Intra- and inter-annual variability of nitrification in the rhizosphere of field-grown bioenergy sorghum

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\textbf{Abstract}
Biological nitrification inhibition (BNI) and plant–microbe competition for ammonium (NH$_4^+$) by sorghum (\textit{Sorghum bicolor} (L.) Moench) have the potential to suppress nitrification, reducing nitrate (NO$_3^-$) and nitrous oxide (N$_2$O) production for more sustainable bioenergy feedstock production. However, it is unknown how variability in environmental factors, field management, and plant growth affect the suppression of nitrification. We conducted a field trial with four genotypes of energy sorghum and four fertilization rates in central Illinois, USA, and measured soil N pools, potential nitrification and denitrification rates, and microbial community composition in bulk and rhizosphere soils to assess nitrification suppression throughout the 2018 and 2019 growing seasons. Concentrations of NO$_3^-$ and NH$_4^+$ were very low in rhizosphere soil regardless of fertilization level, suggesting strong N demand by plants and microbes. Potential nitrification was lower in the rhizosphere soil than bulk soil, and this suppression was strongest mid-season ~2 months after planting in both years (20% suppression in 2018 and 58% in 2019). Since precipitation was lower during the mid-growing season of 2019 compared to 2018, we speculate that hydrophilic BNI root exudates accumulated in the rhizosphere and suppressed nitrification more than in 2018 when soil moisture was higher. Unfertilized plots had greater nitrification suppression than fertilized plots during the mid-season in 2018, but otherwise nitrification suppression was insensitive to fertilizer treatment. Potential denitrification was stimulated in the rhizosphere compared to bulk soil in both study years, suggesting that heterotrophic activity was stimulated by plant carbon inputs, possibly further suppressing slower-growing chemoautotrophic nitrifying microbes. Overall, we found inter- and intra-annual variation in nitrification suppression in the rhizosphere of field-grown biomass sorghum, suggesting that plant phenology and environmental conditions should be considered when devising strategies to improve the nitrogen sustainability of this annual bioenergy crop.

\textbf{KEYWORDS}
bioenergy, biological nitrification inhibition, denitrification, nitrification, rhizosphere, sorghum
1 INTRODUCTION

A nearly fourfold increase in synthetic fertilizer application in the second half of the 20th century (Howarth et al., 2002) has led to elevated nitrate (NO$_3^-$) leaching into surface and groundwater as well as increased nitrous oxide (N$_2$O) emissions to the atmosphere (Gelfand et al., 2016; Van Meter et al., 2017). Nitrification is a key biogeochemical process regulating ecosystem nitrogen (N) loss because it transforms ammonium (NH$_4^+$) to NO$_3^-$, which is a highly mobile form of N susceptible to leaching and denitrification to N$_2$O (Farquharson, 2016). Synthetic nitrification inhibitors can effectively reduce N losses from agricultural fields (Di & Cameron, 2002; Gilsanz et al., 2016) but cost is prohibitive for widespread use (Yang et al., 2016). Nitrification inhibition can also occur naturally through direct biological nitrification inhibition (BNI) by plant root exudates and indirect nitrification suppression due to plant N uptake and immobilization of N by heterotrophic microbes in the rhizosphere. Some pasture grasses and cereal crops exude biological nitrification inhibition (BNI) compounds from their roots (Coskun et al., 2017a; Subbarao et al., 2007), so breeding or engineering crops for BNI presents another option for managing agricultural N losses (Coskun et al., 2017b; Subbarao, Sahrawat, et al., 2013). However, land managers have less control over suppression of nitrification by plants than application of synthetic nitrification inhibitors, and plant development and climate conditions could affect nitrification suppression in the field. Thus, we must better understand the occurrence and magnitude of suppression of nitrification by plants across intra- and inter-annual variability in field conditions to control field N losses and increase crop sustainability.

Sorghum (Sorghum bicolor (L.) Moench), an important grain crop worldwide and an emerging candidate bioenergy feedstock, can suppress nitrification through both direct and indirect mechanisms. The production and release of secondary metabolites from sorghum roots directly inhibits nitrification in laboratory and greenhouse settings (Nardi et al., 2020; Sarr et al., 2019; Subbarao, Nakahara, et al., 2013; Tesfamariam et al., 2014; Weston et al., 2013; Zakir et al., 2008). Notably, inhibited activity of the model nitrifying bacterial isolate Nitrosomonas europaea reflects direct BNI by sorgoleones, sakuranetin, and methyl 3-(4-hydroxyphenyl) propionate (MHPP; Subbarao, Nakahara, et al., 2013). Sorgoleones, a class of hydrophobic p-benzoquinone compounds (Czarnota et al., 2001), also suppress the relative abundance of ammonia-oxidizing archaea (AOA) within the nitrifying microbial community in the rhizosphere of greenhouse-grown sorghum (Sarr et al., 2019). However, estimation of sorghum BNI in the field is nearly absent, with only one study measuring end-of-season BNI in the field to complement a set of laboratory experiments (Tesfamariam et al., 2014). In addition to direct BNI by root exudates, other rhizosphere processes can also indirectly suppress nitrification. Root exudates can serve as sources of labile carbon (C) to fuel the activity of faster-growing heterotrophic microbes (Kuzyakov, 2002), which are better competitors for NH$_4^+$ than relatively slow-growing autotrophic nitrifying bacteria and archaea (Verhagen et al., 1995). Increased heterotrophic activity along with root uptake of NH$_4^+$ (Herman et al., 2006) can indirectly suppress nitrification rates since NH$_4^+$ supply is considered a primary control over nitrification rates (Booth et al., 2005; Stienstra et al., 1994). Given the strong seasonal patterns in root production by annual crops (Black et al., 2017) and changes in root exudate composition and amount throughout plant development (Gransee & Wittenmayer, 2000), temporal dynamics of BNI compound production, root N uptake, and rhizosphere heterotrophic activity could exert substantial control over rhizosphere nitrification.

Given that farmers typically apply N fertilizer early in the growing season and that the highest NO$_3^-$ leaching flux occurs prior to the establishment of annual crops (Stenjem et al., 2019), seasonal variation in plant-induced nitrification suppression could affect its impact on ecosystem N losses. Root exudate amount and chemical profile vary with plant age and nutrient demand (Chaparro et al., 2013; Oburger & Jones, 2018). In sorghum, sorgoleone production and direct BNI increase later in the plant phenology with the strongest BNI effects after a month or more of growth (Sarr et al., 2019; Zakir et al., 2008). Sorghum only exerts significant influence over its rhizobacterial community after approximately a month of growth (Schlemper et al., 2017), with this pattern potentially associated with changes in root exudate profile or simply with the time needed for community structure to change in response to root exudates. Additionally, annual crop root biomass and N uptake increase as the plants develop (Black et al., 2017; van Oosterom, Borrell, et al., 2010), making indirect pathways of nitrification suppression more important later in the growing season. Therefore, rhizosphere nitrification suppression may be temporally decoupled from peaks in ecosystem N losses that occur earlier in the growing season.

