



Crop rotational complexity affects plant-soil nitrogen cycling during water deficit

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ABSTRACT

One of the biggest environmental challenges facing agriculture is how to both supply and retain nitrogen (N), especially as precipitation becomes more variable with climate change. We used a greenhouse experiment to assess how contrasting histories of crop rotational complexity affect plant-soil-microbe interactions that govern N processes, including during water stress. With higher levels of carbon and N cycling hydrolytic enzymes, higher mineral-associated organic matter N concentrations, and an altered microbial community, soils from the most complex rotation enabled 80% more corn N uptake under two moisture regimes, compared to soil from monoculture corn. Higher levels of plant N likely drove the changes in corn leaf gas exchange, particularly increasing intrinsic water use efficiency by 9% in the most complex rotation. The water deficit increased the standing pool of nitrate 44-fold in soils with a history of complex crop rotations, compared to an 11-fold increase in soils from the corn monoculture. The implications of this difference must be considered in a whole cropping systems and field context. Cycling of ¹⁵N-labeled fresh clover residue into soil N pools did not depend on the water regime or rotation history, with 2-fold higher recovery in the mineral vs. particulate organic N pool. In contrast, the water deficit reduced recovery of clover ¹⁵N in corn shoots by 37%, showing greater impacts of water deficit on plant N uptake compared to organic N cycling in soil. This study provides direct experimental evidence that long-term crop rotational complexity influences microbial N cycling and availability with feedbacks to plant physiology. Collectively, these results could help explain general observations of higher yields in more complex crop rotations, including specifically during dry conditions.

1. Introduction

In order to support crop productivity while minimizing N pollution, agricultural soils must be able to both supply and retain nitrogen (N). Addressing this challenge will be more difficult as climate change progresses and precipitation becomes more variable, which could increase the agricultural N losses that impair water quality (Bowles et al., 2018; Sinha et al., 2017). Precipitation not only influences the hydrologic processes that transport N, but also all processes in the plant-soil N cycle, including microbial N transformations, decomposition of organic N, and plant N uptake. For instance, if low water availability limits plant N uptake (Gonzalez-Dugo et al., 2010), substantial buildup of soil

inorganic N can result (Bowles et al., 2018; Gentry et al., 1998; Mroczek et al., 2000). This buildup can in turn lead to leaching losses and decreased water quality as rainfall returns (Loecke et al., 2017). Identifying agricultural management that promotes more resilient plant-soil N functioning is needed to decrease such risks.

Agricultural management that builds soil organic matter (SOM) increases the size of the soil N pool and promotes an active microbial community, in turn enhancing both the supply and retention of bioavailable N (Drinkwater and Snapp, 2007). For instance, increasing crop rotational complexity — which can be attained by increasing crop species richness and/or duration of crop cover (e.g. with cover crops) — affects the quality and quantity of SOM fractions and the composition,

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size, and activity of the microbial community (McDaniel and Grandy, 2016; Tiemann et al., 2015; Venter et al., 2016). Crop rotations also affect decomposition of fresh plant residues, with the extent of N immobilization or mineralization depending on resource stoichiometry (McDaniel et al., 2014a). With sufficient carbon (C) availability, microbial communities may also be able to quickly break down fresh organic N sources, allowing them to accrue in N pools with longer mean residence times for subsequent release (Drinkwater and Snapp, 2007).

An active soil microbial community also helps to provide plants access to N contained in mineral associated organic matter (MAOM). MAOM-N typically constitutes the largest pool of N in soil (Jilling et al., 2018; Liebig et al., 2004), mainly as organic N in the form of proteins and amino acids. Recent developments in understanding of plant-mineral-microbe interactions suggest that MAOM may actually be an important source of bioavailable N in mineral soils, while also acting as a sink for N inputs (Daly et al., 2021; Jilling et al., 2018). Since minerals retain a thin water film in all but the driest conditions, MAOM may also be able to supply bioavailable N through sorption-desorption processes during dry periods (Daly et al., 2021). Higher levels of particulate organic matter (POM), typically composed of less-processed plant residues with higher C:N ratios than MAOM, can act as important sources of labile C that increase microbial activity and demand for N (Wander, 2004).

Plant-soil-microbe interactions that promote access to bioavailable N support the plant physiological processes that sustain plant productivity under water stress. With higher shoot N concentrations, plants, including corn, can produce more photosynthetic machinery with higher maximal photosynthetic rates (Vos et al., 2005). This may lead to higher intrinsic water use efficiency (i.e., the amount of water lost per unit of C gained) during water stress. Plant N limitation increases susceptibility to drought for corn under field conditions, possibly through restrictions in root growth and capacity to extract soil water (Markelz et al., 2011). Higher levels of associations with the root symbionts arbuscular mycorrhizal fungi affects plant-water relations in a number of ways (Augé, 2001), including greater responsiveness to abrupt changes in soil moisture and higher leaf gas exchange at similar soil water potential (Augé et al., 2015; Bowles et al., 2016). Yet few studies directly address how agricultural management that changes soil chemical and biological properties feedback to influence plant physiological processes underlying productivity and responses to water stress (Marriott et al., 2017).

Crop rotation is an ancient strategy for maintaining and building soil health, especially in grain-based cropping systems (Karlen et al., 1994). At the center of soil health are building SOM and promoting active microbial communities (Lehman et al., 2015), which together influence myriad soil functions (Kibblewhite et al., 2008). Increasing cash crop diversity and the duration of crop cover through cover cropping, which together we consider increasing crop rotational complexity, are both important for increasing SOM (Jian et al., 2020; McDaniel et al., 2014b; Poeplau and Don, 2015). Legume cover crops appear to play a particularly important role in increasing soil C (Jian et al., 2020), perhaps due to higher quality residues supporting microbial growth efficiency (Kallenbach et al., 2015). Relatively less is known regarding the extent to which crop rotation affects the distribution of SOM across soil fractions (Poffenbarger et al., 2020), though recent work shows that cover cropping can rapidly increase the N content of both POM and MAOM (Jilling et al., 2020). Crop rotational diversity also alters microbial community structure (Alvey et al., 2003; Trivedi et al., 2015) and can increase soil microbial community diversity (Bünemann et al., 2004; González-Chávez et al., 2010; Jiang et al., 2016; Suzuki et al., 2012).

