed loess (Luehmann et al., 2016) and of co-mingled Kalamazoo (fine-loamy, mixed, semiactive) and Oshtemo (coarse-loamy, mixed, active) series (Crum and Collins, 1995). The predominant land use in the region is cultivated and successional fields, woodlots, residences, lakes, and wetlands. Fields used are outlined in Figure 4.1.

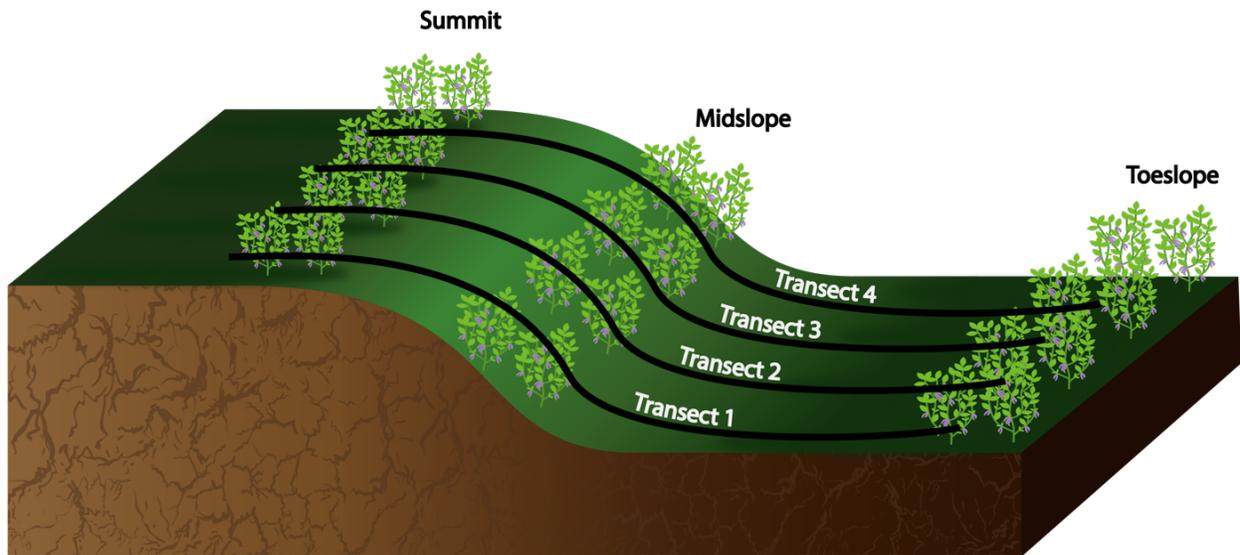
The experiments were conducted on conventionally tilled fields used for corn, soybean, and wheat planted to soybeans during the year of this study. These rainfed fields were managed as per regional practice, including fertilization with potassium ( $K_2O$ ), phosphorus ( $PO_4^-$ ), and lime as recommended by soil tests. All fields were planted at  $150,000$  seeds  $ha^{-1}$  in 15-inch rows. Glyphosate was used to control weeds during soybean years.

For all experiments I quantified soybean BNF by  $^{15}N$  natural abundance using nodulating and non-nodulating isolines, as described below.



**Figure 4.1:** Kellogg Biological Station aerial view with fields used in 2015 in red; fields used in 2016 in green; fields used in 2017 outlined in yellow; and fields used in 2018 outlined in light blue.

### *Toposequence Experiment (Question 1)*



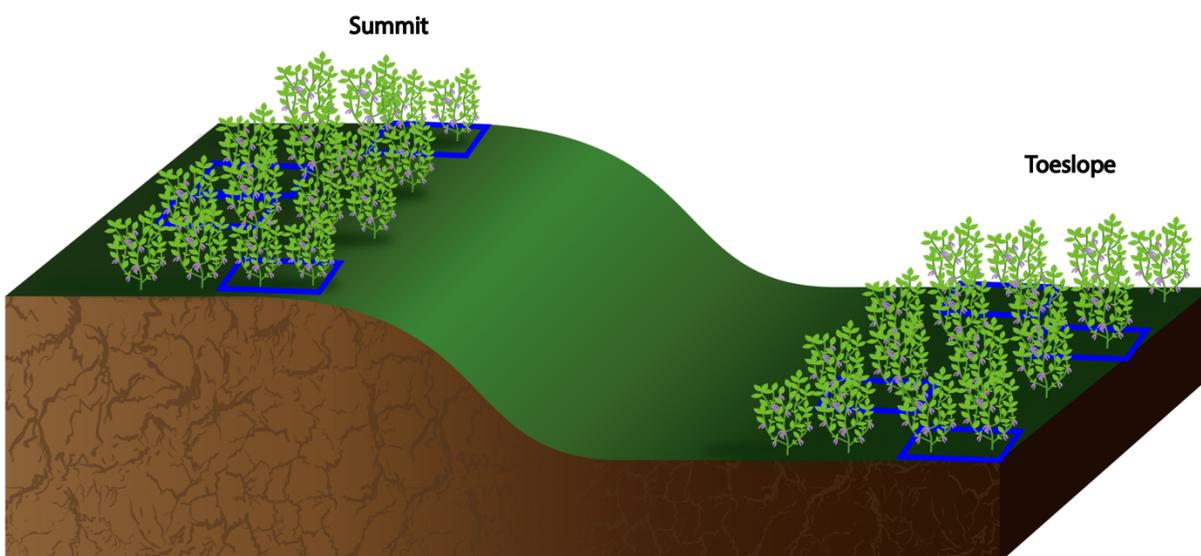
**Figure 4.2:** A illustration showing positions of transect, landscape positions, and soybean plot locations for the toposequence experiment.

