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The impact of short-term flooding on soil microbial communities, soil nitrogen and maize productivity in clay loam soils of Ohio, United States

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Abstract

Short-term flooding from extreme rainfall is becoming more frequent, with implications for aboveground and belowground agroecosystem function. However, few studies have explored how flooding affects the soil microbiome and plant growth in agroecosystems. We investigated how a four-day flood influenced soil microbial communities (bacteria, archaea and fungi), soil nitrogen and maize (*Zea mays* L.) productivity in a split-plot randomized complete block design field trial in Custar, Ohio. The irrigation-induced flood treatment was the main factor (flood, control) and fertilizer treatment was the subplot factor (220 kg N ha^{-1} urea, unfertilized). Soil samples were collected immediately before flooding (when maize was in the V4 growth stage) and 3, 8 and 93 days after the flood treatment ended. Soil microbial community structure was not impacted by the flood or fertilizer treatments in contrast to soil N and maize biomass at the R6 growth stage. Averaged across fertilizer treatments 8 days after flooding, autoclaved citrate extractable (ACE) protein, a measure of organically bound soil N, was lower in the flood treatment compared to the control. Soil fungal community structure correlated with soil moisture, nitrate and ACE protein in vector analyses, whereas bacterial and archaeal community structure was not correlated with these soil properties. More fungal and bacterial taxa were differentially abundant in the flood treatment than the control when comparing communities before flooding to each subsequent timepoint. Taken together, short-term flooding increased soil bacterial and fungal variability and also reduced N availability. This work advances understanding of agroecological responses to flooding in field conditions.

KEYWORDS
bacteria, climate change, disturbance, flood, fungi

1 | INTRODUCTION

Extreme precipitation events are becoming more common due to climate change and frequently result in drought and floods (Intergovernmental Panel on Climate Change, 2022). While numerous studies have uncovered how drought impacts ecosystem function (Bogati & Walczak, 2022; Van der Molen et al., 2011; Williams & de Vries, 2020), the ecological impacts of flooding are not yet fully understood (Sprunger et al., 2023). Flooding following extreme rainfall events has economic and environmental consequences for primary productivity and nutrient cycling in agroecosystems (Lindsey et al., 2024). In fact, extreme rainfall events cause yield losses of a comparable magnitude to drought in maize (*Zea mays* L.) production systems in the Midwest United States, with excessive rainfall reducing yields by up to 34% compared with a 37% reduction under drought (Li et al., 2019).

Flooding disrupts both aboveground and belowground ecosystem services that regulate agroecosystem productivity. Flood events can disrupt plant metabolic processes, such as respiration, root growth, production of exudates and nutrient uptake (Bailey-Serres et al., 2012; Bowles, 2022; Fagerstedt et al., 2024; Martínez-Arias et al., 2022), and the activity of soil microorganisms as regulators of nutrient availability to plants (Chen et al., 2022; Schimel & Schaeffer, 2012). In particular, yield loss from flooding is commonly attributed to soil nitrogen (N) limitations and nitrate leaching (Kaur et al., 2020). Flooding also increases bacterial denitrification, lowering the plant-available nitrate in soil and producing nitrous oxide, a greenhouse gas (Achtnich et al., 1995; Conrad, 1996; Tiedje, 1988). Soil microbes also regulate plant-available N through decomposition, mineralization and nitrification processes that slow under oxygen limitation (Haddad et al., 2013; Pengthamkeerati et al., 2006). The combined lower levels of oxygen under flooded conditions paired with environmental N losses likely increase competition between plants and microbes (Kuzyakov & Xu, 2013; Moreau et al., 2019). However, the impact of a flood disturbance on soil microorganisms, soil N and primary productivity in N-fertilized row-crop agroecosystems is largely unknown (Sprunger et al., 2023).

Previous field-based studies on the effects of flooding on soil microorganisms have primarily focused on soil bacterial and archaeal communities in ecosystems subject to prolonged soil waterlogging, such as rice paddies, wetlands, floodplains and riparian zones (Breidenbach & Conrad, 2015; Doering et al., 2021; Huang et al., 2023; Jiao et al., 2019; Martínez-Arias et al., 2022; Sao et al., 2023). These studies generally report increased abundance of strict and facultative anaerobic bacteria and methanotrophic archaea with flooding, while plant-growth promoting bacteria are less abundant. Research on the influence of flooding on fungal communities is comparatively scarce, especially in agricultural systems, despite its implications for soil fungal biomass and community richness (Das et al., 2025). In particular, yield loss in maize after flooding can be caused by reduced symbiosis with vesicular-arbuscular mycorrhizal fungi that enhance nutrient uptake (Ellis, 1998). Further research on how flooding impacts the entire soil microbiome, including bacteria, archaea and fungi, and agroecosystem productivity will contribute to a more holistic understanding of soil food web dynamics under climate stress (Sprunger et al., 2023).

We address the knowledge gap on how short-term flooding affects soil bacterial, archaeal and fungal communities in field conditions, and its relation to above- and belowground agroecosystem productivity by assessing the effects of a four-day flood on soil microbial communities, soil N and primary productivity in fertilized and unfertilized maize production systems. A four-day flood duration is representative of short-term weather stress in the U.S. Midwest and prior studies show that maize largely recovers after 2 days of flooding but that growth is severely impeded after 6 days (Lindsey et al., 2024; Dill et al., 2020; Liu et al., 2013). Our first objective was to evaluate changes in soil microbial community structure after the flood, over both short-term (3 and 8 days after flooding) and long-term (3 months after flooding) periods. We hypothesize that flooding will alter soil microbial community structure through increased abundance of anaerobic bacteria and archaea and decreased abundance of plant-growth promoting bacteria and fungi, with more rapid changes documented in bacteria and archaea than fungi. Our second objective was to investigate the relationship between soil microbial community structure and agroecosystem function in fertilized and unfertilized systems, with respect to soil N and maize growth, after the flood. We hypothesize that the effects of short-term flooding on soil microbes, soil N and maize growth will be more predominant in unfertilized systems than fertilized systems, with greatest competition between plants and microbes for oxygen and N. We also hypothesize that soil N and maize growth will be lowest in unfertilized, flooded systems and highest in fertilized, control systems, signalling compounding effects of nutrient and water management for agroecosystem productivity.