To test how the history of crop rotational complexity affects soil N cycling and plant responses to water stress, we conducted a greenhouse experiment with corn using soil collected from a long-term cropping system experiment in the U.S. Midwest. We focused on four cropping systems ranging in crop diversity from one (corn monoculture) to five crops (corn-soybean-wheat rotation with a two-species cover crop), and

with and without cover cropping. Previous research showed how increasing crop diversity at this experiment increased soil organic C, total N, and microbial activity (Tiemann et al., 2015), and increased the rate of residue decomposition and net N mineralization (McDaniel et al., 2014b). Corn yields were linearly related to the number of crops in rotations, corresponding strongly to early season soil inorganic N availability (Smith et al., 2008). Corn yields were also substantially higher in the five-species rotation compared to the corn monoculture during putative drought years (Bowles et al., 2020), though the plant-soil feedbacks underlying this effect remain unknown.

Our goal was to determine how changes in soil chemical and biological properties resulting from long histories of contrasting crop rotational complexity affected soil and plant N processes and plant physiology during water stress. Specifically, we asked: 1) How does the proportion of crop N taken up from new litter vs. SOM vary among rotations and with water stress; 2) How does the history of crop rotation impact plant physiological parameters related to water relations, nutrient uptake, and productivity; and 3) How does the pattern of soil N cycling vary among rotations and in response to water deficits, making N either more or less available to plants and more or less vulnerable to environmental losses? We hypothesized that higher rotational complexity would increase C and N in POM and MAOM fractions, alter microbial community composition and increase microbial activity and abundance. In turn, we hypothesized that these changes would accelerate the processing of new organic N inputs and reduce the impact of water stress on corn N uptake and biomass production. To address these questions, we traced ^{15}N -labeled red clover residue into corn biomass and size-based soil fractions following a greenhouse experiment with corn. The corn was grown in soil from contrasting histories of crop rotational complexity and subjected to two water regimes. We also measured soil enzyme activities, phospholipid fatty acids (PLFA), inorganic N concentrations, and corn leaf gas exchange. Our goal was to capture how the history of rotational complexity impacted plant-soil-microbe interactions critical for responding to a future with more variable water availability.

2. Methods

2.1. Overview

This study was conducted using soil from a long-term crop rotation field experiment at the W.K. Kellogg Biological Station in Michigan, USA that began in 2000 (Smith et al., 2008). The Biodiversity Gradient experiment was explicitly designed to test the functional role of crop diversity in grain cropping systems. No external inputs of fertilizers or pesticides are used, and soils are chisel ploughed annually to a depth of 15 cm. Annual rainfall at the KBS LTER averages 1005 mm yr^{-1} with a mean annual temperature of $10.1 \text{ }^\circ\text{C}$. Soils at the experiment are a mixture of Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs) sandy loams. Soil was collected from four crop rotation treatments: corn monoculture (C); corn and soybean two-year rotation (CS); corn with a late summer/winter leguminous cover crop, red clover (C/c); and a corn, soybean, wheat three-year rotation with both red clover and rye cover crops (CSW/rc). Soils were sampled in late summer from the corn phase of all treatments to focus on the longer-term impact of rotation on soil rather than the current crop or recently incorporated cover crop residue. Soil cores, 15 cm deep by 15 cm diameter, were collected on September 20, 2016 from five locations in each of the four replicate plots and shipped to the University of New Hampshire for further processing.

Soil subsamples from within a plot were composited, mixed, and sieved to 7.5 mm and then mixed with autoclaved sand in a 7:3 ratio (soil:sand) in order to prevent excessive compaction and waterlogging in pots with soil only. The soil:sand mix was packed in pots 15 cm in diameter by 45 cm in height to a bulk density of 1.4 g cm^{-3} , similar to field conditions. The water holding capacity of the pots was determined

by measuring soil gravimetric water content 24 h after adding DI water until water freely drained from the pots.

^{15}N -labeled red clover residue was incorporated to surface soil in each of the pots. The clover was grown during spring 2016 in sand using a 1/10 strength Long-Ashton solution with 10 atom percent ^{15}N enriched ammonium and nitrate. Aboveground clover biomass was dried and cut into ~ 3 mm pieces. It contained 3.00% N with 1.498 ^{15}N at % and was thoroughly mixed into the top 5 cm of soil in the pots at a rate of 4.34 g clover pot $^{-1}$ (130.6 mg N pot $^{-1}$). This was the only supplemental N source applied in order to maximize plant dependence on interal plant-microbe-soil interactions that provide bioavailable N.

2.2. Greenhouse experiment

Seeds of the same variety of corn seed as used in the field experiment (Viking, variety O.44–86N) were surface sterilized, germinated, and then planted 2.5 cm deep in each pot on October 14, 2016. Pots were arranged in the greenhouse in a randomized complete block design with four replicates, maintaining the same blocks as in the field plots. From planting until October 31, 2019, soil was maintained close to 80% of water holding capacity by weighing pots and replacing water lost with DI water every 2–3 days. Two different watering regimes began 17 days after planting when plants were at the V4 stage with a height of 47 cm. The control watering regime continued to receive water every 2–3 days, maintaining $\sim 60\%$ water holding capacity. The deficit watering regime received half as much water as the control treatment on every third watering; thus, both the pattern and the amount of watering differed in the deficit treatment. This watering regime resulted in deficit treatments equilibrating at $\sim 50\%$ water holding capacity (WHC) two weeks after the onset of differential watering. Daily water loss averaged 81.9 mL pot $^{-1}$ and 67.8 mL pot $^{-1}$ in the control and deficit watering regime, respectively.

