In Silico Evaluation of Plant Nitrification Suppression Effects on Agroecosystem

Nitrogen Loss

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ABSTRACT

Nitrification regulates potential for nitrogen (N) loss from ecosystems because it converts ammonium to nitrate, which is susceptible to leaching and gaseous emissions. Crops can suppress the microbes that perform nitrification by exuding nitrification-inhibiting compounds from their roots and taking up available ammonium, the substrate for nitrification. However, the effect of nitrification suppression on agroecosystem N losses remains poorly characterized, and a lack of temporal synchrony between nitrification, N losses, and nitrification suppression by plants could limit the effect of nitrification suppression. We used the DayCent-CABBI model to evaluate the effectiveness of the suppression of nitrification by sorghum to reduce N₂O emissions and nitrate leaching in an energy sorghum/soybean rotation at the Energy Farm in Urbana-Champaign, IL. We simulated nitrification suppression at the measured levels (measNS) and at the maximum measured level applied to the entire growing season (maxNS), and we also explored ways to better utilize nitrification suppression by altering the timing of UAN fertilizer applications. Model experiments showed that most nitrification occurred immediately after fertilizer was applied, whereas nitrification suppression begins to ramp up more than a month after planting. On an annual basis, measNS experiments showed a 1-2% reduction in annual N₂O emissions relative to no nitrification suppression (noNS), and maxNS experiments showed a 4-9% reduction in annual N₂O emissions relative to noNS. Both nitrification suppression levels showed < 1% reduction in nitrate leaching. Altering the timing of fertilizer applications to better synchronize nitrification suppression with high soil ammonium levels had mixed effects on annual N₂O emissions and nitrate leaching and sometimes resulted in increased N losses. The timing of simulated N₂O emissions shifted with the timing of fertilization, and N₂O emissions from denitrification increased when N₂O emissions from nitrification decreased. Increasing N retention during the non-growing season may be more effective and growing-season nitrification suppression for reducing annual N losses in the rainfed Midwest, particularly for NO₃⁻ leaching in the early spring. Optimizing the timing of nitrification

suppression alongside off-season N retention strategies would best improve the N sustainability of agroecosystems.

INTRODUCTION

Controlling nitrification, the microbial process primarily responsible for soil nitrate (NO_3^-) production, is key to reducing nitrogen (N) losses from agroecosystems. Leaching of NO_3^- from agricultural fields reduces available N to support crop yield and represents a significant environmental pollutant that contributes to downstream eutrophication (Van Meter et al. 2017). Nitrification also contributes to soil emission of nitrous oxide (N_2O), a potent greenhouse gas and precursor to stratospheric ozone-depleting compounds, by directly producing N_2O as a by-product and fueling denitrification through the production of its substrate, NO_3^- (Farquharson 2016). Synthetic nitrification inhibitors can reduce nitrification rates and thus NO_3^- leaching and N_2O emissions from agricultural fields (Di and Cameron 2002b, Gilsanz et al. 2016) but the cost of the inhibitors relative to variable gains in yield has hindered their widespread use (Yang et al. 2016). In recent years, the suppression of rhizosphere nitrification by plants, including biological nitrification inhibition (BNI) by plant root exudates, has received considerable attention as a more cost effective alternative (Subbarao et al. 2013b, Coskun et al. 2017, Nardi et al. 2020). While BNI has been demonstrated in laboratory and greenhouse settings (Subbarao et al. 2013a, Janke et al. 2018), the effect of plant nitrification suppression on N_2O emissions and NO_3^- leaching has remained uncertain (Teutscheroová et al. 2021, Burnham et al. 2022).

Plants can both indirectly and directly suppress nitrification in the rhizosphere, the zone of soil within approximately 3 mm of fine roots (Finzi et al. 2015). Plant roots indirectly suppress nitrification by creating conditions in the rhizosphere that reduce the activity of nitrifying bacteria and archaea. For example, plant roots and microbes compete for mineral nitrogen in the rooting zone, which can

significantly reduce the availability of ammonium (NH_4^+) (Inselsbacher et al. 2010) and lead to lower nitrification rates in the rhizosphere (Stienstra et al. 1994, Booth et al. 2005). In addition, heterotrophic microbes can potentially outcompete autotrophic nitrifiers for NH_4^+ in the rhizosphere as a result of dead roots and root exudates contributing to high organic carbon availability that favors heterotrophic activity (Elrys et al. 2021). Plants can also exhibit BNI through the release of root exudates demonstrated to directly inhibit nitrifier activity in laboratory assays in which collected root exudates are applied to soil or pure cultures of the ammonia oxidizer, *Nitrosomonas europaea* (Subbarao et al. 2013a, Coskun et al. 2017, Janke et al. 2018). Sorghum (*Sorghum bicolor* (L.) Moench) is one of the best studied plants with BNI capability, directly inhibiting nitrifiers by exuding sorgoleone, sakuranetin and methyl 3-(4-hydroxyphenyl) propionate (MHPP) from its roots (Subbarao et al. 2013a). Other grass species, including wheat, rye, and wild grasses, also have the capacity for direct BNI, suggesting that the trait may be widespread (O'Sullivan et al. 2016, Coskun et al. 2017, Subbarao et al. 2021). These indirect and direct effects of plants on nitrification can be difficult to partition (Teutscheroová et al. 2021), particularly in field studies (Burnham et al. 2022), so we consider them together as plant nitrification suppression.

Nitrification suppression is dynamic throughout the growing season over the lifespan of a plant (Nardi et al. 2020, Burnham et al. 2022), contributing to uncertainty about its effects on N losses from agricultural fields. Plant N demand changes based on growth stage (Moll et al. 1982, van Oosterom et al. 2010, Ciampitti and Vyn 2011), resulting in temporal variability in plant competition for NH_4^+ in the rhizosphere and indirect suppression of nitrification (Burnham et al. 2022). Furthermore, changes in the amount and composition of root exudates throughout annual crop development can lead to greater direct BNI later in the plant's life cycle (Zakir et al. 2008, Chaparro et al. 2013, Sarr et al. 2019, Nardi et al. 2020). Finally, as root biomass of annual crops increases over the growing season (Black et al. 2017), increasing rhizosphere volume presumably leads to greater indirect and direct suppression of nitrification at the field scale as the growing season progresses. Together these factors suggest little

nitrification suppression early in the growing season, with increasing nitrification suppression as the crops develop over the growing season.

