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Distinct N-cycling microbial communities contribute to microtopographic variation in soil N_2O emissions from denitrification

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ABSTRACT

Climate change is increasing the frequency and intensity of large precipitation events that flood soils and establish anoxic conditions that promote microbial denitrification, a predominant source of atmospheric nitrous oxide (N_2O, a strong greenhouse gas). Denitrification may be favored within topographic depressions in otherwise flat fields that are prone to ponding, establishing "hotspots" of N_2O emissions. The location of N_2O hotspots may also depend on the distribution of soil microbial communities that are responsible for the production and consumption of N_2O in soils. Yet, relating soil microbial community composition to N_2O emissions remains challenging. To assess how spatial variation in soil microbial communities affects N_2O emissions, we measured the community composition of active microorganisms using amplicon-based sequencing of cDNA generated from mRNA transcripts associated with N-cycling processes in response to experimentally flooding and draining soils in the lab. We also used stable isotope tracers to relate microbial communities to process rates. Consistent with the hypothesis that denitrifying microbial communities are not functionally redundant, we found that the diversity of microbial taxa expressing nitrite reduction genes (*nirK*) and N_2O reduction genes (Clade I *nosZ)* were correlated with denitrifier-derived N_2O emissions. Depressional soils had more diverse active N_2O consuming communities (assessed using Clade I *nosZ*) under flooded conditions, limiting net N_2O emissions compared to upslope soils. Our results show that depressional soils maintain distinct microbial communities that likely promote higher rates of N_2O reduction compared to upslope soils. Soil microtopography can, therefore, select for distinct microbial communities that emit different amount of N_2O in response to large precipitation events.

1. Introduction

Climate change is increasing the occurrence of large precipitation events globally (Min et al., 2011; Papalexiou and Montanari, 2019), leading to increased surface ponding of upland soils (Gleason, 2008; Paul et al., 2020). Surface ponding of topographic depressions can establish hotspots of denitrification that disproportionately contribute to soil nitrous oxide (N_2O) emissions, a potent greenhouse gas (Turner et al., 2016; Lawrence et al., 2021). However, rainfall can also stimulate N_2O emissions from nearby upslope areas and suppress N_2O from depressional areas, such that the location of N_2O hotspots change with environmental conditions (McClain et al., 2003; Krichels and Yang, 2019). The location of N_2O hotspots may depend on the distribution of soil microbial communities that are responsible for the production and consumption of N_2O in soils (Philippot et al., 2009). Yet, it is not clear how microtopographic variation in soil microbial communities contributes spatial variation in soil N_2O emissions.

Distinct microbial communities in depressional versus upslope soils may emit different amounts of N_2O in response to ponding. Ponding can prevent the diffusive resupply of O_2 into soil pore space and create suboxic conditions that favor anaerobic microbial processes like denitrification (Estop-Aragonés et al., 2013; Jarecke et al., 2016; Krichels

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et al., 2019). In addition to establishing suboxic conditions, repeated ponding over time can select for functionally distinct microbial communities that may emit N_2O under different environmental conditions (DeAngelis et al., 2010; Suriyavirun et al., 2019). Yet, variation in soil microbial community composition does not always relate to variation in ecosystem process rates (Bier et al., 2015; Rocca et al., 2015; Graham et al., 2016), limiting our understanding of how spatial variation in microbial communities affects soil N_2O emissions.

Predicting variation in soil N_2O emissions may depend on relating microbial communities to the specific processes that they carry out. Much of the N_2O emitted from agricultural fields is derived from the reduction of NO_3^-, likely via denitrification (Krichels et al., 2019; Tian et al., 2020; Liang and Robertson, 2021). Denitrification is a multi-enzymatic process where NO_3^- is sequentially reduced to nitrite (encoded by *narG* or *napA* genes), nitric oxide (*nirS* or *nirK* genes), N_2O (*nor* genes), and finally dinitrogen gas (*nosZ* genes) (Firestone et al., 1980; Knowles, 1982). While some microorganisms can only carry out individual steps of denitrification, soil microbial communities can collectively carry out the entire reaction (Graf et al., 2014). Dissimilatory nitrate reduction to ammonium (DNRA; the key enzyme nitrite reductase encoded by the *nrfA* gene) can also reduce NO_2^- and NO_3^-, competing with denitrifiers for N to potentially slow denitrifier-derived N_2O emissions (Putz et al., 2018). Furthermore, N_2O can be reduced to N_2 by highly diverse populations that harbor Clade I or Clade II *nosZ*, including both non-denitrifiers and canonical denitrifiers (Sanford et al., 2012). However, it is not clear whether microorganisms with Clade I or Clade II *nosZ* reduce more N_2O in flooded agricultural soils (Hallin et al., 2018; Chee-Sanford et al., 2020; Shan et al., 2021). While various microbial genes are involved in the production and consumption of N_2O, the abundance of these genes—assessed using quantitative polymerase chain reaction, or qPCR—is rarely correlated to net soil N_2O emissions (Rocca et al., 2015). Because gene abundance does not necessarily relate to gene activity, studies have instead assessed the expression of these genes in the soil by measuring the abundances of transcripts expressed from N-cycling genes using reverse transcription qPCR, or RT-qPCR (Liu et al., 2019; Tosi et al., 2020). Even though transcript abundance should be more closely related to enzyme activity, N-cycling transcript abundances still fail to predict much of the variation found in soil N transformations (Rocca et al., 2015), suggesting that the abundance of active N-cycling communities do not necessarily correlate with soil N_2O emissions.

Rather than the abundance of a given gene or transcript in the soil, microbial community composition may be a better predictor of soil N_2O emissions. Higher microbial diversity can increase the number of functional traits in a community to favor faster process rates (Allison and Martiny, 2008). For example, because denitrifiers can have distinct responses to environmental factors (Aanderud et al., 2015; Lycus et al., 2018), greater diversity of denitrifying microorganisms can increase soil denitrification rates in dynamic soil environments (Philippot and Hallin, 2005; Graham et al., 2016). However, microbial community composition may not relate to ecosystem process rates if communities are so diverse that functional overlap among taxa allows taxonomically disparate communities to function at similar rates (Finlay et al., 1997; Bier et al., 2015; Rocca et al., 2015). Indeed, denitrification occurs over a wide phylogenetic range of microorganisms (Shapleigh, 2013), and higher denitrifier diversity may not increase process rates if the communities are functionally redundant (Attard et al., 2011; Rocca et al., 2015). Whether population diversity leads to functional redundancy or to increased process rates may determine if the composition of active N cycling microbial communities predicts soil N_2O emissions.

Microbial transcript diversity may be a more useful measure of functional diversity than microbial gene diversity because taxa that may not be highly abundant in DNA-based community analyses (e.g. amplicon-based sequencing of genes) can disproportionately transcribe specific N-cycling genes (Aanderud et al., 2015; Liu et al., 2019). As more taxa transcribe a given gene, corresponding functional traits that control process rates may also be expressed, such that variation in soil N_2O emissions may be controlled by the diversity of taxa actively expressing different N cycling genes (i.e., transcript diversity). Sequencing of complementary DNA (cDNA) derived from mRNA can be used to measure transcript diversity specific to genes involved in N cycling processes (Chee-Sanford et al., 2020). Because there are multiple genes involved in the sequential reduction of NO_3^- to N_2O and N_2, the diversity of transcripts encoding any one of these steps may best explain specific N transformation rates.

Here, we asked: how does the diversity of active N cycling microorganisms contribute to microtopographic variation in soil N_2O emissions? To answer this question, we sequenced N-cycling genes (*nirS, nirK, nosZ,* and *nrfA*) and cDNA derived from transcripts in response to flooding upslope and depressional soils from three agricultural fields in central Illinois, USA. We also used stable isotope tracers to measure the contribution of NO_3^- reduction and NH_4^+ oxidation to N_2O emissions and to assess competition between denitrification and DNRA for soil NO_3^-. We hypothesized that the diversity of N-cycling transcripts would contribute to variation in soil N_2O emissions between depressional and upslope soils. From this hypothesis, we predicted that the diversity of transcripts involved in early steps of denitrification (*nirK* and *nirS*) would be positively correlated to net conversion of NO_3^- to N_2O, while the diversity of transcripts involved in N_2O reduction (Clade I *nosZ*) would be negatively correlated to N_2O emissions under flooded conditions. It is not clear how the diversity of Clade II *nosZ* should relate to N_2O emissions since controls over N_2O reduction by Clade II *nosZ* remain poorly understood (Shan et al., 2021). We also predicted that *nrfA* transcript richness would be negatively correlated to N_2O emissions due to competition with denitrifiers for NO_2^- and NO_3^-. While DNRA can produce some N_2O, its prevalence as an N_2O source in soils has yet to be determined (Butterbach-Bahl et al., 2013; Putz et al., 2018). Finally, we predicted that the diversity of active N cycling communities would differ between depressional and upslope soils, contributing to spatial variation in net N_2O emissions.

