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RESEARCH ARTICLE

Genetic variation in *Zea mays* influences microbial nitrification and denitrification in conventional agroecosystems

Alonso Favela · Martin O. Bohn · Angela D. Kent

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Abstract

Background and Aims Nitrogenous fertilizers provide a short-lived benefit to crops in agroecosystems, but stimulate nitrification and denitrification, processes that result in nitrate pollution, N_2O production, and reduced soil fertility. Recent advances in plant microbiome science suggest that genetic variation in plants can modulate the composition and activity of rhizosphere N-cycling microorganisms. Here we attempted to determine whether genetic variation exists in *Zea mays* for the ability to influence the rhizosphere nitrifier and denitrifier microbiome under "real-world" conventional agricultural conditions.

Methods To capture an extensive amount of genetic diversity within maize we grew and sampled the rhizosphere microbiome of a diversity panel of germplasm that included ex-PVP inbreds (*Z. mays* ssp. *mays*), ex-PVP hybrids (*Z. mays* ssp. *mays*), and teosinte (*Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis*). From these samples, we characterized the microbiome, a suite of microbial genes involved in nitrification and denitrification and carried out N-cycling potential assays.

Results Here we are showing that populations/genotypes of a single species can vary in their ecological interaction with denitrifers and nitrifers. Some hybrid and teosinte genotypes supported microbial communities with lower potential nitrification and potential denitrification activity in the rhizosphere, while inbred genotypes stimulated/did not inhibit these N-cycling activities. These potential differences translated to

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A. Favela (✉)
School of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA
e-mail: alonsof@arizona.edu

M. O. Bohn
Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

A. D. Kent
Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

A. D. Kent
Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

A. D. Kent
Center for Advanced Bioenergy and Bioproducts Innovation, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

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functional differences in N_2O fluxes, with teosinte plots producing less GHG than maize plots.
Conclusion Taken together, these results suggest that *Zea* genetic variation can lead to changes in N-cycling processes that result in N leaching and N_2O production, and thereby are selectable targets for crop improvement. Understanding the underlying genetic variation contributing to belowground microbiome N-cycling into our conventional agricultural system could be useful for sustainability.

Keywords Microbiome · Rhizosphere · Maize · Selection · Nitrogen Cycle · Agro-ecosystem · Sustainability · Nitrification · Denitrification

Introduction

More than half the world's population depends on crops grown with synthetic nitrogen (N) fertilizers (Bowles et al. 2018). Regrettably, most of these synthetic N fertilizer inputs escape the agroecosystem, degrade natural areas, and harm human health (Vitousek et al. 2013; Zhang et al. 2015). Stimulation of soil nitrogen cycling microorganisms (i.e. nitrifiers and denitrifiers) is a major contribution to "leaky" agricultural systems (Kuypers et al. 2018). To improve the sustainability of agricultural systems, we need to understand how ecological drivers, such as genetically controlled plant–microbe interactions play out under "real world" conditions to influence soil's nitrogen cycling microorganisms and the movement of nitrogen, and how this can be managed to reduce nutrient losses (Moreau et al. 2015, 2019; Favela et al. 2023).

Genetic variation within crop species has been shown to play a significant role in plant-microbiome assembly and recruitment (Peiffer et al. 2013b; Bouffaud et al. 2016; Walters et al. 2018). Across large-scale and multi-year field trials, researchers find consistent sets of heritable core microbial taxa associated with specific plant genotypes (Walters et al. 2018; Xu et al. 2018). These community composition differences are functionally relevant, as microorganisms contain a diverse biochemical repertoire that allows plants to escape nutrient, drought, and pathogen stress (Philippot et al. 2013a, b; Compant et al. 2019; Trivedi et al. 2020). In the rhizosphere, plants exude chemical cocktails of metabolites, the production of which is directed by the plant genome. These exudate traits, in tandem with root phenology and physiology, act as an ecological filter that has direct fitness consequences for the surrounding soil microbial communities (Huang et al. 2019; Canarini et al. 2019; Favela et al. 2023; Baggs et al. 2023). Soil microorganisms that are phytochemically competent to the ecological filter of the rhizosphere are able to survive and persist near the plant root (Philippot et al. 2013a, b). It follows, then, that the ecological filtering for rhizosphere occupancy that arises from genotypic variation directing microbiome selection should have consequences for the biogeochemical cycling activities carried out by the rhizosphere microbiome.

Rhizosphere nitrifying and denitrifying microorganisms are key contributors to essential ecosystem functions. Processes such as nitrification and denitrification are controlled by microorganisms in the soil interacting with the abiotic environment and can result in a considerable loss of nitrogen from an ecosystem (Philippot et al. 2007; Davidson et al. 2012). An extensive amount of work in nitrifiers and denitrifiers has shown that edaphic soil history and agricultural management plays a significant role in structuring the functions of these communities (Qian et al. 1997; Hayatsu et al. 2021; Raglin et al. 2022). Furthermore, it is well known that the carbon/nitrogen inputs and uptake from plants can structure the functions of these critical N-cycling taxa (Haller and Stolp. 1985). In addition to this we know that the cultivated crop above these soils contributes to their nitrogen functions (Weier et al. 1993; Lucas et al. 2023). Furthermore, emerging research is beginning to show that genetic variation between species and within plant species can have a considerable role in driving both community composition and the activity of biogeochemically-relevant microorganisms (Subbarao et al. 2013; Pérez-Izquierdo et al. 2019). For example, it has been shown that historic selection on plant genotype can drive the assemblage of the nitrogen cycling rhizosphere microbiome, both by changing the taxonomic composition and representation of nitrogen-cycling functional genes (Bouffaud et al. 2016; Favela et al. 2021). Yet we lack evidence linking genotype-driven microbiome changes to altered nitrogen cycling processes within the complex and more microbially diverse conventional agricultural systems. For example, large research efforts have

focused on showing plant inhibition of nitrification under highly-controlled culture conditions with only a few microbial isolates (Kaur-Bhambra et al. 2022; Otaka et al. 2023; Petroli et al. 2023). While these results are mechanistically interesting, they are no guarantee for how diverse soil communities will behave. Here, we attempted to address this gap in an agroecological field setting. Specifically, we sought to determine whether plant genetic variation involved in root phenotypes is an important explanatory variable for understanding changes in the soil microbiome, particularly microbial functional groups, and to connect variation in this extended phenotype back to changes in important nitrogen cycling processes.

To evaluate the influence of plant genetic variation in root phenotypes on nitrogen cycling microbial functional groups, we grew a diverse panel of *Zea mays* (including elite inbreds, their hybrids, and wild teosintes) and measured their contribution to differences in microbial community assembly and nitrogen cycling processes. By doing this in a single field, we were able to control for edaphic factors and estimate the plant genetic contribution driving soil microbiome function in an agronomically relevant field setting. Previously, our greenhouse research in elite inbred maize suggested that breeding of maize has resulted in changes to microbiome recruitment, with more modern cultivars recruiting fewer N-fixing taxa and more denitrifiers/nitrifiers; outcomes that we would predict would change nutrient retention, and thereby shape sustainability (Favela et al. 2021, 2022). Informed by this prior research, we included maize hybrids to estimate if microbiome functions would be regained through heterosis (Wagner et al. 2021). In addition to this, within *Zea mays*, we found microbiome recruitment and N-cycling functional groups differed the most between modern inbred maize and wild teosinte (Favela et al. 2022). Therefore, wild *Zea* was included as an outgroup to evaluate the influence of pre-domestication genetics and their ecological filtering traits on microbiome assembly and function (Brisson et al. 2019; Schmidt et al. 2020). These treatments give us an understanding of how much plant genetic variation is necessary to induce changes in the microbiome of soils and provide insight into how domestication and breeding for performance in high N environments altered microbiome functions. With this information, we hoped to better understand how genetic alterations in maize can have cascading effects in plant microbiome recruitment and nitrogen cycling activity in conventional agricultural settings. This work in maize serves as a model for optimizing sustainable N cycling in other modern crops such as sorghum, which is an emerging bioenergy feedstock crop. Understanding the potential for plant genetics to contribute to these functional processes in an agroecological field setting is critical for improving the sustainability of maize production.