The effectiveness of plant nitrification suppression in reducing agroecosystem N losses likely depends on the synchronization of temporal dynamics in nitrification rates and nitrification suppression over the growing season. In the agriculturally-intensive Midwestern US, the majority of fertilizer N (~75%) is applied at or before the planting of annual crops (Cao et al. 2018), when plant N uptake is low or non-existent. This leaves microbes as the main consumers of soil NH_4^+ prior to crop establishment, with nitrification rates many-fold higher than plant uptake or microbial assimilation (Inselsbacher et al. 2010). Plant root biomass and BNI compound release are also lower early in the growing season (Zakir et al. 2008, Black et al. 2017, Sarr et al. 2019), so both direct and indirect mechanisms of nitrification suppression by plants are minimal at the time of fertilizer-induced stimulation of nitrification. In addition, spring and early-summer rainfall account for approximately one-third of total annual precipitation in this region (Huff and Angel 1992), with the intensity and frequency of early growing season rain events increasing over the past few decades (Dai et al. 2016). This combination of fertilizer application and wet soil conditions in the early growing season could minimize the potential for plant nitrification suppression to reduce NO_3^- production and the resulting N_2O emissions and NO_3^- leaching losses from intensively-managed annual cropping systems.

Here we use in silico experiments to test the hypothesis that greater synchronization between plant nitrification suppression and gross nitrification rates leads to greater reductions in agroecosystem N losses via soil N_2O emissions and NO_3^- leaching. We specifically evaluated the potential for nitrification suppression by energy sorghum to reduce field-scale nitrogen losses in the Midwest U.S. Energy sorghum, an emerging bioenergy feedstock crop, is a high-yielding hybrid of biomass and sweet sorghum optimized for high vegetative growth and stem juice production. We calibrated and validated

the DayCent-CABBI process-based biogeochemical model for energy sorghum using field measurements from two energy sorghum experiments at the University of Illinois Energy Farm in 2018 and 2019, including estimates of plant nitrification suppression through the growing season (Moore et al. 2020b, Quinn 2021, Schetter et al. 2021, Burnham et al. 2022). To test how the timing of nitrification suppression affects N losses, we compared model experiments all simulating conventional fertilizer application at the time of planting but with different timing and magnitude of nitrification suppression (i.e., no nitrification suppression, with measured levels of nitrification suppression that changed over the growing season and varied inter-annually, and with a consistently high level of nitrification suppression starting one month after planting). To test how the timing of fertilizer-induced stimulation of nitrification affects N losses, we compared model experiments all simulating measured levels of nitrification suppression but with different timing of fertilizer application (i.e., conventional fertilizer application at the time of planting, with delayed fertilization by 3-weeks and by 5-weeks, and with split fertilizer application). In addition to evaluating annual N losses in the 2018 and 2019 sorghum years, we also examined how long-term weather variability affects nitrification suppression in a sorghum-soybean rotation through 2030.

METHODS

Site description

Field data were collected at the University of Illinois Energy Farm in Urbana, IL, USA (N 40.063607, W 88.206926). The predominant soil type at this site is the very deep, poorly drained Drummer silty clay loam (fine-silty, mixed, superactive, mesic Typic Endoaquolls). At this location, the mean annual temperature (2010-2019) was 9.0° C and the mean annual precipitation was 1023 mm, with a mean growing season temperature and precipitation of 17.0° C and 598 mm, respectively (NOAA National Climatic Data Center station ID USC00118740, 3.6 km from the field site). Daily weather data

(2008-2020) for model simulations were obtained from a weather station onsite at the Energy Farm. Mean annual N deposition was estimated at $8.5 \text{ kg N ha}^{-1} \text{ y}^{-1}$ based on $5.0 \text{ kg N ha}^{-1} \text{ y}^{-1}$ wet N deposition ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) from 2000-2019 obtained from the National Atmospheric Deposition Program for Bondville, Illinois, which is approximately 15 km from the study site (<http://nadp2.slh.wisc.edu/data/sites/siteDetails.aspx?net=NTN&id=IL11>, accessed December 4, 2020), and an assumption that dry deposition was 70% of wet deposition (McIsaac et al. 2002; USEPA, 2007).

Field experiment data

Field experiment data on mineral nitrogen pools, nitrification suppression, and aboveground biomass for calibrating the model simulations were obtained from a two-year fertilization field trial of energy sorghum conducted in 2018 and 2019. Field trial descriptions and methods for sample collection and analyses are provided in detail by Schetter et al. (2021) and Burnham et al. (2022). In brief, because sorghum is grown in annual rotation with soybean, the two years of the field trial were established on two separate but adjacent fields (200 m apart) with similar soils but slightly different historical management (Table 1, Table 2). This allowed each year of the field trial to occur in sorghum plots preceded by soybean the previous growing season. Within each field, replicated plots ($n = 4$) were set up in a randomized block design with four different levels of urea-ammonium-nitrate (1:1:1) (UAN) fertilizer that was applied within a week of planting: 0, 56, 112, and $168 \text{ kg N ha}^{-1} \text{ y}^{-1}$. These trials also included four energy sorghum genotypes, TX17500, TX17600, TX17800, and TX08001. However, we did not detect any differences in nitrification suppression between genotypes (Burnham et al. 2022), and all genotypes had similar growth curves and biomass yields (Schetter et al. 2021); thus, we averaged our data across the four genotypes. To evaluate changes in soil mineral nitrogen pools after the growing season, we measured potassium chloride (KCl) extractable NO_3^- and NH_4^+ in the top 30 cm of soil prior to fertilization and from 0-10 cm, 10-30 cm, and 30-50 cm depth after sorghum harvest. Nitrification

suppression measurements occurred three times each growing season, the first occurring about a month after sorghum was planted (late June 2018, early July 2019), the second in the middle of the growing season (late July 2018, early August 2019), and the last near the end of the growing season (late August 2018, early September 2019). We performed *ex situ* potential nitrification assays on bulk and rhizosphere soils using a method adapted from Belser and Mays (1980), estimating nitrification suppression rates as the difference between rhizosphere and bulk soil potential nitrification rates (Burnham et al. 2022).