2 | MATERIALS AND METHODS

2.1 | Site description

A field trial was established in 2021 at The Ohio State University's Northwest Agricultural Experimental Station in Custar, Ohio at 41°13′N, 83°45′W. The primary soil series is a Hoytville clay loam and the taxonomic class is a fine, illitic mesic Mollic Epiaqualf (Soil Survey Staff, 2010). The elevation is 211 m. Weather was monitored at the weather station on site (The Ohio State University Weather System, 2024). The experiment was a split-plot randomized complete block design with four replicate blocks. The main plot factor was the flood treatment and the subplot factor was the fertilizer treatment. Main plots were 7.6 $\times$ 24 m, and subplots were 7.6 $\times$ 3 m. This present study focuses on two fertilizer treatments: 'urea' and 'unfertilized'. In the urea treatment, urea (46-0-0; Andersen brand) was applied at 220 kg N ha^{-1} on May 17. The unfertilized treatment served as a control with zero N added. Immediately after fertilizer applications, all plots received shallow tillage (10 cm depth) using a disk to facilitate N incorporation; the unfertilized treatment was also tilled. Three days after fertilizer applications on May 20, a commercial maize (*Z. mays* L.) hybrid of common maturity for Ohio (P0506AM, Corteva AgriScience) was planted. The four-day flood treatment began June 15. The flood treatment was implemented with overhead sprinkler irrigation where 1 in. (25.4 mm) of irrigation water was applied each day to four main flood plots. The other four main control plots did not have overhead sprinkler irrigation and remained at ambient conditions. Blocks were separated with a 4-in.-tall soil berm created with a modified moldboard plough. The previous crop was soybean. Baseline soil test data, final yields and additional agronomic details about the field experiment are available in Novais et al. (2025).

2.2 | Soil and plant sampling

2.2.1 | Soil sample collection over the course of the growing season

Soil samples were collected four times across the 2021 growing season at four distinct timepoints ($n = 16$ per timepoint, $n = 64$ in total). The first pre-flood (PF) soil sample collection occurred on June 15 immediately before the flood treatment began. Samples were also collected 3, 8 and 93 days after the flood treatment ended (3DAF, 8DAF and 93DAF) on June 22, June 27 and September 20. Maize was in the V4 growth stage on June 14 and the R6 growth stage at 93DAF (Abendroth et al., 2011). At each sampling timepoint (i.e., PF, 3DAF, 8DAF, 93DAF), six soil samples (0–20 cm depth) from each subplot were collected with a 1.9 cm diameter hand probe and homogenized by subplot. A subsample was sieved using a 2 mm sieve and stored at -80°C for DNA extractions. The remainder of each sample was stored at 4°C for soil moisture and N analyses.

2.2.2 | Plant biomass collection

Aboveground biomass was collected on September 30 at the R6 growth stage by harvesting six maize plants at the base of the plant from each subplot (Abendroth et al., 2011). Total aboveground plant biomass included leaves, stalk, husk and cob. Grain biomass consisted solely of kernels produced.

2.3 | Soil and plant analyses

2.3.1 | Soil moisture and nitrogen analyses

All 64 soil samples were analysed for soil moisture and N pools including autoclaved-citrate extractable protein (ACE protein), ammonium (NH_4^+)-N and nitrate (NO_3^-)-N. Soil moisture was measured as gravimetric water content in 45 grams of soil (Reynolds, 1970). A subsample was oven-dried at 60°C to a constant weight and ground to 2 mm for analysis of N pools. ACE protein, an indicator of soil health and organically bound N (Naasko et al., 2024), was measured using methods adapted from Hurisso et al. (2018). Briefly, 24 mL of sodium citrate was added to 3 grams of soil. The solution was shaken for 5 min, autoclaved for 30 min at 121°C, cooled for 40 min, shaken for 30 min and then 1.5 mL of the solution in a microcentrifuge tube was centrifuged for 3 min. ACE protein was quantified using the colorimetric bicinchoninic-acid assay (Thermo Scientific, Pierce, Rockford, IL) in a 96-well spectrophotometric plate reader (BioTek, Agilent Technologies, Santa Clara, CA) at 562 nm. Soil inorganic N was extracted by adding 30 mL 2 M potassium chloride to 3 grams of soil. The solution was shaken for 30 min, and then centrifuged at 2000 rpm for 3 min. Nitrate-N and NH_4^+-N were quantified colorimetrically using the methods of Doane and Horwáth (2003) and Sinsabaugh et al. (2000), respectively, and read on a spectrophotometric microplate reader at 480 and 630 nm, respectively.

2.3.2 | DNA extraction, amplicon sequencing and data processing

Soil microbial community composition was investigated via amplicon sequencing. From each sample, DNA was extracted using the FastDNA Spin Kit for Soil according to manufacturer instructions (MP Biomedicals, Heidelberg, Germany). DNA concentration and quality were measured using a Nanodrop spectrophotometer (Thermo Scientific, Pierce, Rockford, IL). High-quality DNA samples were shipped to the North Carolina State University Genome Sequencing Laboratory (Raleigh, NC) for library preparation and sequencing. Libraries for amplicon sequencing targeted the bacterial/archaeal (16S ribosomal RNA, primers 515F and 860R) (Apprill et al., 2015; Parada et al., 2016) and fungal (internal transcribed spacer [ITS] ribosomal RNA, primers ITS1F and ITS2R) (Bellemain et al., 2010) communities. Libraries were sequenced using an Illumina NovaSeq 6000 instrument on an SP 250PE flow cell.

