Long term crop rotation effect on soybean yield explained by soil- and root-associated microbiome and soil health indicators

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Research

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Abstract

Background

Crop rotation is an important management tactic that farmers use to manage crop production and reduce pests and diseases. Long-term crop rotations may select groups of microbes that form beneficial or pathogenic associations with the following crops, which could explain observed crop yield differences with different crop sequences. To test this hypothesis, we used two locations each with three long-term (14 year), replicated, crop rotation treatments: continuous corn (*Zea mays*) (CCC), corn/corn/soybean (SCC), and corn/soybean (CSC); both CSC and SCC had each phase present each year. In Year 15, we grew soybean (*Glycine max*) in each plot, so that soybean replaced corn in CCC and in the CSC phase where soybean grew in Year 14, and took data from soybeans following CCC (14 years of corn), SCC (two years of corn), CSC (one year of corn), and SCS (one year of soybean). Soybean yield and soil health indicators were measured, along with the bulk soil microbiome and soybean root-associated microbiome.

Results

Soybean yields were significantly higher following CCC than in the other three treatments at both locations. Soil protein as a soil health indicator was also higher following CCC than in the other treatments. Differential abundances of bacterial and fungal taxa were related to yield differences in a site-specific manner. Uncultured bacterial taxa in family JG30-KF-AS9 was enriched in the high-yielding CCC plots in Monmouth, whereas *Microvirga* , *Rhodomicrobium* , and *Micromonosporaceae* were enriched in the low-yielding SCS plots. Members of the fungal phylum Ascomycota were informative in explaining yield differences among treatments mostly as pathogens, but *Tumularia* , *Pyrenochaetopsis* and *Schizothecium* were enriched in the CCC plots, suggesting a role as either corn pathogens or beneficial fungal taxa for soybean. Multivariate analysis associated soil health indicators with the rotation regimes and some of the differentially abundant microbial taxa.

Conclusions

Our finding of associations between soil health indicators related to soil microbial populations and soybean yield following different cropping sequences has wide-ranging implications, opening the possibility of both monitoring and manipulating soil microbial populations as a way to improve crop yield potential.

Background

Both the composition and function of microbial communities can be substantially affected by management tactics [1]. Cumulative management effects can be identified by long-term experiments which help to identify problems that threaten future productivity as an early warning system [2], explain the reasons behind existing agricultural production problems [3], and assist in formulating solutions. Additionally, it is important to understand the cumulative effects of enduring management strategies in

order to sustain optimum soil properties [4], specifically the effects on microbial communities and soil health as a result of crop rotation sequences.

Shifts in plant or soil-associated microbial communities are driven by a myriad array of legacy and emerging factors such as plant genetics, soil chemical/physical properties and environmental conditions or soil processes[5]. Because the microbiome is an integral part of almost all soil processes [6], the structure of the microbial communities associated with soil and plants can be directly affected by management strategies such as crop rotation sequences [7]. In the Midwest U.S., corn (Zea mays) and soybeans (Glycine max) cover about 75% of acres used to grow crops[8] and are commonly grown in rotation, which generally improves yields of both crops. However, shifts in the resulting microbial communities due to this crop rotation and variations in crop sequencing are unclear, and may explain yield differences as well as provide new knowledge for future yield improvements.

A list of recommended standard methods for use as soil health indicators that include biologically-influenced metrics has been released by USDA/NRCS recently[9]. The amount of permanganate-oxidizable carbon (POXC) represents the labile portion of the organic carbon which is the most reactive and dynamic driver of carbon mineralization within the pool of soil organic carbon (SOC) [10,11]. Labile organic carbon as measured by POXC has been directly associated with soil carbon (C) and nitrogen (N) mineralization [12], and may promote plant productivity due to its positive influence on soil activities and nutrient availability [13]. Positive correlations have been found between POXC and soil-microbial parameters, comprising microbial biomass and, in particular organic C [10,14]. Therefore, POXC is the recommended method for carbon food source of microbes. A second soil health indicator, the Autoclaved Citrate Extractable (ACE) Protein Content, refers to bioavailable N in the soil organic matter (SOM) [15]. The largest organic N pool in the soil is represented by proteins [16–18]. The labile organic N pool is used to evaluate soils' capacity to provide N [19] by promoting N mineralization in soils [20]. Regarding plant growth and development, N mineralization is a critical process in the soil to provide an adequate amount of N for the use of the plant [21]. Since protein content is an indicator of biological and chemical soil health, especially for SOM quality, it is directly linked to general soil health status [22]. Soil protein includes and an N-linked glycoprotein, glomalin, which is produced by arbuscular mycorrhizal fungi hyphae [23,24]. Glomalin has been reported to enhance soil structure, drainage, microbial activity, and carbon sequestration in soil ecosystems [25] and is sensitive to crop rotation and tillage [22,26–28]. A third soil health indicator, β-glucosidase, is an enzyme which plays a central role in the carbon cycle in soil [29] and serves as an important indicator of general microbial activity [30]. In terms of the carbon cycle, the importance of soil microorganisms in many ecosystems hinges on breaking down cellulose in plant cell walls [31]; cellulose is one of most common organic compounds in the biosphere [32]. β-glucosidase, which has a role in the final stage of cellulose degradation in soils, supplies important energy sources, like simple sugar, for microorganisms [33]. A variety of microorganisms are involved in β-glucosidase activity in soils including filamentous fungi [34–42], yeast [43], and bacteria [44,45].

The lack of strong correlations between rotation-induced crop yield differences and soil chemical and physical properties suggest that soil-associated and plant-associated microbiomes could be determinants for these differences [4,46]. Since soil bacteria and fungi directly mediate the carbon and nitrogen cycle, and regulate the nutrient availability for plants, soil health indicators are expected to be correlated with members of the soil microbiome. We hypothesized that crop yield differences that result from crop rotation would correspond to soil health indicators and soil or root-associated microbiome. To test this hypothesis, we used two sites, each with three replicated, long-term (14 year) crop rotations: continuous corn (CCC), corn/corn/soybean (CCS), and corn/soybean (CS), with each phase of CCS and CS present each year. In Year 15, soybean was planted in all plots, producing treatments with 14 (CCC), 2 (SCC), and 1 (CSC) year of corn, and of one year (SCS) of soybean. Samples were collected for analyses of soil and root-associated microbiome, soil health indicators, and soybean yields.