Methods

Field design

Field plots were located at the Crop Sciences Research and Education Center (CSREC)—South Farms at the University of Illinois, Urbana-Champaign, IL (40°03′30.4"N 88°13′50.4"W). We used a panel of *Zea* cultivars that encompassed modern ex-PVP inbreds, their hybrids, and the wild progenitor of maize, teosinte (27 genotypes in total: Description of genotypes in Table S1). Germplasm was obtained from USDA-ARS Germplasm Resources information Network (https://www.ars-grin.gov/). Teosinte was represented by the subspecies *Zea mays* spp. *mexicana* and *Zea mays* spp. *parviglumis*, with three genotypes per subspecies). Across our field, each cultivar was replicated four times. The replications were arranged in a randomized complete block design (Fig. S1-2). Each maize plot contained four rows with 15 plants per row. Maize inbreds and hybrids were machine planted on 4/30/2017 while teosinte cultivars were hand planted 5/19/2017. Due to seed limitations teosinte was planted in 2-row plots. Fields are managed by the CSREC in a Corn-Soy rotation, tilled yearly, and plant density of 32,000 plants/acre. Fertilization application was applied homogenously across all plots and match those on a typical conventional agricultural farm in Illinois (82 N kg/acre, 35 P kg/acre, 23 K kg/acre). CSREC staff carried out fertilization the week before planting (4/19–4/24). Urea-ammonium-nitrate (28%) solution was applied to fields at a rate of 60 gallons/acre.

Sample collection

Plots were sampled three times. Plants were sampled in approximately in the V4 (Maize and teosinte: 6/5/17), V6 (Maize: 6/20/17, Maize and teosinte:7/20/17), and R2 (Maize:7/12/17, Teosinte: 8/11/17) growth stages across the growing season (Nielsen et al. 1993). Staggered planting and sampling of teosinte and maize was carried out to control for differential growth rates among these genotypes. Staggered sample collection was accounted for in statistical models for nitrification and denitrification by modeling growth stage as a fixed factor and sampling date as a random factor. We found minor influence ($R^2 < 1\%$) of both. At each sampling event, four individual plants per plot were sampled and combined into a composite sample. Individual plants were not resampled. To accommodate the size of the experiment, true "rhizosphere samples" were not collected. Instead, root zone soil was collected as a proxy of the rhizosphere. Root zones soils here consisting of a mixture of rhizosphere and bulk soil present in the immediate proximity of the plant. This method allowed us to sample enough soil for nitrogen cycling assays and molecular work without having to carry out time consuming rhizosphere soil extractions from the roots. Samples consisted of a soil core (10 cm depth) obtained from the root zone of the plant (2 cm away from base of stem). Four root zone soil cores were collected from each plot and combined into a composite sample. Composite samples were placed on ice until they were transported to the lab. Processing of soil cores before assays and molecular work consisted of removal of all root tissue present in sample and homogenization of soil cores. Homogenization was performed by hand, for a minute per samples. Sieving homogenization was avoided as to not simulate mineralization and aeration. Once in the lab, soils were stored at 4 °C awaiting potential nitrification and potential denitrification assays (within 5 h). Aliquots for DNA extraction were frozen immediately. The frozen DNA aliquots were placed into 15 mL centrifuge tubes and lyophilized before DNA extraction (0.5 g total) using the FastDNA for Soil DNA extraction kit (MPBio, Solon, OH).

Soil samples were collected at the end of the season (9/15/17) for soil chemistry analyses carried out by Waypoint Analytical (Champaign, IL, USA). Analysis included buffer pH, nitrate, phosphorus, potassium, sulfur, manganese, copper, organic matter, estimated nitrogen release as estimated, cation exchange capacity, pH, sodium, iron, and boron. These were selected as they are typical reliable indictors of soil nutrition and would give us insights into how the soil nutrient profile was by the end of the season. Buffer pH measures the resistance of the soil to change pH and is done by adding a 7.5 pH solution to a soil and measuring the relative change. Estimated nitrogen release is calculated from the total SOC and is the amount of N thought to be stored with carbon. Cation exchange capacity and pH measure the number of cations in the soil and H+ions in the soil. Finally, all other measurements constitute macro and micronutrients important to plant nutrition.

Potential nitrification assay

The potential nitrification assay was developed and modified from (Schinner et al. 1996). This assay was performed at substrate saturation and values presented should be interpreted as the maximum potential rate of the transformation of ammonium to nitrite, the first-rate limiting step of nitrification. In principle, this assay uses ammonium sulfate (0.19 M, pH 8.5) as the substrate for the first step of nitrification during a 5-h incubation. Nitrite products released during the incubation period were extracted with potassium chloride and concentration is determined colorimetrically at 520 nm. Sodium chlorate (1.5 M) was added to the assay to inhibit nitrite oxidation during the incubation period. Sample tubes were incubated at room temperature on an orbital shaker for 5 h and control tubes were stored at -20 °C for 5 h. After incubation and thawing, KCl was used to extract nitrite from both samples and controls. Potential nitrification rates were arithmetically adjusted by initial soil moisture, soil weight, % dry matter, and initial nitrite in the sample. Potential nitrification data analyses are presented as (log (ng N g d.w soil $^{-1}$ h $^{-1}$)) and percent change ((population mean – genotype mean) divided by population mean).

Potential denitrification rates by acetylene-inhibition assay

Potential denitrification enzyme assays (here by referred to DEA) were carried out using a modified

version of previously described assays (Schinner et al. 1996; Peralta et al. 2016). Field-moist root-zone soil samples were incubated under anaerobic conditions in the presence of 10% acetylene or 100% helium for 3 h at 25 °C. The assay was performed on 25 g of root-zone soil in 125-ml glass Wheaton bottles. Incubations were carried out at substrate saturation of carbon (dextrose 500 mg/L) and nitrogen (nitrate 720 mg/L). Chloramphenicol (10 mg/L) was added to the incubation to act as a bacteriostatic agent to prevent further microbial growth and protein synthesis. The incubation bottles were purged of oxygen with either helium or acetylene. Helium samples were used to estimate the amount of incomplete denitrification produced during the assay (referred to potential (N_2O) DEA). Acetylene purged samples were used to measure complete + incomplete denitrification (referred to as potential ($N_2O + N_2$) DEA). Acetylene is a commonly known inhibitor of nitrous oxide reduction. Potential complete DEA (N_2) was calculated by subtracting (N_2O) DEA from ($N_2O + N_2$) DEA. These results are present in the supplemental materials (Table S8.7–9). Initial and final gas samples were collected at the start and end of the incubation period. Initial and final nitrous oxide in gas samples were quantified using a GC-2014 Gas Chromatograph (Shimadzu, Kyoto, Japan) with an electron capture detector (GC-ECD). Potential denitrification rates were arithmetically adjusted by initial soil moisture, soil weight, % dry matter, sample volume, and headspace. Potential denitrification data is presented as (log (ng N g d.w soil $^{-1}$ h $^{-1}$)) and percent change ((population mean – genotype mean) divided by population mean).

Carbon substrate utilization

Carbon substrate utilization assay was carried out using Biolog EcoPlates (Biolog Inc., Hayward, CA, USA). Biolog EcoPlates are a simple method to characterize the metabolic functions of microbial populations. Plates contain 31 different carbon substrates that can be used as the sole source of carbon. Each substrate is bound to a tetrazolium dye that changes colors once carbon compound is degraded. Assays were carried out with soils collected from the 7/20/17 sampling timepoint (represented by the R2 timepoint). Root zone soils (0.5 g) were diluted (1:4) in PBS, vortexed and centrifuged. Soil mixture supernatant (600 µL) was further diluted (1:25) in PBS. The diluted soil mixture was then added to the microplates and incubated for 5 days at room temperature. Absorbance at 590 nm was measured every 24 h using an Epoch microplate spectrophotometer (Santa Clara, CA, USA). Microbial metabolism was calculated as suggested in (Classen et al. 2003). This comparison focused on inbred B73, hybrid check1, and PI566677 teosinte (4 replicates per genotype). Data used in analysis consisted of carbon substrate usage (average well development) across 4 technical replicates at end of 5-day incubation. The SIMPER procedure in the 'vegan' R package was used to determine differences in substrate utilization across treatments (Oksanen 2017).