Leaf gas exchange measurements were taken on the most recently mature, fully expanded leaves with a field portable open flow infrared gas analyzer (model 6400, LI-COR Inc., Lincoln, NE, USA). It was set up with a 6-cm 2 leaf-chamber, the CO $_2$ reference set at 400 $\mu\text{mol mol}^{-1}$ and a light intensity of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using a light-emitting diode source. Measurements were taken between 10:00 and 12:00 h on four dates, 34, 36, 41, and 43 days after planting. Data presented are presented as a mean of these dates.

The experiment was harvested 45 days after planting. Plants were cut at the soil surface and dried at 60 °C. Soil and roots were divided into three depths (0–5, 5–15, and 15–40 cm) and mixed, with a focus here on the top depth for soil responses and the whole depth profile for root biomass. Subsamples of soil were removed, stored at 4 °C overnight and then sieved to 2 mm prior to soil biogeochemical and microbial analyses. Roots from the remaining soil were washed and dried at 60 °C.

2.3. Soil and plant analyses

Inorganic N was extracted from moist soils with 1M KCl (soil:solution ratio of 2.5) and analyzed colorimetrically for NH $_4^+$ and NO $_3^-$ (Foster, 1995; Miranda et al., 2001). Soil dissolved organic carbon was measured in the same 1 M KCl extracts using a TOC-L CPH/CPN analyzer (Shimadzu, Kyoto, Japan). Microbial biomass C was determined using chloroform fumigation extraction (Brookes et al., 1985). Samples were fumigated in a desiccator under chloroform atmosphere for 24h in the dark and then extracted with 1 M KCl. Fumigated samples were measured on the TOC-L CPH/CPN analyzer and microbial C was calculated as the difference of fumigated samples and KCl extracts of fresh soil samples. Since only relative comparisons are made and we have not calculated a soil-specific extraction efficiency, microbial biomass C is presented without the use of a correction factor.

Potential extracellular enzyme activities were measured, with adaptations, as described in Schnecker et al. (2015). Soils were suspended and homogenized in a 100 mM sodium acetate buffer at pH 5.5 using a

commercially available blender (Magic Bullet, Alchemy Worldwide, Sherman Oaks, California, USA). The soil slurry was transferred into black microtiter plates and amended with MUF (4-methylumbelliferyl) labeled substrates: β -D-glucopyranoside for β -glucosidase (BG), β -D-cellobioside for cellobiohydrolase (CBH) or N-acetyl- β -D-glucosaminide for N-acetyl-glucosaminidase (NAG). L-alanine-7-amido-4-methyl coumarin was used as substrate for alanine-amino-peptidase (AAP). Activity was measured fluorometrically after 200 min of incubation (excitation 365 nm and emission 450 nm; Biotek Synergy HT, Biotek Instruments, Winooski, Vermont, USA). Phenoloxidase (POX) and peroxidase (PEX) activities were measured photometrically using L-3,4-dihydroxyphenylalanine (DOPA) as substrate and addition of H $_2$ O $_2$ for determination of PEX activities. POX activities were then calculated as the increase in color during the incubation time of 20 h. PEX activities were calculated as the increase in color during the incubation time from the results of the wells that received H $_2$ O $_2$ minus the results of the wells without H $_2$ O $_2$ addition.

Subsamples of soil were freeze-dried and frozen at -20 °C for subsequent analysis of phospholipid fatty acids (PLFA). Samples were shipped to a commercial laboratory (Microbial ID, Newark, DE, USA) for analysis using the method of Buyer and Sasser (2012). Fatty acid biomarkers of microbial groups included: 18:1 ω 7c, cy17:0cy and cy19:0 for Gram negative bacteria; i15:0, a15:0, i16:0, i17:0, and a17:0 for Gram positive bacteria; 15:0 and 16:1 ω 7c for general bacteria; 18:1 ω 9c, 18:2 ω 6c, 18:3 ω 6c for fungi; 16:1 ω 5c for putative arbuscular mycorrhizal fungi (AMF); and 10Me16:0, 10Me17:0, and 10Me18:0 for actinobacteria (Zelles, 1999). Total PLFA biomass was calculated by adding all markers.

Soil subsamples were air dried at 60 °C and sieved to 2 mm. A 10-g subsample was divided into three fractions (>53 μm , <53 μm and >20 μm , $<20\mu\text{m}$) using a method adapted from Cambardella and Elliott (1992), based on dispersion in a 5% solution of hexametaphosphate overnight on a reciprocating shaker and subsequent sieving.

For total C and N and $\delta^{15}\text{N}$ analysis, soil and plant samples collected at harvest were dried at 60 °C for 24 h and finely ground in a ball mill, and packed in tin capsules. Samples were analyzed at the UC Davis Stable Isotope Facility via an Elementar Vario EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

2.4. Statistical analysis

Mixed model analysis of variance (ANOVA) was performed using the lme4 and lmerTest packages in R (Bates et al., 2015; Kuznetsova et al., 2016). Rotation and water regime were treated as fixed effects while block was considered random to account for the randomized complete block design. Transformations were used as needed to meet assumptions of homoscedasticity and normality. Multivariate analyses of PLFAs and soil enzyme activities were conducted using the vegan package in R (Oksanen et al., 2012). For PLFAs, non-metric multidimensional scaling (NMDS) with the Bray dissimilarity metric was used. Permutational multivariate analysis of variance (perMANOVA; Anderson, 2001) was also used to assess differences in soil microbial communities based on rotation and the water treatment, implemented using the *adonis()* function in the vegan package. For soil enzyme activities, principal components analysis was used. Confidence ellipses (95%) were calculated for each rotation for display on the ordinations.