Field experiment data on soil N₂O emission and root biomass for calibrating the model simulations were obtained from five sorghum plots located within 200-1000 m of the fertilization field trial and on the same soil series as the field trial. These plots include one 8.7 ha plot and four replicated 1.7 ha plots planted with the TX17500 genotype, which was also planted within the sorghum field trial, and fertilized with UAN at 112 kg N ha⁻¹ y⁻¹ rate at planting, similar to the field trial. We measured N₂O fluxes using the static flux chamber method 12 times throughout the 2018 growing season (May-October), and cumulative growing season N₂O emissions were calculated by linearly interpolating daily N₂O flux rates between sample dates. Details about these measurements and plots are provided by Moore et al. (2020b). Root biomass was measured at 0-10 cm, 10-20 cm, 20-30 cm, 30-50 cm, and 50-100 cm depth increments in soil cores collected from 2 locations near the center of each plot, with one core taken from an alley and one taken from a planted row. Fine (<2 mm) and coarse root biomass was separately quantified after manually extracting roots from soil cores and drying at 50°C for 72 hr (Quinn 2021).

DayCent-CABBI model calibration and validation

We used DayCent, a daily time-step, process based biogeochemical model (Parton et al. 1998) that has been extensively calibrated and validated to simulate the effects of agricultural management

on crop growth, long-term soil organic carbon dynamics (Six et al. 2004, Ogle et al. 2010) and N trace gas emissions (Del Grosso et al. 2005, Del Grosso et al. 2010). It is widely applied for quantification of greenhouse emissions budgets of varying production systems (Del Grosso et al. 2002, EPA 2018). The model was recently applied by to assess the sustainability of forage sorghum in the semiarid southwest (Duval et al. 2015) and to investigate energy sorghum production emissions relative to corn at 3,265 sites across the rainfed United States (Kent et al. 2020). For the model experiments in this research, we used a new version of DayCent that was developed by the Center for Advanced Bioenergy and Bioproducts Innovation (DayCent-CABBI) based on the DayCent-Photo model version (Straube et al. 2018) and applied to simulate switchgrass and maize at the Energy Farm by Moore et al. (2020a).

The model was set up to simulate the historic management of the site (Table S1, S2). Sorghum 2018 and sorghum 2019 were on separate plots with somewhat different cropping history from 2011 - 2017, each sorghum crop was preceded by and followed the next year by a soybean crop (Table S1, S2). Correctly simulating crop yields, root biomass, and C:N ratios of plant material is necessary to accurately estimate plant N-uptake from soils and plant residue inputs to soils. The model was calibrated using measured sorghum yields, C:N of sorghum yields, measured root biomass, C:N of root biomass, cumulative N₂O emissions during the 2018 growing season, and pre- and post-growing season ammonium and nitrate levels in the soil. To validate the model, parameters determined from the calibration were applied using all available 2019 data, including sorghum biomass yields, C:N of sorghum yields, and pre-growing season ammonium and nitrate levels. The model assumed a maximum rooting depth of 60 cm for both sorghum and soybeans with the majority of roots between 5 and 30 cm (Benjamin and Nielsen 2004, Quinn 2021). The distribution of roots affects both plant N uptake and transpiration. The simulated values used for calibration and/or validation were those from the measNS model experiments (measured nitrification suppression levels with no fertilizer delay, described below) since those experiments most closely replicated management that occurred in the field experiments.

188

189 *Model Experiments*

190   We performed six primary model experiments to quantify the effect of nitrification suppression
 191   on N₂O emissions (from both nitrification and denitrification), NO₃⁻ leaching (nitrate exiting the soil
 192   profile), and sorghum aboveground net primary productivity (ANPP). In each primary experiment, we
 193   ran simulations for each sorghum growing season (2018 and 2019) with four levels of fertilizer
 194   applications (N-rates; 0, 56, 112, and 168 kg N ha⁻¹ y⁻¹) for each primary experiment (Table 3). To run the
 195   model experiments over a wider range of weather conditions, the model experiments were continued to
 196   the year 2030, starting with 2019 sorghum and alternating sorghum and soybean so that sorghum
 197   occurs on the odd calendar years and soybeans on the even calendar years. Weather from 2009-2018
 198   was used for the 2021-2030 scenarios. Nitrification suppression levels during sorghum years were
 199   equivalent to those measured in sorghum in 2019, and there was no nitrification suppression or
 200   fertilization during soybean years.

201   The first set of three primary model experiments focused on different scenarios of the
 202   magnitude and timing of nitrification suppression: no nitrification suppression (noNS), measured
 203   nitrification suppression (measNS), and maximum nitrification suppression (maxNS). The noNS
 204   experiment represents actual crop management but assumes that no nitrification suppression occurs.
 205   This experiment serves as a baseline against which nitrification suppression effects on crop yield and
 206   agroecosystem N losses are assessed. The measNS experiment simulates the actual crop management
 207   and nitrification suppression levels that were measured in the field by Burnham et al. (2022) and begin
 208   approximately one month after planting sorghum (Table S2). The purpose of this model experiment is to
 209   evaluate reductions in N losses by measured levels of nitrification suppression, which peaks in the mid-
 210   growing season. The maxNS experiment simulates actual crop management but assumes a consistently

high nitrification suppression level, a 70% reduction in nitrification that begins one month after planting sorghum (same date as the measNS experiment) and continues to the end of the growing season in mid-October (18 weeks). The purpose of this experiment is to evaluate the maximum possible benefit of BNI if it were able to achieve its highest measured level for the entire growing season. The effects of nitrification suppression (measNS and maxNS) are reported relative to no nitrification suppression (noNS) experiment results.

The second set of three primary model experiments focused on evaluating the potential for fertilizer management (timing) to maximize N loss reduction benefits from measured levels of nitrification suppression. We investigated three scenarios of delayed or split fertilizer application aimed at better synchronizing the fertilizer-induced stimulation of nitrification with the onset of nitrification suppression, which starts approximately 1 month after planting: 3-week delayed, 5-week delayed, and split fertilizer application. The 3-week delayed experiment simulated actual crop management except that the UAN fertilizer application occurred three weeks later than it was actually applied in the field experiments. The 5-week delayed experiment simulated actual crop management except that the UAN fertilizer application occurred five weeks later than it was actually applied in the field experiments. The split application experiment simulated actual crop management except that half the UAN fertilizer was applied on the date it was actually applied in the field, and the other half was applied four weeks later. The effects of altered fertilizer management are reported relative to the measNS experiment which differs from these three experiments only in the timing of fertilizer application.

RESULTS

Model calibration and validation

The means of measured values of harvested sorghum biomass increased with N-rate, but these means were not significantly different from one another in either 2018 or 2019 (Figure S2 A, B).

