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

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Abstract

Purpose

Expected changes in rainfall patterns will affect the timing of N-mineralization and other N transformations, potentially promoting or suppressing biological nitrogen fixation (BNF). We test the hypotheses that BNF is more sensitive to changing rainfall patterns in summit vs. toeslope positions and in till vs. no-till consistent with patterns of soil texture and organic matter.

Methods

At a site in the upper Midwest USA, we measured soybean BNF ^{15}N natural abundance at different landscape positions with and without supplemental rainfall and in till vs. no-till rainfall exclusion shelters to lengthen the dry periods between rainfall events.

Results

Soybean BNF was 41% higher at summit than toeslope positions, consistent with lower soil OM and coarser texture at summits. When precipitation was increased by 20%, BNF decreased at summit positions and was unaffected at toeslope positions. In a separate tillage experiment, with 3-week (but not 2-week) rainfall intervals, %BNF decreased 15% under conventional tillage and increased 14% under no-till.

Conclusions

Changing rainfall patterns affected BNF differentially depending on landscape position and tillage in well-drained Alfisols. BNF was greater in summit than in toeslope positions and decreased with added rainfall. BNF under conventional tillage was more sensitive to longer rainfall intervals than was BNF under no-till. 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_2 fixation (BNF). These inputs, most notably the use of fertilizer, account for dramatic increases in reactive N (Nr) on Earth over the last 100 years (Vitousek et al. 2013). When not taken up by plants or soil microorganisms, Nr can be lost from cropping systems and become pollutants in water and the atmosphere. BNF contributes $\sim 25\%$ to Nr inputs to the biosphere (Vitousek et al. 2013) (Rao et al. 1994); soybeans (*Glycine max* L.) in particular contribute $\sim 10.4 \text{ Tg } \text{Nr} \text{ yr}^{-1}$, representing $\sim 18\%$ 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 Nr require less N fertilizer and tend to have lower hydrologic Nr 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).

Long-term studies of farms and fields planted to soybeans show a wide range of BNF rates across management and N fertility 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 also influence BNF including redox state and water availability. Chemical controls include, in addition to the availability of inorganic N, and BNF cofactors such as iron (Fe), molybdenum (Mo), and vanadium (V). Plant genotype and growth rates; soil rhizobia numbers, diversity, and effectiveness; and pests and pathogens also can affect rates of BNF (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).

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 BNF in white clover (*Trifolium repens*) ranged from 0 to 100% of total plant N across different soil textures with rates 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 26–81% (Hauggaard-Nielsen et al. 2010). And at sites in Central New York, USA, soybean BNF differed by soil type (Schipanski et al. 2010).

Climate change, particularly changes in rainfall intensity and amount, may also affect BNF. The US Midwest, responsible for > 80% of US soybean production (USDA NASS 2018), has experienced increasingly more intense and frequent heavy rainfall over the past few decades (Pryor et al. 2014), resulting in longer dry periods between rainfall events. 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). Because 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 the N nutrition of subsequent crops, it seems prudent to examine potential impacts of future climates on BNF. Since BNF appears related to soil organic matter (OM) and texture, two primary determinants of soil moisture and N availability, one might expect the strength of BNF responses to changing rainfall patterns to vary across fields with as OM and texture, particularly along toposequences. For example, impacts at summits may be greater than at toeslopes, where greater OM, clay, and water holding capacity and may buffer against rainfall variability.

In general, BNF rates are lower under drought conditions due to nodule sensitivity to decreased phloem flow (Serraj et al. 1999). Although most studies investigating drought effects on BNF have been performed in greenhouse settings, where plants are protected from normal environmental conditions and the rhizobia-plant symbiosis does not reflect the impact of rainfall changes. Furthermore, artificial environments alter nodules, as well as nodule depth and distribution (Pueppke 1986). Field experiments are needed to better understand BNF response to natural variability and that imposed by climate change. Particularly needed is an understanding of soil by rainfall interactions that may become more pronounced with climate change. Incorporating this knowledge into quantitative models will allow for better predictions of BNF and its global consequences.