2. Methods

2.1. Study site

This experiment was conducted on soils collected in July 2018 from paired upslope and depressional plots in three agricultural fields across Champaign County, Illinois that are managed in conventional maize-soybean rotations: South Farm, Energy Farm, and Cardinal Road. In 2018, the Energy Farm and Cardinal Road fields were planted in maize while the South Farm field was planted in soybean. A depressional area was identified in each field based on visual observations of ponding following rainfall of at least 30 mm in a 24-h period. The paired upslope plot was identified in a well-drained elevated area approximately 100 m from the depressional plot in the same field. All depressional soils are Typic Endoaquolls and mapped in the Drummer series. Upslope soils from South Farm and Cardinal Road fields are Aquic Argiudolls mapped in the Flanagan series, and the upslope soils from the Energy Farm field are Oxyaquic Argiudolls mapped in the Dana soil series (Soil Survey Staff, 2019).

2.2. Experimental design

To assess how microbial community composition contributes to spatial variation in soil N_2O emissions, soil N transformations and microbial community composition were measured from upslope and depressional soils collected from each of the three sites in response to a simulated flooding and drainage event in the lab. Soils were either kept at field moisture, flooded for two days, or flooded for two days and allowed to drain for two days prior to measurements. For each measurement, DNA and RNA in the soil was extracted to measure microbial community composition and transcript richness from select N-cycling

functional genes, and stable isotopic tracing was used to measure rates of specific N transformations. Together, these data allowed us to assess if transcript richness of the selected genes explains variation in soil N_2O emissions, and if topographic position selects for distinct microbial communities that contribute to variation in soil N_2O emissions.

To maintain field-relevant environmental conditions while also minimizing fine-scale variation in soil microbial communities, measurements were conducted on homogenized soils that were collected within one week of beginning the experiment. Re-packed soil cores were used in this experiment to reduce the heterogeneity of soil microbial community composition that can occur across small spatial scales (Upton et al., 2019). Ten soil samples (0–10 cm depth) were collected from each plot using a 5 cm diameter auger. The soil samples were sieved to 8 mm and pooled by plot to produce homogenized soil from each plot. The composited soil was then split and packed into plastic tubes (5 cm diameter by 10 cm depth) at field bulk density (~1.1 g cm^{-3}) to produce 30 re-packed soil cores from each plot. Each plastic tube had 0.5 mm mesh window screening glued to the bottom of the cylinder with a watertight plastic cap fitted over top of the window screening. This allowed the cores to retain water when flooded and drain when the cap was removed. The soils were then loosely covered with aluminum foil and left on the benchtop for two days to allow for acclimation to ambient conditions prior to beginning the experiment.

Five cores from each plot were destructively harvested every other day during the experiment to measure microbial process rates, soil chemical properties, and soil microbial community composition. This was done a total of three times, representing pre-flooding (field moisture), flooded, and drained conditions. On the first day of the experiment—immediately after destructively harvesting the first subset of cores—the remaining cores were flooded with 80 mL of deionized water. To evenly distribute the water, 20 mL of deionized water was injected into each core four times using a 10 cm spinal tap needle. At the beginning of the third day of the experiment—immediately after destructively harvesting the second subset of cores and approximately 48 h after flooding—the cap was removed from the bottom of the remaining cores so that they could drain. This last set of cores were placed in a plastic tub with a 3 cm layer of autoclave-sterilized silica sand to facilitate drainage prior to the final destructive harvest on the fifth day of the experiment, approximately 48 h after the bottom caps were removed.

2.3. Soil property assays

On each measurement day, the destructively harvested cores were assayed for a suite of soil properties that can affect microbial N transformations. After a soil core was extruded into a plastic bag and gently mixed by hand, the homogenized soil was subsampled for determination of initial NO_3^- and NH_4^+ concentrations, gravimetric soil moisture, and pH. Soil NO_3^- and NH_4^+ concentrations were measured by extracting 10 g dry weight equivalent soil in 30 mL of 2 M KCl. The filtered KCl extracts were analyzed colorimetrically for NO_3^- and NH_4^+ concentrations using a SmartChem 200 discrete analyzer (KPM analytics, Westborough, MA). Initial gravimetric soil moisture was measured by drying ~12 g soil at 105 °C for 48 h and measuring water loss. Soil pH was measured in a 1:1 mixture of deionized water to dry soil mass equivalent. Immediately prior to beginning the N cycling measurements on each of the three measurement days, two 1.5 g soil subsamples were preserved in 1.5 mL of RNA preservative solution (Qiagen LifeGuard Soil Preservation) and were immediately frozen at – 20 °C.

2.4. N cycling measurements

Stable isotope tracer techniques were used to quantify denitrifier-derived net N_2O fluxes, nitrifier-derived net N_2O fluxes, DNRA rates, and gross N mineralization rates. We assumed that $^{15}N_2O$ produced from soils that received $^{15}NH_4^+$ resulted primarily from nitrification, whereas $^{15}N_2O$ produced from soils that received $^{15}NO_3^-$ resulted primarily from denitrification (Van Groenigen et al., 2015). We note that we measured soil N_2O emissions as the net soil-atmosphere flux of N_2O (i.e., the net release of N_2O from soils over the course of the incubation) and did not separate gross N_2O production from gross N_2O consumption. Rates of DNRA were determined from $^{15}NH_4^+$ production by soils that received $^{15}NO_3^-$, as described by Silver et al. (2001). Gross N mineralization rates were measured from the isotopic dilution of the $^{15}NH_4^+$ pool between 15 min and 4 h after adding the $^{15}NH_4^+$ label (Kirkham and Bartholomew, 1954; Hart et al., 1994).

Each destructively sampled and homogenized soil sample was split into two ~105 g dry weight subsamples for a 4-h incubation with ^{15}N. These incubations were done on the morning of the first, third, and fifth day of the experiment, representing field moisture, two days post-flooding, and two days post-drainage. Different ^{15}N label concentrations were added to soils from each field sampling location to target 10 atom % ^{15}N enrichment, based on measurements of initial soil NO_3^- and NH_4^+ concentrations. 1 mL of 99 atom % ^{15}N enriched NH_4Cl (Cambridge Isotope Laboratories, Inc., Andover, MA) was added to the first subsample at a solution concentration ranging from 5.5 to 22 μg-N mL^{-1}. 1 mL of 99 atom % ^{15}N enriched KNO_3 was added to the second subsample at a solution concentration ranging from 105 to 258 μg-N mL^{-1}. To measure the initial ^{15}N enrichment of NO_3^- and NH_4^+, ~25 g dry weight equivalent of each soil sample was extracted in 100 mL of 2 M KCl at 15 min after adding ^{15}N label. The remaining soil (~80 g dry weight equivalent) was sealed in a 250 mL canning jar with a lid fitted with a rubber septum. After 4 h, a 90 mL gas samples was collected from the jar headspace; ~25 g dry weight equivalent of the remaining soil in the jar was extracted in 2 M KCl to determine the final ^{15}N enrichment of NO_3^- and NH_4^+.

Nitrogen cycling process rates were calculated from changes in N concentration and ^{15}N enrichment between the initial (15 min) and final (4 h) sampling time points. Soil NO_3^- and NH_4^+ in the KCl extracts were prepared for ^{15}N isotope analysis by acid-trap diffusion (Herman et al., 1995) and analyzed for ^{15}N isotopic composition on an IsoPrime 100 isotope ratio mass spectrometer (IRMS) interfaced to a Vario Micro Cube elemental analyzer (Isoprime Ltd, Cheadle Hulme, UK; Elementar, Hanau, Germany). The 90 mL gas samples were analyzed for N_2O concentrations on a Shimadzu GC-2014 equipped with an electron capture device (Columbia, MD), and for ^{15}N–N_2O using the IRMS interfaced with a trace gas preconcentration unit (Isoprime Ltd., Cheadle Hulme, UK). Net CO_2 and N_2O emissions were calculated assuming a linear change in gas concentration over the course of the 4 h soil incubation. Denitrification-derived net N_2O fluxes were estimated from the $^{15}N_2O$ production rate by $^{15}NO_3^-$-labeled soils scaled by the mean ^{15}N enrichment of the NO_3^- pool measured at the initial and final time points (Krichels et al., 2019). Nitrification-derived net N_2O fluxes were similarly estimated from the $^{15}NH_4^+$-labeled soil samples. DNRA rates were determined using $^{15}NO_3^-$ as a tracer based on the calculations described by Silver et al. (2001).