For individual 16S and ITS datasets, the raw data were processed using nf-core/ampliseq version 2.7.1 (Straub et al., 2020; Straub et al., 2024) of the nf-core collection of workflows (Ewels et al., 2020), utilizing reproducible software environments from the Bioconda (Grüning et al., 2018) and Biocontainers (da Veiga Leprevost et al., 2017) projects. The pipeline was executed with Nextflow v23.10.0 (Di Tommaso et al., 2017). Data quality was evaluated with FastQC (Andrews, 2010) and summarized with MultiQC (Ewels et al., 2016). Sequences were processed with DADA2 (Callahan et al., 2016) and taxonomic identification was performed using the SILVA database v138 including exact species (Quast et al., 2013) for 16S, and the UNITE database v10 (Abarenkov et al., 2024) for ITS. For individual 16S and ITS datasets, representative sequences of each amplicon sequence variant (ASV) were aligned using MAFFT v7.505 (Katoh et al., 2002), and a phylogenetic tree was built using FastTree v2.1.10 (Price et al., 2010). The ASV table, the taxonomic information for each ASV, the sample metadata and the ASV phylogenetic tree were integrated using the 'phyloseq' package (McMurdie & Holmes, 2013) in R version 4.2.2 (R Core Team, 2022).

2.3.3 | Plant analyses

Total aboveground plant and grain biomass were dried at 60°C to a constant weight and ground using a 2 mm mesh mill (model 3379-K05). Yield (Mg ha^{-1}) was calculated using the per plant biomass or grain weight (g plant^{-1}) and the average measured plant stand value biomass (plants ha^{-1}) of 83,333 plants ha^{-1} with the equation below:

$$\text{Yield}\left(\text{Mg ha}^{-1}\right) = \frac{\text{g biomass}}{\text{1 plant}} \times \frac{\text{1 Mg}}{\text{1,000,000 g}} \times \frac{\text{83,333 plants}}{\text{1 hectare}}.$$

2.4 | Data analyses

Data analysis was conducted using R version 4.2.2 (R Core Team, 2022) and figures were produced using ggplot2 (Wickham, 2016). Soil moisture and N pools were analysed for fixed effects of sampling timepoint, flood treatments, fertilizer treatments, all possible interactions, and random effects of replicate block, main plot nested within block, and subplot nested within main plot, using linear mixed-effects models built with the 'lmer' function in the 'lme4' package (Bates et al., 2015). There was no autocorrelation over repeated measures, tested using spatial power and compound symmetry analyses with the 'nlme' package (Pinheiro et al., 2022), and also no correlation or variance within subplots. Thus, final models included fixed effects of sampling timepoint, flood treatments, fertilizer treatments, all possible interactions, and random effects of replicate block and main plot nested within block. Model diagnostics were carried out with the 'resid_panel' function in the 'ggResidpanel' package (Goode & Rey, 2022). Inorganic soil N concentrations were log-transformed; after transformations, each model met assumptions of a Normal residual distribution and constant variance. Maize biomass and grain yields were analysed using linear mixed-effects models for fixed effects of flood treatments, fertilizer treatments, their interaction, and random effects of replicate block and main plot nested within block. Each model was tested for the significance (p < .05, F-tests) of individual and interaction effects in analyses of variance, using the 'Anova' function in the 'car' package (Fox & Weisberg, 2019). Marginal means were estimated using the 'emmeans' function and compared with the Least Significant Difference (LSD) test using the 'contrast' function in the 'emmeans' package (Lenth, 2023); degrees of freedom for contrasts were estimated using the Satterthwaite approximation. For transformed variables, statistical tests and LSD values were calculated on the transformed scale and back transformed to the reported values.

Soil microbial community structure was assessed for individual 16S and ITS datasets. Prior to downstream analyses, one 16S sample with low reads (<1000) (plot 214, 8DAF) and one outlier 16S sample (plot 313, 8DAF) were removed. Singletons and sequences identified as 'chloroplast' or 'mitochondria' were discarded. Alpha diversity metrics of observed richness, Shannon's diversity and Simpson's dominance were calculated using the 'alpha' function from the 'microbiome' package (Lahti & Shetty, 2019). Faith's phylogenetic diversity index was calculated with the 'pd.' function in the 'picante' package (Kembel et al., 2010). Each alpha diversity metric was analysed using linear mixed-effects models built identical to those for soil properties. Prior to beta diversity and differential abundance analyses, 16S and ITS ASVs were normalized using cumulative sum scaling with the 'cumNorm' function from the 'metagenomeseq' package (Paulson et al., 2013). To evaluate beta diversity, Bray–Curtis dissimilarity matrices were created using the 'distance' function and visualized as ordinations through non-metric multidimensional scaling (NMDS) with the 'ordinate' function from the 'phyloseq' package (McMurdie & Holmes, 2013). Ordinations were tested for effects of sampling timepoint, flood treatments, fertilizer treatments and all possible interactions in permutational multivariate analyses of variance (PERMANOVA) using the 'adonis2' function from the 'vegan' package (Oksanen et al., 2019). Vector analyses between ordinations and soil properties were performed with the 'envfit' function from the 'vegan' package (Oksanen et al., 2019). Differential abundance was used to evaluate the ASV-level changes between the pre-flood sampling timepoint and each subsequent timepoint in the flood treatment separately from the control treatment, using the 'Maaslin2' function in the 'Maaslin2' package (Mallick et al., 2021). To isolate flood treatment effects, and given the absence of fertilizer treatment effects on soil microbial community diversity, differential abundance analyses were performed using data from both of the fertilizer treatments. Main plot and fertilizer treatment were set as random factors to account for variability between and within main plots, respectively. The 'Maaslin2' package calculates fixed effect p-values based on a t-statistic using the Satterthwaite approximation (Mallick et al., 2021). The Benjamini–Hochberg false discovery rate procedure was used to adjust p-values (Benjamini & Hochberg, 1995).