Microbial community amplicon sequencing

For this experiment, we characterized the microbiome and diagnostic functional genes related to transformations that occur in the nitrogen cycle: nitrification, and denitrification. Amplicon sequencing was performed on bacterial and archaeal 16S rRNA genes, fungal ITS2, bacterial *amoA,* archeal *amoA, nirS, nirK,* and *nosZ* genes. The Fluidigm Access Array IFC system was used to prepare sequencing amplicons. This method allows for the simultaneous amplification of target functional genes using multiple primer sets (Fluidigm, San Francisco, CA). DNA sequencing was performed for bacterial, archaeal, and fungal amplicons using an Illumina NovaSeq Sp flowcell with 2 × 250 bp reads (Illumina, San Diego, CA). Primer information is provided in supplemental Table S2. Fluidigm amplification and Illumina sequencing were conducted at the Roy J. Carver Biotechnology Center, University of Illinois (Urbana, IL, USA). Fast Length Adjustment of Short reads (FLASH) (Mag and Salzberg 2011) software was used to merge paired-end sequences from bacterial and archaeal 16S rRNA genes. Due to the amplicon size for some functional genes, only forward read sequences were used. Once FLASH merging was performed, files were filtered by quality using the FASTX-Toolkit (Hannon 2014). Reads that did not have a minimum quality score of 30 across 90% of the bases were removed. Using the FASTX-Toolkit, *nirK* sequences were trimmed to the amplicon size of 165-bp (as

the *nirK* amplicon size (165 bp) was smaller than the read length of 250 bp).Once quality preprocessing was performed, FASTQ reads were converted to FASTA format. Using USEARCH-UPARSE version 8.1 (Edgar 2010), sequences were binned into discrete OTUs based on 97% similarity and singleton DNA sequences were removed. Quantitative Insights into Microbial Ecology (QIIME) was used to generate OTU tables for downstream statistical analysis and to assign taxonomic information, this is done with a combination of the UCLUST algorithm and SILVA 138.1 database (DeSantis et al. 2006; Caporaso et al. 2010; Edgar 2010). Once taxonomy was assigned, chloroplast and mitochondrial OTUs were removed from the dataset. Rarefaction was performed to correct for differential sequencing depth across samples. Singleton OTUs were filtered prior to statistical analysis. Functional gene sequences were also assigned using QIIME (Caporaso et al. 2010) with the BLAST algorithm (Altschul et al. 1997) and custom gene-specific databases generated from reference sequences obtained from the FunGene repository (Fish et al. 2013). All OTU tables used in statistical analyses were generated in QIIME.

The number of raw reads generated from sequencing run, reads present after quality filter, and the rarefaction level are reported in supplemental Table S3. Rarefaction level was determined by calculating the rarefaction curve asymptote. Amplicon sequence data for 16S rRNA genes, fungal ITS2 region, and N-cycling functional genes is available for download on the NCBI SRA database at accession number: PRJNA789877. (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA789877/). Code for sequence processing and statistical analysis is available in GitHub (https://github.com/favela3/Maize.N-cycle.Function).

Quantifying nitrogen cycling functional groups

Quantitative PCR (qPCR) was carried out to determine the abundance of functional genes in each of the root zone microbial communities. Specific target amplification (STA), explained in Ishii et al. (2014), was carried out on samples and standards to increase template DNA for amplification. STA and qPCR master mix recipes from (Edwards et al. 2018) were used for all samples. STA product and qPCR master mix were loaded into the Dynamic Array™ Microfluidics Fluidigm Gene Expression chip, where amplification and quantification of functional genes were carried out simultaneously (Fluidigm, San Francisco, CA). All samples and standards were analyzed in 12 technical replicates. Fluidigm Real-Time PCR Analysis software version 4.1.3 was used to calculate gene threshold cycles (C_T). C_T values were converted to gene copy number using gene length and standard curves. All Fluidigm qPCR was conducted at the Roy J. Carver Biotechnology Center (Urbana, IL, USA). The final copy number of each functional gene amplicon was standardized by the ng of template DNA in the qPCR reaction.

In situ N_2O flux measurements

Net soil-atmosphere N_2O fluxes were measured weekly from 6/20/17 to 8/23/17, samples were collected for a total of 6 weeks. As gas flux measurements are laborious and time consuming, sampling was targeted during peak primary productivity (plant growth) and focused on the plant treatments that were hypothesized to have the largest effect on the microbiome function based on previous studies and known plant nutrient demands (Gentry and Below 1993). Specifically, the comparison focused on the B73 inbred, check1 hybrid, and PI566677 teosinte. Flux measurements were measured using static flux chambers as described in USDA-ARS GRACEnet Project protocol (Parkin and Venterea 2010). Chambers were installed in the field during the first sampling timepoint and remained in place throughout the maize growing season. Chambers consisted of two-pieces: PVC pipe with a 30 cm diameter (base installed 20 cm into soil), and sampling lids (10 cm in height). Gas sampling events occurred in the mornings between 10 am-noon; during this time 15 mL of gas were collected from chambers every 10 min for 30 min. Samples were stored in evacuated aluminum crimp-top glass vials with a chlorobutyl stopper and sealed with clear silicone to prevent sample leakage. Gas samples were later quantified using a GC-2014 Gas Chromatograph with an electron capture detector (GC-ECD) (Shimadzu, Kyoto, Japan). Standard curves were used to quantify the amount of N_2O in gas sample. N_2O

samples were corrected using ambient temperature and moisture conditions of the collection day. Temperature and moisture data was collected in the field and validated using Illinois Climate Network (https://www.isws.illinois.edu/warm/weather/). Four sampling timepoints were used to determine the rate of N_2O flux (mg m^{-2} min^{-1}). Linear interpolation was used to estimate cumulative N_2O production (mg L^{-1}) over the growing season (Parkin 2008).

Statistical analysis

Statistical analysis was performed in R, with the packages 'Vegan', 'ASReml', and 'WGCNA'. 'Vegan' was used to perform multivariate statistical comparisons for microbiome data among experimental treatments (Oksanen et al. 2007; Langfelder and Horvath 2008; Gilmour et al. 2017). 'ASReml' was used to perform univariate comparisons among genotypes and cultivar classes (inbred, hybrid, and teosinte) for potential nitrification, potential denitrification, and copies of nitrogen cycling genes as assessed by qPCR. Weighted Gene Correlation Network Analysis (WGCNA) was carried out to compare multivariate microbiome data to univariate nitrogen cycling function data. Model factors used in statistical analyses were growth stage, sampling date, the location of the block, the row of block position, range of block position, the genotype within the block, and the interaction between genotype and time (combined growth stage and sample date). A typical model of analysis is displayed below:

$$\begin{aligned} Microbiome = {} & Plant\ Genotype + Time + Block + Range \\ & + Row + Range : Row\ Position + Genotype \\ & \times Time\ Interaction + Residuals \end{aligned}$$

In PerMANOVA models, block factor was constrained in permutations. For the PerMANOVA models, sampling date and growth stage are combined as a time factor. In the ASReml mixed effect models for potential nitrification and denitrification, plant genotype and the genotype × growth stage interaction were treated as fixed factors, while all other factors (block, range, row, sampling date) were treated as random factors. Furthermore, a simple ASReml mixed effect model was generated to compare soil chemistry at the end of the growing season, plant type was treated as a fixed factor, while all other factors (range, row, block) were treated as random factors.

Results

Nitrogen cycling microbial functional groups

From our analysis of nitrogen cycling functional genes, we observed 210 archaeal *amoA* OTUs, 98 bacterial *amoA* OTUs, 21,022 *nirK* OTUs, 2607 *nirS* OTUs, and 7294 *nosZ* OTUs (DNA sequencing quality is described in Table S3). In response to genotype, the overall microbiome and 4 of 5 nitrogen cycling genes showed statistically significant changes in community membership (Fig. 1 and Table S4), 0 of 5 nitrogen cycling genes changed in abundance (Table S4.1). Conversely, plant classification (i.e., inbred, hybrid, teosinte) affected the composition of 4 of 5 nitrogen cycling genes, and the abundance of 1 of 5 nitrogen cycling genes (Table S4.2). Additionally, genotype classification interactions with time had a significant effect on the composition N-cycling microbial communities and functional gene abundance (Table S4.1–2; Fig. S5-6).