Soil moisture dynamics can also mediate direct and indirect rhizosphere nitrification suppression pathways. Hydrophobic BNI compounds, such as sorgoleones, may remain concentrated near roots, whereas hydrophilic BNI compounds, such as sakuranetin and MHPP, may more readily diffuse away from roots under moist soil conditions. When low soil moisture limits solute diffusion through soil pore water (Raynaud, 2010), even hydrophilic compounds may have restricted movement, leading to...
greater direct BNI in the rhizosphere. Lower soil moisture could also further reduce NH$_4^+$ diffusion into the rhizosphere and thus increase the contribution of indirect competitive pathways to rhizosphere nitrification suppression. The potential for seasonal variability in BNI compound production combined with seasonal and inter-annual soil moisture variability could lead to temporally variable nitrification suppression in the rhizosphere of field-grown crops.

Plant-induced nitrification suppression may also respond to soil N availability. While it is not known exactly why plants direct resources to BNI compound production, reducing nitrification and leaching of NO$_3^−$ could be a mechanism employed by plants to retain soil N under N-limited conditions (Subbarao et al., 2015). Plants adapted to low-N environments exhibit the greatest levels of BNI (Subbarao et al., 2007), but BNI compound production requires a low level of ammonium (NH$_4^+$) in solution (~1 mM; Subbarao, Nakahara, et al., 2013; Subbarao et al., 2007, 2015; Zakir et al., 2008). This suggests that plants actively regulate BNI compound production in response to availability of NH$_4^+$, the nitrification substrate pool. Also, since substrate availability strongly controls nitrification levels (Booth et al., 2005; Herman et al., 2006; Stienstra et al., 1994; Wendeborn, 2020), it is likely that reduced competition for NH$_4^+$ between nitrifiers and roots or heterotrophic microbes in heavily fertilized fields would minimize the effects of these indirect nitrification suppression pathways. Indeed, despite nitrification inhibition by plant root exudates, typical agronomic levels of fertilizer addition still stimulate nitrification in the sorghum rhizosphere (Sarr et al., 2019). The dependence of nitrification in the rhizosphere on plant N status and soil NH$_4^+$ concentration suggests that management of fertilizer N inputs could serve as an important control on suppression of nitrification in the rhizosphere.

Here, we assessed rhizosphere nitrification suppression by field-grown energy sorghum under different fertilizer application rates and across two growing seasons varying in precipitation amounts. In the first year of the study, we included four energy sorghum genotypes grown for bioenergy production to explore the possibility of genotypic variation in BNI. Biological nitrification inhibition cannot be measured in situ in the field, so we inferred nitrification suppression by testing for differences between potential nitrification in rhizosphere soil, the soil that clung to excavated root systems, and in bulk soil collected between planted sorghum rows. The potential nitrification assay allowed us to test for differences in the nitrification capacity of the soil microbial community in different soil environments throughout the growing season, encompassing both direct and indirect suppression of nitrifiers. We expected that plant phenology, variable environmental conditions between seasons, and field N management would interact to control the magnitude of nitrification suppression in rhizosphere soil. Specifically, we hypothesized that (1) the suppression of nitrification would be greatest mid-season during the period of highest sorghum growth, N demand, and exudation of BNI compounds, (2) varying environmental conditions, especially soil moisture, between growing seasons lead to inter-annual variation in the magnitude of the suppression of nitrification in the rhizosphere, (3) increasing available N through fertilizer addition would reduce the suppression of nitrification in the rhizosphere, and (4) denitrification rates would be stimulated in the rhizosphere and rhizosphere microbial communities would be distinct from bulk soil, indicating higher heterotrophic microbial activity and indirect suppression of nitrification via competition for NH$_4^+$.

## MATERIALS AND METHODS

### 2.1 Site description

We conducted a 2-year field study (2018–2019) of biomass sorghum grown for bioenergy feedstock production at the University of Illinois Urbana-Champaign (UIUC) Energy Farm (N 40.063607, W 88.206926). The soil within this site is predominantly Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls), which is very deep and poorly drained. The 2010–2019 mean annual temperature and mean annual precipitation for this site were 9.0°C and 1023 mm, respectively, and growing season (May 1–October 31) mean temperature and precipitation were 17.0°C and 598 mm, respectively (NOAA National Climatic Data Center station ID USC00118740, 3.6 km from the field trial).

### 2.2 Experimental design

We leveraged a sorghum agronomy trial that included four sorghum genotypes grown in factorial combination with four fertilizer application levels in a randomized block design ($n = 4$; Schetter et al., 2021). Each of the 64 treatment plots consisted of eight rows, 12 m in length and spaced 76 cm apart, resulting in an overall plot size of ~72 m$^2$. The plots were planted at a population rate of 185,325 seed ha$^{-1}$. The germplasm was obtained from the Texas A&M University sorghum breeding program. The four genotypes used (TAM08001, TAM17800, TAM17600, and TAM17500) varied in photoperiod sensitivity (Schetter et al., 2021), with TAM17600 and TAM17800 typically flowering earlier than TAM17500 and TAM08001. This potential difference in plant
phenology with respect to flowering could influence plant N dynamics by changing the timing of N demand and uptake from the soil (van Oosterom, Borrell, et al., 2010; van Oosterom, Chapman, et al., 2010). The four fertilizer treatments included 0, 56, 112, and 168 kg N ha\(^{-1}\), knifed into soil in the center of the alley between planted rows at a depth of 6–7 cm as 28% liquid 1:1:1 urea–ammonium–nitrate, hereafter referred to as N-0, N-56, N-112, and N-168. In 2018, the plots were planted on May 18 and fertilized on May 22. In 2019, the plots were planted on June 1 and fertilized on June 10. The timing of planting and subsequent fertilization depends on field conditions and anticipated rainfall to distribute the fertilizer into the soil.