Here we examine the hypotheses that BNF is vulnerable to changing rainfall regimes in summit vs. toeslope landscape positions and, separately, under till vs. no-till management. In both cases we expect BNF responses to changes in rainfall to be attenuated where OM and texture favor water availability. We address three specific questions:

1. How does soybean BNF vary by topographic position and associated patterns of OM, N-mineralization, and water availability;
2. Does BNF in summit and toeslope positions differ in response to added precipitation; and
3. How does soybean BNF vary by till vs no-till systems and associated patterns of OM and water availability?

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⁻¹ in February to 100 mm month⁻¹ 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.

The experiments were conducted on conventionally tilled rainfed fields used for corn, soybean, and wheat planted to soybeans during the years of each experiment (Fig. 1). These fields were managed as per regional practice, including fertilization with potassium (KCl), phosphorus (potash), 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 we quantified soybean BNF by ¹⁵N natural abundance using nodulating and non-nodulating isolines, as described below.

Toposequence Experiment (Question 1)

For the toposequence experiment, we used three fields of 6–8 ha each (KBS fields 30 – 1, 38, and 79 – 8 south; Fig. 1). Paired 1 · 1 m plots were placed along four transects in three landscape positions (summit, midslope, and toeslope) in each field (Table 1). In one plot of each pair we planted in 2015 five non-nodulating soybean plants (described below) at the same density as in the rest of the field. 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.

Table 1
Field identification, summit elevation, toeslope elevation, length of slope (horizontal distance from highest to lowest elevation), and slope degree for study fields.

Field	Highest elevation (m)	Lowest elevation (m)	Length of slope (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 2

Planting date, harvest date, growing season ambient rainfall, rainfall added, and precipitation increase compared to added for Experiment 2.

				Added rainfall	
Year	Planting date	Harvest date	Ambient rainfall (mm)	Amount (mm)	% Increase
2016	June 22	September 28 and 29	158	40	20.2
2017	June 22	September 28 and 29	331	37.5	10.2

Table 3

Planting date, harvest date, ambient rainfall, and additional irrigation for rainfall intensity experiment (Experiment 3).

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

Rainfall Amounts by Landscape Position Experiment (Question 2)

For the rainfall addition experiment, we 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). At four toeslope and four summit locations in each field; a 2 × 2 m plot 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 (Table 1). Water application was allowed to infiltrate completely so water did not spread to neighboring plants. Soybean plants were harvested aboveground and separated into seed and vegetative biomass.

Rainfall Intervals by Tillage Experiment (Question 3)

We conducted the rainfall intervals by 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 we imposed rainfall intervals of two weeks for the period between soybean planting and harvest; adjacent control subplots received ambient rainfall. Rainfall was excluded by complete-exclusion rainfall manipulation shelters (5 × 5 × 1.5 m high as described in Hess et al. 2019) and irrigated with captured rainwater delivered via overhead sprinkler heads. Rainfall interval plots received the same total amount of rainfall as ambient plots. The experiment was repeated in 2018, following wheat (*Triticum aestivum* L.) in 2016 and corn (*Zea mays* L.) in 2017, with three-week intervals. Soybeans plants (five non-nodulating) were harvested aboveground and separated into seed and vegetative biomass.

Materials And Methods

Biological Nitrogen Fixation

We quantified soybean BNF by using ^{15}N natural abundance in nodulating (Pioneer P22T69R) and non-nodulating (PI 547695, seed source: 04U-3266; Horosoy variety) isolines obtained from the USDA Soybean Germplasm Collection (USDA-ARS, Urbana, IL). To plant the non-nodulating isolines, we removed nodulating seeds from the soil immediately following planting 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 the R6.5 stage (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.

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 the remainder of the 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>)

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

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.

Soil organic matter (SOM) was measured by the Cornell Nutrient Analysis Laboratory (Ithaca, NY) 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.