For the toposequence experiment, I used three fields of 6-8 ha each (KBS fields 30-1, 38, and 79-8 south). Soybeans were planted in 2015 along four transects in three landscape positions (summit, midslope, and toeslope) in each field (Figure 4.2; Table 4.1). Plots were  $1 \times 1$  m with five non-nodulating plants per plot, at normal planting density and the same density as in the rest of the field. For this 2015 topography experiment, at physiological maturity plants were harvested both above and below ground and separated into above-ground vegetative biomass, belowground biomass, and seeds as detailed below.

Field	Highest Elevation (m)	Lowest Elevation (m)	Length of Slope (horizontal distance from highest to lowest elevation) (m)	Slope (°)
30-1	288.3	276.7	194.6	6.0
38	288.3	282.6	324.4	1.8
79-8 north	288.3	284.4	116.6	3.4
79-8 south	287.7	280.5	310.0	2.3
80	280.6	274.0	202.5	3.3
82-1	282.7	273.4	235.1	4.0
87	281.3	272.8	136.0	6.2
89+90	285.1	279.8	204.0	2.6
94	282.5	272.1	494.1	2.1

**Table 4.1:** Field identification, summit elevation, toeslope elevation, length of slope, and slope degree of fields used.

***Rainfall Amounts by Landscape Position Experiment (Question 2)***



**Figure 4.3:** A illustration showing positions the landscape positions and rainfall addition plot locations for the rainfall amounts by landscape position experiment.

For the rainfall addition experiment, I used three fields of 2-12 ha each (KBS Fields 79-8 north, 82-1, and 87 in 2016; and 80, 89+90, 79, and 94 in 2017). Soybeans were planted at four toeslope and four summit locations in each field; there were not transects as in the previous experiment (Figure 4.3). Each location had a plot  $2 \times 2$  m with 15 nodulating plants and 15 non-nodulating plants, at the same density as the rest of the field. From planting to harvest one half of

each plot was rainfed and the other half was irrigated with 2.5 mm of water when dry for three days. Water application was allowed to infiltrate completely so water did not spread neighboring plants. Planting date, harvest date, ambient rainfall, and additional rainfall are shown in Table 4.1. Soybean plants were harvested aboveground and separated into seed and vegetative biomass. Roots were not sampled.

Year	Planting Date	Harvest Date	Ambient Rainfall (mm)	Additional Rainfall (mm)	Precipitation Increase (compared to ambient rainfall) (%)
2016	June 22	September 28 and 29	158	40	20.2
2017	June 22	September 28 and 29	331	37.5	10.2

**Table 4.2:** Planting date, harvest date, ambient rainfall, and additional irrigation for 2016 and 2017.

***Rainfall Intervals by Tillage Experiment (Question 3)***



**Figure 4.4:** A illustration showing how rainfall intervals were conducted, with and without a shelter, in tilled and no tillage soils for the rainfall intervals by tillage experiment.

I conducted the tillage experiment in four replicate 1 ha plots in each of the conventional and no-till treatments of the KBS LTER main cropping system experiment (MCSE; Robertson and Hamilton, 2015). In 2015, in one 4 x 4 m subplot per replicate I imposed a rainfall interval of

two weeks for the period between soybean planting and harvest; adjacent control subplots received ambient rainfall (Figure 4.4). Rainfall was excluded by complete-exclusion rainfall manipulation shelters ( $5 \times 5 \times 1.5$  m high as described in Hess et al. 2019) and irrigated with captured rainwater delivered via overhead sprinkler heads. The experiment was repeated in 2018 but with a three-week interval. Soybeans plants were harvested aboveground and separated into seed and vegetative biomass. Roots were not sampled.

Year	Planting Date	Harvest Date	Ambient Rainfall (mm)	Rainfall Interval
2015	May 19 - 21	October 2	659	2 Weeks
2018	May 25 - 28	September 29	387	3 Weeks

**Table 4.3:** Planting date, harvest date, ambient rainfall, and additional irrigation for 2015 and 2018.

## Methods

### *Biological Nitrogen Fixation*

I quantified soybean BNF by using  $^{15}\text{N}$  natural abundance in nodulating (Pioneer P22T69R) and non-nodulating (PI 547695, seed source: 04U-3266; Horosoy variety) soybean isolines obtained from the USDA Soybean Germplasm Collection (USDA-ARS, Urbana, IL). To plant the non-nodulating isolines, I removed nodulating seeds from the soil and replaced them with non-nodulating seeds. Seeds were planted in late May and early June and plants harvested in late September and early October at R6.5 (physiological maturity).

### *Soil Analyses*

Soil was sampled in all experiments by compositing four 2.5 cm diameter  $\times$  25 cm depth push-probe soil cores on each sample date in each subplot replicate. Soils were passed through a 4 mm mesh screen and analyzed for texture, inorganic N, N mineralization, organic matter, gravimetric moisture, and pH at the time of peak N fixation mid-season (Table 4.4).

<b>2015</b>	<b>2016 and 2017</b>	<b>2018</b>
<b>Topography Study</b> -BNF of all plant parts separately -N mineralization -organic matter -pH -gravimetric moisture	<b>Topography Study with Rainfall</b> -BNF of seeds only -soil texture -organic matter -pH	<b>Conventional Tillage vs No-till by 3-day vs 21-day interval</b> -BNF of seeds only -soil texture -organic matter -pH
<b>Conventional Tillage vs No-till by 3-day vs 14-day interval</b> -BNF of seeds only -soil texture -organic matter -pH		

**Table 4.4:** Experiment of each year with associated measurements.

Soil texture was measured using the hydrometer method

(<https://lter.kbs.msu.edu/protocols/108>). Forty g of air-dried soil were shaken for 24 hours in 200 mL jars using sodium hexametaphosphate as a dispersant. The slurry was then put in 1 L cylinders and supplemented with water. Hydrometer and temperature readings were taken over 8 hours. Since sand falls out of solution too quickly to accurately record density changes, to supplement hydrometer readings sand from a separate sample was sieved out with a 53 µm mesh.