In the annual crop rotation at this site, sorghum is planted following a soybean crop the season prior. To maintain this rotation, the 2018 and 2019 trials were planted in different fields located approximately 200 m apart and on the same soil type. Both fields were planted in corn two years prior and soybean one year prior to sorghum. Pre-trial surface (0–10 cm) soil N concentrations were similar between years with slightly higher NO\(_3\)\(^-\) concentrations in 2018 (Table 1), and soil N concentrations did not differ between blocks within either year. We only measured mineral N concentrations in the top 10 cm of soil in 2018, but in 2019 we also measured soil N from 10 to 30 cm soil depth (0.5 mg NH\(_4\)\(^+\)-N kg\(^{-1}\) soil, 5.7 mg NO\(_3\)\(^-\)-N kg\(^{-1}\) soil). Using bulk density measurements from adjacent fields, we estimate that the pre-fertilization mineral N stocks in the 2019 trial field were ~28 kg N ha\(^{-1}\) in the top 30 cm of soil. Although we did not quantify soil texture, we observed no soil texture differences within trial or between the two nearby trial fields, which were on the same soil type and ~200 m apart. A 4 ha plot adjacent to both trial fields had surface soil textures ranging from 5% to 13% sand, 51% to 64% silt, and 30% to 36% clay, and so it is unlikely that our two trial fields differ significantly in soil texture. Pre-trial soil pH did not differ between fields (Table 1), and more soil properties are reported by Schetter et al. (2021).

In each year of the study, we selected a subset of treatments from the sorghum field trial. In 2018, we sampled soils from all four genotypes across two fertilization levels (N-0 and N-168) to evaluate genotypic variation in nitrification suppression and its potential interaction with management practices. In 2019, we selected only TAM08001, the genotype with nitrification suppression most sensitive to fertilization based on 2018 results. We sampled this genotype across all four fertilization levels in 2019 to better characterize the effect of soil N availability on nitrification suppression.

### 2.3 Field sampling

We collected bulk and rhizosphere soils from 0 to 10 cm depth to assess sorghum effects on N cycling potential rates, soil properties, and microbial community composition. To collect rhizosphere soil from each plot, two plants were uprooted from different rows in the plot using a shovel. After knocking off loose soil, soil clinging to the root ball of each plant was hand-collected and pooled as a single rhizosphere soil sample for each plot. On the first sampling date of each growing season, when the plants were still small, we sampled more than two plants as needed to obtain sufficient soil for all analyses. Bulk soil was collected between planted rows within each plot to minimize belowground plant influence while controlling for similar plot conditions experienced by the rhizosphere soil. Since N fertilizer was knifed in directly between planted rows, we sampled bulk soils approximately 25–30 cm into the alley from the row where rhizosphere soils were collected. After collection, soils were split in the field, with one portion stored at 4°C within 2 h of collection for up to 48 h prior to analysis of potential N cycling rates and soil properties. The other portion was initially stored at 4°C within 2 h of collection and then freeze-dried and stored at −20°C within 48 h for later soil microbial analyses. To evaluate the response of BNI to changes in plant phenology and environmental conditions, we sampled in the early-, mid-, and late-growing season. This corresponded to 40, 68, and 96 days since sowing in 2018, and 37, 65, and 94 days since sowing in 2019, or V6, V12, and R growth stages of sorghum.

### 2.4 Soil analyses

We inferred the suppression of nitrification as the difference in potential nitrification between bulk and rhizosphere soils. Although measuring potential nitrification in field-collected soils does not yield in situ field nitrification rates, it does allow us to compare the microbial capacity for nitrification between different soil environments and within and across growing seasons to infer variability in the suppression of nitrification in field rhizosphere soil. To estimate potential nitrification rates, we measured

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**Table 1** Pre-trial average soil pH and mineral N concentrations (±SE) in the field sites for 2018 and 2019 sorghum trials

|         | pH     | NH\(_4\)\(^+\) (mg N kg\(^{-1}\) soil) | NO\(_3\)\(^-\) (mg N kg\(^{-1}\) soil) |
|---------|--------|--------------------------------------|--------------------------------------|
| 2018    | 6.05 ±0.02 | 0.67 ±0.26                           | 12.45 ±1.50                         |
| 2019    | 6.00 ±0.05 | 0.80 ±0.10                           | 9.71 ±0.35                          |
nitrification rates in aerobic soil slurries under excess NH$_4^+$ substrate availability using a method adapted from Belser and Mays (1980). Sodium chloride was also added to the soil slurries to inhibit nitrite (NO$_2^−$) oxidation such that nitrification rates could be measured as the accumulation of NO$_2^−$ over the incubation period. Briefly, for each soil sample, three 5 g subsamples were each added to 20 ml of 1 mM NH$_4$N as ammonium sulfate along with 0.1 ml of 1.5 M sodium chloride. Two subsamples were treated as technical replicates for which nitrification occurred over 5 h; these subsamples were incubated on an orbital shaker at room temperature to promote the distribution of substrate throughout the slurries. The third subsample was frozen at $-20°C$ as a control to account for background soil NO$_2^−$ under minimal microbial activity. After incubation, 5 ml of 2 M potassium chloride (KCl) was added, and NO$_2^−$ concentration of the filtered supernatant was quantified using a colorimetric reaction with 40 g L$^{−1}$ sulphanilamide and 2 g L$^{−1}$ N-(1-naphthyl) ethylenediamine dihydrochloride in a buffer of 6.8 g L$^{−1}$ imidazole and 0.02 g L$^{−1}$ copper (II) sulfate adjusted to pH 7.8 (Griess-Ilosvay method; Nydahl, 1976; Shinn, 1941) read with a Genesys 30 visible spectrophotometer (ThermoFisher Scientific) at 520 nm. Production of NO$_2^−$ was thus the difference in NO$_2^−$ concentrations between the room temperature test samples and chilled control samples after the 5 h soil incubation and was calculated as a daily rate of NO$_2^−$ production. Thus, potential nitrification rates are the accumulation rate of NO$_2^−$ and are expressed as mg N kg$^{−1}$ dry soil day$^{−1}$.

To estimate potential denitrification rates, we measured denitrification rates in anaerobic soil slurries under excess NO$_3^−$ substrate availability using a method adapted from Groffman et al. (1999). Acetylene was added to the slurry incubation headspace to inhibit N$_2$O reduction such that denitrification rates could be estimated from the accumulation of N$_2$O over the incubation period. Briefly, for each soil sample, a 25 g subsample was added to 25 ml of 10 mM NO$_3^−$-N as potassium nitrate and 60 mM dextrose-C in a 125 ml airtight media bottle. After flushing the bottle headspace with ultra-high purity helium, 15 ml of pure acetylene gas was added and the solution was shaken vigorously for 30 s to mix in the acetylene. Headspace gas samples were collected every 10 min for 30 min, and subsequently analyzed for N$_2$O concentration via gas chromatography equipped with an electron capture detector (Shimadzu GC-2014, Shimadzu Scientific Instruments, Inc.). Potential denitrification rates were calculated from the linear rate of increase of N$_2$O after accounting for aqueous-phase N$_2$O using Bunsen's constant at the assay temperature of 20°C. All potential denitrification rates are expressed as mg N kg$^{−1}$ dry soil day$^{−1}$.