3. Results

The history of crop rotation affected the concentration of C and N in two of the three size-based SOM fractions isolated from the sand:soil mixture at the time of harvest (Fig. 1). Specifically, rotational complexity increased the concentration of N in POM (>53 μm) and MAOM (<20 μm), and increased the concentration of C in MAOM,

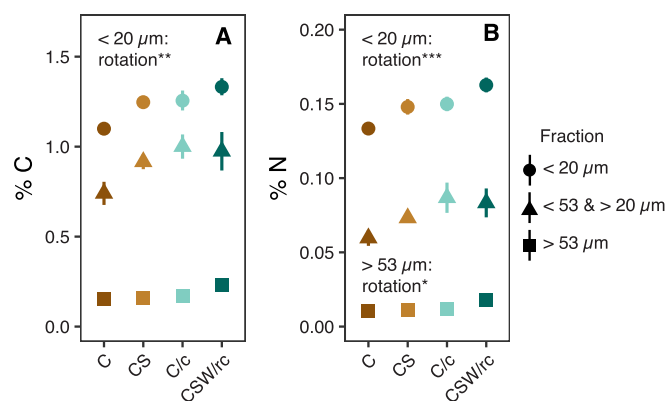


Fig. 1. Soil C and N concentrations across three size-based fractions, MAOM (<20 μm); coarse silt (<53 & >20 μm); and POM (>53 μm), in soils with contrasting histories of crop rotation subjected to a control and deficit water regime: A) Carbon; and B) Nitrogen. Shown are back-transformed means \pm standard errors of the mean ($n = 4$). Inset text in each panel indicates the significance of main and interaction effects: *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

especially between the corn monoculture and the other three rotations. Water regime did not affect C and N concentrations in SOM fractions.

Soil microbial community composition, assessed by PLFA indicators, differed significantly across rotations (Fig. 2A; perMANOVA R^2 of rotation = 0.27) but was not impacted by the deficit water regime. Fatty acids 17:0 10-methyl, 19:0 cyclo (9/10), and 15:0, indicative of actinobacteria, Gram negative bacteria, and general bacteria, respectively, were most associated with the first NMDS axis that separated the CSW/rc rotation from C and CS, with C/c intermediate. Significantly higher total PLFAs in CSW/rc compared to the other rotation soils (Fig. 2A inset), corresponded to higher microbial biomass C in that rotation as well (Table S1). These indicators of soil microbial abundance were not impacted by the water deficit.

Soil potential enzyme activities were generally higher in CSW/rc and C/c rotation soils compared to C and CS soils, as shown along the first axis of the principal component analysis (Fig. 2B), and corroborated by examining each enzyme individually (Table S1). Separation of CSW/rc from C and CS soils along the second axis, with C/c intermediate, indicated different microbial foraging strategies. The separation was related

to higher activity of hydrolytic enzymes BG, CBH, AAP, and NAG in CSW/rc vs. relatively higher activity of oxidative enzymes PEX and POX in C and CS soils. The water deficit did not impact soil potential enzyme activities (Table S1).

The deficit watering treatment reduced maize shoot dry biomass by 25%, similarly across all soils with contrasting histories of crop rotation (Table S2). Considering the water treatments together, corn shoot biomass was similar in CS, C/c, and CSW/rc rotation soils but was significantly lower (17%) in soil with a history of corn monoculture. Shoot N concentration was significantly higher in CSW/rc soils, with similar values for plants in C, CS, and C/c (Fig. 3A). Reflecting the biomass reduction, plant N content (shoot and root) was lower in the deficit water regime in all rotation soils. Plants grown in soils with a history of greater rotation complexity had increasingly higher N content (Fig. 3B), with 80% higher N content in corn grown in CSW/rc soils compared to C soils. The deficit water regime reduced plant uptake of ^{15}N derived from labeled cover crop residue by 33% (Fig. 3C), considering all rotation soils together. Rotation soil did not affect plant uptake of ^{15}N derived from the labeled cover crop, but rotation soils did affect total N content, the proportion of ^{15}N labeled residue in plants being highest in C soils and lowest in the CSW/rc, with other rotation soils intermediate (Fig. 3D). The deficit water regime also reduced the amount of ^{15}N recovered in plants.

Rotation history and the water deficit regime affected photosynthesis, stomatal conductance, and intrinsic water use efficiency (Fig. 4). The water deficit reduced both photosynthesis and stomatal conductance, although relatively less so for photosynthesis, leading to higher intrinsic water use efficiency in the water deficit. Generally, more complex rotations had higher rates of gas exchange and higher intrinsic water use efficiency under both control and deficit water regimes. Although the pattern is not statistically clear, one exception was photosynthetic and conductance rates for the corn monoculture under the deficit treatment. These rates did not change much relative to the control treatment, and were numerically higher than the CS and C/c rotations.

Soil NH_4^+ concentrations at the end of the experiment were low (mean of $0.7 \mu\text{g N g}^{-1}$ soil) and were not affected by rotation soil or water regime (Fig. 5A). Soil NO_3^- concentrations were low in the control water regime and substantially higher in the deficit water regime, with contrasting responses across rotation soils. In the corn monoculture soil, soil NO_3^- concentration increased 11-fold in the deficit vs. control water regime, whereas it increased 44-fold in the CSW/rc, with other rotations