Soil pH was measured for two duplicate subsamples. A slurry of 15 g of field moist soil and 30 mL deionized water was shaken by hand for 10 seconds than allowed to settle for 30 minutes before measuring pH (VWR International, Randor, PA)

(<https://lter.kbs.msu.edu/protocols/163>)

For 2015, Fe, K, P contents were analyzed on composite soil samples by atomic absorption following extraction with 0.1 M hydrochloric acid by the MSU Soil and Plant Nutrient Laboratory (East Lansing, MI). Soils were also by comparable treatment and analyzed

for Fe, K, P by the Cornell Nutrient Analysis Laboratory (Ithaca, NY) using the Modified Morgan analysis.

Inorganic N was measured via a 1 M KCl 100 mL extraction of 10g of fresh soil performed in duplicate. A flow injector analyzer (QuickChem 8500 Series 2, Lachat Instruments, CO, USA) was used to determine nitrate and ammonium concentrations (<https://lter.kbs.msu.edu/protocols/33>).

Potential N mineralization was measured via a 28-day laboratory incubation where 10 g of soil were held at 60% WFPS in a 25 °C incubator. Inorganic N was measured by extracting 10 g of soil in a 100 mL solution of 1 M KC, followed by shaking and filtration.

Organic matter was measured via loss on ignition whereby soil was weighed into crucibles and placed in a 500° C oven for two hours. Weights were recorded before and after combustion and adjusted for soil moisture.

For gravimetric soil moisture 40 g of fresh soil were oven-dried at 60°C for 48 hours (<https://lter.kbs.msu.edu/protocols/24>).

### ***Plant Analysis***

Whole plants were harvested at physiological maturity (R6.5) to determine total nitrogen fixed and soybean biomass. For root harvest in 2015 (toposequence experiment), coarse and fine roots were collected within a 25 × 25 × 25 cm soil volume. Soil was then carefully shaken from roots in the field and roots were examined for the presence or absence of nodules. Roots were then placed on a 0.1 mm screen and washed gently with water to remove adhering soil. All plants from the same replicate subplot were composited and dried to a stable weight in a 60° C forced air oven. Plant material was divided into grain, aboveground vegetative biomass (stem, leaves, and pods, less grain), and, when sampled, belowground biomass (roots including nodules).

Biomass was weighed and ground to pass through a 1 mm sieve and 3-5 mg of homogenized plant material was weighed into tins. Packed tins were analyzed for  $^{15}\text{N}$  and %N at the University California - Davis and Michigan State University isotope facilities. The ash correction was determined by burning subsamples in a muffle furnace at  $550^\circ\text{C}$  for four hours and weighing the remaining residue.

### ***BNF calculations***

The  $^{15}\text{N}$  abundance method for BNF allows for end-of-season sampling to represent growing season fixation (Unkovich et al., 2008). The  $^{15}\text{N}$  is used to distinguish plant N derived from soil vs. air (air contains very little  $^{15}\text{N}$  compared to soil). The fraction of N derived from the atmosphere (fNdfa) is:

$$\text{fNdfa} = (\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{fix}}) / (\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{b}}),$$

where  $\text{N}_{\text{ref}}$  represents tissue from non- $\text{N}_2$ -fixing soybean isolines,  $\text{N}_{\text{fix}}$  represents tissue from  $\text{N}_2$ -fixing soybean isolines, and  $\text{N}_{\text{b}}$  represents tissue from  $\text{N}_2$ -fixing soybean isolines grown with atmospheric  $\text{N}_2$  as the only N source.

I used the  $\delta^{15}\text{N}_{\text{b}}$  value determined by Gelfand and Robertson (2015), who used the same commercial variety grown in N-free sand culture in a KBS greenhouse with N-free Hoagland's solution (0-7-5 NPK with micronutrients; GreenCare Fertilizers, Chicago, IL, USA).

### ***Statistical Analysis***

#### **Project 1:**

All statistical analyses were performed using R software version 3.6.1 (R Development Core Team 2019) and with a significance value of  $P < 0.05$ . I fit the BNF data with a linear mixed

model using the “lme4” package with landscape positions as a fixed factor, and fields and transects as random factors. To compare BNF differences among landscape positions, pairwise T-tests were conducted with the “lsmeans” package.

### **Project 2:**

The statistical model included two rainfall treatments and two landscape positions and the interactions between them were considered fixed factors. Fields were considered a random factor. Landscape position was specified as the whole plot factor, the interaction between fields and landscape position were considered a random factor, and this interaction was used to test landscape position effects. Analysis of variance was used by considering landscape positions as a whole plot factor, and rainfall treatments as a subplot factor.

### **Project 3:**

The statistical model included two rainfall treatments and two tillage treatments and the interactions between them were considered fixed factors. Blocks were considered a random factor. Tillage treatment was specified as the whole plot factor, the interaction between block and tillage treatment was considered a random factor, and this interaction was used to test tillage treatment effects. Analysis of variance was used by considering crops as a whole plot factor, and rainfall-interval treatments as a subplot factor.