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 were weighed into tins. Packed tins were analyzed for ¹⁵N 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° C for four hours and weighing the remaining residue.

BNF calculations

The ¹⁵N abundance method for BNF allows for end-of-season sampling to represent growing season fixation (Unkovich et al., 2008). The ¹⁵N is used to distinguish plant N derived from soil vs. air. The fraction of N derived from the atmosphere (fNd_{fa}) is:

$$fNd_{fa} = (\delta^{15}N_{ref} - \delta^{15}N_{fix}) / (\delta^{15}N_{ref} - \delta^{15}N_b),$$

where N_{ref} represents tissue from non-N₂-fixing soybean isolines, N_{fix} represents tissue from N₂-fixing soybean isolines, and N_b represents tissue from N₂-fixing soybean isolines grown with atmospheric N₂ as the only N source.

We used the $\delta^{15}N_b$ 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

For Question 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. We 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.

For Question 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.

For Question 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 questions, 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 (Fig. 2a). Backslope positions were intermediate with a BNF contribution of $62.6 \pm 4.4\%$. Aboveground primary productivity was highest in toeslope positions ($115.3 \pm 13.5 \text{ g plant}^{-1}$) and lowest in summit positions ($47.7 \pm 8.1 \text{ g plant}^{-1}$) (Fig. 2b). We found no differences in BNF contributions to aboveground or belowground vegetative tissues (Supplementary table 1 and 2); only in seeds was there a BNF difference by landscape position effect, with a higher %N from BNF in summits. The %NPP from BNF showed no significant trend with landscape position (Fig. 2c).

Potential N mineralization (Fig. 3) was highest in toeslope positions at $1.4 \pm 0.1 \text{ mg N kg}^{-1} \text{ soil day}^{-1}$ and substantially lower in backslope and summit positions at 0.5 ± 0.1 and $0.7 \pm 0.1 \text{ mg N kg}^{-1} \text{ soil day}^{-1}$, respectively.

Rainfall Amounts by Landscape Position Experiment (Question 2)

In rainfed plots, BNF contributed $37.7 \pm 6.5\%$ to the N content of grain in toeslope positions and $75.7 \pm 5.7\%$ in summit positions (Fig. 4). Added rainfall did not affect %BNF in toeslope positions ($33.3 \pm 7.0\%$) but decreased %BNF in summit positions ($51.7 \pm 3.7\%$).

Rainfall Intervals by Tillage Experiment (Question 3)

In 2015, in the ambient rainfall treatment BNF contributed from $66.1 \pm 6.5\%$ and $63.7 \pm 2.9\%$ to the grain N content in tilled and no-till plots, respectively. In soybeans experiencing 2-week rainfall intervals, BNF's contribution to the N content of grain was $68.3 \pm 4.4\%$ and $68.9 \pm 3.6\%$ in tilled and no-till plots, respectively (Fig. 5).

In 2018, in the ambient rainfall treatment BNF contributed $81.0 \pm 4.8\%$ and $68.4 \pm 5.1\%$ to grain N content in tilled and no-till plots, respectively (Fig. 5). In soybeans experiencing 3-week rainfall intervals, BNF's contribution to the N content of grain was $66.8 \pm 2.3\%$ and $82.4 \pm 1.0\%$ in tilled plots and no-till plots, respectively (Fig. 5)..

Soil Properties: Soil Texture, SOM, Mo, Fe, K, P, and pH

The contribution of BNF to plant N content linearly increased with soil sand content and decreased with silt content (Fig. 6) and OM (Fig. 7). Percent sand was higher in summit positions and lower in toeslope positions (Fig. 6). Fe concentrations expressed the least range in concentrations from 0.8 to 5.1 mg kg⁻¹. Likewise, P differences ranged from 0.9 to 8.2 mg kg⁻¹. K concentrations expressed the greatest range, from 26.3 to 141.4 mg kg⁻¹. We found no relationship between BNF and soil Mo⁺³, Fe⁺², P, or K concentrations or pH (Supplementary table 3).