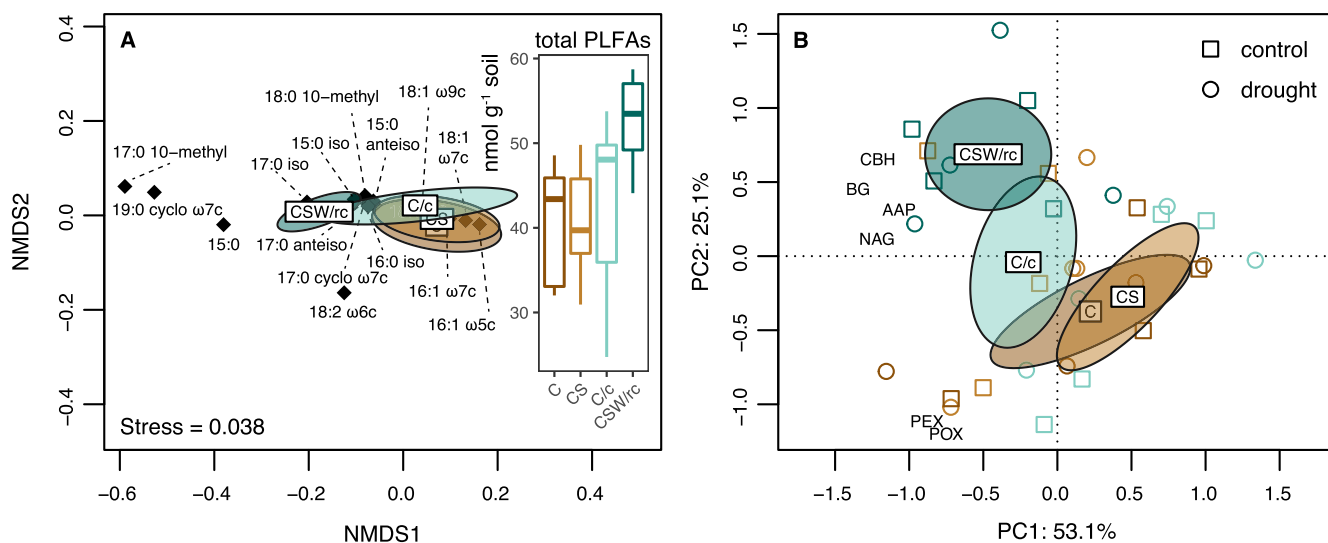


Fig. 2. Soil microbial community composition and soil potential enzyme activities in soils with contrasting histories of crop rotation subjected to control and deficit water regimes: A) Ordination by non-metric multidimensional scaling (NMDS) of indicator phospholipid fatty acids (PLFAs), with inset of the abundance of PLFAs across rotation soils; B) Principal components analysis of potential activities of soil C and N cycling enzymes. Shown are 95% confidence ellipses for rotation soils.

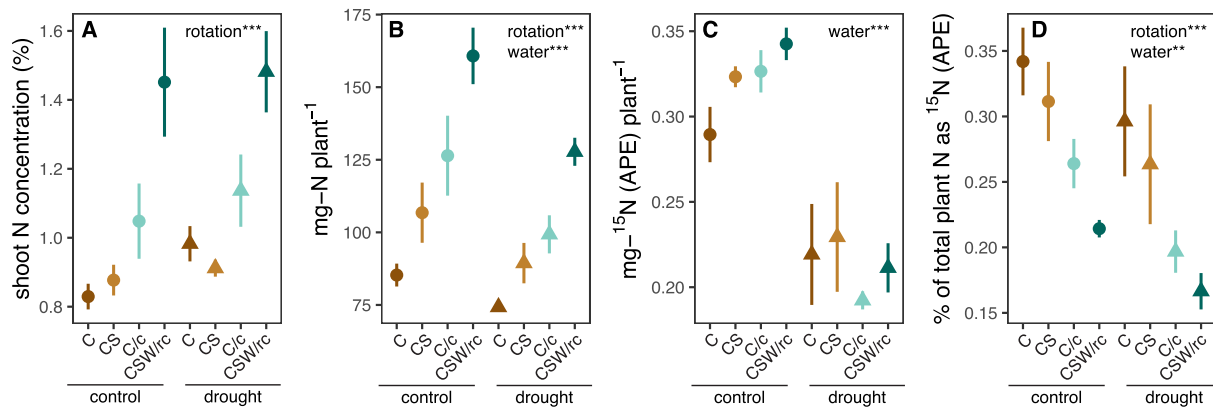


Fig. 3. Nitrogen (N) and N derived from ¹⁵N-labeled rye cover crop residue in corn grown in soils with contrasting histories of crop rotation subjected to control and deficit water regimes: A) shoot N concentrations; B) total plant N content (root and shoot); C) plant ¹⁵N concentration; and D) percent of total plant N as ¹⁵N. Shown are means±standard errors of the mean (n = 4). Inset text in each panel indicates the significance of main and interaction effects at: *, *p* < 0.05, **, *p* < 0.01, ***, *p* < 0.001.

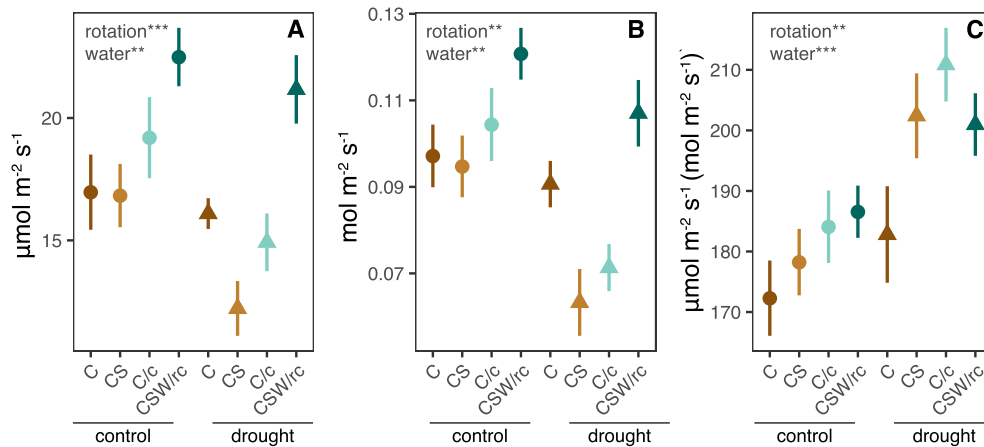


Fig. 4. Leaf gas exchange: A) Photosynthesis; B) Stomatal conductance; and C) Intrinsic water use efficiency. Shown are means±standard errors of the mean (n = 4). Inset text in each panel indicates the significance of main and interaction effects at: *, *p* < 0.05, **, *p* < 0.01, ***, *p* < 0.001.