### **For all:**

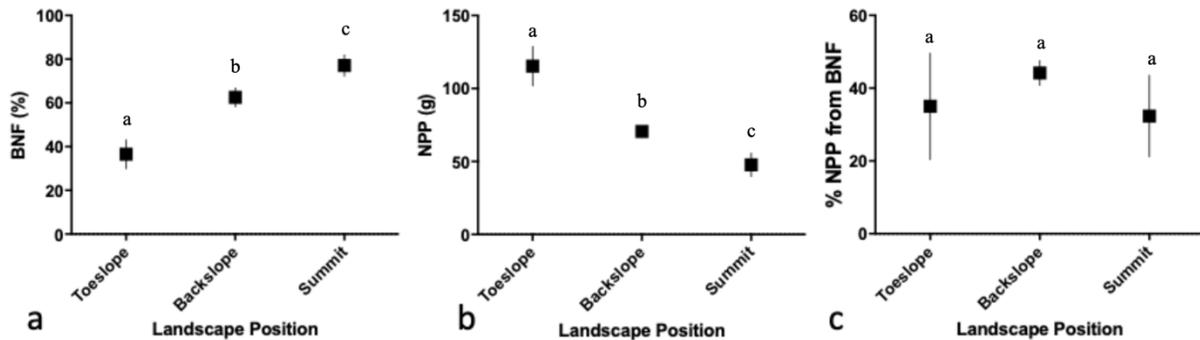
Normality of residuals was visually checked by plotting residuals against fitted values, and no violations of assumptions were found. Homogeneity of variance assumptions were examined by the “leveneTest” function in the “car” package and no heterogeneous variance was detected by Levene’s test. Simple linear regressions were used to determine the relationship

between %BNF and soil texture, OM, pH, and fixation cofactors, holding %BNF as the dependent variable.

## **Results**

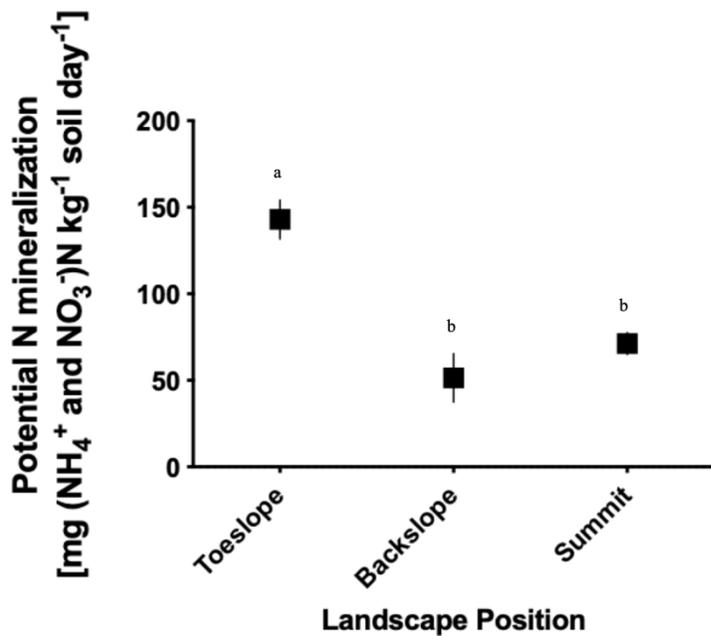
### ***Toposequence Experiment (Question 1)***

BNF contributed  $77.1 \pm 4.9\%$  (standard error of the mean) to the N content of grain in summit positions and  $36.5 \pm 6.6\%$  at toeslope positions. Backslope landscape positions were intermediate with a BNF contribution of  $62.6 \pm 4.4\%$  (Figure 4.5a). Measured net primary productivity was highest in toeslope positions ( $115.3 \pm 13.5 \text{ g plant}^{-1}$ ) and lowest in summit positions ( $47.7 \pm 8.1 \text{ g plant}^{-1}$ ) (Figure 4.5b). I found no differences in BNF contributions to aboveground or belowground vegetative tissues; only in seeds was there a BNF by landscape position effect. The %NPP from BNF showed no significant trends with landscape position (Figure 4.5c).



**Figure 4.5:** Percent BNF by landscape position (a), NPP by landscape position (b), and %NPP from BNF (c). Error bars represent standard errors of the mean, n=4 replicate plots. Asterisks indicate significant effects (P<0.05).

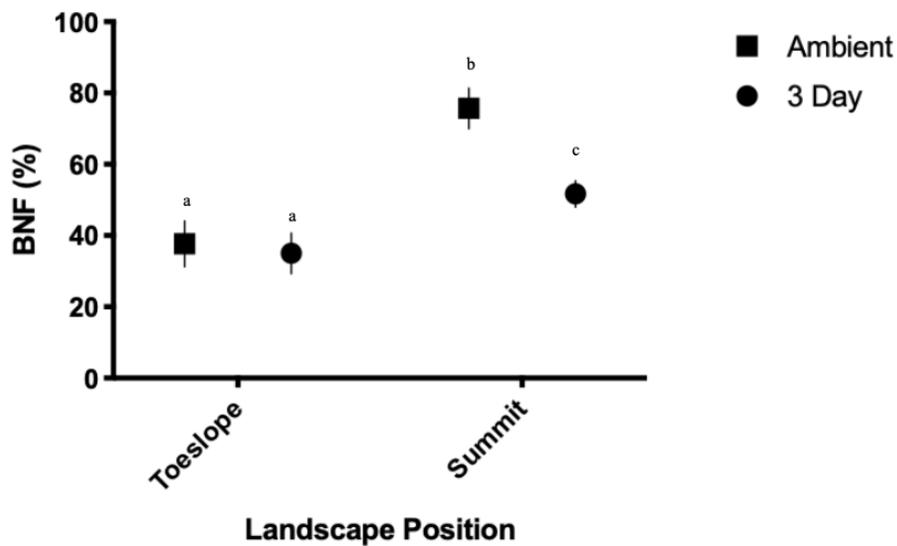
Potential N mineralization was lowest in backslope positions at  $51.4 \pm 14.1$  (mg  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) N  $\text{kg}^{-1}$  soil  $\text{day}^{-1}$  and highest at toeslope positions and lowest at  $143.0 \pm 11.3$  (mg  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) N  $\text{kg}^{-1}$  (Figure 4.6).



**Figure 4.6:** N mineralization potentials at toeslope, backslope, and summit positions. Error bars represent standard errors of the mean, n=4 replicate plots. Asterisks indicate significant effects (P<0.05) with soil texture.