Results

Soybean yield and soil health indicators

Data for soybean yield, soil protein, POXC and β-glucosidase are summarized in Table 1. At both sites there were significant differences in soybean yields following the four long-term (14 year) crop sequences - CCC, SCC, CSC, and SCS. Soybean yields from CCC (5,614 and 5,255 kg ha^{-1} at Urbana and Monmouth, respectively) were significantly higher than those in SCC, CSC and SCS at both sites, and at Urbana, SCC (with two previous years of corn) yielded more that SCS, with the previous crop of soybean. At the Monmouth site, there were no significant differences among the CCS, CS and SCS treatments, but yields trended lower as the number of years of corn prior to this soybean crop dropped, and were lowest where soybean was the previous crop. Across both sites, the rotations with a higher frequency of corn (CCC, SCC) produced higher yields of soybean compared than those with corn in alternate years (CSC, SCS).

Soils in the CCC treatment had higher protein content compared with other crop sequences at Urbana. The CCC (7,360 mg per kg soil) treatment was significantly greater than SCC (5,627 mg per kg soil), CSC (5,417 mg per kg soil) and SCS (5,613 mg per kg soil) treatments ($p < 0.05$). There were no significant difference among the SCC, CSC and SCS treatments. At the Monmouth location, we found no significant differences in protein content among the CCC (5,842 mg per kg soil), SCC (5,426 mg per kg soil), CSC (5,453 mg per kg soil) or SCS (5,469 mg per kg soil) crop sequences, although CCC had the highest (numerically) average protein. Distribution analysis showed that protein content was normally distributed.

Soil β-glucosidase enzyme activity was significantly higher in SCS (Urbana: 1.19 and Monmouth: 1.73 mg p-nitrophenol per gm of soil) treatments compared with other rotations ($p < 0.05$) at both sites. We found no differences among the CCC (0.93 mg p-nitrophenol g $soil^{-1}$), SCC (0.91 mg p-nitrophenol per gm of soil) and CSC (0.87 mg p-nitrophenol per gm of dried soil) rotations at the Urbana site. At Monmouth, SCS (1.73 mg p-nitrophenol per gm of soil) and CSC (1.73 mg p-nitrophenol per gm of soil) has more β-glucosidase enzyme activity produced than CCC (1.51 mg p-nitrophenol per gm of soil) and

SCC (1.24 mg p-nitrophenol per gm of soil). Distribution analysis showed that soil β-glucosidase activity values were normally distributed.

After 14 years of the above-mentioned crop rotation regimes, we found that POXC was significantly greater in CC (816 mg C per kg soil) and CCS (736 mg C per kg soil) ($P < 0.001$) than CS and SCS treatments (Table 1). Also, there was no difference between CS (606 mg C per kg soil) and SCS (585 mg C per kg soil) at the Monmouth location. At the Urbana site, POXC was significantly higher in CC (647 mg C per kg soil) and CCS (777 mg C per kg soil) compared to the SCS (562 mg C per kg soil) and CS (490 mg C per kg soil) treatments ($P < 0.05$). No significant differences were observed between the CC and CCS, and there was no difference found among the CC, CS and SCS treatments.

There were no significant differences in soybean yield, soil protein and POXC between the Urbana and Monmouth locations when comparing the same treatments. However, β-glucosidase enzyme analysis was significantly different for the same treatments at the two locations.

Bulk Soil Microbiome

Based on ANCOM results in the bulk soil data from Monmouth, an uncultured bacterium belonging to the order of JG30-KF-AS9 within the Chloroflexi phylum had a descending relative abundance order of CCC > SCC > CSC > SCS (Fig. 1A). In the fungal community in the bulk soil from the same site, the relative abundance of Ascomycota effectively discriminated among the four crop sequences. Specifically, the Macrophomina genus was detected as the most abundant in the SCS crop sequence with a decreasing order of abundance as SCS > SCC > CSC > CCC (Fig. 1B). The relative abundance of the genus of Corynespora was significantly higher in the SCS rotation with a decreasing relative abundance in the order of SCS > SCC > CSC > CCC rotations. The genus of Mycoarthris was more abundant in the SCC and CCC rotation groups; and less abundant in the two-year rotation treatments CSC and SCS.

In the bulk soil from the Urbana site, the bacterial genus of Microvirga, belonging to family Methylobacteriaceae under class Alphaproteobacteria, was an informative taxa distinguishing the four treatments, with higher abundance in low-yield rotation groups (CSC and SCS) compared to high-yield (CCC and SCC) rotation groups (Fig. 1C). An uncultured fungus belonging to family Corynesporascaceae in order Pleosporales under class Dothideomycetes in the phylum of Ascomycota was found significantly different in relative abundance separating the four crop sequences, with the order of SCS > CSC > CCC > SCC at the Urbana site (Fig. 1D). From the same order, a taxa under family Pleomassariaceae and genus Tumularia has been found significantly different in relative abundance in decreasing order of CCC > SCC > CSC > SCS. Two additional taxa from genus Clonostachys and Idriella under class Sordariomycetes and phylum Ascomycota were found to be significantly different in terms of relative abundance. Clonostachys was found to be in a decreasing order of SCS > SCC > CCC > CSC, while Idriella was higher in abundance in high-yield groups in the decreasing order of CCC > SCC > CSC > SCS.

Root-Associated Microbiome

The most informative root-associated bacterial genus at the Monmouth site was Rhodomicrobium under class Alphaproteobacteria which has significantly different in relative abundance among the four crop sequences in the order of SCC > SCS > CSC > CCC (Fig. 1E). While for fungal community from the same site, three taxa all belonging to phylum Ascomyota under order Pleosporales (genus Pyrenochaetopsis), Leotiomycetes (genus unknown) and Sordariales (genus Schizothecium) were found to be significantly different in relative abundance with the order of CCC > SCC > CSC > SCS, SCS > CSC > CCC > SCC and CCC > CSC > SCC > SCS, respectively (Fig. 1F). At the Urbana site, root associated bacterium that was differentially-abundant among the rotations was Micromonosporaceae under phylum Actinobacteria with the order of SCS > CSC > SCC > CCC (Fig. 1G). A root-associated uncultured fungus belonging to family Chaetothyriaceae in order Chaetothyriales under class Eurotiomycetes in the phylum of Ascomycota was found to be the only differential fungal taxa at the Urbana site and had the greatest abundance in the SCS crop sequence and a decreasing abundance order of SCS > SCC > CSC > CCC (Fig. 1H).