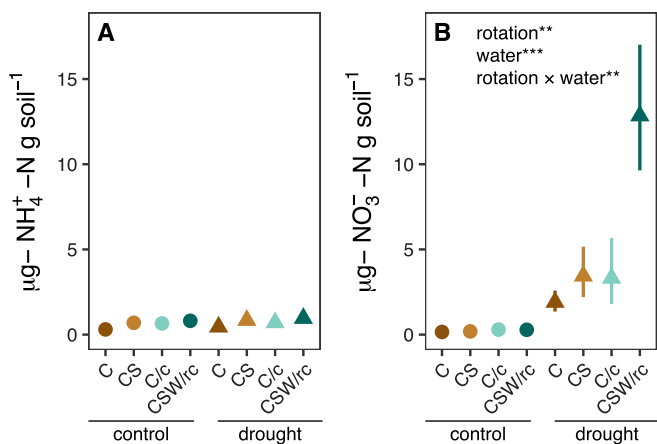


Fig. 5. Soil inorganic N concentrations at the end of the greenhouse experiment growing corn in soils with contrasting histories of crop rotation subjected to a control and deficit water regime: A) soil NH₄⁺ concentration; and B) soil NO₃⁻ concentrations. Shown are back-transformed means±standard errors of the mean (n = 4). Inset text in each panel indicates the significance of main and interaction effects at: *, *p* < 0.05, **, *p* < 0.01, ***, *p* < 0.001.

intermediate (Fig. 5B).

Recovery of ¹⁵N-labeled clover residue in corn shoots was sharply reduced in the deficit water regime (37% lower in drought vs. control), but did not depend on rotation soil (Table 1). Little cover crop ¹⁵N was recovered in corn roots, with slightly higher recovery in CS soils (2.8%) compared to CSW/rc (1.8%). Recovery in MAOM was much higher than in POM and coarse silt fractions though recovery in SOM fractions was not affected by the water deficit or rotation. In bulk soil (which includes these fractions) recovery was higher in the deficit water regime (55.5%) compared to the control (48.0%, *p* = 0.013). Total ¹⁵N recovery in plant and surface soil pools averaged 65.5% and was not affected by the water deficit or rotation.

4. Discussion

Our study shows that crop rotational complexity alters the plant-soil-microbe interactions that govern soil C and N cycling and availability, which in turn influence plant growth and leaf gas exchange. With higher levels of hydrolytic C and N cycling soil enzymes, higher POM-N and MAOM-N concentrations, and an altered microbial community, soils from the most complex rotation enabled substantially more plant N uptake in this experiment (in which no supplemental inorganic fertilizer was provided). Higher levels of plant N likely drove the changes in corn leaf gas exchange, particularly increasing intrinsic water use efficiency by increasing photosynthetic capacity relative to stomatal conductance.

Table 1

Percent recovery of ^{15}N from cover crop residue in plant, bulk SOM, and three SOM fractions. Standard error of the mean is shown in parentheses ($n = 4$). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

water	rotation	shoot	root	>53 μm	<53 μm , >20 μm	<20 μm	Bulk soil (0 – 5cm)	Total (plant + bulk soil)
control	C	12.3 (± 1.1)	2.5 (± 0.3)	6.9 (± 0.9)	3.5 (± 0.4)	19.7 (± 0.9)	52.7 (± 3.0)	67.5 (± 2.3)
control	CS	13.8 (± 0.1)	2.8 (± 0.3)	7.9 (± 2.2)	3 (± 0.5)	15.3 (± 1.4)	44.8 (± 3.0)	61.4 (± 2.7)
control	C/c	13.8 (± 0.5)	2.9 (± 0.3)	6.8 (± 1)	4 (± 0.8)	16.7 (± 0.5)	46.8 (± 2.3)	63.6 (± 2.8)
control	CSW/rc	15.5 (± 0.7)	2.1 (± 0.2)	7.3 (± 1.9)	3.2 (± 0.5)	15.4 (± 1)	47.9 (± 4.3)	65.4 (± 4.7)
drought	C	8.7 (± 1.2)	2.5 (± 0.3)	7.7 (± 1.4)	3.1 (± 0.2)	20.1 (± 3.2)	52.4 (± 5.7)	63.7 (± 5.8)
drought	CS	8.9 (± 1.4)	2.8 (± 0.3)	8.7 (± 1.1)	2.8 (± 0.2)	17.5 (± 1.5)	55.5 (± 2.1)	67.3 (± 1.9)
drought	C/c	7.9 (± 0.5)	1.9 (± 0.4)	6.6 (± 2.5)	2.4 (± 0.8)	13 (± 4.2)	53.5 (± 3.0)	63.2 (± 3.2)
drought	CSW/rc	9.3 (± 0.8)	1.5 (± 0.1)	10.6 (± 2)	2.8 (± 0.5)	15.5 (± 1.7)	60.6 (± 5.5)	71.4 (± 5.3)
	water	$F_{1,21} = 80.5^{***}$	$F_{1,21} = 3.9$	$F_{1,21} = 1.0$	$F_{1,21} = 2.9$	$F_{1,21} = 0.0$	$F_{1,21} = 7.4^*$	$F_{1,20.4} = 0.5$
	rotation	$F_{3,21} = 2.0$	$F_{3,21} = 4.8^*$	$F_{3,21} = 0.8$	$F_{3,21} = 0.3$	$F_{3,21} = 2.2$	$F_{3,21} = 0.5$	$F_{3,20.4} = 0.6$
	water X rotation	$F_{3,21} = 1.0$	$F_{3,21} = 1.7$	$F_{3,21} = 0.5$	$F_{3,21} = 0.8$	$F_{3,21} = 0.7$	$F_{3,21} = 1.1$	$F_{3,20.4} = 0.8$

This change in plant physiology could help explain general observations of higher yields in more complex crop rotations (Smith et al., 2008), including specifically during dry conditions (Bowles et al., 2020; Gaudin et al., 2015b; Renwick et al., 2021). Plants grown in soils with a history of more complex rotations relied less on N from fresh organic matter inputs as a proportion of total plant N, again reflecting the higher bioavailability of native soil N in these rotations. Rotation impacts on soil parameters in turn affected bioavailable N cycling under different water regimes, with a substantially greater standing pool of nitrate during water deficit in soils with a history of complex crop rotation. While this provides experimental evidence for the risks of inorganic N accumulation during drier and more variable rainfall (Bowles et al., 2018), conclusions about higher risks of inorganic N buildup in more complex rotations must be considered in a whole systems and field context.