Simulated sorghum yield also increased with N-rate, and simulated values (one value per fertilization level per year) were not significantly different from mean observed values except at no fertilizer in 2019 (Figure S2 A, B). The C:N ratio of measured and simulated sorghum harvested biomass (2018) decreased with N-rate, and simulated values were within one standard error mean of measured values (Figure S2 C). The peak simulated root biomass for both N-rates (0.0 and 168 kg N ha⁻¹ y⁻¹) in 2018 were also within one standard error of the measured means (Figure S1 A), as was the simulated C:N ratios of root biomass (Figure S1 B).

Cumulative measured N₂O emissions began May 5, 2018 (Time = 2018.34) for the 112 kg N ha⁻¹ y⁻¹ fertilizer treatment; therefore, simulated cumulative N₂O emissions for sorghum 2018 from the measNS model experiment at the same N-rate that began on this same date were compared to the measured values (Figure S3). Though simulated cumulative emissions initially increased at a greater rate than measured emissions, simulated emissions are within the lower and upper bound of measured emissions by the beginning of June (Time = 2018.42). By the end of the growing season in mid-October (Time = 2018.8), simulated cumulative N₂O emissions are comparable to the mean of measured emissions.

Pre-growing season measured ammonium and nitrate levels were made for the sorghum plots on May 18, 2018 and June 7, 2019, while post-growing season measurements were made on October 1, 2018 (Table S3). The depths over which measurements occurred varied and were not always directly comparable to DayCent results because DayCent simulates a single soil ammonium pool assumed to occur in the top 20 cm of soil while simulated nitrate pools exist for each of the 13 soil layers and extend through the soil profile down to 180 cm. Pre-growing season measured ammonium levels in the 0-15cm layer in 2018 (0.8 ± 0.3 kg N ha⁻¹) and in the 0-30cm layer in 2019 (2.2 ± 0.2 kg N ha⁻¹) were less than

DayCent's estimates of total soil ammonium (2.9 and 3.6 kg N ha⁻¹ in 2018 and 2019, respectively) (Table S3). Pre-growing season measured nitrate levels in the 0-15 cm layer in 2018 (14.6 ± 1.3 kg N ha⁻¹) were greater than DayCent's estimate for the same depth (7.4 kg N ha⁻¹). Pre-growing season measured nitrate levels in the 0-30cm layer in 2019 (25.6 ± 0.7 kg N ha⁻¹) were close to DayCent's estimate over the same depth (23.0 kg N ha⁻¹). DayCent showed greater rooting zone depletion of soil ammonium and nitrate by the end of the growing season than measurements indicated. The 2018 post-growing season measurements of ammonium (25 ± 1.7 kg N ha⁻¹) and nitrate (25 ± 7.6 kg N ha⁻¹) in the top 50 cm of soil were greater than DayCent's estimates, 2.0 kg N ha⁻¹ for total ammonium and 1.2 kg N ha⁻¹ (0-45 cm) for nitrate, however DayCent showed high nitrate levels deeper in the soil profile where measurements were not taken (Table S3). On average across all simulations, DayCent showed that approximately 35% of all soil nitrate existed in depths from 105 - 180 cm.

Nitrification suppression effects on sorghum nitrogen losses in 2018 and 2019

On an annual basis, the measNS and maxNS experiments showed little to no reductions in gross nitrification rates, N losses, and ANPP relative to the noNS experiments across all four fertilizer application rates. In 2018 and 2019, measNS reduced gross nitrification rates for sorghum by 1% and 2-3%, respectively. In contrast, maxNS led to about 10 times greater effects on nitrification, reducing gross rates for sorghum by 11-14% and 7-12% in 2018 and 2019, respectively (Figure 1 A, B; Figure 2 A, B). Patterns in annual N₂O emissions mirrored those in annual gross nitrification rates, with the measNS experiment showing a 1-2% reduction and the maxNS experiment showing a 4-10% reduction, with greater reductions as fertilizer application rates increased (Figure 1 C, D; Figure 2 C, D; Table S4). However, sorghum NO₃⁻ leaching did not respond to nitrification level suppression in 2018 as NO₃⁻ leaching was limited to ~ 2.7 kg N ha⁻¹ due to relatively dry conditions in the mid-growing season (Figure

1 E; Figure 2 E). However, even in 2019 when more typical rainfall amounts led to NO_3^- leaching of ~16-17 kg N ha⁻¹, both measNS and maxNS showed less than 0.5% reduction in NO_3^- leaching (Figure 1 F; Figure 2 F, Table S4). In both 2018 and 2019, simulated ANPP from the noNS, measNS, and maxNS experiments were comparable for a given fertilizer level (Figure S4).

Most nitrification occurred prior to establishment of annual sorghum crops and suppression of nitrification in both 2018 and 2019, regardless of fertilization rate (Figure 3). In fertilized plots, fertilizer was applied within a week of planting sorghum (Table S2) and nitrification increased dramatically immediately after fertilization in both in sorghum 2018 and 2019 (Figure 3 B). For the measNS model experiment with a fertilizer application rate of 112 kg N ha⁻¹ y⁻¹, 83% of annual gross nitrification had occurred by the time nitrification suppression was first measured on June 27th, and in 2019 88% of annual gross nitrification had occurred by the time nitrification suppression was first measured on July 8th (Figure 3B). In both fertilized and unfertilized plots, little nitrification occurred after mid-season (Figure 3). Despite very low levels of nitrification after the early-season, nitrification was lower in the maxNS experiment than either noNS or measNS throughout the growing season, and this difference was greater in fertilized plots than unfertilized plots (Figure 3). Likewise, most cumulative nitrification, N₂O emissions, and NO_3^- leaching occurred early in the season in 2018 and 2019, and only the maxNS experiment maintained lower cumulative totals throughout the remainder of the season (Figure 2).

Fertilizer timing effects on sorghum nitrogen losses in 2018 and 2019

Delaying fertilizer application affected simulated above-ground net primary production (ANPP) of sorghum in both years but splitting fertilizer applications had minimal effects on simulated ANPP compared to one-time fertilizer applications at a given total fertilizer application rate (Figure S4 C, D). In 2018 and 2019, delayed fertilizer applications increased ANPP by 3 - 6% for N-rates of 56 and 112 kg N

ha⁻¹ y⁻¹, but decreased ANPP by as much as 7% for N-rates of 168 kg N ha⁻¹ y⁻¹, while splitting fertilizer applications increased ANPP from 0.0 to 2% relative to conventional fertilizer application around the time of planting (Figure S4 C, D).