Nitrification genes and potential function

Bacterial and Archaeal nitrifiers (indicated by gene sequences for bacterial and archaeal ammonia monooxygenase – *amoA*) responded differently to plant genotype and plant classification. Plant genotype explained a small but significant amount of variation for archaeal *amoA* ($R^2 = 0.08$, $p < 0.001$, Figs. 1, Tables S5.1–3), there was not a significant change in community composition of bacterial ammonia oxidizers in response to genotype (perMANOVA $p = 0.16$, Table S4, S5.4–6). Regarding abundance, neither archaeal nor bacterial ammonia oxidizers were significantly influenced by plant genotype (archaeal *amoA* $p = 0.61$, bacterial *amoA* $p = 0.99$, Table S6.1–7). Plant classification showed the same patterns as genotypes (Fig. 2d-e, S5, Table S4, S5, S6). Potential nitrification rate (log (ng N g d.w soil $^{-1}$ h^{-1})) of root zone soils was influenced by both growth stage, genotype, and plant classification (Fig. 3a, S3a, Table S7.1–2). Specifically, teosinte genotypes had lower potential nitrification rate by 9% compared to population mean, on average, stimulated potential nitrification rates by 4% (means difference of 13% between inbred and teosinte, $p < 0.05$, Fig. 2d, S9.1). It should also be noted that some amount of variation in potential

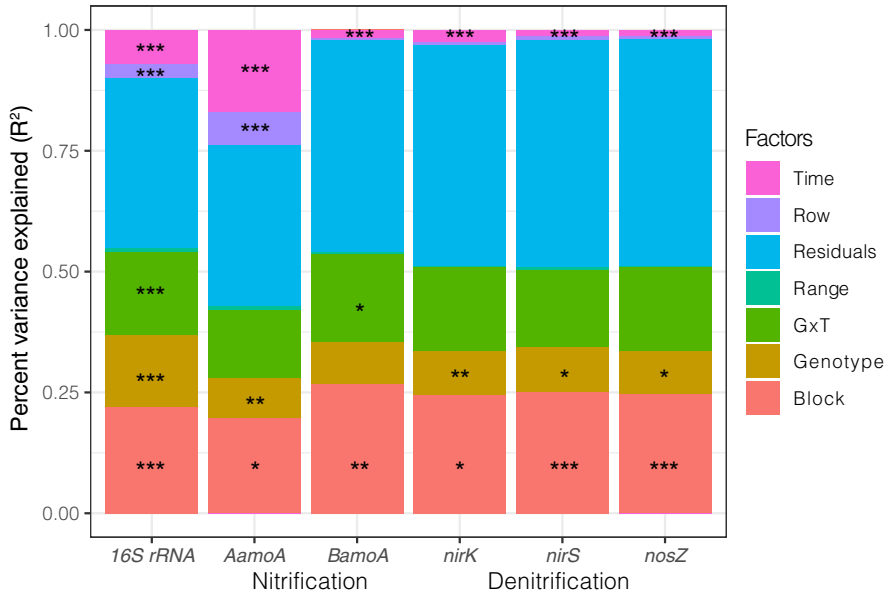

Fig. 1 PerMANOVA results for the overall microbiome (16S rRNA) and different nitrogen cycling functional genes included in this study (nitrification: *AamoA* – archaeal *amoA* [ammonia monooxygenase], *BamoA* – bacterial *amoA*; denitrification: *nirS* and *nirK* – nitrite reductase, *nosZ* – nitrous oxide reductase). The y-axis shows $R.^2$, percent variance explained by the treatment factor, and the x-axis shows the functional genes tested. (* – $P<0.05$, ** – $P<0.01$, *** – $P<0.001$)

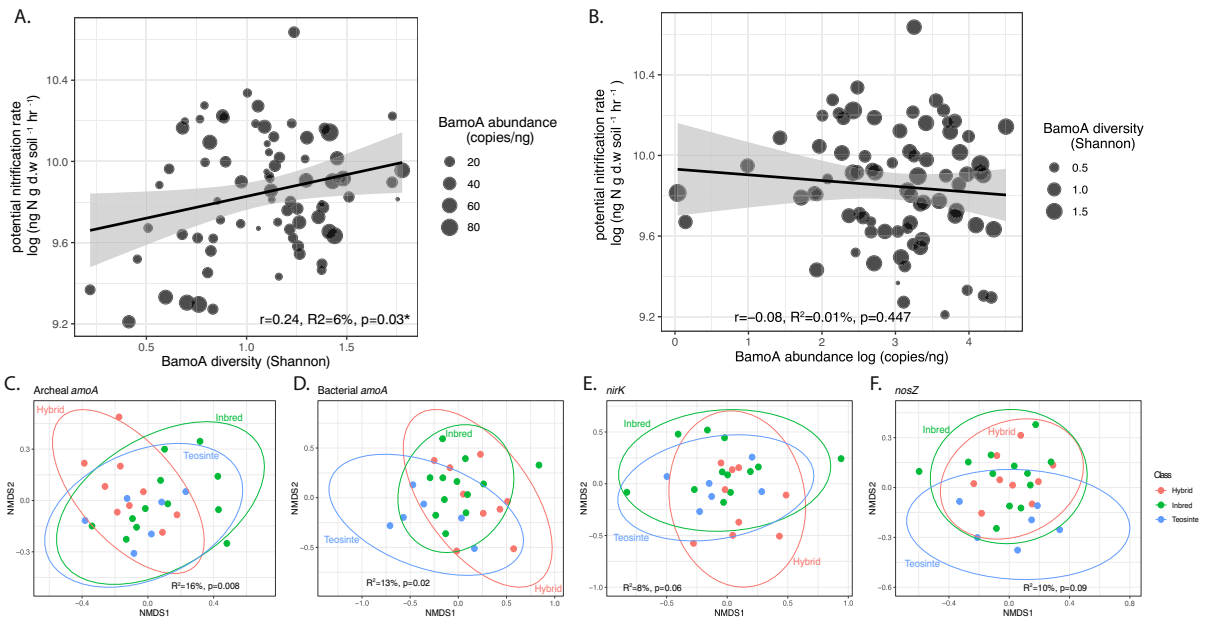

Fig. 2 The relationship between functional bacterial *amoA* diversity, abundance, and potential nitrification function (A-B) and NMDS ordinations based on Bray–Curtis dissimilarity (C-F) display functional gene composition. A. Shannon diversity of bacterial *amoA* regressed against potential nitrification function. Note size of point is gene abundance. B. Bacterial *amoA* qPCR abundance regressed against potential nitrification function. Note size of point is gene diversity. (C-F) Shows that inbred, hybrid, and teosinte maize lines host different microbial taxa in the root zone under the same environmental conditions within for timepoint 1 (young plants V2-V4). Each point represents a genotypic mean (within mean n = 4) of the microbial community. C. Bacterial *amoA,* D. displays archaeal *amoA,* E. displays *nirK,* F. displays *nosZ* (note: *nirS* was not presented here as $p>0.10$. Statistic presented in the ordination is the PerMANOVA of the classification model on genotypic means. For Temporal patterns in N-cycling gene communities please refer to supplemental materials (Fig S11)