The uniformly low inorganic N concentrations in the well-watered regime shows that plant demand for N was high, especially since corn N concentrations across all soils were well below thresholds for N sufficiency during corn vegetative growth (Ziadi et al., 2008). By contrast, the accumulation of soil nitrate under the water deficit regime indicates that soil N mineralization and nitrification persisted while plant N uptake declined. Soil and plant N processes respond to water stress at different thresholds. Microbes tolerate soil water potential as low as -14 MPa, while many plants cease activity near -1.5 MPa (Manzoni et al., 2012). The much higher levels of nitrate in the most complex crop rotation soil indicates that rates of inorganic N production were higher than other rotations, especially the corn monoculture. It is possible this is due to buildup of MAOM-N in the complex rotation soils, which has recently been postulated as an important source of inorganic N under dry conditions (Daly et al., 2021). This result corresponds with higher levels of microbial biomass and hydrolytic soil C and N enzyme activities in the more complex rotation. Changes in soil microbial community composition across rotation soils may have also influenced N production under water stress. For instance, soil fungi and actinobacteria are generally considered more tolerant of low soil moisture compared to gram-negative bacteria (Manzoni et al., 2012; Zenova et al., 2007). The PLFA marker most associated with the first axis of the NMDS separating CSW/rc from the other rotations (10Me17:0) is indicative of actinobacteria (Zelles, 1999), which have been linked to higher soil net N mineralization (Zhang et al., 2019).

The higher residual nitrate concentrations in the more complex crop rotation soil under water deficit suggest higher potential for N losses with more variable rainfall. Yet several considerations must be made. First, in a field context, cropping systems that include winter cover crops, as the CSW/rc rotation does, could recover residual soil inorganic N prior to spring rainfall when substantial N leaching often occurs (Tonitto et al., 2006). Second, the experiment from which these soils were collected was designed specifically to assess crop rotational complexity in the absence of external inputs. Under typical field management, synthetic fertilizer inputs would have built up as plant N

uptake declined, including in the corn monoculture rotation, as has been observed in field conditions (Gentry et al., 1998; Loecke et al., 2017; Morecroft et al., 2000). Third, since more complex rotations typically require fewer synthetic N inputs (Davis et al., 2012; Gaudin et al., 2015a), the risks of fertilizer N accumulation would be reduced relative to simplified rotations. Fourth, depending on rainfall intensity, the standing N pool could support greater plant N uptake and growth following rewetting. Finally, mixing field soil with sand for this greenhouse experiment may have increased the desorption potential of MAOM-N and increased N mineralization.

The ~ 2 -fold higher ^{15}N accumulation in the MAOM-N pool compared to the POM-N pool shows that MAOM is the main organic matter fraction sink for organic N inputs (Bosshard et al., 2008; Kölbl et al., 2006; Vanlauwe et al., 2010), even after only 45 days of the experiment. In contrast to our expectations, neither the history of crop rotational complexity nor an altered water regime affected the partitioning of N from fresh organic N inputs into SOM fractions, in spite of higher C and N enzyme activities in the more complex rotations. We had expected that with greater microbial biomass and activity, soils with a history of more complexity crop rotations would depolymerize and solubilize these organic N inputs more rapidly, cycling the N from the cover crop residue more quickly into soil N fractions with longer mean residence times. Yet Bosshard et al. (2008) showed no effect of conventional vs. organic farming systems on incorporation of ^{15}N from organic and mineral fertilizers into soil fractions, in spite of higher biological activity in the organic soils. Thus, the rate of N incorporation into MAOM from fresh organic matter may not depend on biological activity. We had also anticipated that lower soil water content could slow these processes, and create physical disconnections between breakdown of the organic residues and association with mineral surfaces. While this did not occur, the reduction of plant uptake of cover crop ^{15}N under water deficit shows plant N uptake was compromised by water availability in the deficit regime, reinforcing the notion that plant processes responded more strongly to short-term water deficits than soil processes.

In contrast to the lack of impacts on soil processes, leaf gas exchange was sharply reduced by the water deficit, though without a discernibly different pattern across rotation soils (i.e. no significant water \times rotation interactions). Higher rates of photosynthesis and stomatal conductance as rotational complexity increased were likely mediated by higher plant N nutrition (Vos et al., 2005). Since this effect was somewhat stronger for photosynthetic rates than stomatal conductance, intrinsic water use efficiency also increased as rotational complexity increased. These differences persisted under the water deficit. Higher intrinsic water use efficiency may be one component of the increased resistance to drought observed in corn grown in more complex rotations (Bowles et al., 2020; Gaudin et al., 2015b; Renwick et al., 2021).

Which aspects of biodiversity most impact ecosystem functioning remains a fundamental ecological question (Hooper et al., 2005). This is especially true in agricultural systems that differ from natural systems in

terms of temporal vs. spatial diversity and planned vs. associated biodiversity (Isbell et al., 2017). Unlike in natural systems in which communities assemble from both stochastic and deterministic processes, in agricultural systems farmers select rotation crops with complementary functions. Annual cropping systems also vary in terms of the duration of plant cover, depending on the type of crops grown and whether cover crops are used. Crop rotational complexity encompasses both these aspects of diversity and cover. In this study, including legumes undoubtedly had a large impact on belowground functioning relative to the corn monoculture, especially since the long-term experiment did not include external fertilizer inputs. Including a cover crop also likely played an important role, given differences between the CS and C/c rotation in soil enzyme activities, PLFA profiles, and corn N uptake. The most complex rotation — which had the strongest impact on microbial communities, N uptake, and corn physiology — had a lower proportion of total crops as legumes compared to CS and C/c, and a similar level of plant cover as C/c, but had the highest species diversity. Thus, we suggest that the inclusion of legumes, maintaining plant cover, and higher diversity all played a role in differentiating CSW/rc from the other rotations studied here.