In the fertilizer timing experiments, simulated gross nitrification increased immediately when UAN fertilizer was applied, both in sorghum 2018 and 2019 (Figure 4). Subsequently, the timing of fertilizer-induced gross nitrification was shifted so that it occurred when measured nitrification suppression commenced (Figure 4). However, delaying fertilizer application by three or five weeks increased annual gross nitrification rates 4.3-7.0% for sorghum 2018 relative to no delay in fertilizer application at all (Figure 5 A; Table S5). This led to a 3 – 9% increase in annual N₂O emissions but only small changes (≤ 1%) in NO₃⁻ leaching during 2018 (Figure 5 C, E; Table S5). In contrast, in 2019 when measured nitrification suppression levels were higher, delayed fertilizer application consistently decreased simulated gross nitrification rates by 1– 10% (Figure 4 B; Figure 5 B; Table S5). Additionally, the decrease in gross nitrification rates with delayed fertilizer addition in 2019 was greater at higher fertilization rates (Table S5). When 56 kg N ha⁻¹ fertilization was delayed 3-weeks and 5-weeks, annual gross nitrification was 0.3% higher and 2.7% lower than without fertilization delay, respectively. However, when 168 kg N ha⁻¹ fertilization was delayed 3- and 5-weeks, annual gross nitrification was 6.5% and 10.2% lower than without fertilization delay, respectively. At the same time, altering fertilization timing had variable effects on annual N₂O emissions, which decreased 0.3-3.2% when 56 kg N ha⁻¹ fertilization application was delayed but increased 1.0-1.5% when 112 or 168 kg N ha⁻¹ application was delayed (Table S5). Delayed fertilizer application reduced NO₃⁻ leaching by 0.5–3%, again with greater decreases in leaching with longer delays in application and higher application rates relative to fertilization at planting.

How split fertilizer application affected N losses from sorghum also differed between 2018 and 2019. In 2018, split application of 56 kg N ha⁻¹ led to a 2.0% decrease in gross nitrification rate relative to

fertilization at planting, but split application of higher amounts of fertilizer increased gross nitrification 0.8-0.9 %, and a similar pattern was observed in the effect of split fertilizer application on N₂O emissions (Table S5). Also in 2018, split fertilizer application led to consistent 0.5-1.1% decreases in NO₃⁻ relative to fertilization at planting, with greater decreases at higher fertilization rates. In 2019, split fertilizer application consistently reduced annual gross nitrification, annual N₂O emissions, and NO₃⁻ leaching rates. by 2-7%, annual N₂O emissions by 1– 4%, and NO₃⁻ leaching by < 1% (Table S5). Split fertilizer application of 56 kg N ha⁻¹ reduced gross nitrification 7.0% and N₂O emissions 4.0% compared to fertilization at planting, but these reductions declined to 1.6-2.7% for nitrification and 0.8-1.6% for N₂O emissions with higher fertilizer application rates (Table S5). In contrast, 2019 NO₃⁻ leaching was consistently reduced by 0.4-0.6% by splitting fertilization, regardless of application rate.

Cumulative N₂O emissions increased immediately at the time of fertilizer application and were 80% nitrification-derived by mid-June 2019 (Figure S5). However, for a given fertilizer application rate, cumulative annual N₂O emissions converged across all three timing experiments by the end of the growing season. Increases in denitrification-derived N₂O emissions compensated for reductions in nitrification-derived N₂O emissions earlier in the growing season (Figure S5). Conditions suitable for denitrification increased in the latter part of the 2019 growing season as soil moisture increased whereas soil moisture was lowest during July and August (Figure S6). By the end of 2019, 78% of simulated N₂O emissions were nitrification-derived and 22% were denitrification-derived.

Sorghum legacy effects on soybean nitrogen losses

Although no nitrification suppression or fertilizer application was simulated during the soybean years (2019 and 2020), we observed residual effects from the sorghum years (2018 and 2019) on N losses from soybean (Figure 1; Table S6). N₂O emissions, NO₃⁻ leaching, and gross nitrification all

increased with the previous year's fertilization rate (Figure 1). However, there were only small effects of nitrification suppression from the previous year on either N₂O emissions, NO₃⁻ leaching, or gross nitrification (Figure 1). Across all 2019 sorghum fertilization rates, 2020 soybean N₂O emissions were stimulated by up to 0.2% in the measNS experiment and up to 2% in the maxNS experiment (Table S6). In contrast, 2020 soybean NO₃⁻ leaching was consistently reduced in the measNS and maxNS experiments by 0.2-0.6% and 0.6-3.0%, respectively (Table S6).

Altering the timing of sorghum fertilizer application in 2019 generally reduced N₂O emissions and NO₃⁻ leaching in 2020 soybean. Soil N₂O emissions were reduced by 0.5 to 3% relative to fertilizer addition at planting in all but the 168 kg N ha⁻¹ y⁻¹ split fertilizer addition. Nitrate leaching was reduced by 2-6% across all fertilizer application rates in all three fertilizer timing experiments.

Long-term effects of nitrification suppression and fertilizer timing on nitrogen losses

Over the period of 2019-2030, with sorghum simulated in odd years and soybean simulated with no fertilization or nitrification suppression in even years, nitrification suppression reduced cumulative N losses by a small amount. The measNS experiment showed a 0.5-1% reduction in cumulative N₂O emissions and <1% reductions in cumulative NO₃⁻ leaching relative to the noNS experiment (Table 2). In contrast, the maxNS experiment showed a 1.5-5% reduction in cumulative N₂O emissions and a 1-3% reduction in cumulative NO₃⁻ leaching compared to the same fertilizer application rate in the None experiment; the N loss reductions increased with application rate (Table 2).

Fertilizer timing experiments showed mixed effects on cumulative 2019–2030 N losses. The 3-week and 5-week delayed experiments showed changes from -2% to +2% in cumulative N₂O emissions relative to no fertilizer delay at the same fertilizer application rate (Table 2). For fertilizer application

rates of 56 and 112 kg N ha⁻¹ y⁻¹, the delayed experiments reduced total nitrate leaching 2–4% relative to no fertilizer delay; however, total nitrate leaching increased by 16–18% when fertilizer application rate was 168 kg N ha⁻¹ y⁻¹ (Table 2). The split fertilizer experiment showed a 1–4% reduction in cumulative N₂O emissions and a 2–4% reduction in cumulative NO₃⁻ leaching compared to the same fertilizer application rate in the no delay experiment (Table 2).