3 | RESULTS

3.1 | Impact of the irrigation-induced flood treatment on soil moisture and coincidence with ambient precipitation

In this experiment, sprinkler irrigation was used to impose a four-day flood in maize production systems early in the 2021 growing season. Soil moisture significantly differed in the interaction between flood treatment and sampling timepoint (p < .01, F-test; Table S1). At 3DAF, there was standing water in the flood treatment (Figure 1b), which had received 190 mm of combined precipitation (89 mm) and irrigation water (102 mm) since planting (Figure S1). Averaged across fertilizer treatments at 8DAF, the flood treatment had 4.3% higher soil moisture than the control treatment (p < .01, LSD test; Figure 1a). Across treatments, soil moisture levels increased between PF and 3DAF and 8DAF (Figure 1a), which coincides with 17 mm of precipitation on the third day of the flood, 7 mm 2 days after the flood and 8 mm on 8DAF (Figure S1). The field site received an additional 226 mm of precipitation between 8DAF and 93DAF, and 84 mm before aboveground biomass collection on September 30 (Figure S1).

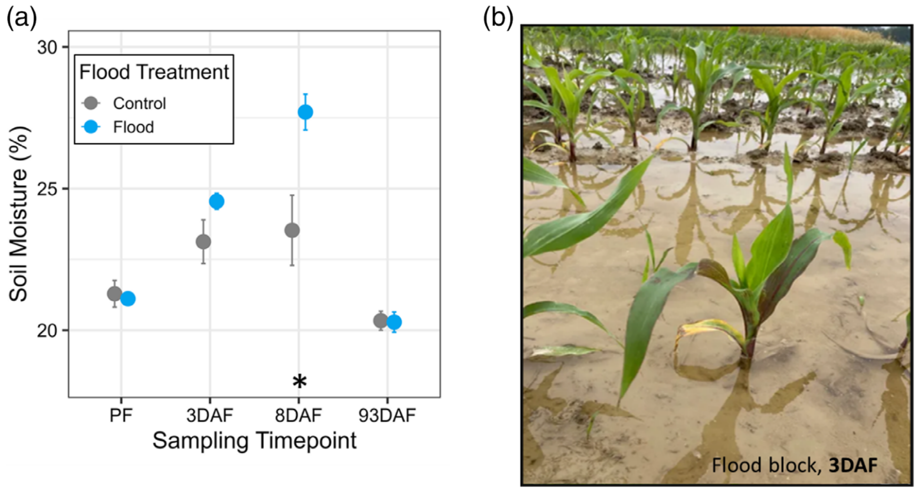

FIGURE 1 Impact of the flood treatment on (a) gravimetric soil moisture (%) of the flood and control treatments at the sampling timepoints, and (b) standing water at 3DAF in the flood treatment at the field experiment. Points and error bars represent means ($n = 8$) and standard errors in the flood and control treatments at each timepoint averaged across fertilizer treatments. The asterisk indicates significant differences between flood treatments on 8DAF ($p < .01$; LSD = 1.84; residual df = 47). DAF, days after flood; PF, pre-flood.

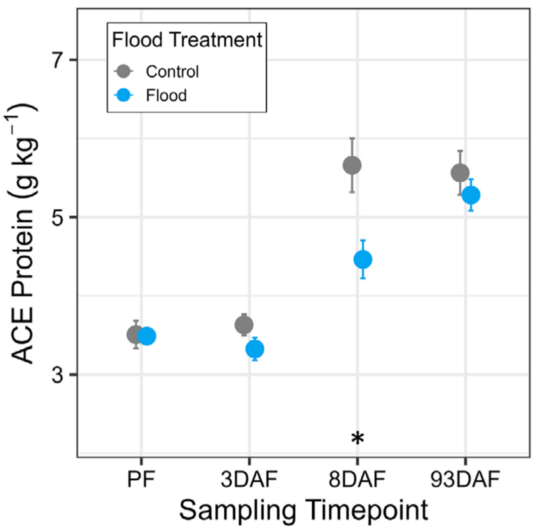

FIGURE 2 Impact of the flood treatment on autoclaved citrate extractable protein (ACE protein, g kg^{-1}) at the sampling timepoints. Points and error bars represent means ($n = 8$) and standard errors in the flood and control treatments at each timepoint averaged across fertilizer treatments. The asterisk indicates significant differences between flood treatments on 8DAF ($p < .01$; LSD = 0.61; residual df = 24.9). ACE protein, autoclaved-citrate extractable protein; DAF, days after flood; PF, pre-flood.