2.5. Soil nucleic acid extraction

Total soil DNA and RNA were simultaneously extracted from preserved soil samples to characterize the diversity of the extant microbial community and the active microbial community in response to flooding and drainage. Extractions were performed within two months of the experiment using a modified phenol-chloroform extraction method (Tsai and Olson, 1991; Chee-Sanford et al., 2020). Following extraction of the nucleic acid pool, an aliquot of the crude extract was treated with DNase (I Turbo DNA-free kit, Thermo Scientific) at 37 °C for 1 h to digest all DNA in the sample. This DNase treatment was repeated twice on all samples. To ensure that no DNA remained in the sample, we confirmed that PCR amplification of 16S rRNA genes produced no products.

Next, reverse transcription reactions were carried out to arithmetically convert specific mRNA transcripts to corresponding cDNA prior to

sequencing. Reverse transcription was done using SuperScript IV Reverse Transcriptase (Thermo Fisher) following manufacturer instructions. Briefly, a pool of reverse primers targeting the diversity within *nosZ-I, nrfA*, *nirS,* and *nirK* (Table S1) and dNTPs was added to each RNA sample (<5 μg) to create a final solution concentration of 0.5 μM for each primer and 0.5 mM dNTP. We used one reverse primer to target all clades of *nosZ-II* genes in this same reaction (Jones et al., 2013). This mixture was then heated for 5 min at 65 °C to anneal the primers to the mRNA and put on ice for ~1 min. Each RT reaction mixture comprised SuperScript IV reverse transcriptase (200 U), 1X SSIV buffer, DTT (5 mM), and Superase-In/RNase Inhibitor (20 U), and T4 gene protein 32 (62.5 μg/ml) as recommended by the manufacturer. Each sample was then heated at 50 °C for 10 min followed by 85 °C for an additional 10 min to complete the reverse transcription reaction.

Following reverse transcription, a preamplification step using a low number of cycles was used to increase the concentration of cDNA prior to sequencing. To pre-amplify cDNA, a separate 14-cycle PCR amplification was run on separate subsamples of the cDNA using each primer pair targeting the specific functional genes (Table S1). The pre-amplified cDNA from each of the seven PCR reactions was pooled for each sample. Each pooled cDNA sample was purified using the Qiagen MinElute 96 UF PCR Purification Kit (Qiagen) prior to submission for amplicon-based sequencing.

2.6. Sequencing and bioinformatics

Pooled cDNA along with simultaneously extracted DNA from the corresponding samples were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign for multiplex amplification of target sequences using the Fluidigm Access Array system followed by sequencing of the amplicons using the Illumina NovaSeq 6000 SP Flow Cell platform. Seven different primer sets included Cluster I *nirK*, Cluster II *nirK,* Cluster I *nirS,* Clade I *nosZ,* Clade II (A) *nosZ,* Clade II (B) *nosZ,* or *nrfA*, generating amplicon lengths ~248–483 bp (Table S1). We use the terms "Cluster" and "Clade" as designated by the original designers of these primer pairs; both terms refer to distinct but related functional gene sub-communities within the class of a given gene. We note that the efficiencies of the Clade II (A) *nosZ* and Clade II (B) *nosZ* primers have not been widely tested and may underestimate Clade II *nosZ* diversity. Furthermore, there are other subclades of Clade II *nosZ* that these primers do not target and which may account for a large portion of microorganisms that contain Clade II *nosZ* (Orellana et al., 2018).

To process amplicon sequence data, paired end reads (250 bp each) were stitched together for each of the primer sets and filtered to the expected amplicon length using mothur software (Schloss et al., 2009). Sequences from each sample were analyzed using mothur with the appropriate aligned reference sequence file for each of the gene targets of interest (Chee-Sanford et al., 2020). Due to the large number of sequences generated on the NovaSeq platform, sequences were subsampled to 20% to facilitate data processing. Output files included a listing and the number of unique OTUs found in each sample based on 97% sequence similarity and a representative of each OTU in FASTA format. DNA and cDNA data were processed together so that OTU labels could correspond accordingly between the two nucleic acid pools analyzed for each sample.

2.7. Statistical analyses

We used analysis of variance (ANOVA) to assess if initial soil properties differed based on topographic position, site, or experimental treatment. For each model either pH, moisture, NO_3^-, or NH_4^+ was included as the response variable and topographic position, site, and experimental treatment were included as additive predictor variables. To account for pseudo-replication, the five replicates from each sampling location were averaged, leading to a sample size of 18 (3 sites x 2 topographic positions x 3 treatments). The same modeling approach was used to determine if denitrification-derived N_2O emissions, nitrification-derived N_2O emissions, and DNRA rates differed based on topographic position, site, or experimental treatment.

DNA sequence data for each of the functional genes were analyzed using ordination methods in the vegan package in R (Oksanen et al., 2013; R Core Team, 2019). First, the OTU tables for each gene were rarefied based on the mean of 100 rarefication runs. The rarefied table was then normalized using a Hellinger transformation. A Bray-Curtis dissimilarity matrix was made from the rarefied and normalized OTU. We averaged dissimilarity scores from the five samples from each sampling location on each day to account for pseudo-replication and used these values for subsequent statistical analyses. We then used permutational analysis of variance (Oksanen et al., 2013; R Core Team, 2019) to determine if the microbial community composition differed based on site or topographic position. Results were visualized using nmds.

We used the cDNA sequence data to calculate the Shannon-Wiener species diversity index from each N-cycling primer. The cDNA OTU tables were not rarefied prior to analysis because not all samples are expected to have cDNA present; rarefying the OTU table would remove these samples even though the lack of a transcript is ecologically meaningful. We calculated the Shannon-Wiener species diversity index—hereafter referred to as transcript diversity—from each primer set in each sample using the diversity function in R (vegan package; Oksanen et al., 2013; R Core Team, 2019).

Transcript diversity of each primer pair as well as soil moisture, NO_3^-, NH_4^+, and pH were included as predictor variables in models of denitrification-derived N_2O emissions and DNRA rates throughout the experiment (Tables 1 and 2). We focused on denitrification-derived N_2O emissions because hotspots of N_2O emissions from agricultural fields are mostly driven by NO_3^- reduction (Turner et al., 2016; Krichels et al., 2019; Lawrence et al., 2021; Liang and Robertson, 2021), while nitrification may only be a major source of N_2O when soil NH_4^+ concentrations are high in the weeks following fertilization (Edwards et al., 2018; Hartman et al., 2022). All variables were assessed for normality using a Shapiro Wilkes test, and log transformations were applied to correct for non-normal distributions. All the transformed variables were included as fixed factors is a mixed effect linear model run using the lme function (Pinheiro et al., 2017). For each model, either denitrification-derived N_2O emissions or DNRA rates were the response variable. Topographic position and site were included as random error terms to account for the sampling design. Next, we selected which model terms to keep by comparing Akaike Information Criterion scores corrected for small sample sizes (AICc (Bartoń, 2023);). We report the model terms that were retained by the AICc selection process as well as the marginal and conditional pseudo-R^2 of the model.

Table 1
Results from linear mixed-effect models predicting denitrification-derived N_2O emissions throughout the entire experiment.

	value	P-value	Marginal R^2	Conditional R^2
Moisture	1.36	<0.001	0.41	0.63
NO_3^-	0.07	0.06		
Cluster II *nirK* transcript diversity	0.12	0.004		
Clade I *nosZ* transcript diversity	−0.10	0.02		

Only fixed effects that were chosen using AICc model selection are shown. Value represents estimated model coefficients, P-value shows statistical significance, marginal pseudo-R^2 estimates how much variance the fixed effects explain (out of 1.0), and conditional pseudo-R^2 estimates how much variance the total model explains (out of 1.0). The original model included soil moisture, NO_3^-, NH_4^+, pH, and transcript diversity from all seven primer pairs used in this study. Transcript diversity represents the Shannon-Weiner diversity index calculated from cDNA sequences for a given primer pair.

Table 2
Results from linear mixed-effect models predicting DNRA rates throughout the entire experiment.

	Value	P-value	Marginal pseudo-R^2	Conditional pseudo-R^2
NO_3^-	0.12	<0.001	0.63	0.63
Moisture	0.31	0.04		
Cluster I *nirK* transctipt diversity	0.04	0.06		
Cluster II *nirK* transctipt diversity	−0.04	0.05		
Cluster I *nirS* transctipt diversity	0.06	0.03		

Only fixed effects that were chosen using AICc model selection are shown. Value represents estimated model coefficients, P-value shows statistical significance, marginal pseudo-R^2 estimates how much variance the fixed effects explain (out of 1.0), and conditional pseudo-R^2 estimates how much variance the total model explains (out of 1.0). The original model included soil moisture, NO_3^-, NH_4^+, pH, and transcript diversity from all seven primer pairs used in this study. Transcript diversity represents the Shannon-Weiner diversity index calculated from cDNA sequences for a given primer pair.