Changes in 2019–2030 cumulative N₂O and NO₃⁻ leaching from altering fertilizer N application rates were a magnitude greater than in any other N loss mitigation experiment (Table 2). Compared to corresponding experiments with no UAN fertilizer, experiments with 56 kg N ha⁻¹ y⁻¹ fertilization increased N₂O emissions by 18–22% and cumulative NO₃⁻ leaching by 13–17%; experiments with 112 kg N ha⁻¹ y⁻¹ fertilization increased N₂O emissions by 45–50% and cumulative NO₃⁻ leaching by 31–37%; experiments with 168 kg N ha⁻¹ y⁻¹ fertilization increased N₂O emissions by 81–93% and cumulative NO₃⁻ leaching by 65–100%.

DISCUSSION

We hypothesized that the effectiveness of nitrification suppression in reducing agroecosystem N losses via soil N₂O emissions and NO₃⁻ leaching would depend on the synchronization between nitrification suppression and the fertilizer-induced spike in nitrification. As expected, our model experiments showed that, with conventional timing of fertilizer application with planting, nitrification suppression led to relatively low (< 9%) reductions in annual N losses for energy sorghum. Consistent with rapid nitrification of NH₄⁺ applied in fertilizer (Norton and Ouyang 2019), gross nitrification rates peaked before nitrification suppression started in the fifth week after planting (Figure 3). Our fertilizer timing model experiments showed that, as expected, delayed and split fertilizer application delayed the fertilizer-induced spike in nitrification to better synchronize it with measured seasonal patterns in

nitrification suppression levels. However, we found that the effect of fertilizer timing on gross nitrification and N loss rates depended on temperature and soil moisture around the time of fertilizer application such that these rates could increase or decrease, depending on the weather in a given year. Moreover, we found greater effects of nitrification suppression on annual rates of soil N₂O emissions than NO₃⁻ leaching, likely due to differing environmental controls on these two N loss pathways. Here we discuss potential mechanisms leading to these patterns in nitrification suppression and fertilizer timing effects predicted by the DayCent-CABBI model and the implications of our findings for the potential use of nitrification suppression to reduce agroecosystem N losses in the Midwest U.S. and elsewhere.

Nitrification suppression may have little effect on annual cumulative gross nitrification rates regardless of the timing of nitrification suppression or fertilizer application. Our model experiments show that delaying or splitting fertilizer application reduces early growing season gross nitrification rates by shifting later the fertilizer-induced spike in nitrification and by synchronizing the spike with start of nitrification suppression to reduce the magnitude of the spike (Figure 4). However, this reduction is offset by increased gross nitrification rates later in the growing season, likely by shifting elevated soil NH₄⁺ concentrations to when warmer summer temperatures stimulate microbial activity in general (Agehara and Warncke 2005, Ouyang et al. 2017, Norton and Ouyang 2019). Therefore, altering fertilizer timing had little effect on cumulative gross nitrification rates at the annual time scale (Figure 2EF). In irrigated systems, higher moisture and temperature later in the season could further increase annual gross nitrification when fertilization is delayed (Agehara and Warncke 2005). Similar to our findings, altering fertilizer timing had inconsistent effects on losses of both NO₃⁻ leaching (Eagle et al. 2017) and N₂O emissions (Zebarth et al. 2008, Phillips et al. 2009, Zebarth et al. 2012, Han et al. 2017) in past field studies. These results suggest that environmental controls on nitrification should be taken into account

when considering potential effects of nitrification suppression and fertilizer timing on annual cumulative gross nitrification rates.

Simulated effects of nitrification suppression and fertilizer timing on N₂O emissions mirrored effects on gross nitrification. This is largely due to the predicted role of nitrification as the dominant N₂O source at our site and the source of NO₃⁻ for denitrification. Our model experiments show that nitrification is responsible for 78% of annual soil N₂O emissions, consistent with some empirical studies in cropping systems (Skiba et al. 1993, Bremner 1997, Stevens et al. 1997, Toyoda et al. 2011, Tierling and Kuhlmann 2018) but inconsistent with other empirical studies showing denitrification as the dominant N₂O source (Müller et al. 2014, Li et al. 2016, Liang and Robertson 2021). In particular, nitrification appears to be a significant source of N₂O when NH₄⁺ substrate is abundant and soils are not fully saturated (Bremner 1997, Toyoda et al. 2011), which is consistent with the significant increase in both gross nitrification and N₂O emissions after fertilization in our study. If N₂O is mostly nitrification-derived after fertilization, delaying fertilizer addition to better align N availability with the suppression of nitrification should lead to reduced N₂O losses (Ferrari Machado et al. 2020). However, because delayed or split fertilizer timing also led to high soil NO₃⁻ concentrations later in the growing season when soil moisture conditions were more conducive for denitrification (Bremner 1997) (Figure S6), greater late season denitrification-derived soil N₂O emissions partially offset earlier season reductions in nitrification-derived soil N₂O emissions (Figure S5). Therefore, reductions in annual soil N₂O emissions due to nitrification suppression or changes in fertilizer timing were smaller than reductions in cumulative annual gross nitrification rates.

Our model experiments suggest that NO₃⁻ leaching is dominantly controlled by water flux such that changes in soil NO₃⁻ concentrations caused by nitrification suppression or different fertilizer management have limited effects on annual NO₃⁻ leaching rates. In the Midwestern US, a large