Followed the ANCOM analysis, differential abundances of taxa between high-yield (CCC and SCC) and low-yield (CSC and SCS) crop rotation groups using balances in Gneiss were analyzed. In the bulk soil from Monmouth, members of the genus Rubrobacter were proportionally higher in the high-yield treatments and Sphingomonas were proportionally higher in the low-yield treatments (Fig. 2A). Fungi from the genus Aspergillus were proportional higher in the low-yield rotation groups (Fig. 2B). At Urbana, bacterial genera Bradyrhizobium, Gemmatimonas, Cellulomonas, family Micrococcaceae, and several uncultured taxa were proportionally higher in high-yield rotation groups (Fig. 2C), while several fungi from genera Plectosphaerella, Tetracladium, Fusarium, Clonostachys, and Purpureocillium were present in higher proportions in low-yield rotation groups (Fig. 2D).

Root-associated microbiome communities from Monmouth, however, showed that Actinobacteria from the family Micromonosporacea and genus Streptomyces were proportionally higher in low-yield rotation groups and bacteria from genus Bradyrhizobium were proportionally higher in high-yield rotation groups (Fig. 2E). Fungi from the family Lasiosphaeriaceae were found to be in higher proportion in low-yielding rotations, including some unidentified fungi in both high- and low-yield rotation groups (Fig. 2F). At Urbana, several bacteria belonging to genus Streptomyces, family Micromonosporaceae, which are both under phylum Actinobacteria, and order Sacharimonadales were in higher proportions in low-yield treatments (Fig. 2G). Root-associated fungi from Urbana, such as genera Tausonia, Solicoccozyma and Cladophialophora in high-yield groups were found to be enriched, whereas Leptosphaeria and several uncultured fungal taxa were more abundant in the low-yield groups (Fig. 2H).

Rarefaction curves were graphed to visualize the minimum amount of sequencing reads required for the analysis. In Figure S1, the X-axes represent the number of sequences extracted from each sample and the Y-axes represent the alpha diversity based on Shannon index. The rarefaction curves for the four rotation groups plateaued for both soil (Figure S1; A-D) and root-associated (Figure S1; E-H) microbial communities, indicating that the amount of sequencing data used for the analysis was appropriate and any increment in the sequencing data would not have further contributed to the species diversity or discovery of any additional species.

With data combined between the two locations for the analysis of alpha diversity, there were no significant differences among the treatments analyzed with either bulk soil or root-associated microbiome data. Data was also analyzed separately for the two locations, and no differences were found in the bulk soil microbiome based on Shannon index ($P > 0.05$) among the four rotations (Fig. 3; A-D). However, significant differences ($P < 0.05$) were found among the four treatments in Shannon index for root-associated bacterial (Fig. 3E) and fungal (Fig. 3F) communities from Monmouth. Also, root-associated bacterial communities were found to be significantly among treatments at Urbana (Fig. 3G), whereas fungal communities were not significantly different among the four treatments (Fig. 4H). Interestingly, CSC rotation had consistently the least average diversity in the root-associated microbiome.

Beta-diversity analyses of the bacterial communities from bulk soil did not produce separate clusters for the four rotation treatments (Fig. 4; A and C) but did for the fungal communities (Fig. 4; B and D). On the other hand, beta-diversity of both bacterial and fungal communities associated with roots did produce separate clusters for four rotation treatments (Fig. 4; E-H).

To correlate the soil health indicators with bulk soil microbiomes and the crop rotation treatments, the results of canonical correspondence analysis (CCA) was plotted. The high-yield rotation groups (CCC and SCC), were associated with the POX-C and protein, while low-yield groups (CSC and SCS) were associated with β-glucosidase (Fig. 5; A-D). We did not see strong association of significantly abundant microbial taxa with any of the soil health indicators analyzed for Monmouth and Urbana sites (Fig. 5; A-D).

Discussion

Studies have shown that higher yields were observed in soybeans when rotated with other crops instead of growing soybeans continuously (monoculture) [47–50]. Our results showed that following 14 years of continuous corn, soybeans had a higher yield when compared with the other preceding crop sequences. A recent study conducted by Farmaha, et al. [51] stated that soybean yield in an irrigated corn-corn-soybean rotation was higher than in a soybean-corn-soybean rotation. The results from our study expanded the conclusion that two or more previous years of corn crop sequences resulted in increased soybean yield as compared to one previous year of corn crop sequences. However, the underlying cause for the yield increase has been difficult to explain. One study reported that the rotation of corn and soybeans had a neutral effect on above-ground biomass [52]. Whiting and Crookston [53] found that the yield benefit from the rotation of soybean with corn was not due to decreases in the incidence of leaf diseases. It has been speculated that rotation-related increased yields were due to enhanced root function [54–56], decreased soil pathogenic microorganisms or parasites affecting root growth [57,58,59,60].

In the same plots we compared in this study, Zuber, et al. [4] concluded that continuous corn and corn-soybean rotation did not produce significant differences in soil physical and chemical properties, but that effects of crop sequence on soybean yield seemed to be the result of multiple interactive biological components in the soil. Indeed, as we show here, biological properties did have an association with yield, likely because soil microorganisms directly drive the carbon [61,62] and nitrogen cycles [63]. For example, the

present study showed that the labile organic carbon pool measured by POXC was higher in CCC and SCC, both sequences with high proportions of corn in the rotation, which corresponded to higher yields of soybean in those plots.

Based on ANCOM analysis of the bulk soil, an uncultured bacterial taxon under family JG30-KF-AS9 (order Ktedonobacterales), was found to be significantly abundant at the Monmouth site with a decreasing order of CCC > SCC > CSC > SCS. Although JG30-KF-AS9 was associated with high-yield rotation groups in our study, their biological significance is not known. Similarly, we identified differentially-abundant fungal taxa in bulk soil that are of particular interest. Among those, Macrophomina which was observed as a dominant taxa in SCS plots, includes some of the most devastating pathogenic species of crop plant, such as Macrophomina phaseolina [64,65]. Pathogens under genera Macrophomina are also known to infect soybean roots causing charcoal rot disease [66]. Similarly, Corynespora, a genus mostly composed of plant pathogens [67,68], was found to be significantly abundant in low-yield SCS plots in decreasing orders of SCS > SCC > CSC > CCC at Monmouth and SCS > CSC > SCC > CCC at Urbana. The fungal pathogen from the genera Corynespora is the causal agent of soybean for frogeye leaf spot disease [69]. Mycoarthris, a genus under order Helotiales, was another fungal taxon that was significantly abundant at Monmouth and was associated with high-yield rotation groups. However, its biological significance is not well-studied.