Discussion

As hypothesized, the contribution of BNF to total plant N (% BNF) was highest in summit positions and lowest in toeslope positions, coincident with finer texture and organic matter contents at toeslope positions. Added rainfall at summit positions suppressed BNF. Tillage differences were less consistent: in 2015 neither tillage nor rainfall intervals affected %BNF, in 2018 %BNF was higher under conventional than no-till, and with longer rainfall intervals %BNF decreased under conventional tillage and increased under no-till.

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 along our toposequence 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, we observed a significant effect of landscape position on BNF, correlated with soil texture and N-mineralization rates. %BNF was more than twice higher (77%) at summit positions where soils were coarser and N mineralization rates were lower (Fig. 2). Interestingly, however, 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 (Fig. 2c).

The differential response of N₂-fixing and non-N₂-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.

We found no differences in %BNF in roots by landscape position, though this assumes that our 0–25 cm depth roots adequately represent roots throughout the profile. 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 reflects 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% N). Vegetative and belowground biomass have much lower N contents (~ 0.8% and ~ 1.3%, respectively) and %BNF (~ 4.5% and ~ 15%). Thus, the introduction of N_r to the environment through soybean production is mostly associated with the grain.

Higher %BNF in summit positions is correlated with lower N mineralization rates (Fig. 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 available 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 N₂-fixing soybeans may indicate the ability of N₂-fixing soybeans to allocate carbon belowground and induce N mineralization in low fertility soils, such as those at summit positions (Schipanski 2010). We found soil N assimilation by both non-nodulating and N₂-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 N₂-fixing soybeans compared to the non-nodulating plants at low soil N availability.

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, lower BNF at toeslope positions may also be due to lower oxygen availability insofar as soils with more clay and higher microbial activity have lower oxygen available (Layzell and Hunt 1990).

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 ~ 30% with additional rainfall at summit positions, but not at toeslope positions. The most likely explanation for suppressed BNF with additional rainfall at summit positions is increased

inorganic N supply. Inorganic N pools can suppress BNF (Schipanski et al. 2010), and it's likely that added water stimulated N mineralization, which in turn suppressed BNF. Toeslope positions, with their higher ambient water contents and N mineralization potentials (Fig. 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 under drier conditions. Thus, one might have predicted additional rainfall to have increased rather than attenuated %BNF. N_2 fixation is more sensitive to soil conditions than to plant stress (Abdelhamid et al. 2011); additionally by dry conditions that can lead to excess solutes in the root zone, restricting water availability to rhizobia (Walsh 1995). That BNF was not inhibited by drier ambient conditions is likely because summit soils were not sufficiently dry: In an Illinois study, 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 (<https://lter.kbs.msu.edu/datatables/7>).

The timing of dry conditions can also affect %BNF, which occurs differentially throughout plant stages (Gan et al. 2003). Furthermore, low nodulation response to rainfall variation could persist throughout the growing season despite temporarily improved soil moisture conditions and areas in the field with greater water holding capacity. If dry conditions are severe enough to inhibit BNF stages where BNF rates are high, there will be lower total 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, 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 will conventional tillage management. In fact, counter to expectations, BNF under conventional tillage increased under 3-week droughts from 68 to 80 %BNF, rather than decreased. Were the hypothesis supported, we would expect BNF under no-till to change little following drought and BNF in tilled soil to decrease.

There are several possible 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; which 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}N through the soil profile with a higher distribution of ^{15}N in the upper 10 cm than in the lower depths (Nadelhoffer 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}N uptake patterns than soybeans in tilled plots because of associated $\delta^{15}\text{N}$ patterns. If so, then this apparent difference would have been observed in 2015 as well, instead of just 2018.