### ***Rainfall Amounts by Landscape Position Experiment (Question 2)***

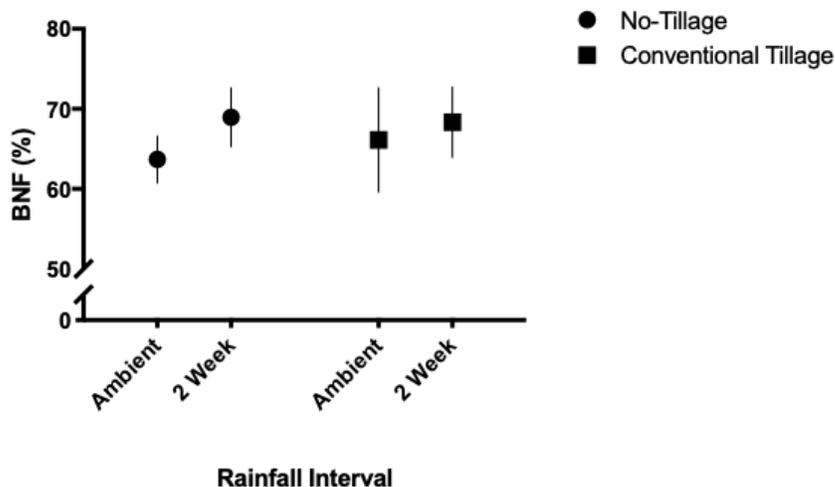
In rainfed plots, BNF contributed 37.7% to the N content of grain in summit positions and 75.7% in toeslope positions. In irrigated plots, BNF contributed 51.7% to the N content of grain in summit positions and 35.0% in toeslope positions (Figure 4.4).



**Figure 4.7:** BNF (%) increased at summit positions with more precipitation. Error bars represent standard errors of the mean, n=4 replicate plots. Asterisks indicate significant effects (P<0.05) with soil texture.

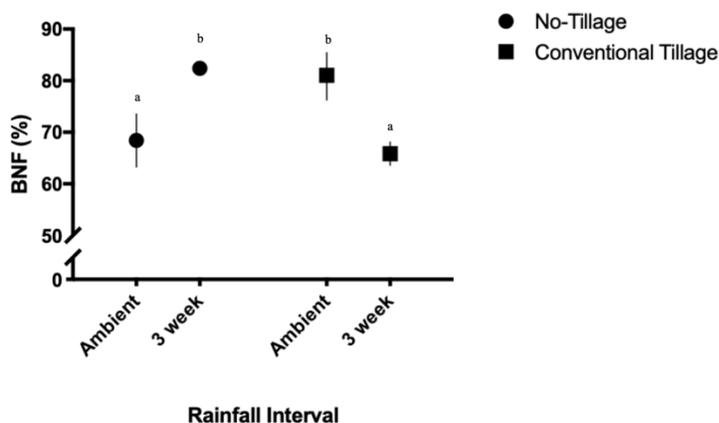
### ***Rainfall Intervals by Tillage Experiment (Question 3)***

In 2015, in the ambient rainfall treatment BNF contributed from  $66 \pm 6\%$  and  $64 \pm 3\%$  to the N content of grain in tilled and no-till plots, respectively. In soybeans experiencing 2-week rainfall intervals, BNF's contribution to the N content of grain was  $68 \pm 4\%$  and  $69 \pm 4\%$  in tilled and no-till plots, respectively (Figure 4.5).



**Figure 4.8:** Percent BNF in no-till and conventional tillage with ambient and 2-week rainfall intervals. Error bars represent standard errors of the mean, n=4 replicate plots.

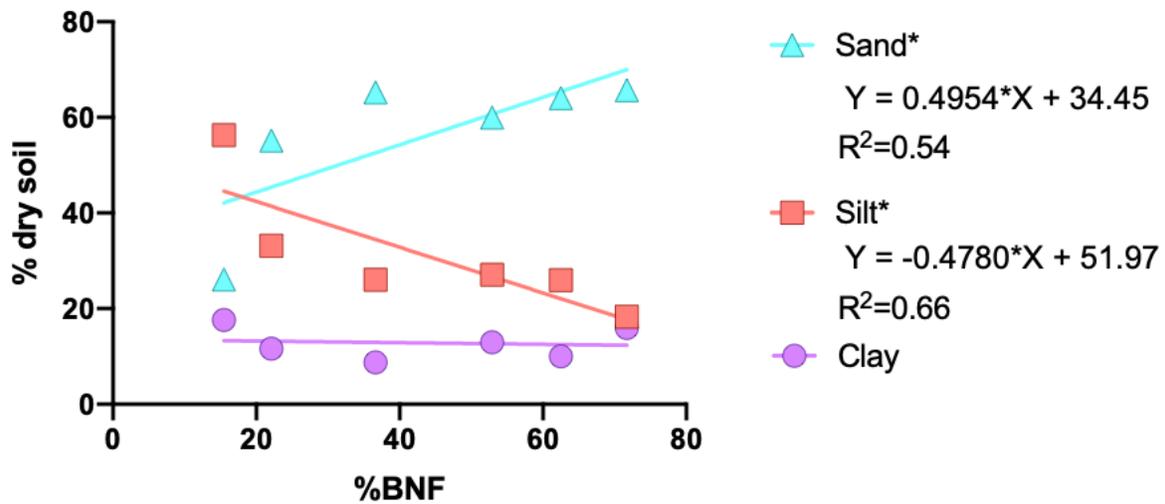
In 2018, with 3-week rainfall intervals, the BNF contributions to the N content of grain in tilled and no-till plots were  $66 \pm 3\%$  and  $82 \pm 1\%$ , respectively. In ambient rainfall intervals, the BNF contributions were correspondingly  $81 \pm 5\%$  and  $68 \pm 5\%$ , respectively (Figure 4.5).