At Urbana, Ascomycota belonging to family Chaetothyriacea (root-associated) were among the significantly more abundant fungal taxa. Although majority of the Chaetothyriacea genera are saprobes and only a very few are known to be plant pathogens and host specific parasites [70–72], they were associated with low-yield with higher abundance in SCS rotation.

Based on both ANCOM and Gneiss analysis, root-associated bacterial family Micromonosporaceae under order Micromonosporales and phylum Actinobacteria was associated with low yield. Micromonosporaceae are known to act as plant saprophytes or symbionts that thrive under anaerobic conditions[73], and their association with the low-yield rotation groups suggests they are enriched to degrade soybean residues. Gneiss analysis showed several bacterial and fungal taxa present in higher proportions in high and low-yield rotation groups that were not found to be significantly higher by ANCOM analysis. Among those, two bacterial taxa, Streptomyces which were proportionally higher in low-yield and Bradyrhizobium which were proportionally higher in high-yield are particularly interesting. Although mostly symbionts, some Streptomyces species are known to produce extracellular hydrolytic enzymes that can break down highly stable organic compounds inaccessible to other microbes, infect living plant cells and cause diseases of roots [74,75]. Bradyrhizobium helps plants with nitrogen fixation, P and K solubilization [76,77], thus affecting the overall yield. Based on our data, Streptomyces only were associated with roots, whereas Bradyrhizobium were also present in bulk soil.

The multivariate CCA analysis revealed that the yields of high-yield rotation groups (CCC and SCC) were associated with certain soil health indicators, specifically, protein and POX-C; while low-yield rotation groups (CSC and SCS) seem to be associated with β-glucosidase. Bacterial taxa JG30-KF-AS9, enriched

in the CCC plots, was negatively correlated with beta-glucosidase in Monmouth, whereas Microvirga, enriched in low-yield treatment, was positively associated with beta-glucosidase in Urbana. Both the Tumularia and Idriella, enriched in the high yield CCC plots, are positively associated with soil protein in Urbana. Some of the differentially abundant fungal taxa from the bulk soil samples were associated with the soil health indicators, such as Mycoarthris correlates positively with POXC, whereas Macrophomina and Corynespora negatively with POXC and soil protein in Monmouth. Notably, Corynespora also negatively correlates with POXC in Urbana.

The 14-year long-term rotational treatments provided the opportunity to determine the impact of crop rotation and sequencing on specific microbial taxa and their relationship with soybean yield following these 14 year crop sequences. Notably, alpha diversity was not significantly different between treatments, which means continuous corn did not result in a less richness of microbiome than the other regimes. Although alpha-diversity analysis of microbes does not indicate differences in species richness between high and low-yield rotation groups, we still see changes in relative species abundances between the rotation groups based on the separation of clusters with a multidimensional scaling analysis, particularly with regards to the root-associated data. Also, some of the bacterial and fungal taxa were found in higher abundance between the rotation groups based on ANCOM and Gneiss analyses.

Soybeans following 14 years of continuous corn (CCC) yielded significantly more than the other three (SCC, CSC, SCS) crop rotations at both locations. The application of custom crop rotation systems in the field could provide many important benefits enhancing soil C concentration and fertility, improving soil physical properties, providing diverse bacterial and fungal communities, and increasing crop yields. This study provided evidence that soil biological properties, including POXC, protein content, specific bacterial 16S rDNA and fungal ITS sequence relative abundances, could be significantly correlated with yield. This finding is particularly important given that measuring chemical and physical properties did not provide an adequate explanation for soybean yields differences following the different crop sequences [4]. The results suggest that soybean pathogen populations may be determinants, as well as some uncultured bacterial taxa, which still require efforts in culturing and further characterization. Culturability of bacteria has been greatly improved in recent years, and our finding that adding preparations of bacteria, such as those under the order of JG30-KF-AS9 could perhaps be used as a way to increase soybean yields even in fields were soybeans are grown more frequently than once every three or more years.

Conclusion

Crop rotation and sequencing are management tactics that can increase crop yields. The current study found that differential abundances of bacterial and fungal taxa were related to yield differences in a site-specific manner. Multivariate analysis result indicates that soil- and root-associated microbiome members contribute towards some of the observed yield differences that correlates well with different indicators of soil health. Pathogens as expected are associated with the low yield, and correlated negatively with soil protein and POXC, whereas taxa selected by the high yield treatment had positive correlation with soil protein and POXC.

Methods

Fields Descriptions and Soil and Soybean Root Sampling

Field conditions were described in a previously published article [78].The Urbana soil site structure is on Flanagan silt loam and the Monmouth site soil structure is on Muscatune silt loam. Soils were sampled in July 2017 at a 0–15 cm depth from 14-year long-term rotation plots (Northwestern Illinois and Agricultural Research Center of the University of Illinois, Urbana-Champaign) with two locations (Monmouth IL (GIS: 40.931–90.722) and Urbana IL (GIS:40.048–88.232)). The experiment had 4 treatments: T1: Continuous corn (CCCCCCCCCCCCCC-S), T2: 2-yr of corn (CCSCCSCCSCCSCC-S), T3: 1-yr of corn (SCSCSCSCSCSCSC-S) and T4: 1-yr of soybean (CSCSCSCSCSCSCS-S) ahead of soybean x 4 rep (block) x 3 subsamples/plot in 2016. The field layout followed the random complete block design at both locations. Soybean roots were sampled from the corresponding plots in July 2017. Samples were kept cool during transportation on ice and stored in a -80 °C horizontal freezer immediately until further processing.

Determination of Permanganate Oxidizable Carbon (POXC)

The procedure defined by Weil et al. [10] was followed for the measurement of the POXC. The colorimetric method was used to measure the absorbance by a microplate reader (BioTek Synergy 2 Multi-Mode Microplate Reader) at the wavelength of 550 nm. Sample exceeding the range of the standard curve were diluted with water.

Soil protein Index

Soil protein content was measured in triplicate for each soil sample by following a protocol modified from Wright and Upadhyaya (1996) and Moebius-Clune et al. (2016) [15,79].[15,79] by autoclaving (121 °C @ 15 psi for 30 minutes) a citric acid (pH 7.0) soil extraction and measuring protein in the extract by colorimetric methods (Pierce™ BCA Protein Assay Kit, LOT: TB263610, Thermo Scientific). Protein assays were performed in 96 well plates incubated at 60 °C for 30 minutes. After the incubation, the microplate reader (BioTek Synergy 2 Multi-Mode Microplate Reader) was used to obtain the optical density reading at the wavelength of 562 nm.