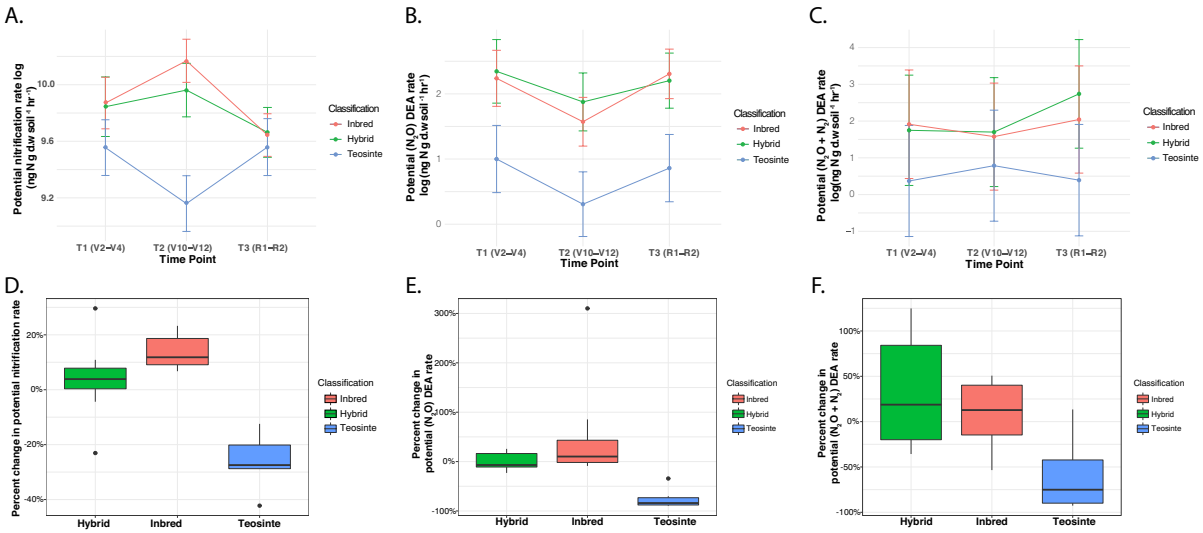

Fig. 3 Seasonal variation in potential nitrification and denitrification rate compared among germplasm group across the season. LS Means and Standard Error were calculated using ASReml-R. **A.** Potential nitrification rate (log (ng N g d.w soil $^{-1}$ h $^{-1}$)) across the three sampling time points averaged over plant classification. **B.** Potential (N_2O) DEA rate(log (ng N g d.w soil $^{-1}$ h $^{-1}$)) across the growing season averaged over plant classification, no differences in among plant classifications was observed, but potential denitrification rates increased slightly across the season. **C.** Potential (N_2O+N_2) DEA (N_2O+N_2) (log (ng N g d.w soil $^{-1}$ h $^{-1}$)) across the growing season averaged over plant classification, no differences in among plant classifications was observed, but potential denitrification rates increased slightly across the season. Lines in figures (A-C) were added easily track changes in potential rates across the growing season, and do not represent collected data in the intermediate time points. Plant classification influences rhizosphere N-cycling activities. **D.** Average genotypic effect across all timepoints of hybrid, inbred, and teosintes genotypes on the potential nitrification (log (n ng N g d.w soil $^{-1}$ h $^{-1}$)) determined across the population. Statistical analysis for both figures can be found in supplemental tables S7-9. E**.** Average genotypic effect across all timepoints of hybrid, inbred, and teosintes genotypes on the log of potential (N_2O) DEA (N_2O) (log (ng N g d.w soil $^{-1}$ h $^{-1}$)) determined across the population. Statistical analysis for both figures is included in supplemental tables S7-9. **F.** Average genotypic effect across all timepoints of hybrid, inbred, and teosintes genotypes on the log of potential (N_2O+N_2) DEA (log (ng N g d.w soil $^{-1}$ h $^{-1}$)) determined across the population. Percent change calculation described in Methods. Statistical tests associated with figures are presented in supplemental materials

nitrification rates could be attributed to plant genotype ($p<0.05$, Fig. 3, Table S7, S9). Furthermore, it appears that teosinte in the *parviglumis* compared to *mexicana* have greater abilities to lower potential nitrification. Furthermore, we regressed both nitrification and denitrification functional gene abundance, composition, and diversity against potential function across all time points and found that only bacterial *amoA* Shannon diversity was important in predicting potential nitrification function ($p<0.05$), while abundance was not (ANOVA $p>0.05$, Fig. 2a-b).

Denitrification genes and potential function

All the denitrification gene composition surveyed were significantly different among genotypes and plant classification (Fig. 1, S6, Tables S4, S5.7–15). Communities of denitrifiers possessing the cytochrome cd_1-type nitrite reductase (encoded by *nirS*) or the copper containing nitrite reductase (encoded by *nirK)* both varied significantly among plant genotypes (*nirS:* $R^2=0.09$, Fig. 1, Table S5.8; *nirK:* $R^2=0.09$, $p=0.003$, Fig. 1, 3e: Genotype means, Table S5.11) In addition, *nosZ,* the gene that encodes typical nitrous oxide reductase and crucial for the consumption of N_2O, was found to be affected by plant genotype ($R^2=0.09$, $p=0.011$, Fig. 1, 2f: Genotype means Table S5.14). Quantitative PCR of denitrification genes showed no difference in the abundance of genes in the root zone across plant genotype and largely for plant classification (Fig. S5-6, Table S4). One exception to this was *nosZ,* which was observed to be altered by plant classification ($p<0.05$, Table S5.13, Fig. 3e, S4e).

Similar to the observations for bacterial *amoA*, the denitrification genes also showed a strong and significant interaction between plant classification and time (Fig. S5-6). The teosinte root zone microbiome contained similar denitrification gene abundance for the first sampling time point (V2-V4), had greater levels of dentification genes during the final (R1-R3) sample point, and finally, had lower numbers of denitrification genes compared to inbred and hybrid maize by the end of season (LS means, $p < 0.05$, Tables S5-6). Full analysis of plant classification effects and additional genotype models on denitrification genes is presented in Supplemental Tables S5-6. Conversely, we found variation in the potential (N_2O) DEA and ($N_2O + N_2$) DEA rates (log (ng N g d.w soil $^{-1}$ h $^{-1}$)) of root zone soils to be consistently influenced by genotype and plant classification, but not growth stage (Fig. 3b, S3b, potential (N_2O) DEA: $p < 0.001$, potential ($N_2O + N_2$) DEA: $p < 0.001$, Table S8.1–4). Complete DEA (N_2) did not significantly differ across genotypes in this study ($p = 0.8$, Table S8.7), but did across plant classification ($p < 0.001$, Table S8.8). For potential (N_2O) DEA (log (ng N g d.w soil $^{-1}$ h $^{-1}$)), teosinte genotypes had lower activity by 75% and inbred genotypes on average stimulated potential (N_2O) DEA denitrification by 32% (mean difference 102%, Fig. 3e, Table S9.3). On average, teosinte genotypes had lower potential ($N_2O + N_2$) DEA by 59% compared to inbred maize, which stimulated it by 4% (mean difference 63%, Fig. 3f, Table S9.2).

Static N_2O flux chambers

To estimate whether our potential denitrification and nitrification rates were reflected in ecosystem fluxes, we placed static flux chambers in blocks with three of our genotypes inbred, hybrid, and teosinte. From these static chambers, we found that over the growing season, that teosinte genotype plots produced significantly less cumulative N_2O production (mg L^{-1}; linear interpolation of N_2O over the season) ($t = 2.01$, $df = 29$, $p = 0.05$, Fig. 4a), and had lower N_2O flux rate (mg m^{-2} min^{-1}) ($t = 2.09$, $df = 33$, $p = 0.04$) compared to the inbred genotype. Hybrid plots were not significantly different in N_2O production from inbred and teosinte (B73: $t = 1.26$, $df = 29$, $p = 0.22$; Teosinte: $t = -0.76$, $df = 30$, $p = 0.25$) or in N_2O flux (B73: $t = 1.74$, $df = 30$, $p = 0.09$; Teosinte: $t = -0.07$ $df = 30$, $p = 0.95$). In addition to this, we observed a dynamic pattern in N_2O flux (mg m^{-2} min^{-1}) across the season – where early season fluxes were similar but diverged by the end of the growing season (Fig. 4b).

Soil nutrient analysis

We found no significant difference in organic matter, estimated nitrogen release as estimated by SOM, cation exchange capacity, pH, sodium, iron, and boron between maize, hybrid, and teosinte plots. Teosinte plots had significantly higher levels of nitrate, phosphorus, potassium, sulfur, and manganese compared to inbred and hybrid maize plots (Wald's test: $p < 0.05$; Table S11). Increased nitrate in teosinte plots may be indicative of the suppressed denitrification. Inbred maize plots had higher levels of buffer pH, copper, and calcium compared to teosinte and hybrid plots (Wald's test: $p < 0.05$; Table S11).

Carbon substrate utilization

We found that plant classification was not statistically significant in explaining variation in microbial carbon substrate utilization (PerMANOVA: $DF = 2$, $R^2 = 0.25$, $p = 0.10$). Between inbred B73, hybrid check1, and teosinte PI566677 genotypes, we found that teosinte root zone microbiomes had greater overall levels of substrate utilization compared to inbred and hybrid microbial communities (t-test; $p < 0.05$, Fig. 4c). We found no significant differences in utilization of individual substrates between inbred maize and teosinte microbial communities, significant differences in utilization of 3 substrates between inbred and hybrid maize, and significant differences in utilization of 2 substrates between hybrid and teosinte microbiomes (ANOSIM, $p < 0.05$). Inbred-hybrid differences include glycogen, D-cellobiose, and L-serine. Teosinte-hybrid differences include L-phenylalanine and tween-80.

Root zone microbiome

In this field experiment, we identified 37,596 different 16S rRNA operational taxonomic units (OTUs, 97% similarity, (rarefied to 100,000 reads per sample), and 2236 fungal OTUs (rarefied to 10,000) were identified from the ITS2 region (Table S2).