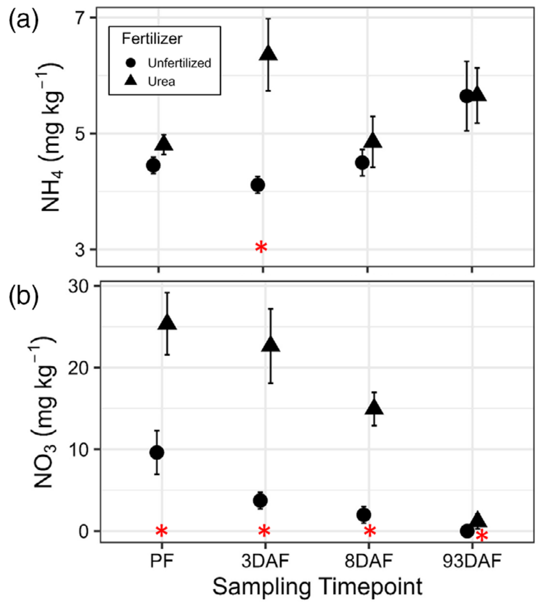

FIGURE 3 Impact of the fertilizer treatments on (a) ammonium and (b) nitrate (mg kg^{-1}) at the sampling timepoints. Points and error bars represent means ($n = 8$) and standard errors in the unfertilized and urea treatments at each timepoint averaged across flood treatments. Asterisks indicate significant differences between fertilizer treatments in (a) ammonium on 3DAF ($p < .01$; LSD = 1.24; residual df = 45), and (b) nitrate on PF, 3DAF, 8DAF and 93DAF (all $p < .05$; all LSD = 2.76; all residual df = 45). DAF, days after flood; PF, pre-flood.

3.2 | Impact of the short-term flood and nitrogen fertilization on soil nitrogen

Autoclaved-citrate extractable protein differed in the interaction between sampling timepoints and flood treatments ($p = .03$, F-test; Table S1). Averaged across fertilizer treatments compared to the control treatment, the flood treatment had 1.2 g kg^{-1} lower ACE protein at 8DAF ($p < .01$, LSD test; Figure 2).

Inorganic N pools of ammonium-N and nitrate-N differed in the interaction between sampling timepoints and fertilizer treatments ($p < .05$, F-tests; Table S1). Averaged across flood treatments compared to the unfertilized treatment, the urea treatment had higher ammonium-N at 3DAF by 3 mg kg^{-1} ($p < .001$, LSD test; Figure 3a), and higher nitrate-N at PF, 3DAF, 8DAF and 93DAF respectively by 16, 19, 13 and 1 mg kg^{-1} (all $p < .05$, LSD tests; Figure 3b).

3.3 | Impact of short-term flooding on soil microbiome diversity

Flooding did not significantly ($p > .05$, F-tests) alter short- or longer-term soil microbial community alpha diversity, with respect to

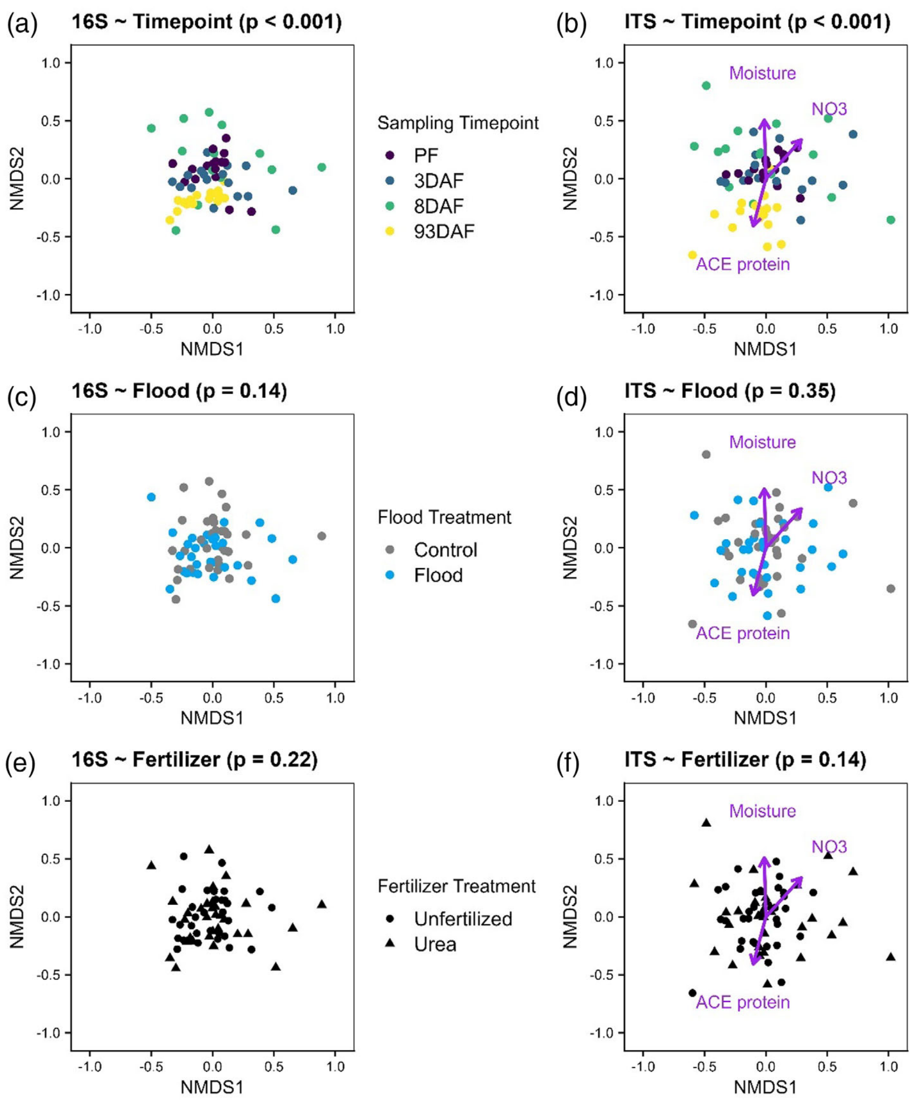

FIGURE 4 Soil bacterial/archaeal (16S) and fungal (ITS) community beta diversities across: (a, b) sampling timepoints, (c, d) flood treatments and (e, f) fertilizer treatments, in relation to soil properties in vector analyses (purple vectors). Timepoint and treatment effects were evaluated using PERMANOVA. The NMDS stress values for 16S and ITS were 0.17 and 0.19, respectively. ACE protein, autoclaved-citrate extractable protein; DAF, days after flood; PF, pre-flood.