Soil β-glucosidase enzyme activity

Soil β-glucosidase enzyme activity was assayed according to the method described by Deng and Tabatabai (1994) [80].[80]. THAM buffer (pH 10) was used to dilute samples at the rate of 1:4 (note: the controls were not diluted) and samples were pipetted into 96 well microplates. The absorbance was measured using a microplate reader (BioTek Synergy 2 Multi-Mode Microplate Reader) at the wavelength of 405 nm.

Soil DNA Extraction

The FastDNA™ Spin Kit (For soil, Cat.No.116560200, MP Biomedicals, Solon, Ohio) was used following the manufacturer's protocol for DNA extraction from soil with some minor modification. Samples were kept at -20 °C until further hexadecyltrimethylammonium bromide (CTAB) purification of DNA for post-extraction cleanup [81].

Bacterial and Fungal DNA Amplicon Sequencing

Bacterial 16S rDNA and fungal nuclear ribosomal internal transcribed spacer (ITS) classifications were amplified and sequenced by University of Minnesota Genomic Center (Minneapolis, Minnesota, USA) using MiSeq-V3 chemistry from a published protocol with a dual-index approach [82].[82]. The 16S V3-V4 and ITS-1 regions were targeted for the bacterial community and fungal community, respectively. Data was deposited in NCBI BioProject SRA accession: PRJNA521547.

Data Analyses

Sample details can be found in Table S1, and soil health indicators data can be found in Table S2. Statistical analyses were performed using R programming. Yields, POXC, protein index and β-glucosidase activities were analyzed by fitting a mixed-effect model with block as a random effect using 'lme4' [83] followed by the posthoc tests of LSMeans Differences with 'emmeans' packages in R. The following assumptions for linear mixed model were also tested: that errors are linear, independent, normally distributed and have homogeneity of variance. The threshold was designated for probability at $P < 0.05$. The classifications of bacteria and fungi were determined using QIIME2 [84], sequences were denoised and filtered using DADA2 [85], resulting feature tables were then rarefied to perfom core diversity analysis, followed by analyses to detect differential abundance of taxa with the ANCOM [86] and Gneiss [87] tests. Taxonomic assignment of representative sequences of fungi and bacteria were performed based on the trained ITS and 16S RNA OTUs clustered at 99% similarities within Unite (version 8) [88] and Silva132 [89] databases, respectively, using the Naïve-Bayes classifiers [90] built in QIIME2. Kruskal-Wallis [91] tests followed by Wilcoxon post hoc tests (where p-values were significant [$P < 0.05$] for omnibus Kruskal-Wallis tests) were performed to determine significant alpha diversity metrics between the rotation types. Rarefaction curves were generated from multiple samplings of the same sample data with QIIME2 adjusting for variation in the sequencing depth. Canonical correspondance analysis (CCA) was performed using 'vegan' package in R. Data was summarized in the supplementary file, named as CCA. High throughput sequencing data has been submitted to NCBI SRA with the accession number available upon revision or the acceptance of the manuscript.

Abbreviations

ANCOM: Analysis of composition of microbiomes

CCC: Continuous corn (CCCCCCCCCCCCCCC-S)

SCC: 2-yr of corn (CCSCCSCCSCCSCC-S)

CSC: 1-yr of corn (SCSCSCSCSCSCSC-S)

SCS: 1-yr of soybean (CSCSCSCSCSCSCS-S)

POXC: permanganate-oxidizable carbon

SOC: Soil organic carbon

SOM: Soil organic matter

Declarations

Ethics approval and consent to participate: Not applicable
Consent for publication: Not applicable
Availability of data and material: Sequence data is publicly available under NCBI BioProject *SRA accession:* PRJNA521547.

Competing interests: The authors declare that they have no competing interests.
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Authors' contributions: AN, SLM, and ZW analyzed the sequencing data, IB, ZW and RML measured the soil health indicators, EN and SLM conceived the study. IB and AN helped to draft the manuscript. All authors read and approved the final manuscript.

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Table

Due to technical limitations, table 1 is only available as a download in the supplemental files section.