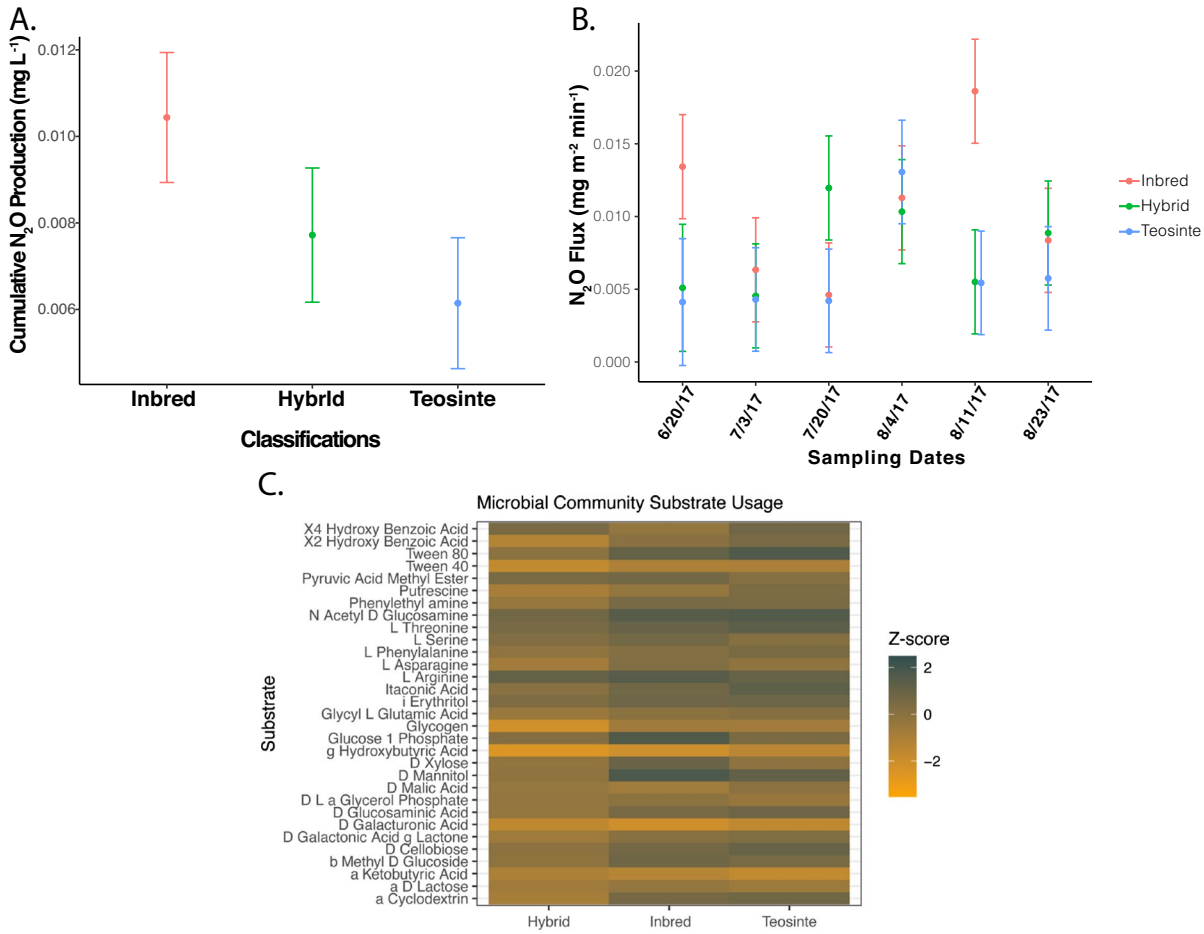

Fig. 4 Cumulative N_2O static flux chamber results over the growing season. **A.** LS means + 95% CI of cumulative N_2O produced (mg L^{-1}) averaged over the season comparing teosinte and B73 maize. **B.** LS means + 95% CI of N_2O production flux (mg m^{-2} min^{-1}) comparing teosinte and B73 maize displaying all timepoints. **C.** Carbon substrate usage (AWD) Z transformed compared among inbred B73, hybrid check1, and teosinte PI566677 (4 replicates per genotype) soil microbiomes measured using a BIOLOG-Eco microarray plates

Within the prokaryotic community (based on 16S rRNA gene sequences), we found that plant genotype, genotype × time interactions, the location of the block, and sampling time explained 74% of the variation within the root zone microbiome; 26% of the variation within the microbial community was unexplained (DF = 26, $p < 0.001$; Fig. 1, S7; Table S10). In total, 34% of the variation in the prokaryotic microbiome was explained by plant genetics; 18% of this 34% variation was independent of temporal effects while 16% was highly linked to the time of sampling (genetics × sampling time). Interestingly, 20% of the variation in the soil microbial community was explained by block location alone. This would mean that across time, 20% of the microbiome was unchanged across the season. Sampling time (independent of genotype) explained 12% of the variation within the microbiome. Roughly, these results suggest that plant genetics explained about a third of the variation in the root zone microbiome. Respectively, spatial and temporal effects seem to explain a third of the variation within the microbiome. Finally, an additional third of variation within the soil microbiome was unexplained.

Across plant classification (inbred, hybrid, teosinte), the most divergent genotype points showed the greatest differences in microbiome recruitment

(Fig. S7-8; Table S10.1–3). Specifically, teosinte and hybrid maize treatments have the strongest effect on the composition of the soil microbiome agroecosystem. Teosinte root zone soils contained greater relative abundance of *Actinobacteria* and *Proteobacteria* (specifically, *Actinomycetales, Burkholderiales)* and less *Acidobacteria* (*iii1-15*, *Solibacteres)* compared to modern maize (Fig. S9-10). Additional analysis was carried out within plant category (i.e., within inbred, within hybrid, within teosinte), and inbred maize was the only category where genotype did not significantly contribute to differential microbiome recruitment.

Fungal communities showed similar results as the prokaryotic communities, except for notably weaker effects of space. This may indicate that fungi are less dispersal-limited than bacterial communities (Table S10).

Relationship between the microbial community and N-cycling function

To further understand the differential contribution of the root zone microbiome to the potential function of a soil sample, we used Weighted Gene Correlation Network Analysis (WGCNA) (Langfelder and Horvath 2008) to identify four unique co-correlated clusters of OTUs (modules) with a significant response to potential function (3 modules that were correlated to potential nitrification, and one module that was correlated to potential (N_2O) DEA (Fig. 5). WGCNA taxa Module 2 was positively correlated to potential nitrification ($r=0.20$, $p<0.001$), while two modules were negatively correlated to potential nitrification (Module 4: $r=-0.25$, $p<0.001$; Module 7: $r=-0.23$, $p<0.001$). Module 2 contained 129 OTUs and was dominated by