2.8. Accession numbers

All sequence data has been deposited in the National Centre for Biotechnology Sequence Read Archive under BioProject accession number PRJNA1101905.

3. Results

3.1. Soil properties

Soil moisture, pH, and NO_3^- all differed between depressional and upslope soils throughout the course of our experiment (Fig. 1). Prior to flooding, soil moisture averaged 0.22 ± 0.017 g water g^{-1} soil in depressional soil and 0.18 ± 0.022 g water g^{-1} soil in upslope soils. Soil moisture increased in response to flooding ($F_{2,12} = 660$, $P < 0.001$) and was slightly elevated in depressional compared to upslope soils throughout the experiment (on average 8% wetter in depressional soils; $F_{1,12} = 15.6$, $P = 0.02$). Soil moisture did not consistently differ among sites across all experimental conditions ($F_{1,12} = 0.47$, $P = 0.64$). Upslope soils were more acidic compared to depressional soils (on average 0.29 pH units lower in upslope soils; $F_{1,12} = 34.8$, $P < 0.001$), and soils from the Energy Farm were more acidic (pH = 5.1 ± 0.28) compared to soils from the South Farm (pH = 6.3 ± 0.17) and Cardinal Road (pH = 7.0 ± 0.17; $F_{2,12} = 493$, $P < 0.001$). Soils became slightly less acidic in response to flooding and drainage ($F_{2,12} = 9.6$, $P = 0.003$). Soil NO_3^- concentrations were consistently elevated in upslope compared to depressional soils ($F_{1,12} = 5.65$, $P = 0.03$). Prior to flooding, upslope soils had higher NO_3^- concentrations compared to depressional soils in the Energy Farm (69.5 ± 3.29 μg N g^{-1} vs 22.7 ± 0.44 μg N g^{-1}) and the South Farm (13.5 ± 0.23 μg N g^{-1} vs 9.60 ± 0.30 μg N g^{-1}). NO_3^- concentrations were also higher in the Energy Farm soil compared to the other two sites ($F_{2,12} = 10.4$, $P = 0.002$) but did not change in response to experimental treatments ($F_{2,12} = 0.61$, $P = 0.6$). Finally, while soil NH_4^+ concentrations did not differ based on topographic position ($F_{1,12} = 0.01$, $P = 0.92$), they were generally higher in soils from the South Farm ($F_{2,12} = 07.75$, $P = 0.007$) and increased in response to flooding and drainage ($F_{1,12} = 11.1$, $P = 0.002$).

3.2. N cycling process rates

Soil N_2O emissions were low at field moisture but increased in response to flooding, especially in upslope soils, and remained elevated after drainage. At field moisture, denitrification- and nitrification-derived N_2O emissions were negligible across all sites, remaining below 0.12 ng N–N_2O h^{-1} g^{-1} regardless of topographic position (Fig. 2). Flooding stimulated both denitrification-derived N_2O emissions ($F_{2,12} = 6.80$, $P = 0.01$) as well as nitrification-derived N_2O emissions ($F_{2,12} = 6.77$, $P = 0.01$). Denitrification-derived N_2O emissions were higher in upslope compared to depressional soils across all experimental conditions ($F_{2,12} = 5.80$, $P = 0.03$). This topographic difference was most pronounced in flooded soils from the South Farms where denitrification-derived N_2O emissions reached 2.7 ± 0.19 ng N–N_2O hr^{-1} g^{-1} in upslope soils compared to 0.04 ± 0 0.01 ng N–N_2O hr^{-1} g^{-1} in depressional soils (Fig. 2b). While nitrification-derived N_2O emissions were generally lower than denitrification-derived N_2O emissions, they were also higher in upslope compared to depressional soils across all experimental conditions ($F_{1,12} = 7.83$, $P = 0.02$).

DNRA rates were consistently higher in the Energy Farm ($F_{2,12} = 1.28$, $P = 0.03$), but did not consistently differ based on topographic position ($F_{1,12} = 1.10$, $P = 0.29$; Fig. 3). While the topographic effect was not statistically significant, DNRA rates were slightly higher in depressional (12.2 ± 1.37 ng N g^{-1} hr^{-1}) compared to upslope (7.20 ± 1.49 ng N g^{-1} hr^{-1}) field moist soils.

Gross N mineralization rates were higher in depressional compared to upslope soils under field moist and flooded conditions ($F_{1,12} = 9.59$, $P = 0.002$; Fig. S1). Flooding stimulated gross N mineralization, averaging 232 ± 10.2 ng N g^{-1} hr^{-1} across all samples, but rates plummeted to 4.94 ± 0.91 ng N g^{-1} hr^{-1} during drainage ($F_{2,12} = 334$, $P < 0.001$).

3.3. Site differences in microbial community composition and transcript richness

Many N-cycling microbial DNA functional gene communities differed between upslope and depressional soils (Fig. 4). Topographic position selected for distinct composition of Cluster II *nirK* ($R^2 = 0.08$, $P = 0.01$) and Cluster I *nirS* genes ($R^2 = 0.09$, $P = 0.01$). However, Cluster I *nirK* genes did not differ based on topographic position ($R^2 = 0.06$, $P = 0.61$). Depressional and upslope soils had distinct N_2O-reducing communities (Fig. 4d,e,f). While Clade I *nosZ* communities may have differed based on topographic position ($R^2 = 0.07$, $P = 0.06$), this difference was not as pronounced as it was for both Clade II (A) *nosZ* ($R^2 = 0.11$, $P < 0.001$) and Clade II (B) *nosZ* ($R^2 = 0.11$, $P = 0.002$). The DNRA microbial community—assessed using the *nrfA* gene—also differed between depressional and upslope soils (Fig. 4g; $R^2 = 0.07$, $P = 0.02$). None of the N-cycling microbial DNA functional gene communities differed in response to experimental treatments ($P > 0.1$) but they all differed among sites ($P < 0.01$; Fig. 4).

Only Cluster I *nirS* transcript richness consistently differed between upslope and depressional soils ($F_{1,12} = 4.8$, $P < 0.05$) across all experimental conditions and sites, with depressional soils having higher transcript diversity compared to upslope soils (Fig. 5). Diversity of transcripts associated with nitrite reduction (Cluster I *nirS,* Cluster I *nirK,* Cluster II *nirK)* increased in response to flooding ($P < 0.05$, Fig. 5). Cluster I *nirS* and Cluster II *nirK* transcript diversity differed by site ($P < 0.05$), with Energy Farm generally having lower transcript diversity compared to South Farm or Cardinal Road (Fig. 5). However, Cluster I *nirK* transcript diversity did not differ by site ($F_{1,12} = 3.6$, $P = 0.06$).

Neither Clade II (A) *nosZ* nor CladeII (B) *nosZ* transcript diversity differed between depressional and upslope soils across all treatments ($P > 0.05$, Fig. 6). While Clade I *nosZ* transcript diversity also did not significantly differ based on topographic position across all treatments, it was higher in depressional soils when flooded soils were considered separately from field moist and drained soils ($F_{1,2} = 250$, $P = 0.004$). Clade I *nosZ* transcript diversity also responded to experimental treatments ($F_{2,12} = 8.0$, $P < 0.001$) and differed between sites ($F_{2,12} = 5.0$, $P = 0.03$); Clade II (A) *nosZ* and CladeII (B) *nosZ* did not ($P > 0.05$, Fig. 6). Clade I *nosZ* transcript diversity was higher in flooded than in drained soils compared to field moist soils and was higher in soils from South Farm and Cardinal Road compared to soils from Energy Farm.