To assess how soil properties and N pools differed among genotypes or treatments, we analyzed mineral N pools (NO$_3^−$ and NH$_4^+$), soil moisture, and soil pH for all soil samples. Mineral N pools were quantified via colorimetric analysis of 2M KCl soil extracts on a SmartChem 200 discrete analyzer (Unity Scientific). Concentrations of NH$_4^+$ were quantified using a method adapted from Weatherburn (1967) for the SmartChem 200 instrument in which NH$_4^+$ reacts with hypochlorite and sodium salicylate in the presence of sodium nitroprusside catalyst. Concentrations of NO$_3^−$ were quantified using a SmartChem 200 adaptation of the Griess-Ilosvay method. Gravimetric soil moisture was determined by drying soils for 48 h at 105°C. Finally, pH was measured in a 1:1 mass ratio of field moist soil and deionized water.

2.5 Molecular analyses

To characterize soil microbial community structure and quantify the relative abundance of nitrifiers, microbial DNA was extracted from freeze-dried soil samples using the DNeasy 96 PowerSoil Pro QIAcube HT Kit. Samples were disrupted for DNA extraction by weighing out 0.05 g of soil into PowerBead Pro Tubes, lysed using a TissueLyser II, and extracted using the QIAcube DNA extraction robot (QIAGEN).

Amplification of the V4 region of the 16S rRNA gene was carried out using a Fluidigm Access Array IFC chip (Fluidigm) with single-index barcoded primers 515F (5’-GTGYCAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACNVGGGTWTCTAAAT-3’; Apprill et al., 2015; Parada et al., 2016), along with barcodes for assigning individual reads to samples. Sequencing was completed using 2 x 250 bp paired-end chemistry on an Illumina NovaSeq 6000 Sequencing System (Illumina) at the Roy J. Carver Biotechnology Center. Paired-end sequences were merged using Fast Length Adjustment of Short reads (FLASH) software (Magoc & Salzberg, 2011), and the FASTX-Toolkit (Hannon, 2014) was used to filter merged sequences using a minimum quality score of 30 across 90% of the bases. USEARCH version 8.1 (Edgar, 2010) was used to remove singletons, chimeras, and cluster OTU sequences based on 97% similarity. Taxonomic classification was assigned with Quantitative Insights into Microbial Ecology (QIIME; Caporaso et al., 2010) using the UCLUST algorithm and GreenGenes database (DeSantis et al., 2006). QIIME was also used to assemble OTU tables. All sequence data are archived in the National Center for Biotechnology Information's (NCBI) Sequence Read Archive (accession number SRP326979, project number PRJNA741261).
2.6 | Statistical analysis

We constructed linear mixed-effects models using the lme4 package in R (Bates et al., 2015) to determine (1) if rhizosphere nitrification suppression or rhizosphere effect on denitrification depended upon sorghum growth stage, (2) genotype and fertilizer effects on nitrification suppression or rhizosphere effect on potential denitrification rate, and (3) genotype and fertilizer effects on the relative abundance of nitrifiers in the soil microbial community. In addition, we used linear mixed-effects models to evaluate growth stage, genotype, and fertilization effects on soil NO$_3^-$ and NH$_4^+$ concentrations. The R package phyloseq (McMurdie & Holmes, 2013) was used to subset nitrifying archaeal and bacterial taxa (Nitrosomonadales, Nitrososphaerales, and Nitrospirae) from the 16S OTU table for calculation of relative abundance. Although the order Nitrosomonadales also contains non-nitrifying organisms, nearly all of this group’s OTUs in our dataset (98%) were identified as Nitrosospiribrio tenuis, an ammonia-oxidizing bacterium (Harms et al., 1976). Additionally, Nitrospirae are nitrite-oxidizing bacteria, and although our potential nitrification assay measures only ammonia oxidation, the indirect suppression of nitrification in rhizosphere soil could also impact nitrite oxidizers. Therefore, we included Nitrospirae when analyzing differences in nitrifier relative abundance between bulk and rhizosphere soils. We also used linear mixed-effects models to determine if the ammonia oxidizer relative abundance affected potential nitrification assay rates. For this analysis, we excluded Nitrospirae because our potential nitrification assay only measured ammonia oxidation to nitrite. A log of the odds ratio (logit) transformation was applied to relative abundance values to adhere to the model assumption of a boundless continuous response variable for statistical testing. Significance was determined as $p < 0.05$ and means are reported ±1 standard error. Models included dependent variables of potential nitrification, potential denitrification, or relative abundance of nitrifier operational taxonomic units (OTUs); fixed effects of bulk versus rhizosphere soil, fertilizer treatment, genotype (for 2018 models only), growth stage, and all interactions between fixed effects; and a random effect of plot nested within block to account for spatial variability across the field. Post-hoc pairwise comparisons of estimated marginal means were computed using the emmeans package in the R statistical environment (R Core Team, 2020). To assess the effects of bulk versus rhizosphere soil, fertilizer addition, genotype (2018 only), and growth stage on the full microbial community composition (non-subsetted community dataset) and nitrifier community composition (subsetted nitrifier dataset), permutational analysis of variance models (PERMANOVA) for each year were used with the adonis function from the community ecology R package vegan (Oksanen et al., 2008). Differences in the PERMANOVA models were visualized by sample date using non-metric multidimensional scaling (NMDS) ordinations and ggpplot2 (Wickham, 2016).

3 | RESULTS

3.1 | Edaphic and climate factors

Growing season precipitation and soil moisture dynamics were distinct between the two years of this study, with 2018 being a wetter year than average and 2019 being drier. From May 1 through October 31, rainfall totaled 666 mm in 2018 and only 580 mm in 2019, compared to a 10-year average of 598 mm from 2010 to 2019 (Figure 1). Much of this difference occurred during the middle of the growing season, as rainfall totaled 122 mm from July 8 to August 20, 2018 but only 36 mm over the same period in 2019. As a result, soil moisture differed significantly between years ($p < 0.01$, Figure 2), with the inter-annual soil moisture difference especially pronounced mid-season (17% gravimetric water content in 2018 vs. 10% in 2019) and late season (24% in 2018 vs. 15% in 2019).