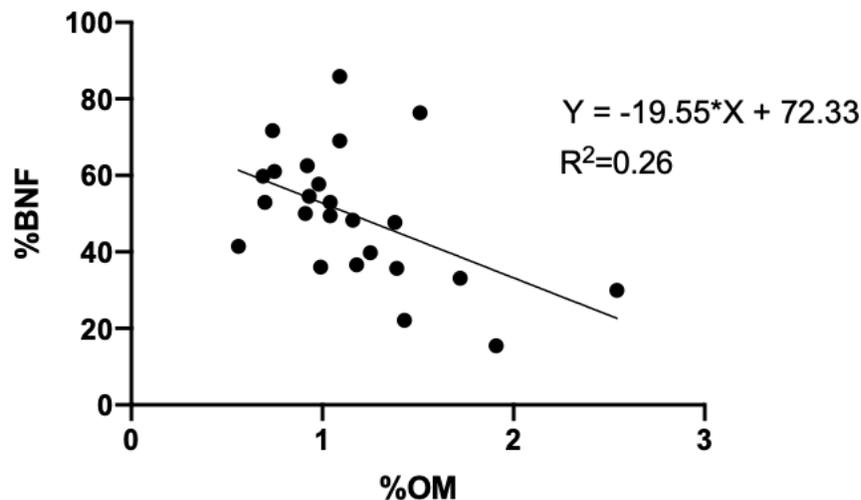
**Figure 4.6:** Percent BNF in no-till and conventional tillage with ambient and 3-week rainfall intervals. Error bars represent standard errors of the mean, n=4 replicate plots. Asterisks indicate significant rainfall interval effects ( $P < 0.05$ ) within tillage systems.

**Soil Properties: Soil Texture, OM, Mo, Fe, K, P, and pH**

The contribution of BNF to plant N content linearly decreased with soil sand content and increased with silt content (Figure 4.6) and OM (Figure 4.7). There was a higher percent sand in summit positions and lower percent sand in toeslope positions (Figure 4.8). Fe concentrations expressed the least range in concentrations from 0.8 to 5.1 mg kg<sup>-1</sup>. Likewise, P differences ranged from 0.9 to 8.2 mg kg<sup>-1</sup>. K concentrations expressed the greatest range, from 26.3 to 141.4 mg kg<sup>-1</sup>. I found no relationship between BNF and soil Mo, Fe, P, or K concentrations. All pH values for all experiments were between 5.7 and 7.1.



**Figure 4.9:** Percent sand, silt and clay by %BNF. Error bars are omitted for clarity, n=4 replicate plots. Asterisks indicate significant effects (P<0.05) with soil texture.



**Figure 4.10:** Percent BNF by %OM. Error bars are omitted for clarity, n=4 replicate plots. P value = 0.0116

## Discussion

As hypothesized, the contribution of BNF to total plant N (% BNF) was highest in summit positions and lowest in toeslope positions, coincident with texture and organic matter. More rainfall at summit positions suppressed BNF, as did 3-week rainfall intervals in tilled plots.

### *BNF by Toposequence (Question 1)*

How does soybean BNF vary by topographic position as affected by OM and its influence on N-mineralization and water availability? Percent BNF ranged from 0 – 94%, reflecting ranges seen in the literature (Salvagiotti et al., 2008; Schipanski et al., 2010) and at a nearby site (Gelfand and Robertson, 2015). Nevertheless, I observed a significant effect of landscape position on BNF, correlated with soil texture and N-mineralization rates. %BNF was higher at summit positions where soils were coarser and N mineralization rates were lower. The differential response of N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing soybeans to the fertility and textural

gradients reveals the influence of soil properties along toposequences on BNF across heterogeneous fields.

Other studies have also revealed in situ soil properties that influence BNF across individual fields. Riffkin et al. (1999) documented higher rates of BNF on sandier soils in Australia, as did Shipanski et al. (2010) in New York. Riffkin et al. (1999) found differences of 7% between light- and medium-textured soils; this compares to my finding of 50% differences, on average. However, in KBS fields, when BNF contribution is scaled by grain yield, there were no differences between landscape positions in the total amount of  $N_r$  supplied through BNF (Figure 4.2c).

I found no differences in %BNF in roots by landscape position, though this assumes that %BNF of roots at all depths are similar to those at the 0-25 cm depth that I sampled. However, this may not have sufficiently captured differences since soybean roots grow deeper than 150 cm (Ordóñez et al., 2018) and root distributions have been known to relate to claypan soil properties across toposequences (Myers et al., 2007). Rooting depths at different landscape positions may have differed due either to water availability or low permeability soil layers caused by tillage or geology.

Whole plant %BNF reflect changes in all parts of the plant, though trends are especially prevalent in seeds since they are the biggest sink for N and have the highest concentrations (~6%). Vegetative matter and belowground biomass have much lower N contents (~0.8% and ~1.3%, respectively) and %BNF (~4.5% and ~15%). Thus,  $N_r$  through soybean production is mostly associated with the grain.

Higher %BNF in summit positions is correlated with lower N mineralization rates (Figure 4.3), congruent with other studies that note lower %BNF where mineralization rates are high

(Schipanski et al., 2010). Soils with higher sand content tend to have less organic matter and N in comparison to soils with more clay (Six et al., 2000), thus sandier soils would be expected to have lower N mineralization rates.