The diversity of *nrfA* transcripts was consistently higher in depressional compared to upslope soils ($F_{1,12} = 6.2$, $P = 0.03$). *nrfA* transcript

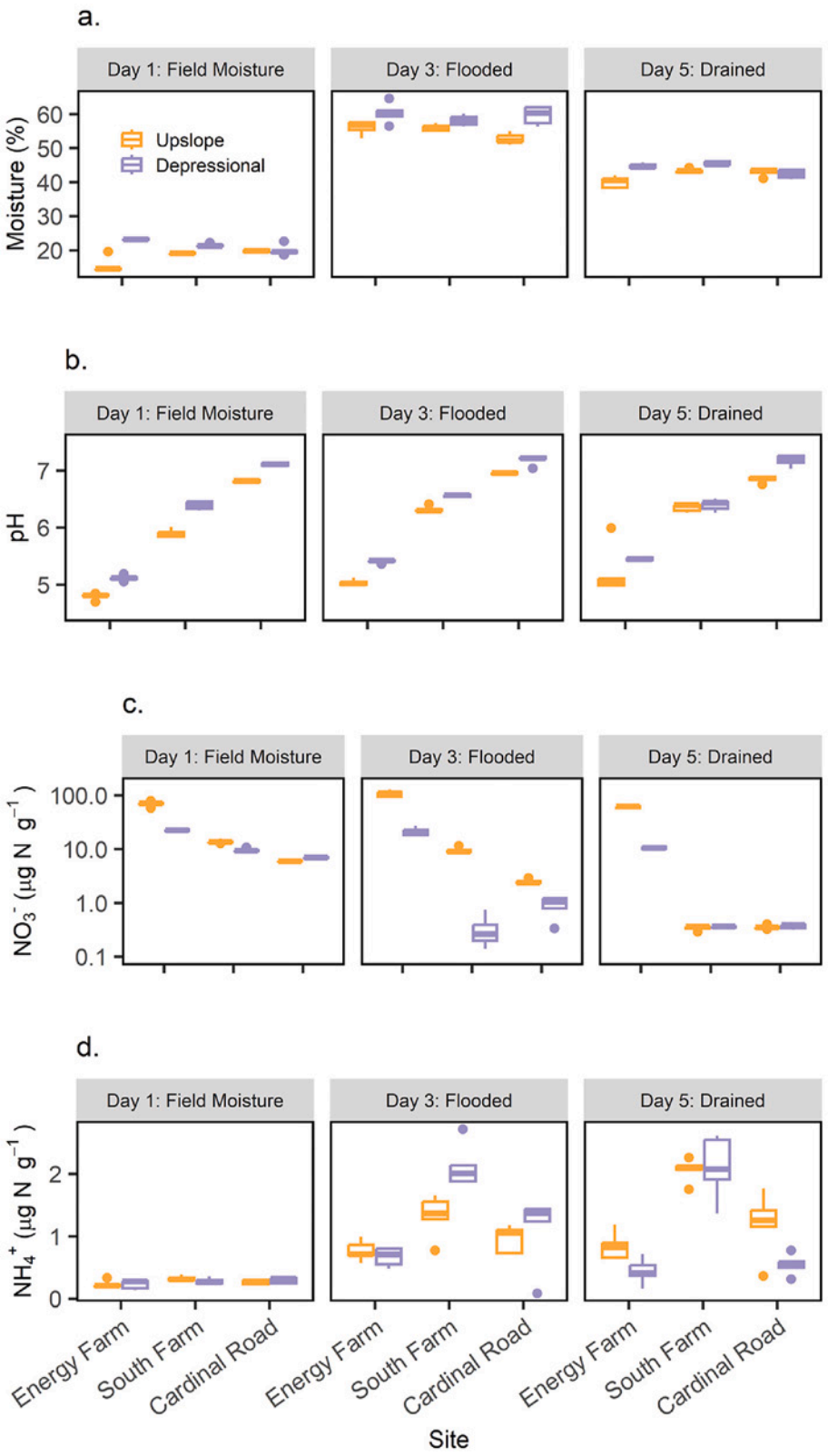

Fig. 1. Soil moisture (a), pH (b), NO_3^- concentrations (c), and NH_4^+ concentrations (d) in soils from all three sites in response to flooding and experimental drainage (n = 5 per experimental unit). The lines in each box plot represent the mean, the upper and lower portions of the box plots correspond to the 25th and 75th percentiles, and dots represent outliers.

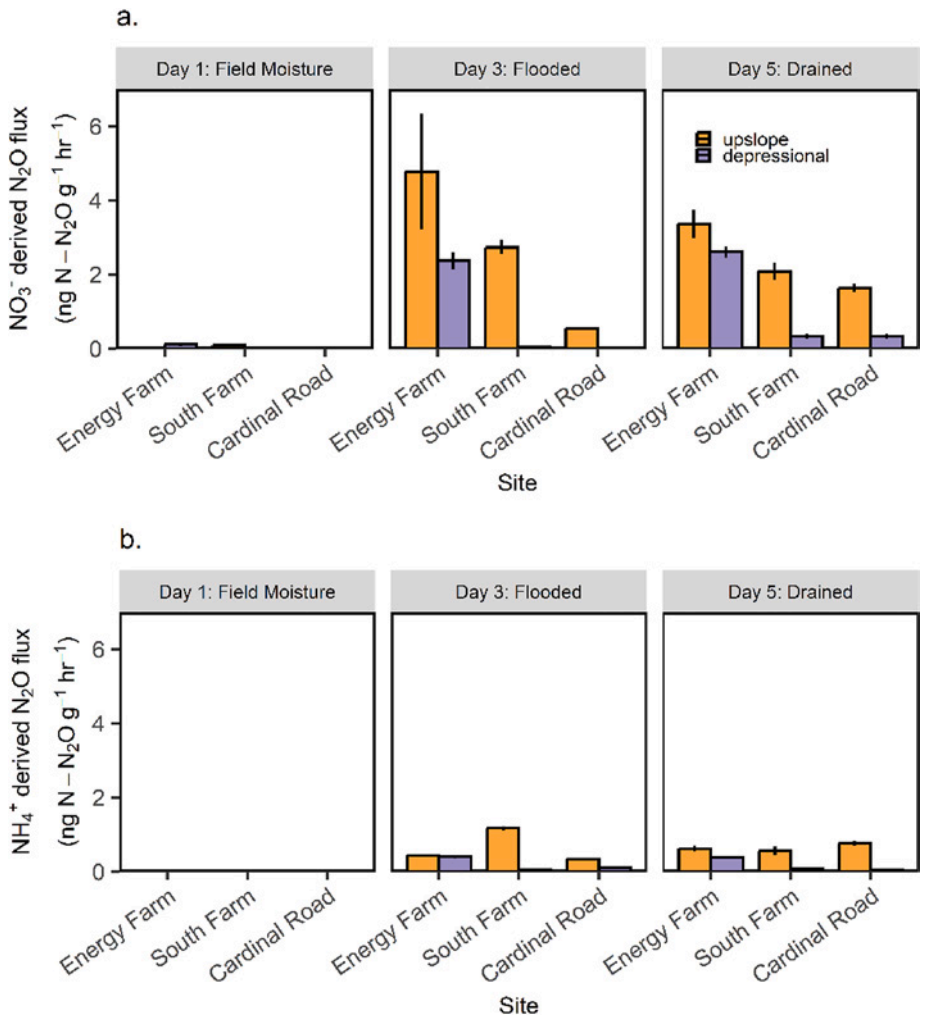

Fig. 2. NO_3^- (denitrification) (a) and NH_4^+ (nitrification)-derived (b) net N_2O flux (±SE) from soils collected from upslope and depressional soils from Energy Farm, South Farm, and Cardinal Road at field moisture, flooded, and drained conditions in the lab. Bars represent means (n = 5) and error bars represent standard error of the mean.

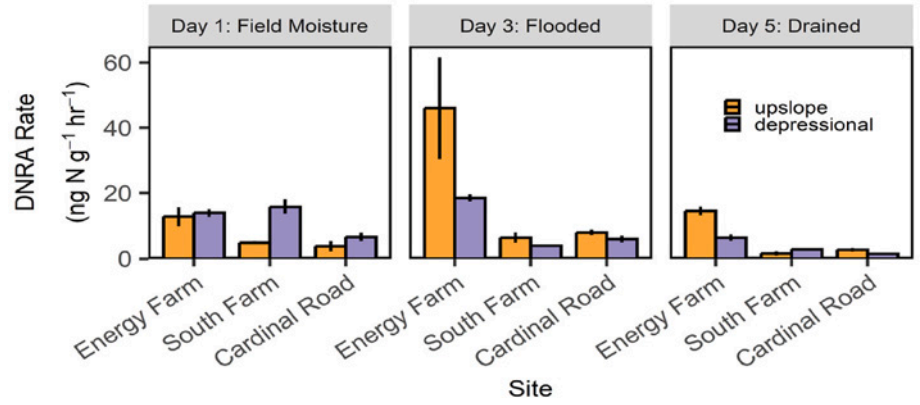

Fig. 3. Rates of dissimilatory nitrate reduction to ammonium (DNRA) (±SE) from soils collected from upslope and depressional soils from Energy Farm, South Farm, and Cardinal Road at field moisture, flooded, and drained conditions in the lab. Bars represent means (n = 5) and error bars represent standard error of the mean.

diversity also increased in response to flooding and drainage ($F_{2,12}$ = 9.0, P = 0.004). Finally, *nrfA* transcript diversity was higher in soils from South Farm and Cardinal Road compared to soils from Energy Farm ($F_{2,12}$ = 11.1, P = 0.002).