Soil moisture dynamics showed similar growing season patterns over both years of the study, but differences between bulk and rhizosphere soil moisture occurred only in 2019 (Figure 2). In both years, soil moisture dropped...
significantly from the V6 to the V12 growth stage and then recovered by the R growth stage (2018, \( p < 0.001; 2019, p < 0.001 \)). Under the drier conditions in 2019, rhizosphere soil exhibited higher moisture than bulk soil in the V12 and R growth stages (growth stage*bulk vs. rhizosphere effect; \( p < 0.001 \); bulk vs. rhizosphere post-hoc Tukey’s test, \( p < 0.001 \)).

Soil NH\(_4^+\) concentrations exhibited different growing season patterns, fertilization effects, and bulk versus rhizosphere soil effects in 2018 versus 2019 (Figure 3, Table 2). In 2018, soil NH\(_4^+\) concentration varied with plant phenology and the effect of sorghum genotype depended upon growth stage, with TAM17600 having higher soil NH\(_4^+\) than TAM08001 and TAM17500 in the V6 stage (growth stage, \( p = 0.03 \); genotype \( \times \) growth stage, \( p = 0.009 \); Figure S1). There were no differences between bulk and rhizosphere soils nor fertilizer effects on soil NH\(_4^+\) in 2018, possibly due to a lack of rainfall immediately following fertilization favoring ammonia (NH\(_3\)) volatilization. In contrast, 15.7 mm of rain fell after fertilization in 2019, which typically decreases NH\(_3\) loss (Fillery & Khimashia, 2016). These effects and the resulting soil N concentrations could also have contributed to lower sorghum tissue N in 2018 (Schetter et al., 2021). In 2019, NH\(_4^+\) concentration increased with fertilizer level in the bulk soil but not in the rhizosphere (\( p = 0.001 \)). There was no difference between bulk and rhizosphere soils by the R growth stage when NH\(_4^+\) was depleted throughout the soil in all plots.

Temporal and treatment patterns in soil NO\(_3^-\) concentrations were similar in 2018 and 2019, although bulk soil NO\(_3^-\) concentrations were higher in 2019 (Figure 3, Table 2). In both years, growth stage, fertilizer treatment, and bulk versus rhizosphere soil affected soil NO\(_3^-\) concentrations (all \( p < 0.001 \), Table 2). In addition, sorghum genotypes differed in soil NO\(_3^-\) concentration in 2018 (\( p = 0.006 \); Figure S1). Fertilizer addition increased NO\(_3^-\) concentrations in the bulk soil but not rhizosphere soil in both years (2018, \( p = 0.035; 2019, p = 0.008 \)). However, bulk soil NO\(_3^-\) was depleted by the R growth stage in 2018 (\( p < 0.001 \)). In 2019, bulk soil NO\(_3^-\) remained elevated through the V12 growth stage when plots fertilized with 168 kg N ha\(^{-1}\) had higher soil NO\(_3^-\) than unfertilized plots (\( p < 0.001 \); Figure 3).

### 3.2 Potential nitrification rates

In 2018, interactions among bulk and rhizosphere soil differences, sorghum growth stages, and fertilizer treatments suggested complex controls on the suppression of nitrification in the field (Table 3). Across all growth stage sampling dates, fertilizer treatments, and genotypes, potential nitrification averaged 17.5 ± 0.5 mg NO\(_3^-\)N kg soil\(^{-1}\) day\(^{-1}\) in bulk soil and 16.0 ± 0.6 mg NO\(_3^-\)N kg soil\(^{-1}\) day\(^{-1}\) in the rhizosphere, with the higher rates in bulk soil compared to the rhizosphere indicating the suppression of nitrification in the rhizosphere (\( p < 0.001 \)). Sorghum suppressed potential nitrification only during the V12 growth stage mid-season, when rhizosphere soil exhibited 20% lower potential nitrification rates than bulk soil across all fertilization levels and genotypes (\( p < 0.001 \); Figure 4).

In 2019, potential nitrification rates were lower overall compared to 2018 but nitrification suppression in the rhizosphere was more pronounced than in 2018 in terms of both absolute and relative reduction of potential nitrification rates in the rhizosphere compared to bulk soil. Across all growth stages and fertilizer treatments, potential nitrification averaged 12.7 ± 0.7 mg NO\(_3^-\)N kg soil\(^{-1}\) day\(^{-1}\) in bulk soil and 8.4 ± 0.5 mg NO\(_3^-\)N kg soil\(^{-1}\) day\(^{-1}\) in the rhizosphere (\( p < 0.001 \)). Similar to 2018, the suppression of nitrification was greatest during the V12 stage mid-season, when rhizosphere potential nitrification was 58% lower than bulk soil across fertilization levels (\( p < 0.001 \); Figure 4, Table 3).

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**FIGURE 2** Gravimetric soil moisture in bulk and rhizosphere soils (yellow and green bars, respectively) by sorghum growth stage in 2018 (a) and 2019 (b). Given no statistically significant fertilizer treatment or genotype effects on any sampling date, all fertilizer treatments and genotypes were averaged for each soil type within growth stage. Asterisks denote significant differences between bulk and rhizosphere soil (\( p < 0.05 \)).
contrast to 2018, significant nitrification suppression was also observed during the R stage, when rhizosphere potential nitrification was 22% lower than bulk soil across all fertilization levels ($p < 0.001$). Fertilizer treatment did not affect the difference between bulk and rhizosphere soil potential nitrification on any sampling date during the 2019 growing season.

**FIGURE 3** Soil NH$_4^+$ (a–f) and NO$_3^-$ (g–l) concentrations in bulk and rhizosphere soils (yellow and green bars, respectively) by fertilization treatment at the V6, V12, and R sorghum growth stages in 2018 (averaged across genotypes) and 2019. 2018 genotype effects are shown in Figure S1. Only two fertilizer treatments, 0 and 168 kg N ha$^{-1}$, were sampled in 2018. Differing letters indicate significant differences in soil N within sorghum growth stage sampling date ($p < 0.05$), and panels with no letters have no significant differences.
3.3 | Potential denitrification rates

In 2018, potential denitrification was higher in the rhizosphere than in bulk soil, but was also affected by growth stage, fertilizer, and genotype. The mean potential denitrification was 0.8 ± 0.07 mg N₂O-N kg soil⁻¹ day⁻¹ in bulk soil and 1.2 ± 0.07 mg N₂O-N kg soil⁻¹ day⁻¹ in the rhizosphere in 2018 across all growth stages, genotypes, and fertilization treatments (p < 0.001, Figure 5, Table 3). Potential denitrification rates changed over the growing season, with V6 stage rates approximately double the later rates (p = 0.001). The greatest difference between bulk and rhizosphere soil occurred during the V12 growth stage when potential denitrification was 171% higher in the rhizosphere than in bulk soil (p < 0.001; Figure 5). Neither genotype nor fertilizer treatment altered the difference between bulk and rhizosphere soils.