Figures

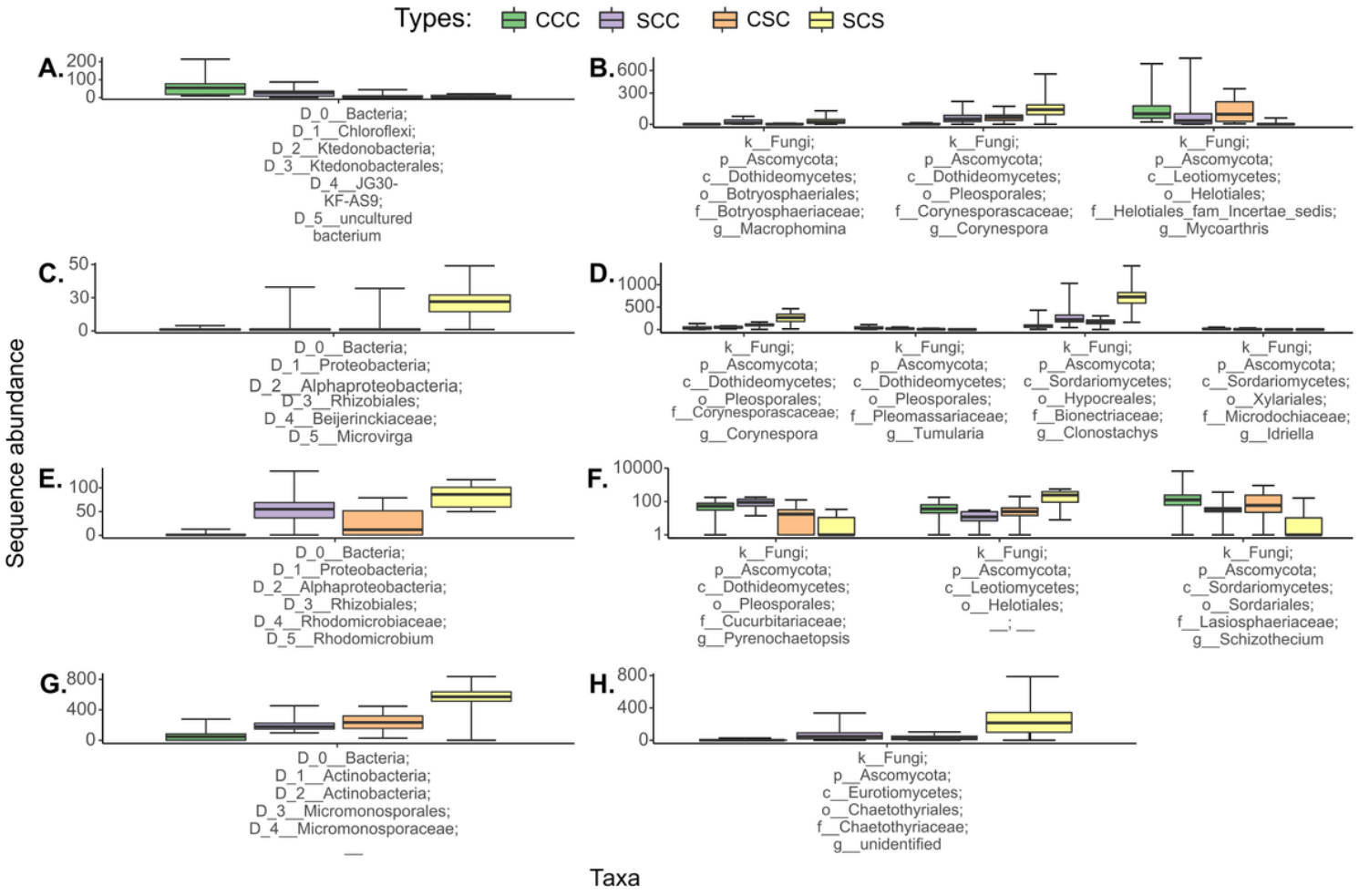

Figure 1

Differential abundance of taxa analyzed by ANCOM approach. Box-and-whisker plots of bacterial (A) and fungal (B) at the Monmouth site, and bacterial (C) and fungal (D) at the Urbana site associated with bulk soil; bacterial (E) and fungal (F) at the Monmouth site, and bacterial (G) and fungal (H) at the Urbana site

associated with roots are shown with relative abundance distributions for the taxa that varied significantly among rotation treatments.

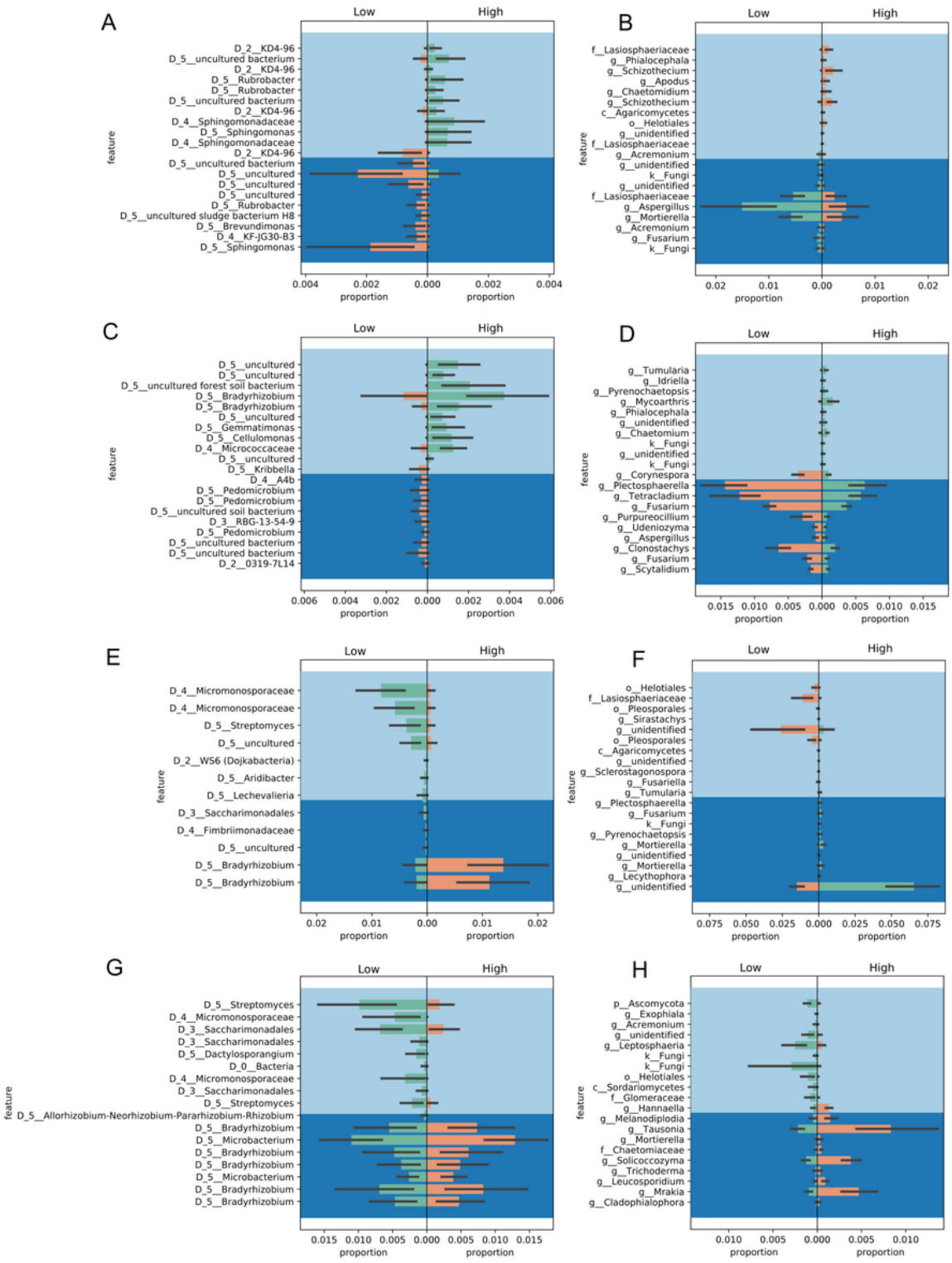

Figure 2

Differential abundance of taxa analyzed by Gneiss approach. Abundances of bacterial (A) and fungal (B) at the Monmouth site, and bacterial (C) and fungal (D) at the Urbana site associated with bulk soil; bacterial (E) and fungal (F) at the Monmouth site, and bacterial (G) and fungal (H) at the Urbana site

associated with roots are shown. The differential abundance analysis was performed based on high- and low-yield groups. The high yield group is CCC (continuous corn) and SCC (two years corn). The low yield group is CSC (one year corn) and SCS (one year soybean).