Comparing N assimilation between non-nodulating and N<sub>2</sub>-fixing soybeans may indicate the ability of N<sub>2</sub>-fixing soybeans to allocate carbon belowground and induce N mineralization in low fertility soils, such as those at summit positions. I found soil N assimilation by non-nodulating and N<sub>2</sub>-fixing soybeans was similarly high in toeslope soils with their finer texture, evidenced by lower % BNF. George et al. (1993) found similar differences across an elevation and fertility gradient, with more soil N uptake in N<sub>2</sub>-fixing soybeans compared to the non-nodulating plants at low soil N availability. This indicates that N<sub>2</sub>-fixing soybeans may be able to circumvent N limitation, through carbon allocation, even as soil N stocks decline.

Roots obtain oxygen from pores in the bulk soil environment. Oxygen availability is an important regulator of nitrogenase activity; legume nodules can have four times the oxygen demand of an equal biomass of roots (Layzell and Hunt, 1990). Soils with lower microbial respiration, then, may have more oxygen-rich environments capable of supporting a high number of nodules, which might promote more BNF. Thus, my finding of lower BNF rates at toeslope positions may also be due to less oxygen where soils have more clay and higher microbial activity. Additionally, disease can be a sign of low oxygen. While I did not observe any evidence of disease pressure, finer textured soils at toeslopes can have an increased prevalence of soybean pathogens (Workneh et al., 1999) due to lack of oxygen. Furthermore, differences in rooting depth between nodulating and non-nodulating plants can influence the accuracy of the methods used, potentially accounting for differences between landscape position (Schipanski et al., 2010).

### ***BNF with Changes in Rainfall Amounts by Landscape Position (Question 2)***

Does BNF in summit and toeslope positions differ in response to added precipitation? In this experiment %BNF decreased with additional rainfall at all summit positions, but not at toeslope positions. The most likely explanation for suppressed BNF with additional irrigation at summit positions is increased inorganic N supply. Inorganic N pools can suppress BNF (Schipanski et al., 2010), and it's possible that added water stimulated additional N mineralization, which in turn suppressed BNF. Toeslope positions, with their higher ambient water contents and N mineralization potentials (Fig. 4.3), may have likewise had BNF suppressed by soil mineral N pools.

Higher %BNF under drier (ambient) conditions in summit positions contrasts with studies that show nodule production, which is closely tied to BNF, generally decreases with reduced precipitation. Thus, one may have predicted additional rainfall to have increased %BNF. BNF is sensitive to soil drying because N<sub>2</sub> fixation is more sensitive to soil conditions than to plant stress during drought (Abdelhamid et al. 2011), compounded by dry conditions that can lead to excess solutes in the root zone, restricting water availability to the bacteria (Walsh, 1995). That BNF was not inhibited under ambient conditions, is likely because summit soils were not sufficiently dry: In a study in Champaign, Illinois, Gray et al. (2013) found that drought stress must be greater than 41% of the historical average to inhibit nodulation. Drought stress at KBS was ~15% in 2016 and nil in 2017.

The timing of dry conditions can also affect %BNF, which occurs differentially throughout plant stages (Gan et al., 2003). Furthermore, low nodulation response to high or low moisture conditions could persist throughout the season despite improved weather conditions and

complex spatial-temporal variation. If dry conditions are severe enough to inhibit BNF, there will be lower BNF values, thus scaling up BNF values from soybeans under drier conditions can potentially lead to an underestimation of BNF (Gelfand and Robertson, 2015).

***BNF with Changes in Rainfall Intervals under Different Tillage (Question 3)***

Do changes in precipitation intensity influence soybean BNF in tilled plots differently from no-tilled plots? The same amounts of BNF occurred in tilled and no-till treatments under 3-day and 2-week rainfall interval treatments in 2015. However, when rainfall was excluded for three weeks in 2018, there was less BNF in no-till plots, where BNF decreased from 82 to 68 %BNF. This response fails to support the hypothesis that no-till management, with its higher organic matter content (Syswerda et al. 2011), will be better buffered against changes in rainfall intensity than conventional tillage management. In fact, counter to expectations, BNF under conventional tillage increased after drought from 68 to 80 %BNF, rather than decreased. Were the hypothesis supported, it would be expected BNF in the no-till to change little following drought and BNF in the tilled soil to decrease.

There are several possible partial explanations for 1) BNF's being greater in no-till than in conventional till systems under ambient intervals; 2) decreasing %BNF in conventional till exposed to the longer dry interval; and 3) increasing %BNF in no-till soybeans exposed to the longer dry interval.

First, BNF could be higher in no-till than conventional due to higher N immobilization in no-till soils due to greater retention of organic matter, resulting in less available plant N following drought. This may be less of an issue in the ambient treatment because more consistent rainfall may have promoted more N mineralization. This is corroborated by past work of in situ

assays in the same plots, which show higher net N mineralization in no till plots vs. conventionally tilled plots (Millar and Robertson, 2015)

Second, biogeochemical processes create a vertical gradient of  $^{15}\text{N}$  through the soil profile with a higher distribution of  $^{15}\text{N}$  than in the upper 10 cm than in the lower depths (Natelhoffer and Fry, 1988). However, when soils are homogenized as they are in tilled plots, this disrupts the naturally occurring patterns of  $\delta^{15}\text{N}$  with soil depth in comparison to no-till plots. Since soybean roots are concentrated in the upper 15 cm (e.g. Böhm et al., 1977; Robertson et al., 1980), it is possible that soybeans in no-till systems may have different  $^{15}\text{N}$  uptake patterns than soybeans in tilled plots because of associated  $\delta^{15}\text{N}$  patterns.