richness, evenness, Simpson's dominance and phylogenetic diversity (Table S2), or beta diversity (Figure 4). However, across flood and fertilizer treatments, beta diversities differed between sampling timepoints: bacteria/archaea ($R^2 = 0.17$; $p < .001$, PERMANOVA; Figure 4a) and fungi ($R^2 = 0.15$; $p < .001$, PERMANOVA; Figure 4b). In vector analyses, soil fungal beta diversity (in terms of NMDS2 coordinates) correlated with soil moisture ($R^2 = 0.26$; $p < .001$, permutation test, $n = 64$), NO_3^--N ($R^2 = 0.20$; $p = .002$, permutation test, $n = 64$), and ACE protein ($R^2 = 0.18$; $p < .01$, permutation test, $n = 64$) (Figure 4b,d,f), in contrast to soil bacteria/archaea (Figure 4a,c,e). For fungi, ACE protein was correlated with 93DAF communities along NMDS2 in the opposite direction to NO_3^--N and soil moisture (Figure 4b,d,f).

3.4 | Changes in soil microbial community composition after short-term flooding across time

The change in soil microbial community composition across time after the flood was evaluated through differential abundance, averaged across fertilizer treatments, using the pre-flood timepoint as a baseline for the flood treatment and control treatment. The flood treatment had a higher number of differentially abundant bacterial and fungal ASVs than the control treatment at each timepoint, and more ASVs were differentially abundant at 93DAF ($n_{flood} = 124$; $n_{control} = 91$) than at 3DAF ($n_{flood} = 22$, $n_{control} = 7$) or 8DAF ($n_{flood} = 10$, $n_{control} = 7$) (Figure 5). Proteobacteria and Ascomycota were the most common phyla to which differentially abundant bacterial and fungal ASVs were respectively assigned (Table 1).

3.5 | Impact of flooding and fertilization on maize biomass and grain yield

Fertilization impacted maize biomass and grain yield at the R6 growth stage ($p < .01$, F-tests) more than flooding (Table S1). Averaged across flood treatments compared to the unfertilized treatment, the urea treatment had higher maize biomass and grain yield levels by 15.5 and 10.5 Mg ha^{-1}, respectively ($p < .05$, LSD test; Table 2).

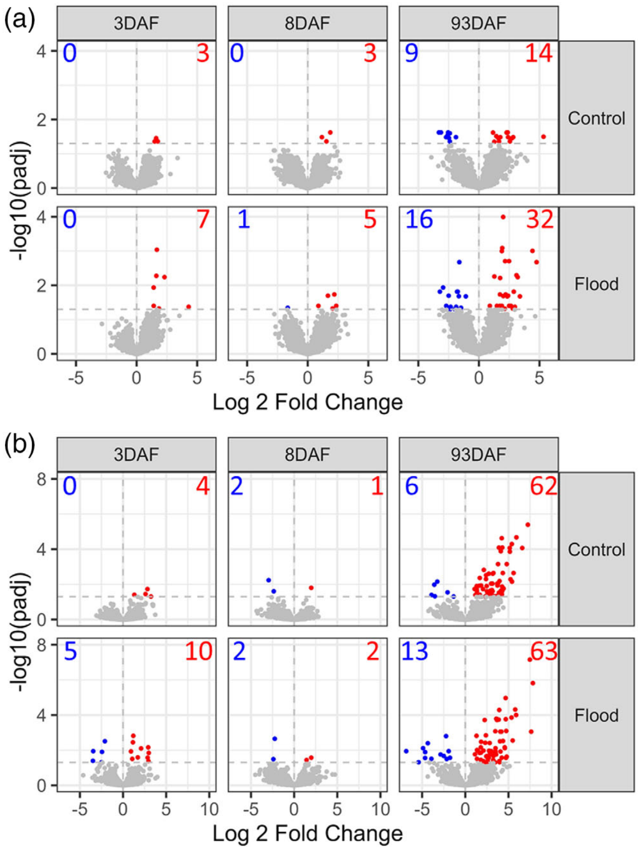

FIGURE 5 Impact of short-term flooding on soil microbiomes across time, with respect to ASV-level differential abundance, from the pre-flood sampling timepoint to each subsequent sampling timepoint, averaged across fertilizer treatments, for (a) bacteria/archaea and (b) fungi. Counts indicate the number of ASVs that were significantly (p_{adj} <.05, *t*-statistic) more abundant at the pre-flood timepoint (blue) or the post-flood timepoint denoted in vertical facets (red). *p*-values were adjusted with the Benjamini–Hochberg false discovery rate procedure. DAF, days after flood.