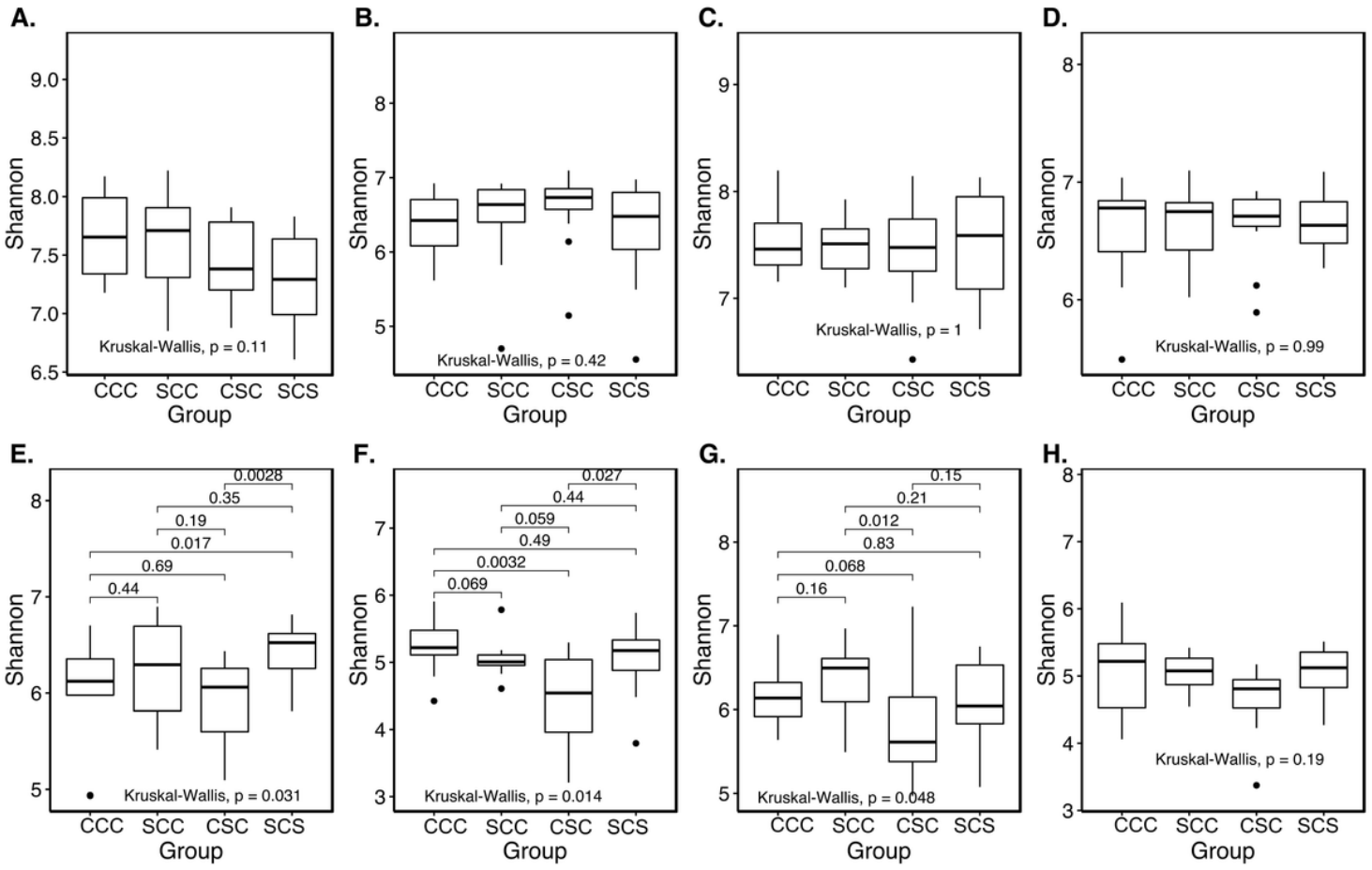

Figure 3

Alpha diversity estimated by Shannon index. Bacterial (A) and fungal (B) diversities at the Monmouth site, and bacterial (C) and fungal (D) diversities at the Urbana site associated with bulk soil; bacterial (E) and fungal (F) diversities at the Monmouth site, and bacterial (G) and fungal (H) diversities at the Urbana site associated with roots are shown.