proportion of annual NO_3^- leaching occurs in the non-growing season, especially April-June, when low evapotranspiration rates lead to substantial water flux through the soil profile and high rates of NO_3^- loss out of the soil (Di and Cameron 2002a, Randall and Vetsch 2005, Liao et al. 2012, Hess et al. 2020). Therefore, suppression of nitrification, either by plants or synthetic nitrification inhibitors applied in-season (Eagle et al. 2017), has a limited impact on annual NO_3^- leaching rates. However, in regions with high precipitation mid-growing season or in irrigated systems, NO_3^- losses could be temporally synchronized with nitrification suppression (Ritter 1989, Nyamangara et al. 2003, Mack et al. 2005, van der Laan et al. 2010), and since nitrification increases with temperature and moisture (Agehara and Warncke 2005), suppression by plants could have a larger effect. Surprisingly, the model predicted little to no effect of fertilizer application rate on NO_3^- leaching in 2018 or 2019 (Figure 2EF), which is inconsistent with past studies in the Midwest showing increased N leaching with higher fertilizer application rates (Helmets et al. 2012, Smith et al. 2013, Hussain et al. 2020). In our study, it appears that the rate of fertilizer addition had a larger impact on NO_3^- leaching the following growing season. For example, despite a lack of fertilizer effect in 2018 sorghum plots, higher fertilization in 2018 increased the amount of NO_3^- leached the following season then the plots were planted with soybean (Figure 1). We also found a net positive effect of fertilization rate on NO_3^- leaching from 2019-2030, which accounts for lag effects in the long-term (Table 2). In addition, the recommended fertilization rate for bioenergy sorghum is 112 kg N ha^{-1} , and we detected significant increases in NO_3^- leaching when we exceeded this recommended rate and applied 168 kg N ha^{-1} (Table 2). However, there are also challenges in measuring the field-scale effects of management practices on NO_3^- exiting the soil profile (Hess et al. 2020, Wey et al. 2021), so empirical datasets for calibrating and validating NO_3^- leaching in models are scarce. Since there is significant water flux through the soil profile during the non-growing season after sorghum harvest and prior to crop establishment the next year, our results underscore the importance of water

flux and variability in precipitation in controlling NO_3^- leaching, especially during fallow periods (Di and Cameron 2002a, Randall and Vetsch 2005, Liao et al. 2012).

Since nitrification suppression by sorghum had only very small effects on N losses, we also found no evidence of legacy effects of sorghum nitrification suppression on N losses in the following soybean growing seasons. In fact, despite low leaching rates of NO_3^- during the 2018 sorghum growing season, much of the NO_3^- that had been stored within the soil was lost during the wetter 2019 soybean growing season (Figure 1). When sorghum-soybean rotations were simulated through 2030 over a realistic range of weather conditions, consistent but small reductions in long-term cumulative N losses (Table 2) illustrate that sorghum nitrification suppression does have some control over N_2O emissions and leaching of NO_3^- . However, the limited magnitude of sorghum's influence further emphasizes the strength of environmental control and impact of mineralization on N losses (Di and Cameron 2002a, Loecke et al. 2017, Leitner et al. 2020). In addition, reducing the amount of N fertilizer applied was more effective in reducing N_2O emissions and nitrate leaching over the long-term than in 2018 and 2019 model simulations, and reducing N inputs had a much stronger effect than any rate of nitrification suppression.

Management strategies targeted at retaining N in the non-growing season may be more effective for reducing annual N losses from agroecosystems in climates such as the rainfed Midwest where spring thaw and fall precipitation drive a large proportion of annual NO_3^- leaching. For example, incorporating winter cover crops into annual rotations eliminates the spring fallow period and is an effective strategy for limiting spring N losses (Di and Cameron 2002a, Jewett and Thelen 2007, Blanco-Canqui et al. 2021). Since bioenergy and biomass sorghum crops are harvested for aboveground biomass rather than grain yield, farmers have more flexibility in the timing of harvest and cover crop planting in the late summer or early fall when sorghum biomass begins to plateau (Schetter et al. 2021).

This could provide a unique opportunity for synergy between nitrification suppression by sorghum during the growing season and uptake of available N during the fall and spring by a cover crop. Combining nitrification suppression during the growing season with off-season management to retain N is likely to achieve lower N losses and better agroecosystem sustainability than either strategy alone.

CONCLUSION

Despite recent advances in our understanding of the ability of some plant species to suppress nitrification in the rhizosphere, the impact on agroecosystem N losses has remained poorly characterized. We show that nitrification suppression in the rhizosphere of energy sorghum has only a limited capacity to reduce field-scale emissions of N_2O and leaching of NO_3^- due to asynchrony between peak gross nitrification in the spring prior to crop establishment and suppression of nitrification by sorghum plants mid-season. Thus, breeding or engineering crops to simply increase nitrification suppression will lead to only modest reductions in N losses in rainfed regions where significant precipitation occurs outside of the growing season. Altering the timing of fertilizer addition to better synchronize N availability with crop N demand and nitrification suppression can reduce N losses. However, delaying fertilization also increases N availability when environmental conditions, especially soil temperature and moisture, favor higher biological activity (i.e., nitrification and denitrification rates), reducing and sometimes negating the effect of altered fertilizer timing on cumulative N losses. Additionally, because the controls on N_2O emissions and NO_3^- leaching are different, the magnitude and direction of effects of nitrification suppression and fertilizer timing on these two N loss pathways can differ. Overall, management strategies targeted at retaining N in the non-growing season may be more effective for reducing annual N losses in the rainfed Midwest, particularly for NO_3^- leaching in the early spring. Given the potential for different timing of N losses in irrigated systems, we suggest that the

effects of nitrification suppression on N₂O emissions and NO₃⁻ leaching be further explored in regions where irrigation is more common. Further research on nitrification suppression should prioritize optimizing its timing and magnitude alongside off-season N retention strategies to improve the N sustainability of agroecosystems.

ACKNOWLEDGEMENTS

The authors thank Adam von Haden and Ryan Quinn for their efforts quantifying root biomass in these trials. We also thank August Schetter and DK Lee for measuring and providing aboveground biomass and yield data. We also thank Avarna Jain, Taylor Bozman, Allison Cook, Ingrid Holstrom, Rachel van Allen, Elle Lucadamo, Sierra Raglin, Rachel Waltermire, and Sandra Simon for their help with measurements of soil N pools and nitrification suppression. We also thank Dr. Bill Rooney for supplying the germplasm via the Texas A&M Sorghum Breeding Program. Finally, we thank Tim Mies and Trace Elliott for their management of planting and fertilization at the UIUC Energy Farm. This work was supported by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420).

AUTHOR CONTRIBUTIONS

MDH led the writing of the manuscript and performed all DayCent model simulations. MB and AF provided field data. All authors contributed critically to the draft and gave final approval for publication.

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Zebarth, B. J., E. Snowdon, D. L. Burton, C. Goyer, and R. Dowbenko. 2012. Controlled release fertilizer product effects on potato crop response and nitrous oxide emissions under rain-fed production on a medium-textured soil. *Canadian Journal of Soil Science* **92**:759-769.

741 Table 1. Summary of model nitrification suppression and fertilizer timing experiments. These treatments applied to years that sorghum was grown starting in
742 2018 or 2019.