4 | DISCUSSION

4.1 | Short-term flooding resulted in limited changes to soil microbial communities

The first main goal of this study was to evaluate the impact of a short-term flood on soil microbial community structure. We hypothesized that flooding would accompany shifts in soil microbial community structure, and that soil bacterial and archaeal communities would have more short-term responses to flooding, whereas fungal community disturbance would be longer-term. Contrary to our predictions, overall soil microbial community structure did not shift with flooding, even at 3DAF when the flooded main plots had standing water. The lack of change in our study contrasts with previous literature (Breidenbach & Conrad, 2015; Das et al., 2025; Doering et al., 2021; Gschwend et al., 2020; Huang et al., 2023; Jiao et al., 2019; Martínez-Arias et al., 2022; Sao et al., 2023). Past studies involved recurrent flooding or waterlogged conditions that may have produced legacy effects, unlike our single, short-term flood in a tilled, annual row crop system. Furthermore, differences have been reported in studies that employed longer flood durations and floods later in the growing season (Francioli et al., 2021; Sao et al., 2023; Shah et al., 2021). Moreover, we assessed bulk soil microbiomes that are less responsive to flooding than rhizosphere and root microbiomes and fungal biomass and activity (Francioli et al., 2021; Graff & Conrad, 2005; Hamonts et al., 2013). It is possible that more prominent flood effects in root-associated and rhizosphere microorganisms such as plant growth-promoting bacteria, archaea and arbuscular mycorrhizal fungi were not captured due to the bulk soil sampling approach employed in our study (Barnes et al., 2018; Ellis, 1998; Francioli et al., 2021; Graff & Conrad, 2005; Hamonts et al., 2013; Pennington, 1986). Although flooding did not shift overall bulk soil microbiome structure at a community level, the flood treatment enhanced variability of soil moisture and the short-term and long-term differential abundance of soil bacterial and fungal taxa across the growing season. Furthermore, fungal community structure correlated with soil moisture in vector analyses, unlike the bacterial/archaeal community, which suggests that fungi were more affected by flooding and moisture variability across the sampling timeline. Thus, we emphasize the importance of considering responses of different soil food web members to flooding across time in future studies.

4.2 | Linking soil microbiomes to agroecosystem function after flooding

In addition to investigating the impact of a short-term flood on the soil microbiome, we also wanted to consider how the soil microbiome related to agroecological functional differences, with respect to soil N and maize productivity, in fertilized and unfertilized systems. We hypothesized that unfertilized systems would have more pronounced differences in soil microbes, soil N and maize productivity than fertilized systems, but the observed relationships were not consistent. Trophic mismatches between aboveground (plants) and belowground (microbe, soil N) components were documented with respect to their response to the flood and fertilizer treatments. More specifically, while soil microbiome structure was not altered by the flood or fertilizer treatments, ACE protein was reduced 8 days after the flood in the flood treatment, and inorganic N, maize biomass, and grain yield were lower in the unfertilized treatment. Ultimately, linking soil microbiome structure and ecosystem function is a grand challenge in ecosystem ecology (Bier et al., 2015). In our study, soil microbiome structural stability could indicate strong physiological adaptation to flood and fertilizer treatments rather than high functional redundancy since there were differences in agroecosystem functionality related to soil N and maize growth (Allison & Martiny, 2008).

We hypothesized incorrectly that the flood and fertilizer treatments would have compounding effects on maize growth and that soil N responses would be congruent with maize growth. The fertilizer treatments drove differences in maize growth and inorganic N, while flood treatments drove differences in ACE protein. Furthermore, our

TABLE 1 Phyla assignment of differentially abundant ASVs at each post-flood sampling timepoint in the flood and control treatments, compared to the pre-flood sampling timepoint.

		Sampling timepoint and flood treatment					
		3DAF		8DAF		93DAF	
Kingdom	Phylum	Control	Flood	Control	Flood	Control	Flood
Bacteria	Proteobacteria		+1			-1, +2	−5, +9
	Chloroflexi	+1		+3	+1	−1	
	Actinobacteriota		+1		+2	−1, +6	−1, +4
	Acidobacteriota	+1	+1		+1	+2	+6
	Bacteroidota					−5, +1	−8, +2
	Planctomycetota	+1			−1	−1	−2, +3
	Gemmatimonadota		+1				
	Verrucomicrobiota		+2			+1	+5
	Myxococcota					+1	+1
	Cyanobacteria					+1	+1
	Methylomirabilota		+1		+1		+1
Fungi	Ascomycota	+2	−1, +4		+2	+22	−6, +23
	Mortierellomycota			−1		+5	+7
	Basidiomycota	+1		−1, +1	−1	−1, +24	−1, +19
	Chytridiomycota	+1	+4			−2, +2	−3, +2
	Glomeromycota					+4	+7
	Rozellomycota		+1				
	Unidentified		−4, +1		−1	−3, +5	−3, +5

Note: Counts indicate the number of ASVs assigned to each phylum that were more (+) or less (−) abundant at the timepoints denoted in the columns relative to PF, across fertilizer treatments.
Abbreviation: DAF, days after flood.

TABLE 2 Impact of fertilizer treatments on maize biomass and grain yield (Mg ha^{-1}).

Fertilizer treatment	Maize biomass (Mg ha^{-1})	Grain yield (Mg ha^{-1})
Urea	26.1 ± 1.6	15.6 ± 1.0
Unfertilized	10.5 ± 1.9	5.2 ± 1.1

Note: Means ($n = 8$) ± standard errors are averaged across flood treatments. Fertilizer treatment differences were significant for maize biomass ($p < .001$; LSD = 3.8; residual df = 5.7) and grain yield ($p < .001$; LSD = 2.2; residual df = 5.7).

prediction that flood treatment effects on maize growth and soil N would be more predominant in unfertilized systems was not supported. In contrast to our R6 maize biomass and grain yield data, maize grain yield data at the end of the growing season, as reported in Novais et al. (2025), showed that urea applications mitigated yield loss after flooding, comparing the urea treatment to the unfertilized treatment, which highlights the importance of water and nutrient management for agroecosystem function (Novais et al., 2025; Yang et al., 2018; Kaur et al., 2017).