Other explanations are also possible. These include the potential for differences in rhizobia populations ability to fix N_2 as well as tillage-related compaction that may have limited rooting development in tilled plots. In tilled plots, compaction caused by repeated tillage could 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>)

Validity of %BNF validity

Various soil and environmental factors can influence the suitability of ^{15}N natural abundance to accurately reflect BNF rates, but three consistent outcomes in this study suggest that the values reported are robust. First, we found no significant relationships between %BNF and soil P, K, pH or soybean yield, 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 occur 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

Several lines of additional research could be useful for further understanding spatial patterns on BNF in the field and responses to changing precipitation patterns. These include a better understanding of BNF with respect to 1) rhizobia strains; 2) other elements of global change than rainfall patterns; and 3) BNF rate differences in other legumes and throughout the growing season.

First, genetic variation among rhizobia strains may be sensitive to topographic position and tillage treatments. Rhizobia inoculants differ, and some are more effective at fixing N_2 than others (Thilakarathna and Raizada 2017). We 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). Certain rhizobia genes are needed to establish symbioses (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. Though we saw difference in spite of different strains, 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 both precipitation amounts and intervals. Understanding the full relationship between BNF and rainfall requires additional experimentation with different precipitation treatments, including both amounts and delivery patterns. For example, heavy rainfall events, particularly in the spring, can delay planting and lead to anerobic conditions that can limit rhizobia's ability to infect roots and thus lead to reduced nodulation (Layzell and Hunt 1990). Elevated CO_2 (van Groenigen et al. 2006, Parvin et al. 2019) and ozone (Morgan et al. 2003) are additional global change factors that may interact with precipitation change and BNF.

Third and finally, more experimentation is needed to understand how timing of drought influences N_2 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. We know, for example, that BNF varies with different stages of soybean crop growth (Córdova et al. 2019).

Implications And Conclusions

Changing precipitation patterns seem likely to influence BNF in predictable ways depending on landscape position and tillage history. We found %BNF highest in summit positions and lowest in toeslope positions, coincident with coarser texture and less soil organic matter at summits. Increased precipitation diminished %BNF at summit positions, but not at toeslope positions. In no-till plots, subjected to longer (3-week) rainfall intervals, %BNF increased, while in conventionally tilled plants %BNF decreased. 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 could lead to more accurate estimates of landscape and regional contributions of BNF to N_r . The creation of regional N_r budgets for BNF are currently performed with values from small plots, likely more similar to toeslope positions than summits, insofar as most

agricultural research is conducted on level ground of higher fertility (Robertson et al. 2007). This would imply that regional estimates of BNF from soybeans are likely underestimated.

Second, despite meta-analyses that show soybean responses to N fertilizer are rarely justified (Mourtzinis et al. 2017), N fertilizer use is promoted in some materials (e.g., Pioneer 2020). Results from rainfall and toposequence experiments suggest that soil organic matter is the best predictor of BNF in tilled fields (Fig. 6). 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 provide N available to soybeans, decreasing the necessity 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. Understanding toposequence differences in soybean BNF provides the opportunity to manage inputs more efficiently by slope position.

Declarations

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Conflicts of interest/Competing interests

There are no known conflicts of interests or competing interests.

Availability of data and material

Data will be available at dryaddata.org upon publication.

Code availability

There is no code repository associated with this research.

Authors' contributions

KRG and GPR conceived the research, designed the experiment, and wrote the paper. KRG performed the experiment and analyzed data.

Compliance with ethical standards

Conflicts of interests/ Competing interests The authors declare no competing financial interests.

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Figures

Figure 1

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

Figure 2

Question 1: (a) percent BNF, (b) NPP by landscape position, and (c) %NPP from BNF by landscape position. Error bars represent standard errors of the mean, n=4 replicate plots. Asterisks indicate significant effects (P<0.05).

Figure 3

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.

Figure 4

BNF (%) at different landscape positions with and without added precipitation. Error bars represent standard errors of the mean, n=4 replicate plots. Letters indicate significant effects (P<0.05).

Figure 5

Question 3: (a) percent BNF in no-till and conventional tillage with ambient and 2-week rainfall intervals and (b) .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

Figure 6

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 7

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

Supplementary Files

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