3.4. Top N cycling transcripts and genes

OTUs that were highly abundant in cDNA pools were often not detected in DNA pools (F igure S2 through S9). For example, of the ten Cluster II *nirK* OTUs that were most abundant in the cDNA pool, eight were not detected in the DNA pool (OTUs 470, 1466, 1792, 394, 539,

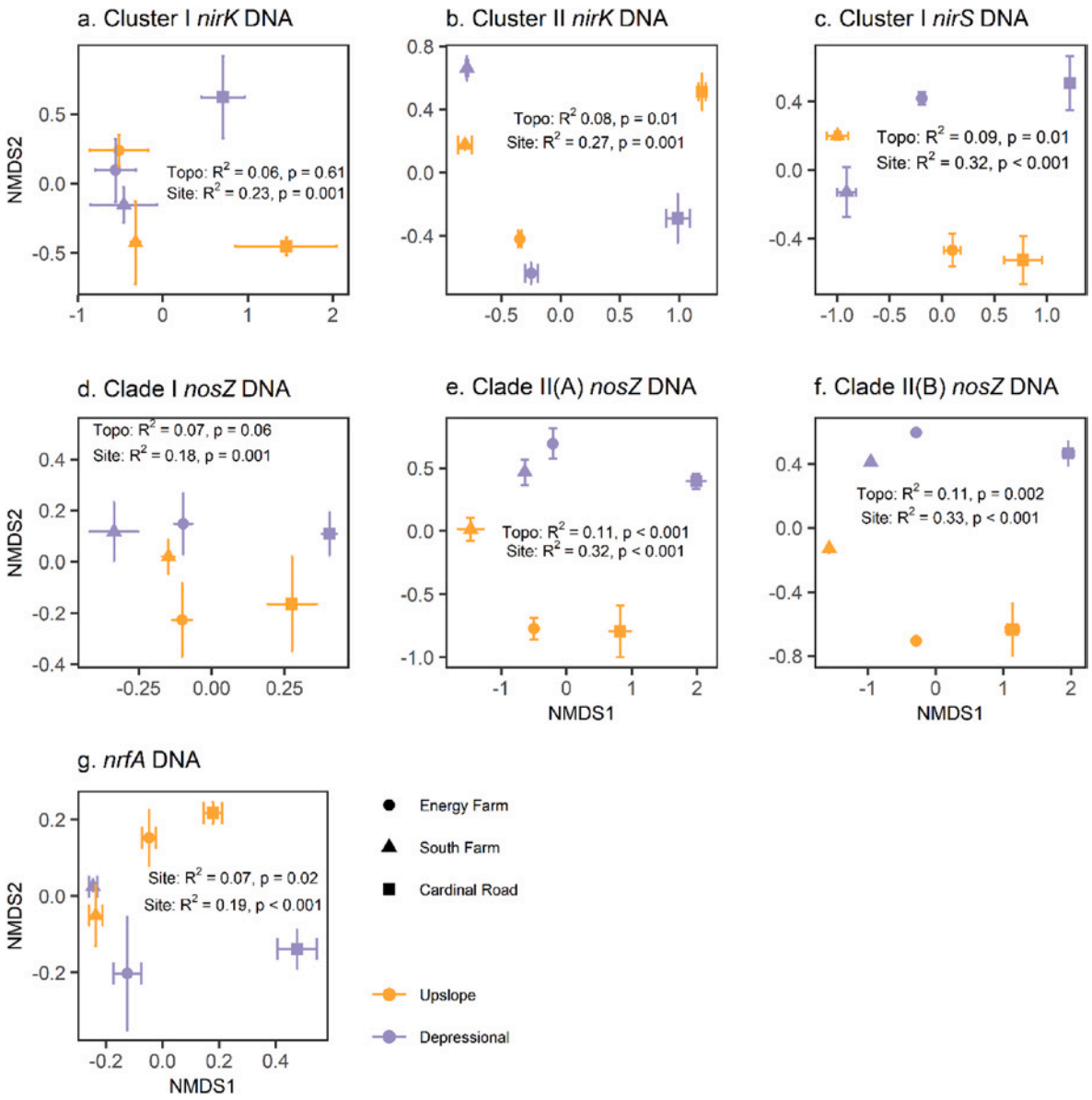

Fig. 4. non-metric multidimensional scaling (NMDS) plot showing distribution of nitrite reductase genes *nirK and nirS* (a–c), nitrous oxide reductase genes (d–f), and DNRA genes (g). Differences in community composition were assessed using PERMANOVA with topographic position and site as the predictor variables; R^2 and p values are reported in each panel. We did not detect a significant effect of experimental treatments, so all three experimental conditions were averaged; error bars represent the standard error of the microbial community across experimental treatments (n = 15).

1272, 1744, and 1610; Fig. S3). Similarly, of the 10 Clade I *nosZ* OTUs that were most abundant in the cDNA pool, three were not detected in the DNA pool (OTUs 1312, 780, and 411 Fig. S5). While some of the top OTUs were detected in both cDNA and DNA pools, the relative abundance of genes rarely matched the relative abundance of transcripts (Fig. S9).

3.5. Controls over denitrification-derived N_2O emissions and DNRA rates

The best model explaining denitrification-derived N_2O emissions across the entire experiment (assessed using AICc) included soil moisture, NO_3^-, Cluster II *nirK* transcript diversity, and Clade I *nosZ* transcript diversity as fixed effects (Table 1). Moisture, NO_3^-, and Cluster II *nirK* transcript diversity were positively associated with denitrification-derived N_2O emissions while Clade I *nosZ* transcript diversity was negatively associated with the emissions. This model had a marginal pseudo-R^2 of 0.41 and a conditional pseudo-R^2 of 0.63 (Table 1).

The best model explaining DNRA rates across the entire experiment included soil moisture, NO_3^- concentrations, Cluster I *nirK* transcript diversity, Cluster II *nirK* transcript diversity, and Cluster I *nirS* transcript diversity as fixed effects (Table 2). Moisture, NO_3^-, Cluster I *nirK* transcript diversity, and Cluster I *nirS* transcript diversity were all positively associated with DNRA rates. Conversely, Cluster II *nirK* transcript diversity was negatively associated with DNRA rates. This model had a marginal pseudo-R^2 of 0.62 and a conditional pseudo-R^2 of 0.63 (Table 2).

4. Discussion

Predicting how soil N_2O emissions respond to changing precipitation regimes may depend on our ability to relate microbial community composition to soil N cycling rates. Yet, relationships between microbial communities and soil N transformations remain poorly constrained. Here, we used cDNA sequencing and ^{15}N tracers to investigate how