Other explanations are also possible. These include the potential for differences in rhizobia populations as well as tillage-related compaction that may have limited rooting development in tilled plots. In tilled plots, compaction caused by repeated tillage could form a tillage pan, which can be difficult for roots to penetrate. Keisling et al. (1995) found soybean roots in no-till systems followed classical taproot trends, but when tillage pans were present, roots followed old root channels and pan fractures. There is also speculation that increased disturbance, like tillage, will decrease the presence of effective rhizobia, though this remains to be tested specifically (Kiers et al., 2002). Trace element deficiencies could also contribute to lower %BNF, but there appear no trace element deficiencies in either conventional or no-till treatments (<http://lter.kbs.msu.edu/datatables/354>)

### ***BNF validity***

Various soil and environmental factors can influence BNF rates and the suitability of  $^{15}\text{N}$  natural abundance to accurately reflect rates, but three consistent outcomes in this study suggest that the values reported are robust. First, I found no relationship between soil P, K, pH and

soybean yield or fixation, which suggests that these factors were not influencing BNF differentially in these fields during the experimental period. Second, calculated BNF values never exceeded 100%, which can happen when isolines do not appropriately represent N uptake under non-BNF conditions. And third, the N content in both nodulating and non-nodulating soybeans responded in the same direction, i.e., increases in the N content of nodulating soybeans were paralleled by increases in the N content of non-nodulating soybeans, further suggesting good correspondence between nodulating and non-nodulating isolines. These three observations warrant connecting the three studies and drawing overall conclusions.

### ***Remaining Questions***

Results here suggest several lines of additional research that could be useful for further understanding spatial patterns on BNF in the field and responses to changing precipitation patterns. Three remaining areas in BNF in order to better understand and manage for topographic, tillage, and climate change issues are: 1) rhizobia strains; 2) other elements of global change; and 3) BNF rate differences in legumes and over space, and time.

First, genetic variation among rhizobia strains may be sensitive to topographic position. I did not examine the distribution of strains, which are known to differ regionally (Batzli et al., 1992) and with management (Kiers et al., 2002). Weese et al. (2015), for example, found less-mutualistic rhizobia evolved in long-fertilized fields and likewise, there may be less-mutualistic rhizobia in toeslope positions where N mineralization is high. This may also be the case with different precipitation regimes, as we know that changes in regimes alter microbial communities (Zeglin et al., 2013). A major assumption of this study is that rhizobia strains are consistent across landscape position and with tillage treatments. However, rhizobia inoculants differ, and some are more effective at fixing N<sub>2</sub> than others (Thilakarathna and Raizada, 2017). Certain

rhizobia genes are needed to establish symbioses and have been identified (Bottomley and Myrold, 2015). Unfortunately, it was not feasible to calculate separate B values for each field or measure rhizobia strains or their effectiveness in different soils. Thus, it would be valuable to understand differences in rhizobia strains at differing landscape positions, potentially justifying rhizobia inoculant for parts of fields.

Second, BNF appears sensitive to changes in precipitation amount. Understanding the full relationship between BNF and rainfall requires additional experimentation with different precipitation treatments, including both amounts of precipitation and delivery patterns. Heavy rainfall events, particularly in the spring, can delay planting and lead to anaerobic conditions that can limit rhizobia's ability to infect roots and thus lead to reduced nodulation (Layzell and Hunt, 1990).

Another global change factor that might affect BNF is elevated concentrations of atmospheric CO<sub>2</sub>, which stimulates N<sub>2</sub> fixation in legumes (van Groenigen et al 2006). However, elevated CO<sub>2</sub> is affected by drought differentially since higher levels of CO<sub>2</sub> stimulate nodulation and nodule density can help maintain N<sub>2</sub> fixation under drought (Parvin et al., 2019).

Third and finally, more experimentation is needed to understand how timing of drought influences N<sub>2</sub> fixation, i.e. how drought effects may depend on the stages of growth and development (Zahran, 1999). There are few experiments that integrate measures of plant physiology and structure to provide an understanding of whole plant function and fixation throughout the growing season alongside changes in precipitation. However, we know BNF varies with different stages of soybean crop growth (Córdova et al., 2019) .

## ***Implications***

Changing precipitation patterns seem likely to influence BNF in predictable ways depending on landscape position and tillage history. As hypothesized in Question 1, %BNF was highest in summit positions and lowest in toeslope positions, coincident with texture and organic matter: summits had coarser (sandier) textures than toeslopes and less organic matter. Increased precipitation diminished %BNF at summit positions, but not at toeslope positions (Question 2). In no-till plots, %BNF decreased with more rainfall events, but only in no-till fields (Question 3). Results have several implications for global assessments of N fixation and soybean N management.

First, results suggest that including information on landscape position and rainfall changes in calculations of field-scale BNF rates would lead to more accurate landscape and regional contributions of BNF to  $N_r$  insofar as soybean BNF rates vary greatly by landscape position and rainfall regime. The creation of regional  $N_r$  budgets for BNF are currently performed with values from small plots. If BNF varies over a landscape,  $N_r$  created through BNF by soybeans may be greatly over or underestimated, especially in areas that are topographically diverse. Differences in %BNF by toposequence, rainfall, and tillage will influence regional and global estimates of BNF contributions to  $N_r$ .

Second, results suggest that irrigation and organic matter can decrease the need for N fertilizer in soybeans. Despite meta analyses that show response to N supplementation are inconsistent (Mourtzinis et al., 2017), N fertilizer recommendations are promoted in some materials (Pioneer 2020). Results from rainfall and toposequence experiments suggest that soil organic matter is the best predictor of BNF in tilled fields (Figure 4.7). With higher organic matter and more frequent rainfall in tilled fields, there are lower BNF rates. Organic matter and

irrigation can supply N through mineralization, thus managing soil organic matter and irrigation can act as substitutes for N fertilizer, avoiding the environmental cost of applying fertilizer N to soybeans.

Third and finally, results suggest that management on a site-specific basis would be helpful. Given increasing soybean acreage and yields in the US, in order to limit inputs, it will be practical to be efficient in inoculation, fertilizer, irrigation and organic matter management when transferring to the larger scale, whether that be a field, the Midwest, different geographical regions or globally. Understanding toposequence differences in soybean BNF provides the opportunity to manage inputs more efficiently by slope position.

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