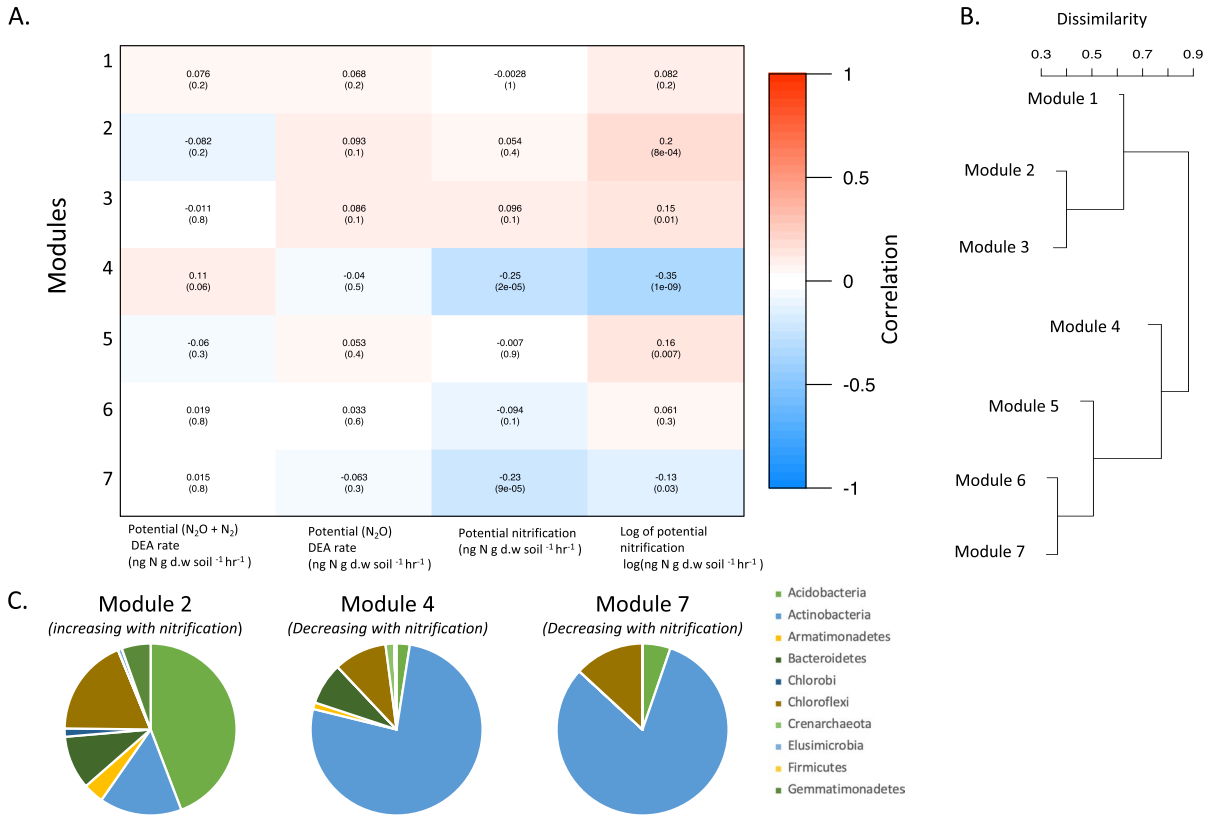

Fig. 5 Weighted Gene Correlation Network Analysis (WGCNA) results between potential denitrification and potential nitrification and microbial community composition. WGCNA starts by clustering microbial OTUs into modules of highly correlated taxa (based on abundance). These modules are then regressed against our explanatory factor (here that is denitrification and nitrification. **A.** Strength and significance of correlation between microbial modules and nitrogen cycling function. **B.** Correlations among microbial modules generated in the clustering process. **C.** Phylum-level composition of modules that were significantly correlated to changes in potential nitrification and denitrification

the presence of *Acidobacteria.* Interestingly, the second most dominant phylum in this module – *Cholorflexi* – was recently shown to have the ability to carry out potential nitrification (Spieck et al. 2020). Module 4 contained 290 OTUs and Module 7 contained 38 OTUs, both modules were dominated by *Actinobacteria.*

Discussion

Microbial N-cycling processes are modulated by crop genetics

Across a variety of environmental conditions and plant species, it has been shown that genetic variation within a plant population can consistently shape the rhizosphere microbiome (Peiffer et al. 2013a, b; Xu et al. 2018; Deng et al. 2021). Additionally, previous studies have also shown that changes in rhizosphere microbiome functional groups resulting from host plant genetics are important to ecosystem processes (i.e., carbon and nitrogen cycling) (Bulgarelli et al. 2015; Mwafulirwa et al. 2021a, b). Furthermore, for decades, researchers have known that plant carbon contributions to soils play a significant role in modulating microbial nitrogen transformations (Haller and Stolp 1985; Qian et al. 1997). However, there is a lack of data showing how host genotype-specific changes in microbiome functional groups influence nitrogen cycling ecosystem processes. This field study demonstrates that the effect of plant genotype extends to modulating functions of the root zone microbiome. Specifically, we observed that the plant genotype influenced the recruitment of functional groups related to nitrification and denitrification along with the potential rates of those ecosystem processes (Figs. 1, 2 and 3). These findings highlight the feasibility of breeding crops for microbiome-associated phenotypes (MAPs) to influence N-cycling microbes and their functions (Oyserman et al. 2018). Ultimately, our results suggest that we can select genetic haplotypes linked to MAPs within populations of agricultural cultivars to promote sustainable ecosystem processes within agroecosystems.

Our study demonstrates that genetic variation within *Zea mays* plays a significant role in both the assembly of the microbiome and the nitrogen cycling capability of the community, even in a stochastic field setting. Our most genetically heterogeneous treatments (wild and domesticated hybrid) had the strongest effects on the composition and function of the microbiome (Smýkal et al. 2018; Favela et al. 2021, 2022; Ren et al. 2022). Our hybrids showed patterns of microbial community recruitment distinct from their inbred parents, suggesting that heterosis plays a role in recruitment and function of the root zone microbiome. Interestingly, others have found that the expression of heterosis for root biomass and germination can be modulated by the presence of a soil microbiome (Wagner et al. 2020, 2021). Understanding the genetic basis of heterosis for MAPs is critical for designing efficient breeding programs for optimizing soil microbiome functions. Furthermore, we found that teosinte genotypes had the strongest influence on the activity of soil microbial taxa. We hypothesize that these differences in the teosinte microbiome were largely driven by a diverse set of belowground phenotypes (Gaudin et al. 2011), which facilitate changes that shape biotic interactions (via, exudate production, composition of metabolites, and root morphology) and abiotic characteristics (pH, moisture, NO_3^-, NH_4^+) which we know to shape nitrogen cycling taxa (Haller and Stolp 1985; Qian et al. 1997). These results suggest that selective reincorporation of traits important to N-cycling would be key MAPs for "rewilding" modern breeding programs for sustainability (Perino et al. 2019; Razzaq et al. 2021). Importantly, this field study confirms that N-cycling functions, as well as composition of the rhizosphere microbiome, are responsive to genetic variation within the plant host.

Potential mechanisms underlying maize genotypes nitrification and denitrification differences

We observed that teosinte and hybrid maize root zones had communities with a lower potential to carry out nitrification compared to inbred maize and shifts in the residing *amoA* genes (Fig. 2a, 3d, 3c-d). While it is clear the function of these nitrifiers is modulated by variation in genotype, it is unclear whether this is a direct or indirect process. It is possible that this alteration to function is caused by an indirect change to the abiotic environment (i.e., mineralization, ammonium, soil moisture, carbon) (Mwafulirwa et al. 2021b). Alternatively, these changes in function could be caused by direct phytochemically mediated biological suppression nitrification. Biological nitrification

inhibition (BNI) has been seen across a variety of different grass species (particularly in wild varieties) (Coskun et al. 2017a, b) with recent work showing that modern maize does have the phytochemical capacity for BNI (Otaka et al. 2022). It has already been shown that teosinte's metabolome and nutrient up take differs from modern maize, perhaps some of these physiological changes are related to changes in nitrification capacity (Wang et al. 2018; Xu et al. 2019). As nitrification can result in major losses of N fertilizer, investigating whether teosinte characteristics that confer lowered nitrification can be reincorporated into modern maize maybe of interest to future breeders (Nair et al. 2020; Subbarao et al. 2021). Evaluating the vast diversity of maize cultivars for the capacity to alter the nitrogen cycle either directly or indirectly using genetic information is a valuable first step toward incorporating and improving these novel MAPs in agriculturally viable germplasm.

Interactions of plant genotypes with denitrifying microorganisms followed a similar pattern to nitrifiers, except with considerably more variation. Over the growing season, genotype and cultivar classification played a significant role in shaping potential denitrification activity and denitrification gene composition (Figs. 2b-c, 3e-f, 3e-f). These results were surprising, as maize is typically grown in aerobic soils and denitrification is an anaerobic process. These results suggest that the genotype can interact with the highly variable hot spots and hot moments typical of denitrification (Krichels and Yang 2019). Interestingly, teosinte appears to support lower potential (N_2O) DEA and (N_2O+N_2) DEA rates leading to the hypothesis that teosinte contains some indirect (e.g., soil moisture, nitrate loads) or direct mechanism to shape denitrification not previously reported. One possible direct mechanism could be, biological denitrification inhibition (BDI), which is hypothesized to have evolved in plants as a mechanism to compete with denitrifying microbes for soil nitrates. BDI work is still in early stages with only a single class of metabolites, procyanidins, being shown to mediate BDI (Bardon et al. 2014, 2016; Galland et al. 2019). Interestingly, outside the context of BDI, a considerable amount of work has focused on a maize depolarized procyanidin (cyanidin) and anthocyanin (a glucoside cyanidin), showing that maize genotypes have considerable variation in cyanidin and anthocyanin production (Sharma et al. 2011; Paulsmeyer et al. 2017). While not quantified in this study, the BDI differences observed here among maize genotypes could potentially be related to differences in cyanidin and anthocyanin exudation in the rhizosphere. It is possible that these rhizosphere cyanidins and anthocyanin (Tselas et al. 1979; Hawes et al. 1998) are acting as anti-reductants, which is known to occur at low pH (Becker 2016), and are competitive inhibitors of denitrification or allosteric inhibitors like procyanidins. Further research needs to be done to determine the abiotic and biotic drivers of these alterations in population level denitrification differences.