Primary experiment (abbreviation)	Sorghum UAN fertilizer application rates and timing for the experiment	Nitrification suppression level
Measured nitrification suppression (measNS)	0, 56, 112, 168 kg N ha ⁻¹ y ⁻¹ within a few days of planting sorghum	nitrification suppression as was measured in the field
No nitrification suppression (noNS)	0, 56, 112, 168 kg N ha ⁻¹ y ⁻¹ within a few days of planting sorghum	no reduction in gross nitrification
Maximum nitrification suppression (maxNS)	0, 56, 112, 168 kg N ha ⁻¹ y ⁻¹ within a few days of planting sorghum	70% reduction in gross nitrification starting 1 month after planting and continuing 16 weeks, to the end of the growing season
Delay one-time fertilizer application 3 weeks (measNS_dly3wk)	56, 112, 168 kg N ha ⁻¹ y ⁻¹ three weeks after actual fertilization date	nitrification suppression as was measured in the field
Delay one-time fertilizer application 5 weeks (measNS_dly5wk)	56, 112, 168 kg N ha ⁻¹ y ⁻¹ five weeks after actual fertilizer date	nitrification suppression as was measured in the field
Split fertilizer application (measNS_split)	56, 112, 168 kg N ha ⁻¹ y ⁻¹ where half is applied a few days of planting sorghum, the other half 4 weeks later	nitrification suppression as was measured in the field

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745 Table 2. Simulated cumulative N₂O emissions and nitrate leaching from 2019-2030, where sorghum was grown in odd years and soybean was grown in even
746 years. Experiments include sorghum nitrification suppression (noNS = no nitrification suppression, measNS = measured nitrification suppression (2019), maxNS
747 = maximum nitrification suppression) and fertilizer timing experiments (dly3wk = 3-week delay, dly5wk=5 week delay, split = split fertilizer application) at four
748 UAN fertilizer application amounts (N-rate = 0, 56, 112, or 168 kg N ha⁻¹ y⁻¹). No nitrification suppression or fertilizer was applied in soybean years.

Treatment (sorghum/soybean 2019-2030)	N ₂ O emissions, 2019-2030 (Total, kg N ₂ O-N ha ⁻¹)	NO ₃ ⁻ leaching, 2019-2030 (Total, kg NO ₃ -N ha ⁻¹)	Emissions change vs. noNS at same N-rate (%)	Leaching change vs. noNS at same N-rate (%)	Emissions change vs. measNS at same N- rate (%)	Leaching change vs. measNS at same N- rate (%)	Emissions change vs. no fertilizer, measNS (%)	Leaching change vs. no fertilizer, measNS (%)
000_noNS	72.5	88.8						
000_measNS	72.2	88.8	-0.5	-0.3				
000_maxNS	71.4	87.6	-1.5	-0.9	-1.0	-0.6		
056_noNS	88.6	104.4						
056_measNS	88.2	104.4	-0.5	-0.3			+22.2	+17.3
056_maxNS	87.1	103.2	-1.8	-1.0			+20.7	+16.5
056_measNS_dly3wk	88.4	102.0			+0.2	-1.8	+22.5	+15.2
056_measNS_dly5wk	86.7	99.6			-1.7	-3.9	+20.1	+12.8
056_measNS_split	84.9	100.8			-3.7	-3.5	+17.7	+13.3
112_noNS	108.6	121.2						
112_measNS	107.6	121.2	-0.8	-0.5			+49.2	+36.6
112_maxNS	105.4	120.0	-2.9	-1.7			+46.1	+35.0
112_measNS_dly3wk	106.5	116.4			-1.1	-4.3	+47.6	+30.7
112_measNS_dly5wk	108.6	118.8			+0.9	-2.3	+50.5	+33.4
112_measNS_split	104.8	117.6			-2.6	-3.4	+45.3	+32.0
168_noNS	137.5	151.2						
168_measNS	136.1	150.0	-1.0	-0.6			+88.6	+69.5
168_maxNS	130.5	146.4	-5.1	-3.0			+80.9	+65.4
168_measNS_dly3wk	139.5	174.0			+2.5	+16.1	+93.3	+96.8
168_measNS_dly5wk	138.2	177.6			+1.5	+17.9	+91.5	+99.7
168_measNS_split	135.0	147.6			-0.8	-1.5	+87.1	+66.9

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Figure Legends

Figure 1. Simulated annual rates of gross nitrification (a, b), N₂O emissions (c, d), and NO₃⁻ leaching (e, f) under maximum (maxNS), measured (measNS), and no (noNS) suppression of nitrification by sorghum at 0 and 112 kg N ha⁻¹ fertilization rates in 2018 (A, C, E) and 2019 (B, D, F) sorghum field trials. All rates were simulated during the sorghum growing season (2018 & 2019) and the following growing season when each trial was planted in soybean.

Figure 2. Simulated cumulative gross nitrification (A, B), N₂O emissions (C, D), and NO₃⁻ leaching (E, F) throughout the 2018 (A, C, E) and 2019 (B, D, F) growing seasons under maximum (maxNS), measured (measNS), and no (noNS) nitrification suppression by sorghum. Simulated rates are shown for noNS in unfertilized plots (red line) and for all simulated nitrification suppression rates in plots fertilized at a rate of 112 kg N ha⁻¹.

Figure 3. Simulated daily gross nitrification rate (kg N ha⁻¹ d⁻¹) during the 2018 and 2019 growing seasons in unfertilized (A) and fertilized (112 kg N ha⁻¹, B) plots at three levels of nitrification suppression.

Figure 4. Simulated daily gross nitrification rate (kg N ha⁻¹ d⁻¹) under measured nitrification suppression (measNS) during the 2018 and 2019 growing seasons with fertilizer addition (112 kg N ha⁻¹) delayed either 3 (A) or 5 (B) weeks. Solid black lines show gross nitrification rates without fertilizer delay, dashed lines show gross nitrification rates with delayed fertilizer addition, and the solid green lines show the timing and level of simulated nitrification suppression (measNS).

Figure 5. Simulated cumulative gross nitrification, N₂O emissions, and NO₃⁻ leaching throughout the 2018 and 2019 growing seasons with 112 kg N ha⁻¹ fertilizer addition delayed 3 weeks or 5 weeks, or split into two additions, under measured (measNS) nitrification suppression by sorghum.

772 Figure 1.

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775 Figure 2.

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Figure 3.

Figure 4.

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