Drier conditions in 2019 led to an order of magnitude lower potential denitrification rates compared to 2018, with 2019 rates averaging only 0.047 ± 0.01 mg N₂O-N kg soil⁻¹ day⁻¹ in bulk soil and 0.075 ± 0.01 mg N₂O-N kg soil⁻¹ day⁻¹ in the rhizosphere (Figure 5). Despite the dry conditions, potential denitrification still differed significantly between bulk and rhizosphere soils, with 63% higher rates in rhizosphere soil than bulk soil across all treatments (p = 0.01, Figure 5). We observed the largest difference between bulk and rhizosphere soil during the R growth stage when rhizosphere potential denitrification was 125% higher than in bulk soil (p = 0.04). In 2019, V12 growth stage potential denitrification was 71% and 79% lower than the earlier V6 and later R growth stage rates, respectively (p < 0.001). Fertilizer treatment did not affect potential denitrification on any sampling date in 2019.

3.4 | Soil microbial community

Soil bacterial and archaeal community composition differed between bulk and rhizosphere soils and by sorghum
### TABLE 3  Results from linear mixed-effects models evaluating effects on potential nitrogen cycling rates and nitrifier relative abundances

| Year | df  | Potential nitrification rate | Potential denitrification rate | Total nitrifier relative abundance | Archaeal nitrifier relative abundance | Bacterial nitrifier relative abundance |
|------|-----|------------------------------|-------------------------------|------------------------------------|--------------------------------------|----------------------------------------|
|      |     | F  | p  | F  | p  | F  | p  | F  | p  | F  | p  | F  | p  |
| 2018 |     |    |    |    |    |    |    |    |    |    |    |    |    |
| Date | 2,120 | 156.2 | <0.001 | 44.0 | <0.001 | 27.8 | <0.001 | 27.7 | <0.001 | 3.4 | 0.035 |
| Bulk versus rhizosphere soil (B-R) | 1,120 | 14.9 | <0.001 | 41.4 | <0.001 | 11.5 | <0.001 | 11.3 | <0.001 | 1.7 | 0.195 |
| Fertilizer | 1 | 0.9 | 0.35 | 0.0 | 0.933 | 0.0 | 0.963 | 0.0 | 0.960 |
| Genotype | 3,21 | 0.6 | 0.601 | 2.2 | 0.115 | 2.2 | 0.114 | 1.9 | 0.155 | 2.0 | 0.139 |
| B-R × Date | 2,120 | 3.4 | 0.035 | 7.3 | 0.001 | 2.7 | 0.069 | 1.2 | 0.291 | 6.7 | 0.002 |
| B-R × Fertilizer | 1,120 | 0.8 | 0.371 | 0.7 | 0.409 | 0.0 | 0.971 | 0.1 | 0.756 | 0.6 | 0.443 |
| B-R × Genotype | 3,120 | 1.6 | 0.205 | 0.2 | 0.921 | 1.0 | 0.388 | 1.4 | 0.236 | 0.4 | 0.769 |
| Date × Fertilizer | 1 | 0.4 | 0.703 | 1.8 | 0.176 | 3.7 | 0.027 | 3.6 | 0.030 | 0.8 | 0.450 |
| Date × Genotype | 6,120 | 0.5 | 0.844 | 1.0 | 0.422 | 0.5 | 0.804 | 0.5 | 0.831 | 0.7 | 0.676 |
| Fertilizer × Genotype | 3,21 | 0.7 | 0.570 | 0.5 | 0.663 | 0.0 | 0.985 | 0.1 | 0.941 | 0.5 | 0.657 |
| B-R × Date × Fertilizer | 2,120 | 2.4 | 0.098 | 0.6 | 0.532 | 1.7 | 0.195 | 1.8 | 0.177 | 0.2 | 0.784 |
| B-R × Fertilizer × Genotype | 3,120 | 0.8 | 0.472 | 0.3 | 0.835 | 0.7 | 0.583 | 0.6 | 0.615 | 0.9 | 0.445 |
| B-R × Date × Genotype | 6,120 | 0.6 | 0.693 | 0.4 | 0.888 | 1.7 | 0.121 | 1.7 | 0.121 | 1.8 | 0.106 |
| Date × Fertilizer × Genotype | 6,120 | 1.3 | 0.251 | 2.4 | 0.029 | 1.5 | 0.178 | 1.4 | 0.236 | 1.2 | 0.294 |
| B-R × Date × Fertilizer × Genotype | 6,120 | 0.3 | 0.920 | 1.6 | 0.146 | 1.0 | 0.440 | 0.8 | 0.550 | 1.2 | 0.322 |

| 2019 | df  | F  | p  | F  | p  | F  | p  | F  | p  | F  | p  |
|------|-----|----|----|----|----|----|----|----|----|----|----|
| Date | 2,60 | 4.1 | 0.022 | 25.2 | <0.001 | 2.3 | 0.112 | 4.6 | 0.014 | 5.4 | <0.001 |
| B-R | 1,60 | 35.4 | <0.001 | 7.1 | 0.010 | 8.7 | <0.001 | 5.4 | 0.023 | 1.6 | 0.206 |
| Fertilizer | 3,12 | 1.3 | 0.320 | 0.5 | 0.705 | 0.8 | 0.543 | 1.0 | 0.444 | 2.1 | 0.167 |
| B-R × Date | 2,60 | 14.5 | <0.001 | 3.6 | 0.035 | 1.3 | 0.272 | 1.7 | 0.196 | 1.4 | 0.252 |
| B-R × Fertilizer | 3,60 | 0.5 | 0.675 | 0.5 | 0.667 | 2.2 | 0.095 | 3.3 | 0.027 | 0.6 | 0.648 |
| Date × Fertilizer | 6,60 | 0.7 | 0.685 | 1.3 | 0.287 | 1.5 | 0.203 | 1.6 | 0.160 | 0.6 | 0.702 |
| B-R × Date × Fertilizer | 6,60 | 0.7 | 0.687 | 1.2 | 0.311 | 0.7 | 0.680 | 0.7 | 0.619 | 1.6 | 0.167 |

Note: All models included a random effect of plot within block to account for the hierarchical experimental layout. Bulk versus rhizosphere soil effect is abbreviated B-R. Significant (p < 0.05) results are indicated in bold.
growth stage and fertilizer treatment. The overall microbial community composition differed between bulk and rhizosphere soils in both 2018 and 2019 ($p < 0.001$, $R^2 = 0.02$, and $p < 0.001$, $R^2 = 0.02$, respectively, Figure 6). Bulk and rhizosphere soil microbial communities diverged more as the season progressed in both years as well (2018, $p < 0.001$, $R^2 = 0.02$; 2019, $p = 0.02$, $R^2 = 0.02$). Soil microbial community composition also varied by fertilizer treatment in both years (2018, $p < 0.001$, $R^2 = 0.01$; 2019, $p < 0.001$, $R^2 = 0.05$; Figure S3).