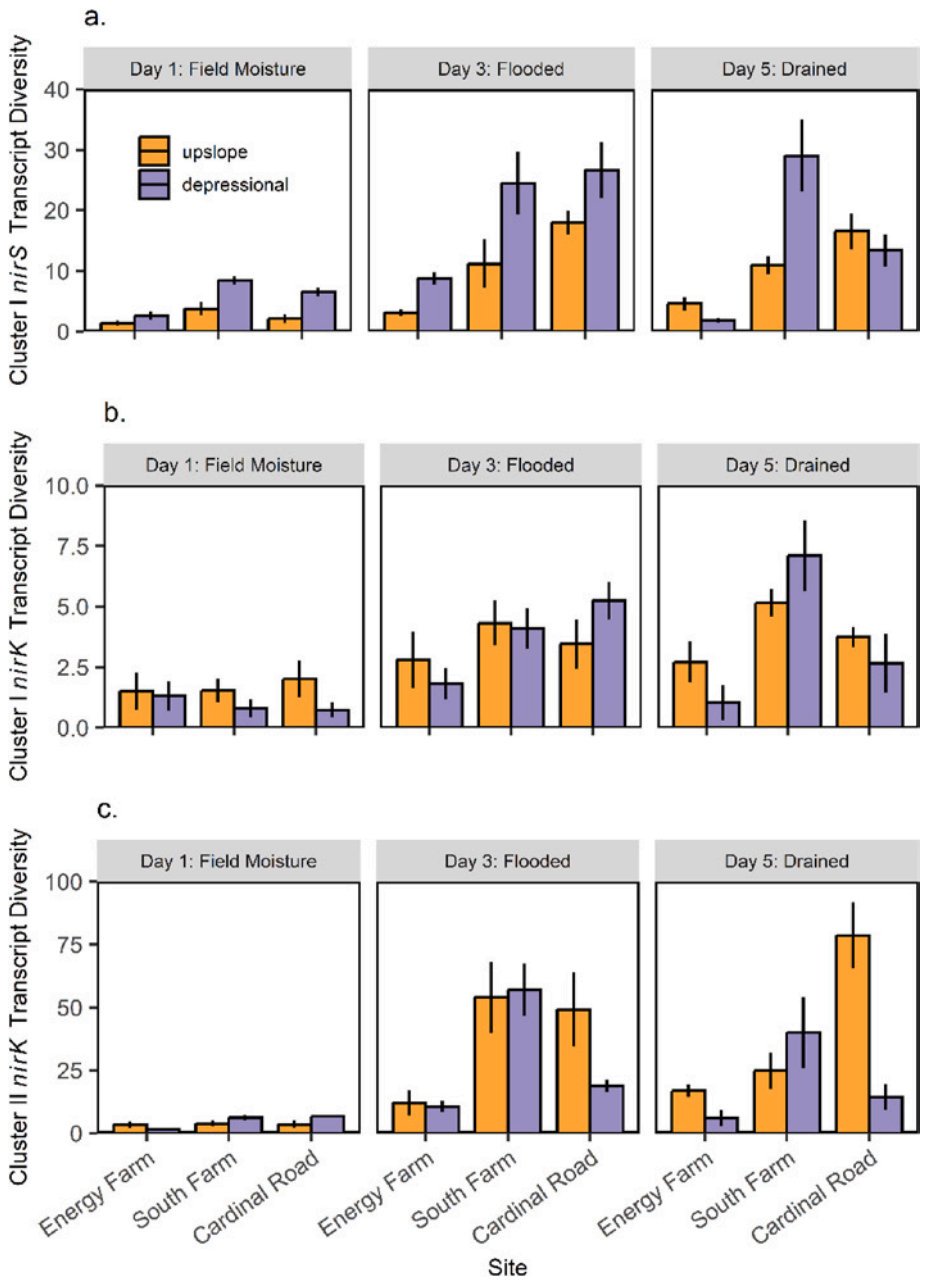

Fig. 5. Mean diversity of transcripts (Shannon-Weiner species diversity index) from Cluster I *nirS* (a), Cluster I *nirK* (b), and Cluster II *nirK* (c). Error bars correspond to standard error of the mean (n = 5). Panels correspond to the day of experiment. Colors correspond to topographic position. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

microbial community composition contributes to variation in N_2O emissions between depressional and upslope soils. We found that the diversity of some measured N-cycling transcripts correlated with denitrification-derived N_2O emissions. Furthermore, the diversity of some transcripts involved in N_2O production and consumption differed between depressional and upslope soils. Together, these results offer support for the hypothesis that the diversity of N-cycling transcripts contributes to variation in soil N_2O emissions between depressional and upslope soils.

4.1. The functional importance of microbial diversity

The diversity of microbial taxa expressing certain N-cycling functional genes affected denitrification-derived N_2O emissions, challenging the idea of functional redundancy in N-cycling microbial communities (Finlay et al., 1997; Bier et al., 2015; Rocca et al., 2015). Specifically, nitrite reductase gene (Cluster II *nirK*) transcript diversity was positively correlated with denitrification-derived N_2O emissions, suggesting that as more taxa express Cluster II *nirK*, more NO_3^- is converted to N_2O (Table 1). Further supporting the functional diversity of N-cycling microorganisms, Clade I *nosZ* transcript diversity was negatively associated with denitrification-derived N_2O under flooded conditions, suggesting that as more taxa transcribed this gene, more N_2O was reduced to N_2 to limit net N_2O emissions. However, transcript diversity of other denitrification genes was not related to denitrification-derived N_2O emissions. Perhaps, these genes were not limiting to denitrification rates, such that Cluster II *nirK* reduced more nitrite than the other nitrite reductase enzymes and Clade I *nosZ* reduced more N_2O than the other N_2O reductase enzymes. While rapid turnover of transcripts may limit our ability to relate transcript diversity to process rates (Liu et al., 2019),

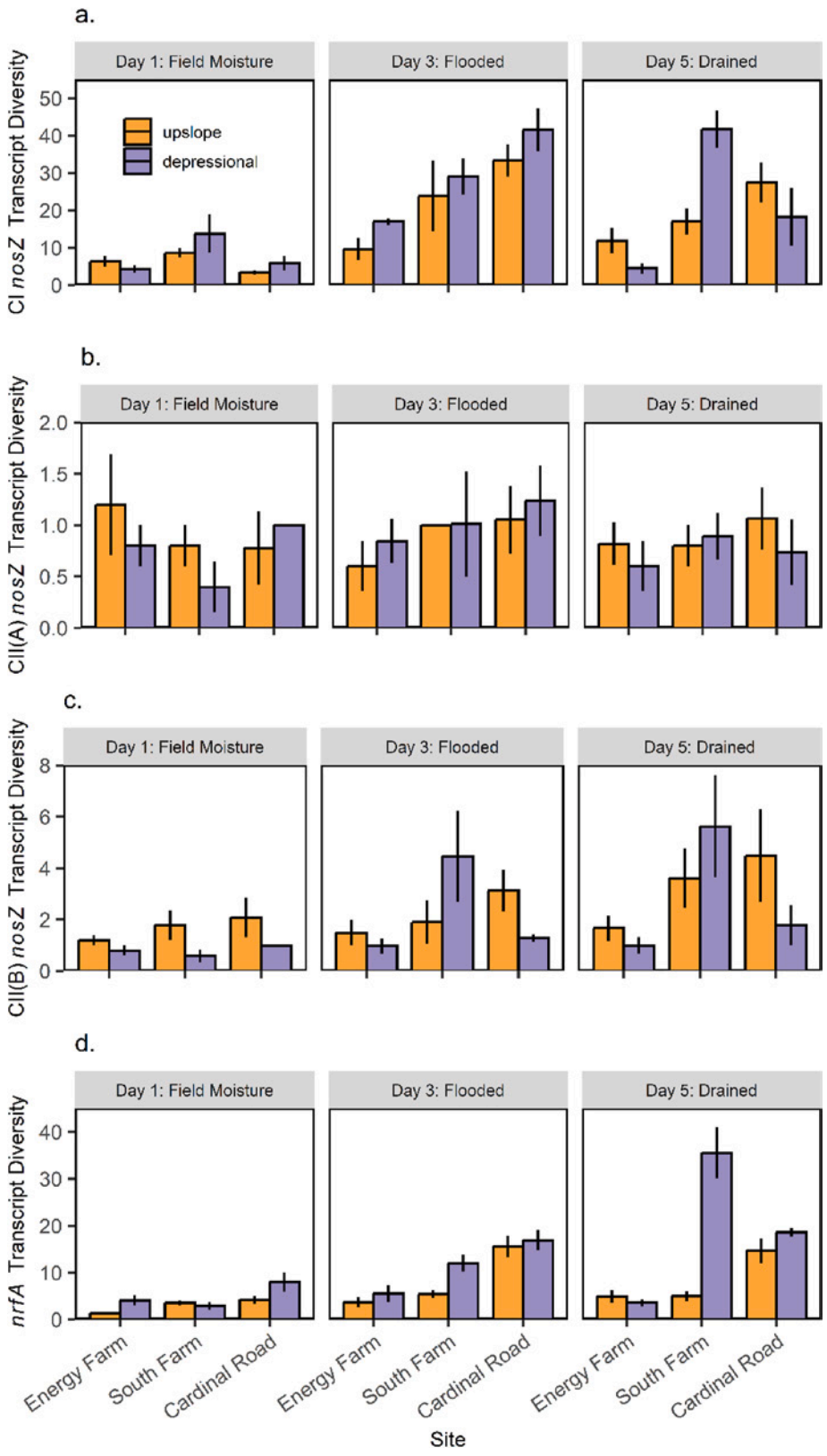

Fig. 6. Mean diversity of transcripts (Shannon-Weiner species diversity index) from Clade I *nosZ* (a), Clade II(A) *nosZ* (b), Clade II(B) *nosZ* (c), and *nrfA* (d). Error bars correspond to standard error of the mean (n = 5). Panels correspond to the day of experiment. Colors correspond to topographic position. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

microorganisms can continue transcribing denitrifying genes in the days following the onset of anaerobic conditions (Liu et al., 2010; Tosi et al., 2020), allowing us to detect active denitrifiers 48 h after the onset of experimental flooding and drainage. Our results illustrate that transcript richness from some N-cycling microorganisms can explain variation in N_2O emissions from upland agricultural soils, supporting the idea of functional diversity in microbial communities.

Rare taxa which are below the threshold of detection using amplicon gene sequencing can be functionally important based on the relative abundance of gene transcripts, highlighting the importance of characterizing the active microbial community to improve our understanding of controls on N_2O dynamics. Indeed, most OTUs that were detected in cDNA sequencing pools for a given sample were not present in corresponding DNA sequencing pools from that same sample (Fig. S9). The overrepresentation of taxa detected in transcript-versus gene-based sequencing supports the argument that rare taxa that make up a small portion of the DNA pool can disproportionately contribute to ecosystem process rates (Aanderud et al., 2015; Liu et al., 2019). Similarly, many OTUs that were highly abundant in DNA sequencing pools were not detected in cDNA sequences, further suggesting that the microorganisms that dominate DNA-based amplicon sequencing techniques are not necessarily active (Lennon and Jones, 2011; Blazewicz et al., 2014; Aanderud et al., 2015). As such, variation in DNA-based microbial community composition may not be useful in predicting ecosystem functions like denitrification or N_2O reduction.