Outcomes of plant modulation of N-cycling processes

From a sustainability perspective, this study highlights a potential avenue to reduce agricultural N losses and GHG emissions generated by soil microorganisms. N_2O static chamber results (Fig. 4a-b) provide support that the potential nitrification and denitrification assays are representative of more variable ecosystem processes. It should be noted that these potential assays and N_2O chambers, have their limitations (Nannipieri et al. 2018; Grace et al. 2020). Potential assays indicate that the maximum function of these N-cycling communities has changed in response to plant host in the root zone. While the N_2O chambers shows that changes in microbiome functional potential may be related to differences in N fluxes at the ecosystem scale. The capability for crop genotypes to reduce N_2O losses is an exciting finding, as agriculture is a major producer of N_2O emissions (Vitousek et al. 1997; Reay et al. 2012), and these results suggest an additional tool to potentially curb production of this potent GHG. While these N_2O results presented are interesting, a major limitation of this study is that we only examined these cultivars in a single field experiment and static flux chambers are known to be extremely variable (Waldo et al. 2019). Further support for our potential nitrification and denitrification assays translating to actual differences in field processes can be seen in our end of season soil nutrient analysis. From this analysis for a single end of season timepoint we observed that teosinte plots had higher levels of nitrates (Table S11) compared to hybrid and inbred plots. These higher nitrate levels could be driven by the plant, but this claim is difficult to support with the current dataset, since we only collected a single physiochemical timepoint. Teosinte's

enhanced ability to mine nutrients may be facilitated by changes in root zone pH driven by carbon exudation, and root density. This conclusion is supported by our finding showing that teosinte plots have a lower buffer pH (residual or reactive pH) compared to hybrid and inbred maize ($p < 0.05$, Table S11). Alteration of soil buffer pH is likely underpinning many of the functional changes we observed in this study. In addition, we observed modest differences in microbial community carbon metabolism between plant classifications (Fig. 4c). Teosinte-derived microbial communities were better at degrading phytochemical precursors (e.g., phenylalanine, oleic acid) in the flavonoid and linoleic acid synthesis pathways (Kaur-Bhambra et al. 2022), while hybrid-derived microbial communities were well-equipped to consume carbohydrates. This result is interesting, as microbes differ in their ability to metabolize different photosynthates exuded into the rhizosphere (Fan et al. 2022), lending support to the hypothesis that differences we are observing across microbiomes may be caused by altered amount and composition of plant exudates. This hypothesis is further supported by work in barley showing that genotypes can vary in rhizodeposition-derived carbon and this variation shapes soil microbial mineralization (Mwafulirwa et al. 2016). The next steps toward incorporating potential nitrogen conservation MAPs into agricultural practices would be research to explore whether suppression of nitrogen transformations is consistent across a wide range of biogeographic environments.

The effects of seasonal phenology and *Zea* genotype × sampling time interaction over the growing season played a major role in microbiome recruitment and function. Maize has different nutrient requirements across the growing season, and these nutrients are extracted from the soil environment (Bender et al. 2013), so, along with genotype-specific biochemistry, plant growth and development likely influence interactions with the soil microbial community. In addition, previous studies have shown that the complexity of the microbiome is built through time (Shi et al. 2016; Emmett et al. 2020; Ajilogba et al. 2022). These temporal effects are important to consider, as they can dramatically influence the conclusions drawn about the interaction between plants and their microbiome. We observed that potential nitrification and potential denitrification were dependent on plant growth stage (Fig. 3). Potential nitrification and denitrification, for example, seemed to peak in the middle of the season, coinciding with plant primary productivity (Fig. 3). Perhaps, during this high productivity phase *Zea* is releasing greater quantities of fresh exudate resulting in the priming of soil organic matter by microorganisms. Furthermore, rhizosphere C exudation has been shown to enhance the release of N (Phillips et al. 2011; Dijkstra et al. 2013; Emmett et al. 2020), perhaps explaining the overall increase of nitrification at this timepoint. Furthermore, these temporal impacts highlight a limitation in this study, whereby we may be overestimating our genotype differences because of differences in growth phenology among the classes of plants. In addition, temporal growth patterns are likely interacting with the measurability of effects in the root zone as root density of the plant is anticipated to change through plant development (Chaparro et al. 2013; Vetterlein et al. 2020; Tkacz and Poole 2021).

Relationship between microbial diversity and N-cycling

We found that different microbial taxa were correlated with functional changes in the microbiome. WGCNA identified four modules of microbial taxa that were significantly associated with both changes of potential nitrification and denitrification (Fig. 5). These results suggest that specific taxa and their interactions play a role in driving the function of the microbiome and that, to some degree, plants can influence the activities of specific microbial groups. Interestingly, we observed that modules positively correlated with higher potential nitrification rates were dominated by gram-negative bacteria within the phylum *Acidobacteria*. In contrast, those that were negatively correlated with nitrification were dominated by gram-positive bacteria within the *Actinobacteria* phylum. Surprisingly, these modules of correlated OTUs were not dominated by known nitrifying taxa, suggesting that nitrification processes may be, in part, dependent on the metabolism of other microbial community members, that nitrification is controlled by the level of transcriptional regulation rather than nitrifier population size, nitrifiers or nitrifier ideal conditions are facilitating habitat alteration that is influencing microbiome structure (e.g. altering pH to enrich for *Acidobacteria*), or that some other microbial interaction (i.e. predation, competition) is controlling nitrification (Baskaran et al. 2020; Spieck et al. 2020; Burian et al. 2021). Determining how ecological

interactions within the microbiome influence microbial functions is critical for understanding and predicting microbially-mediated ecosystem functions (Kuypers et al. 2018). In addition, we carried out analysis to determine how functional gene diversity, abundance, and composition related to potential functions. Among all functional gene comparisons between bacterial *amoA*, and archaeal *amoA*. We only found bacterial amoA diversity to be correlated to potential function, while abundance and composition was not (Fig. 3a-b). These results suggest that at least for potential nitrification the regulation of diversity is important to function. Overall, it is clear that understanding the relationship between microbial characteristics and functions is complex and will require further research to determine the rules of these relationships.

Conclusion

The ability of plants to influence microbial functions in the rhizosphere likely evolved as a mechanism for nutrient retention, enhancing plant competition for available nutrients from the soil matrix (Philippot et al. 2013b; Delaux and Schornack 2021; Lata et al. 2022). It is becoming increasingly clear that plant genetic variation (within and among species) modulates the activities of the soil-associated microbiome and that these alterations can impact soil biogeochemical functions (Falkowski et al. 2008; Morris et al. 2020). Identification of the plant genomic regions that direct recruitment of N-cycling microorganisms or modulation of their activities will enable progress on re-engineering the agroecosystem to reduce its contributions to N pollution (Johnson 2006; Subbarao and Searchinger 2021). That is not to say we suggest growing teosinte in the field, but that these wild cultivars are reservoirs of critical genetic variation that are potentially important to enhancing sustainability. This study thus contributes an important advancement by showing that maize, an agronomically important crop, has genetic variation that contributes to alterations in the microbiomes and N-cycling function – potentially enough variation to breed and incorporate these extended phenotype ecosystem traits into modern hybrid cultivars. Furthermore, we have demonstrated that a small subset of hybrids does appear to have regulation of N-cycling microbiome in a way similar to teosinte. In addition, it should be noted that a limitation of the work presented is that we did not characterize the mechanisms by which we are observing these effects. A great deal of mechanistic work is needed in this area of research. Furthermore, it should be noted that while plant genotypic control of function plays an active role in the growing season other historic edaphic and abiotic effects will play a major role in managing and predicting the agroecosystem N cycle. Integrating sustainability-related microbiome associated phenotypes into our agricultural systems is a way forward to address many agronomic challenges facing society (York et al. 2022; Favela et al. 2023).

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. Sequences and code are available via Github and SRA database. Please references methods for details.

Declarations

Competing interests The authors declare no competing financial interests.

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