The composition of nitrifiers also differed between bulk and rhizosphere soils and by sorghum growth stage and fertilizer treatment. During both years, the nitrifier community differed between bulk and rhizosphere soils (2018, $p = 0.023$, $R^2 = 0.01$; 2019, $p = 0.01$, $R^2 = 0.02$). The nitrifier community also diverged between bulk and rhizosphere soils throughout both growing seasons (2018, $p = 0.005$, $R^2 = 0.01$; 2019, $p = 0.031$, $R^2 = 0.03$). Soil nitrifier community composition varied by fertilizer treatment as well (2018, $p = 0.001$, $R^2 = 0.02$; 2019, $p = 0.001$, $R^2 = 0.10$).

Total (archaea + bacteria) nitrifier relative abundance was lower in the rhizosphere compared to bulk soil in both 2018 and 2019 ($p < 0.001$ and $p < 0.001$, respectively; Figure 7, Table 3). In 2018, the difference between bulk and rhizosphere soils was marginally significantly greater in the V6 and V12 growth stages than the later R stage ($p = 0.07$). Archaeal nitrifier relative abundance

![Figure 4: Potential nitrification rates in bulk and rhizosphere soils (yellow and green bars, respectively) by fertilization treatment in the V6, V12, and R sorghum growth stages in 2018 (a–c) and 2019 (d–f). Given no statistically significant genotype effects, all genotypes were averaged for each soil type within sorghum growth stage sampling date. Asterisks denote significant differences between bulk and rhizosphere soils ($p < 0.05$), with lower rates in rhizosphere soils indicating suppression of nitrification.](image-url)
was also lower in the rhizosphere than bulk soil in both study years, but this effect did not change through the growing season (2018, \( p < 0.001 \); 2019, \( p = 0.02 \)). However, in 2018, the difference between relative abundance of bacterial nitrifiers in bulk versus rhizosphere soil differed among growth stages (\( p = 0.002 \), Figure S4). In the V6 and V12 stages, bacterial nitrifier relative abundance was lower in the rhizosphere, but in the R stage it was higher in the rhizosphere compared to bulk soil. In contrast, in 2019, bacterial nitrifier relative abundance differed among growth stages (\( p < 0.001 \)) but there was no difference between bulk and rhizosphere soils. Sorghum genotype and fertilizer treatment did not affect relative abundances of total nitrifiers, archaeal nitrifiers, or bacterial nitrifiers.

In 2018, the relative abundance of ammonia oxidizers had no relationship with potential nitrification rates across all sample dates. When isolating the mid-season date when rhizosphere effects were the strongest, there was a trend toward lower potential nitrification rate at lower relative abundances of both archaeal (\( p = 0.09 \)) and bacterial ammonia (\( p = 0.09 \)) oxidizers. However, in 2019, when rhizosphere effects on nitrification were much stronger, potential nitrification rate significantly declined with lower relative abundance of total ammonia oxidizers (\( p = 0.006 \)) and both archaeal (\( p = 0.02 \)) and bacterial ammonia oxidizers (\( p = 0.03 \)) individually.

4 | DISCUSSION

The expanding body of literature on BNI suggests that many plant species may be capable of reducing nitrification in the rhizosphere (Chowdhury et al., 2017; Coskun et al., 2017a; Janke et al., 2018; Subbarao et al., 2007). In the agricultural setting, direct and indirect suppression of nitrification could reduce \( \text{NO}_3^- \) losses from annual cropping systems, with the agronomic benefit of increased fertilizer use efficiency and environmental benefits of reduced \( \text{NO}_3^- \) flow into downstream waterways and \( \text{N}_2\text{O} \) emissions into the atmosphere. Across two growing seasons, we detected lower potential nitrification in the rhizosphere soil of field-grown sorghum, a species with direct BNI capacity, with nitrification suppressed 20%–58% during peak growth in the mid-season. This is comparable to the sorghum direct BNI effect of 20%–60% measured in laboratory and greenhouse studies (Sarr et al., 2019; Subbarao, Nakahara, et al., 2013; Tesfamariam et al., 2014). Importantly, we showed that plant phenology exerts substantial control over rhizosphere soil nitrification.
and that the most suppression occurs when plants were growing fastest mid-season. Intra- and inter-annual variation in soil moisture and N availability also affected soil potential nitrification and its suppression in rhizosphere soils. Thus, these factors influence how we can leverage sorghum rhizosphere dynamics to reduce N losses and improve the sustainability of biomass sorghum as a bioenergy feedstock.

Estimating rhizosphere nitrification suppression in an agricultural field over two full growing seasons allowed us
to explore a possible role of plant phenology in controlling rhizosphere nitrification dynamics. Direct BNI likely plays a significant role in suppression of nitrification along growing roots, and production of BNI compounds by sorghum increases as plants develop such that we expected to observe temporal changes in BNI throughout the growing season. In both study years, rhizosphere nitrification potential was reduced during the mid-season V12 sorghum growth stage but not in the early season, consistent with the typical pattern of increasing BNI root exudate production and release as sorghum plants develop (Sarr et al., 2019; Subbarao, Nakahara, et al., 2013; Zakir et al., 2008).

The mid-season nitrification suppression effect was also during the period of maximum growth rate and highest N demand for biomass sorghum (Maughan et al., 2012; van Oosterom, Borrell, et al., 2010; van Oosterom, Chapman, et al., 2010; Schetter et al., 2021), so the increased nitrification suppression during the V12 growth stage of sorghum likely also resulted from plant N uptake reducing NH$_4^+$ availability for nitrifiers. Contrary to our expectation, the four genotypes we used had very similar growth rates and flowering times (differing by only ~1 week), resulting in the lack of any effect of genotype on rhizosphere nitrification. However, investigating different sorghum types, such as grain, forage, and biomass sorghum, could reveal stronger temporal changes in production and release of BNI compounds and NH$_4^+$ competition resulting from plant N uptake. Thus, the temporal dynamics of nitrification suppression within a growing season is likely the result of both indirect suppression of nitrifiers driven by growth rate and N demand as the plant develops, as well as direct suppression of nitrifier activity through increasing release of BNI compounds.