4.2. Implications of microbial diversity for predicting spatial variation in soil N_2O emissions

Cluster II *nirK* denitrifiers may thrive in fertilized agricultural fields regardless of topographic position, such that flooding increased Cluster II *nirK* transcript diversity in all soils but did not contribute to spatial variation in N_2O emissions. Despite higher denitrification-derived N_2O emissions in upslope soils, Cluster II *nirK* transcript diversity did not consistently differ based on topographic position (Fig. 5), suggesting that *nirK* communities may not contribute to spatial variation in soil N_2O emissions. Topographic position may not select for functionally distinct *nirK* communities because *nirK* denitrifiers thrive in soils with high NO_3^- concentrations, which are present across soil microtopography in fertilized agricultural fields (Keil et al., 2011; Schulz et al., 2017; Suriyavirun et al., 2019). Rather, flooding may stimulate transcription of Cluster II *nirK* by diverse microorganisms from both upslope and depressional soils, explaining the positive association between Cluster II *nirK* transcript richness and denitrification-derived N_2O emissions. Other factors besides Cluster II *nirK* transcript diversity must, therefore, contribute to topographic variation in N_2O emissions.

Rather than selecting for distinct N_2O-producing microbial communities, depressional soils harbor distinct N_2O-reducing communities that may limit net N_2O emissions in response to flooding. Clade I *nosZ* transcript diversity was negatively correlated with denitrification-derived N_2O emissions, suggesting that diverse N_2O-reducing communities limited N_2O emissions. Indeed, flooded depressional soils harbored more diverse Clade I *nosZ* transcripts and emitted less N_2O compared to flooded upslope soils (Fig. 6). Perhaps, repeated ponding of depressional soils favors N_2O reduction by diverse microorganisms that can upregulate Clade I *nosZ* more quickly after flooding. While we also detected Clade II (A) and Clade II (B) *nosZ* transcripts, neither were correlated to denitrification-derived N_2O emissions, suggesting that the diversity of active of Clade II N_2O-reducing microorganisms may not vary in response to flooding and drainage and as such may not explain spatial variation in soil N_2O emissions. Our Clade II nosZ primers target organisms, including Anaeromyxobacter spp. and genus Gemmatirosa within the plylum Gemmatimonadete, which are not canonical denitrifiers and may reduce N_2O in the presence of O_2 (Sanford et al., 2002; Jones et al., 2014; Park et al., 2017; Shan et al., 2021). While the detection of Clade II (A) and Clade II (B) *nosZ* transcripts suggests that these microorganisms were active, perhaps they reduce N_2O under diverse environmental conditions and did not respond to flooding. It is also possible that we underestimated the contribution of Clade II *nosZ* to N_2O reduction because the primers that we used do not detect all microorganisms that have Clade II *nosZ* genes. For example, our primers do not amplify Clade II *nosZ* genes from *Bacteriodes*, which are prevalent at our study sites (Orellana et al., 2018). Regardless, the strong association between Clade I *nosZ* transcript diversity and denitrification-derived N_2O emissions suggests that reduction of NO_3^- to N_2 by microorganisms that contain CI *nosZ*, which are often canonical denitrifiers, may limit net N_2O emissions in flooded depressional soils compared to flooded upslope soils at our site (Shan et al., 2021). The dominance of canonical denitrifiers in depressional compared to upslope soils is further supported by higher *nirS* transcript richness in depressional soils (Fig. 5). Microorganisms with *nirS* genes may be more likely to have complete denitrification pathways compared to microorganisms with *nirK* genes, which can lack *nosZ* and emit more N_2O (Hallin et al., 2018). Selection for diverse microbial communities that favor complete denitrification of NO_3^- to N_2 may, therefore, decrease how much N_2O denitrification releases to the atmosphere when depressional soils are flooded.

The diversity and activity of Clade I *nosZ* denitrifiers may be mediated by soil pH. Soil pH was consistently lower in upslope soils, which harbored less diverse Clade I *nosZ* transcripts and emitted more denitrification-derived N_2O while flooded. Furthermore, soil pH was lowest in soils from the Energy Farm, where denitrification-derived N_2O emissions were highest. In contrast, soil pH was highest in soils from Cardinal Road, where denitrification-derived N_2O emissions were lowest. This positive association between the diversity of Clade I *nosZ* transcripts and soil pH suggests that Clade I *nosZ* may reduce more N_2O in soils with higher pH. While denitrification rates are thought to increase with soil pH, so is the proportion of N_2O that is reduced to N_2 (Firestone et al., 1980; Knowles, 1982). This is consistent with our observation of greater Clade I *nosZ* transcript diversity and lower denitrification-derived N_2O emissions in soils that have higher pH. In addition to directly altering rates of N_2O reduction, soil pH can shape microbial community composition (Fierer and Jackson, 2006), potentially selecting for a more diverse community of Clade I *nosZ* taxa in less acidic soils that reduce N_2O when soils are flooded. Given that repeated surface ponding can be associated with higher soil pH (Krichels et al., 2019; Suriyavirun et al., 2019), depressional soils that are more prone to ponding may select for N_2O reducer communities that are more likely to reduce N_2O compared to upslope soils that rarely pond.

Other processes may contribute to spatial variation in N_2O emissions by controlling how much NO_3^- is available to denitrifiers when soils are flooded. Higher NO_3^- availability in upslope soils under dry conditions may have stimulated greater denitrifier-derived N_2O fluxes from the Energy Farm and the South Farm, consistent with the selection of NO_3^- in the model (Table 1). However, gross N mineralization rates were higher from field moist depressional soils from these two sites (Fig. S1), suggesting that N supply does not explain greater N availability in upslope soils. While we did not assess if nitrification rates contributed to differences in NO_3^- availability, nitrifier-derived N_2O emissions were over four times lower than denitrifier-derived emissions, suggesting that nitrifiers did not directly contribute to much spatial variation in N_2O emissions. Alternatively, higher DNRA rates in field-moist depressional soils from the South Farm and the Energy Farm may have limited NO_3^- availability in depressional soils, consistent with DNRA outcompeting denitrifiers for NO_3^- under non-flooded conditions (Silver et al., 2001; Putz et al., 2018; Pan et al., 2020; Egenriether, 2021). While this difference in DNRA rates was not statistically significant, the diversity of *nrfA* transcripts was higher in field-moist depressional versus upslope soils (Fig. 6), and *nrfA* DNA communities consistently differed between depressional and upslope soils (Fig. 4). This suggests that repeated redox fluctuations in depressional soils might select for microorganisms that can carry out DNRA when soils are not flooded. Besides DNRA or other

microbial processes governing N accumulation, increased NO_3^- leaching from wetter depressional soils could also decrease NO_3^- availability compared to upslope soils (Hall et al., 2023). Regardless of the mechanism, our results show that greater NO_3^- availability in dry upslope soils can fuel denitrifier-derived N_2O emissions after rainfall.

5. Conclusion

Our study links microbial community composition to ecosystem process rates to better understand controls over N_2O emissions from flooded soils. We found that elevated diversity of Cluster II *nirK* transcripts was associated with higher denitrification-derived N_2O emissions, suggesting that these denitrifying microorganisms are functionally diverse and may emit much of the N_2O from agricultural soils. Distinct microbial communities between depressional and upslope soils contributed to larger N_2O emissions from flooded upslope soils. Flooded depressional soils harbored a more diverse group of microorganisms that transcribed Clade I *nosZ*, likely favoring N_2O reduction to N_2 and limiting net N_2O emissions compared to upslope soils. Taken together, these results demonstrate that historical environmental conditions select for distinct N cycling communities that can contribute to spatial and temporal variation in soil N_2O emissions.

CRediT authorship contribution statement

Alexander H. Krichels: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Robert A. Sanford:** Writing – review & editing, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Joanne C. Chee-Sanford:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Lynn Connor:** Writing – review & editing, Supervision, Methodology, Investigation. **Rachel Van Allen:** Writing – review & editing, Project administration, Methodology, Investigation. **Angela D. Kent:** Writing – review & editing, Resources, Project administration. **Wendy H. Yang:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Data availability

All data presented in this manuscript will be made publicly available upon publication (https://doi.org/10.5061/dryad.xwdbrv1n4). Reviewers can access the data here: https://datadryad.org/stash/share/Y6z2f6TGzLGc7j8ISTlLD2-QIIf6vuATBUIRTjlWsoA. Click or tap if you trust this link.">https://datadryad.org/stash/share/Y6z2f6TGzLGc7j8ISTlLD2-QIIf6vuATBUIRTjlWsoA. Raw sequence reads are available at the National Centre for Biotechnology Sequence Read Archive under BioProject accession number PRJNA1101905.

Declaration of competing interests

The authors declare no competing interests.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109683.

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