The reduced ACE protein in the flood treatment reflects functional shifts in aboveground and belowground ecosystem processes related to plant-derived organic inputs and nutrient cycling. Autoclaved-citrate extractable protein is a soil biological health indicator that reflects the largest organically bound pool of soil N that may be mineralized into plant-available, inorganic forms, such as nitrate (Hurisso et al., 2018; Naasko et al., 2024). In particular, ACE protein is positively associated with soil organic matter stabilization, soil aggregate stability and plant productivity (Agnihotri et al., 2022; Hurisso et al., 2018). Reduced aboveground biomass production after the flood may have been paired with reduced plant-derived organic matter belowground, such as from roots (Martínez-Arias et al., 2022), resulting in lower ACE protein as an organically bound form of N. Lower ACE protein may have compounded the slowing of soil N transformations with flooding such as N mineralization, as supported by lower ammonium-N and subsequent nitrification, resulting in lower plant-available soil N (Haddad et al., 2013; Pengthamkeerati et al., 2006; Robertson & Groffman, 2015). While we did not measure root biomass or target arbuscular mycorrhizal fungi, we speculate that lower ACE protein in the flood treatment could additionally signal disrupted belowground fungal activities, such as mycorrhizal fungal production of glucoproteins that constitute a portion of ACE protein (Agnihotri et al., 2022; Gillespie et al., 2011; Schindler et al., 2007). Previous studies report strong correlations between ACE protein and maize yield (Sprunger et al., 2019; Svedin et al., 2022; Wade et al., 2020), and yield in other row crop systems (Naasko et al., 2024; Sainju et al., 2022). From these results and ours, ACE protein can be seen as an early indicator of reduced N availability due to flooding with respect to soil N cycling.

The correlations of soil fungal community structure with both ACE protein and nitrate point to the role of soil microorganisms in organic–inorganic N transformations that support agroecosystem function (Robertson & Groffman, 2015). The nitrate and ACE protein vectors were inversely related, with nitrate pointing towards communities on the day before the flood treatment and ACE protein pointing towards 10 days before harvest. This trend coincides with increased ACE protein and decreased nitrate across the sampling timeline that spanned the growing season in our study, which is consistent with other Midwest cropping systems (Naasko et al., 2024). Typically, bacteria and archaea are more closely associated with soil N cycling than fungi, with respect to mineralization of organically bound soil N, such as ACE protein, and subsequent nitrification into nitrate; both of these processes are essential to N supply and demand across the maize growing season with greater organic plant inputs and inorganic N uptake (Robertson & Groffman, 2015). The relationships of soil N with the fungal community in our study point to the important role of soil fungi in inorganic and organic N dynamics. Slow-growing fungal communities could have stronger implications on ACE protein, as a relatively stable pool that serves as a long-term N sink and indicates soil organic matter stabilization (Naasko et al., 2024), compared to faster-growing soil bacteria and archaea, thus exhibiting stronger relationships. Furthermore, the respective impacts of flooding and fertilization on ACE protein and nitrate, and the correlations of these soil N pools with soil fungal community structure signal functional impacts of extreme weather events and nutrient management on the balance of plant supply and demand of organic and inorganic N in agroecosystems.

4.3 | Limitations

One limitation of this study is a lack of soil oxygen or functional microbiome data to support the extent of oxygen limitations and their impact on soil microbiome function, which brings into question the extent of soil microbiome disturbance as a consequence of flooding. Yet, a four-day flood duration resulted in standing water, increased soil moisture and reduced ACE protein. A flood duration of 4 days is representative of flooding events from extreme rainfall that thwart aboveground and belowground maize productivity in the Midwest United States (Novais et al., 2025; Lindsey et al., 2024; Dill et al., 2020; Liu et al., 2013; Purvis and Williamson, 1972; DeBoer and Ritter, 1970; Ritter and Beer, 1969). It is possible that the soil microbiome was limited by nutrients besides N or other unmeasured factors that overpowered the effects of flooding. Furthermore, the absence of soil microbiome compositional differences does not mean that the functional potential is also unaffected. For example, in a separate system, Bennett et al. (2024) observed variation in soil microbiome functional potential, but not in taxonomical composition, as a consequence of the presence of heavy metals. An additional limitation to the study is that it was a one-year study, so long-term effects of short-term flooding cannot be inferred. This present study provides a novel perspective of flooding within row-crop agriculture in situ, by examining the effects of a single occurrence flood on aboveground and belowground dynamics with high temporal distinction by looking at short-term (3 and 8 days) and longer-term (3 months) differences.

4.4 | Conclusions

This study enhances our understanding of the soil microbiome and biological health indicators of N cycling, both crucial for achieving sustainable crop production in a changing climate. We found that a short-term, single occurrence flood had stronger effects on organically bound soil N, measured as ACE protein, than on maize growth or soil microbial community structure. Understanding the impact of climate disturbances on soil microbiome structure, nutrient cycling and agroecosystem function will help predict ecosystem dynamics with ongoing climate change. The sensitivity of ACE protein to flooding shows it can serve as an early indicator of reduced primary productivity and of soil N dynamics in agroecosystems (Naasko et al., 2024). Ultimately, this study reveals that soil N can be more disturbed by short-term flooding than soil microbiome structure, and so we recommend future research focus on functional indicators of soil–plant–microbe interactions or microbiome functional profiling through shotgun metagenomics/metatranscriptomics, over soil microbiome taxonomic structure to inform the management of agroecosystems in a changing climate.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available upon publication. The 16S and ITS raw paired-end FASTQ files will be available in the NCBI Sequence Read Archive as Bioprojects PRJNA1146346 and PRJNA1146347, respectively. The other soil and plant data will be available upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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