Inter-annual variability in the strength of nitrification suppression suggests that there is significant environmental control over rhizosphere nitrification in the field. The main difference between the two years in our study was lower precipitation and soil moisture in 2019, particularly mid-season from early July through mid-August (Figure 3). Soil water content and exudate compound diffusivity are key drivers of the movement of exudates away from roots (Raynaud, 2010) such that hydrophilic compounds are retained and concentrated in closer proximity to the root in dry years. Of the three known BNI compounds exuded by sorghum roots, MHPP and sakuranetin are hydrophilic, while sorgoleones are hydrophobic (Subbarao, Nakahara, et al., 2013). Although sorgoleones have gained more attention for their role in sorghum BNI (Dayan et al., 2010; Sarr et al., 2019), the concentration of hydrophilic BNI compounds in the rhizosphere under the drier soil conditions in 2019 may have led to a greater BNI effect compared to 2018. Additionally, lower soil moisture restricts diffusion of NH$_4^+$ (Agehara & Warncke, 2005; Stark & Firestone, 1995), which likely interacted with plant NH$_4^+$ uptake and microbial immobilization in the rhizosphere to further reduce NH$_4^+$ availability for relatively slow-growing nitrifiers, especially mid-season in 2019. This suggests that inter-annual variability in growing season precipitation and soil moisture should be accounted for when predicting how plants contribute to nitrification suppression and mitigation of ecosystem N losses.

Although we found evidence for phenological and environmental controls over rhizosphere nitrification dynamics, changes in soil N availability across a range of fertilization levels did not affect nitrification suppression as expected. In our study, fertilizer was knifed into the soil between planted rows immediately after planting, so one potential caveat to our design is the possibility that bulk soils were affected more by fertilization than rhizosphere soils, confounding the fertilizer and bulk versus rhizosphere soil effects. However, suppression of nitrification in the rhizosphere did not differ between fertilization levels in either year (Table 3), and NH$_4^+$ concentration did not differ between the bulk and rhizosphere soils in 2018 and was only higher in bulk soil of the highest fertilization treatment in early- and mid-2019 (Figure 3). Thus, plant-induced nitrification suppression rather than fertilizer placement most likely led to our measured nitrification dynamics. Interestingly, our sorghum plants were generally unresponsive to N addition, with minimal effect on biomass yield (Schetter et al., 2021). Since the experimental plots were located on fertile soil in fields historically managed as a fertilized maize-soybean rotation, it is likely that the sorghum stands were not N-limited even in the absence of fertilizer application in the study years. Additionally, direct BNI may be generally unresponsive to plant N status so long as there is at least a low NH$_4^+$ concentration present in soil solution (~1 mM) to stimulate BNI compound production (Subbarao et al., 2017; Subbarao, Nakahara, et al., 2013; Zakir et al., 2008).

As a result, we expect that BNI will occur at agronomic levels of N fertilization, potentially playing a role in reducing fertilizer N loss as NO$_3^−$. Determining whether NO$_3^−$ leaching is reduced by BNI crops across a range of fertilization levels compared to non-BNI crops is important for evaluating the potential for BNI to contribute to sustainable production of high-yielding crops that often require N inputs.

Greater rhizosphere denitrification potential relative to bulk soil during both growing seasons provides support for heterotrophic competition with nitrifiers for NH$_4^+$ contributing indirectly to the suppression of nitrification. Given that denitrifiers represent a diverse group of facultative anaerobes that use organic carbon for aerobic heterotrophic respiration under oxic soil
conditions, the stimulation of rhizosphere denitrification potential starting in mid-2018 and late-2019 suggests that heterotrophic activity may have been broadly stimulated in the rhizosphere by root exudation of labile carbon compounds during the V12 and R growth stages of sorghum. Lower relative abundance of nitrifiers in the rhizosphere compared to bulk soil is also consistent with increased abundance of heterotrophic microbes. Thus, competition for NH$_4^+$ via the combination of stimulated heterotrophic activity and increased plant uptake of N (van Oosterom, Borrell, et al., 2010) likely exerted significant control over rhizosphere nitrification and contributed to the strong seasonal changes in the suppression of nitrification in the rhizosphere. Although our design does not allow us to compare the relative importance of indirect and direct pathways of nitrification suppression, we speculate that the indirect mechanisms are at least as important as direct BNI starting mid-season.

In conclusion, we demonstrated that nitrification suppression in field-grown energy sorghum exhibits considerable intra- and inter-annual variability likely associated with plant phenology and environmental conditions. Since it is not constant throughout the growing season, the potential mismatch between the timing of greatest NO$_3^−$ production and greatest rhizosphere nitrification suppression could reduce the effectiveness or dependability of engineering BNI to mitigate ecosystem N losses. Additionally, climate variability and resulting differences in soil moisture control the magnitude of nitrification inhibition, with weaker nitrification suppression during moist periods when microbial activity and NO$_3^−$ leaching would be the greatest. However, we found that rhizosphere nitrification can be suppressed in fertile soils even with high rates of fertilizer addition, indicating that it can limit NO$_3^−$ production when NH$_4^+$ availability is high, as is often the case in intensively managed annual cropping systems. Together, our results suggest that carefully considering how interactions between plant development, local climate, and rhizosphere microbes affect N cycling and loss would maximize the role of rhizosphere nitrification suppression in reducing agroecosystem N losses. Strategies that match the timing of sorghum nitrification suppression with NH$_4^+$ availability and selecting for greater direct BNI expression earlier in plant development will increase the effectiveness of managing plant-rhizosphere interactions for ecosystem N retention and maximizing the sustainability of biomass sorghum as a bioenergy crop.

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CONFLICT OF INTEREST
The authors have no conflicts of interest.

AUTHORS’ CONTRIBUTIONS
Mark B. Burnham, Wendy H. Yang, and Evan H. DeLucia conceived and designed the project, DoKyoung Lee designed and implemented the field trial, Sandra J. Simon processed the microbial samples, Sandra J. Simon and Angela D. Kent completed bioinformatics and microbial statistical analyses, Mark B. Burnham and Sandra J. Simon completed field and laboratory sampling, Mark B. Burnham analyzed N cycle data, Mark B. Burnham, Sandra J. Simon, and Wendy H. Yang wrote the manuscript, Evan H. DeLucia, Angela D. Kent, and DoKyoung Lee provided significant editorial comments.

DATA AVAILABILITY STATEMENT
Data availability information was provided during article submission, and can also be found below:

Soil data are available in the Illinois Data Bank (https://doi.org/10.13012/B2IDB-3696813_V1). All microbial sequence data are archived in the National Center for Biotechnology Information’s (NCBI) Sequence Read Archive (accession number SRP326979, project number PRJNA741261).

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