5. Conclusions

Crop rotational complexity has long shown clear and consistent benefits for soil health. Recent studies also show higher yields under stressful growing conditions, including drought, compared to simplified rotations. Our study took a first step in connecting these observations by assessing how the changes in soil chemical and biological properties affected soil and plant processes under water deficit. Rotational complexity modulated the buildup of soil nitrate under water stress, with much greater accumulation as rotational complexity increases. This accumulation likely resulted from greater soil N supplying capacity in the more complex rotations as plant N uptake declined. The greater resistance of soil processes to the water deficit compared to plant processes reinforces how drought can lead to biogeochemical disconnects. Otherwise, the changes in microbial communities, enzyme activities, and SOM fractions from long-term management did not affect processing of new organic N inputs or modulate plant processes under water deficit. Rather, positive effects of rotation soils (e.g. for plant N uptake and leaf gas exchange) were similar with both sufficient water and water deficit. Although more challenging to implement, field manipulations of water availability will ultimately be needed to understand the mechanisms underlying the rotation effect on crop responses to stress. In particular, field studies would preserve potentially important changes in soil physical structure that affect water dynamics. Nevertheless, our glasshouse study reveals the complex and contrasting responses of soil and plant processes under water stress and the extent to which the legacies of crop rotational complexity modify them.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2022.108552>.

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1 Supplement

2

3 **Table S1.** Potential activities of soil extracellular enzymes by rotation and results of statistical
 4 analysis. Since there were no significant main effects of water or rotation X water interactions,
 5 means are shown by rotation across water treatments (n = 8). Standard error of the mean is shown
 6 in parentheses. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Variable	Rotation				ANOVA results		
	C	CS	C/c	CSW/rc	Rotation	Water	Rotation X water
cellobiohydrolase (CBH) (nmol h ⁻¹ g ⁻¹ soil)	16.1 (1.3)	15.2 (1.3)	21.7 (1.4)	28.2 (1.3)	F _{3,21} = 22.5***	F _{1,21} = 0.2	F _{3,21} = 0.3
β-glucosidase (BG) (nmol h ⁻¹ g ⁻¹ soil)	63.4 (4.5)	61.1 (4.2)	75.4 (3.7)	88.6 (3.3)	F _{3,21} = 19.2***	F _{1,21} = 0.2	F _{3,21} = 0.7
N-acetylglucosaminidase (NAG) (nmol h ⁻¹ g ⁻¹ soil)	53.9 (4.2)	44.4 (2.2)	57.0 (5.5)	57.4 (5.5)	F _{3,21} = 3.1*	F _{1,21} = 0.4	F _{3,21} = 0.4
alanine-amino-peptidase (AAP) (nmol h ⁻¹ g ⁻¹ soil)	30.0 (1.9)	29.6 (0.7)	33.5 (1.3)	34.4 (1.7)	F _{3,21} = 4.1*	F _{1,21} = 0.0	F _{3,21} = 1.0
peroxidase (PEX) (nmol h ⁻¹ g ⁻¹ soil)	889.1 (145.7)	860.4 (166.1)	927.9 (132.4)	770.8 (122.6)	F _{3,21} = 0.6	F _{1,21} = 0.1	F _{3,21} = 0.9
phenoloxidase (POX) (nmol h ⁻¹ g ⁻¹ soil)	600.9 (53.4)	541.2 (52.4)	594.6 (50.6)	492.9 (23.0)	F _{3,21} = 1.9	F _{1,21} = 0.0	F _{3,21} = 0.6
Labile:Oxidative ratio	0.057 (0.005)	0.061 (0.007)	0.070 (0.009)	0.102 (0.015)	F _{3,21} = 6.7**	F _{1,21} = 0.0	F _{3,21} = 0.2
Microbial biomass carbon (μg C g ⁻¹ soil)	25.8 (1.6)	30.2 (2.5)	29.5 (3.7)	37.7 (3.2)	F _{3,21} = 6.2**	F _{1,21} = 0.2	F _{3,21} = 0.9

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9 **Table S2.** Corn root and shoot dry biomass at harvest and the root:shoot ratio, and results of
 10 statistical analysis. Standard error of the mean is shown in parentheses (n = 4). *, $p < 0.05$, **, p
 11 < 0.01 , ***, $p < 0.001$.

Rotation	Water	Root mass (g dw)	Shoot mass (g dw)	root:shoot ratio
C	control	2.26 (0.09)	8.30 (0.61)	0.28 (0.02)
C	drought	2.01 (0.10)	6.05 (0.38)	0.33 (0.01)
CS	control	2.66 (0.14)	9.84 (0.63)	0.27 (0.01)
CS	drought	2.77 (0.09)	7.50 (0.42)	0.37 (0.02)
C/c	control	2.64 (0.24)	9.86 (0.78)	0.27 (0.02)
C/c	drought	2.31 (0.35)	7.13 (0.16)	0.32 (0.04)
CSW/rc	control	2.29 (0.17)	9.78 (0.71)	0.24 (0.02)
CSW/rc	drought	2.03 (0.31)	7.52 (0.36)	0.27 (0.03)
	Rotation	$F_{3,21} = 4.3^*$	$F_{3,21} = 4.8^*$	$F_{3,21} = 3.2^*$
	Water	$F_{1,21} = 1.9$	$F_{1,21} = 53.8^{***}$	$F_{1,21} = 13.4^{**}$
	Rotation X water	$F_{3,21} = 0.6$	$F_{3,21} = 0.1$	$F_{3,21} = 0.8$

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