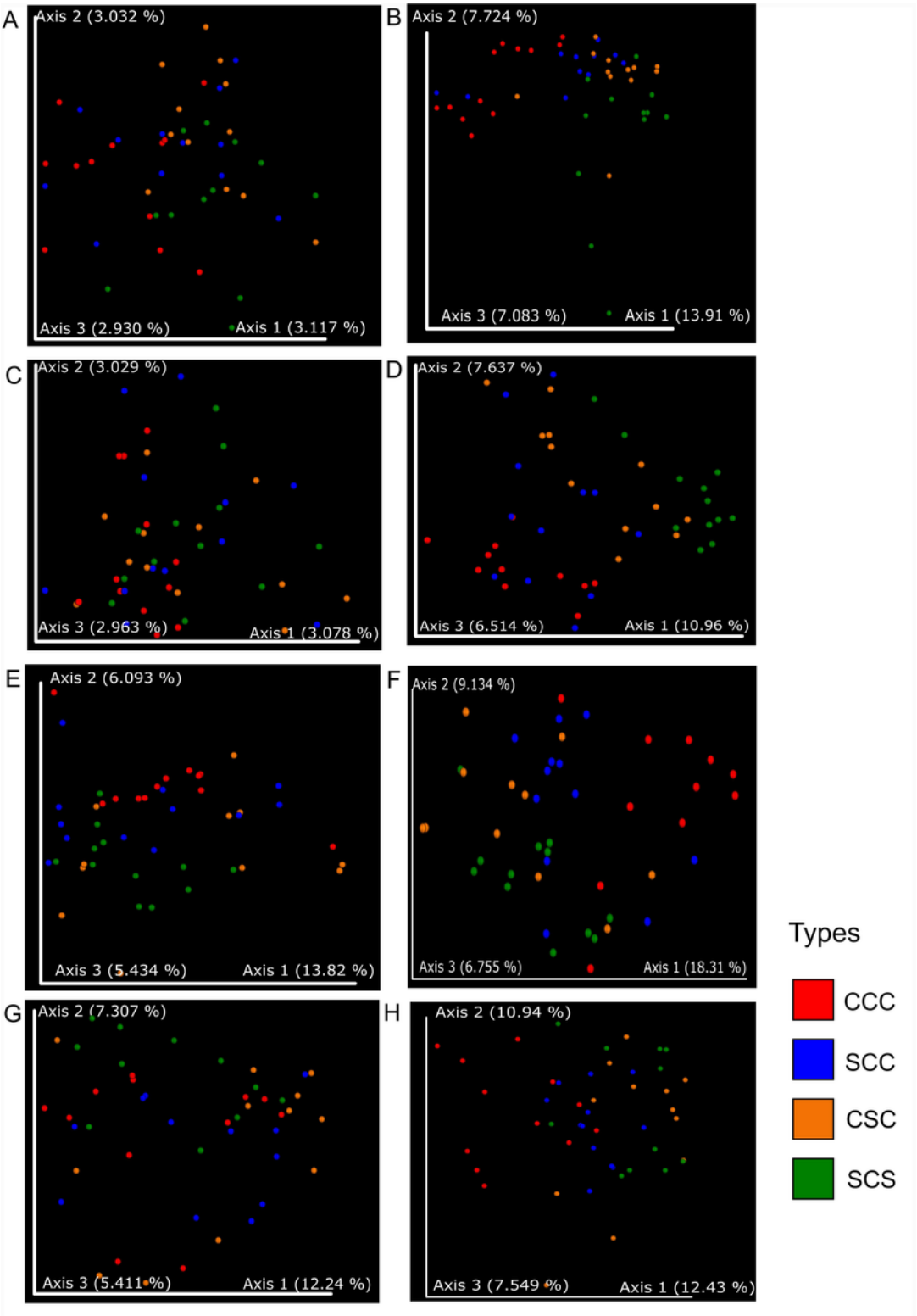

Figure 4

Beta-diversity visualized by EMPeror. Bacterial (A) and fungal (B) diversities at the Monmouth site, and bacterial (C) and fungal (D) diversities at the Urbana site associated with bulk soil; bacterial (E) and fungal (F) diversities at the Monmouth site, and bacterial (G) and fungal (H) diversities at the Urbana site associated with roots are shown.

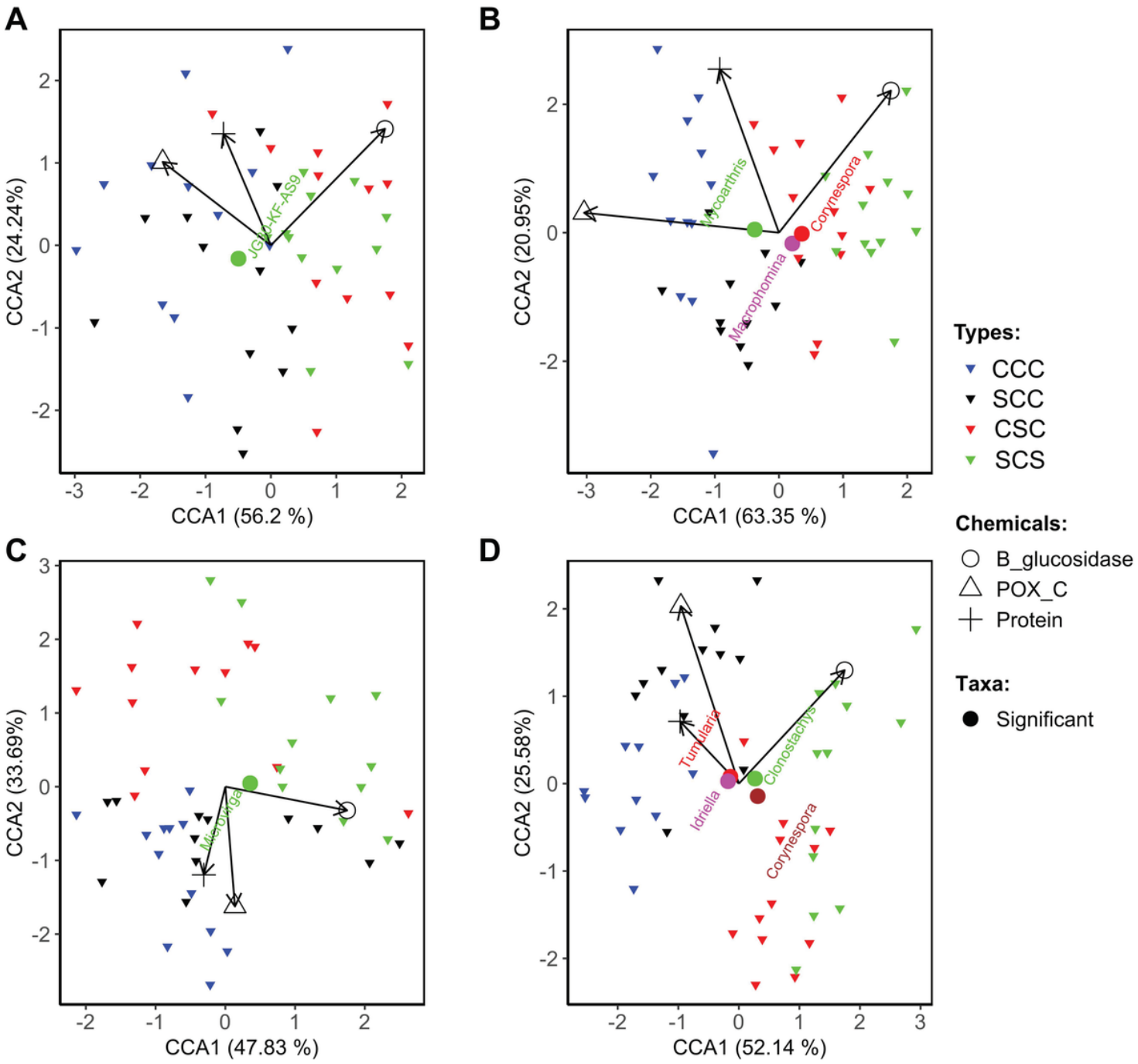

Figure 5

Relationships between operational taxonomic units from different crop rotations and the soil health indicators. A multivariate approach using canonical correspondence analysis (CCA) was used. Only significant taxa are shown by colored-coded circles. Rotation regimes are denoted by color-coded triangles (Treatments) and soil health indicators are denoted by hollow shapes (Indicators). Bacterial (A) and fungal (B) taxa with differential abundances at the Monmouth location, and CCA for bacterial (C) and fungal (D) taxa with differential abundances at the Urbana location are shown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AllSupplemental.pdf
- Table1.pdf