**Supplementary Materials: Microbial community response to drought depends on crop**

**Supplementary Methods**

**Rainout shelter details**

After emergence of both corn and soybean crops, we imposed an under shelter drought from June 28th, 2019 to August 26th, 2019. To account for differences in crop height between corn and soybean, the shelters had sidepost heights of 8.5’ for corn and 4.5’ for soybean. Because we anticipated moisture under shelter up to one meter from the outside perimeter especially in corn, we used a large shelter footprint of 24’ x 26’. The shelters were constructed with 2” x 3” galvanized steel rectangular tubing with vertical supports every five feet. The roof truss network had a slope of 3”/12” so the designed center peak was three feet taller than the sidepost height. The peak run was in the same direction of the row crop, thus the south and north sides had slightly more ambient exposure. The corrugated roofing panels were 50”W by 12’7” long, installation of panels overlapped to prevent any rain intrusion. The corrugated roofing panels (Amerilux Greca Lexan) allow approximately 90% light transmittance above 385 nm. Panels are weather resistant and have a UV protective coating on one side to prevent panel yellowing. A clear Lexan ridgecap was installed to prevent rain intrusion the length of the shelter peak. Each prototype shelter has a base made the same shelter material running the length of the shelters in the same direction as the center peak. A gutter and hose network spanning the length of each shelter was installed to allow any rain collected from the roof to be diverted outside the plot. Gutters are pitched accordingly towards the plot edge. The total rain excluded footprint area was 58 m2 for each shelter. Shelters were removed on 8/26/19 with extended forks and a John Deere tractor.

**Inoculum creation and application methods**

We developed microbial inocula originating from both soil and roots to test the effect of different diverse microbial communities on plant-microbial interactions under drought, and we applied the microbial inocula using plant-based and whole-plot applications to test the effect of different application locations on inoculation success. Both inocula originated from restored prairie soil, because restored prairie soil microbial communities can have higher diversity and stress tolerance than microbial communities from agricultural fields (McKinley et al., 2005; Barber et al., 2017; Upton et al., 2018). We chose to inoculate with diverse microbes because multiple microbes are less likely to overlap functionally with existing microbes (Hu et al., 2016; Rivett et al., 2018)) and inoculating with multiple strains can increase the environmental range that inocula increase plant growth (Bai et al., 2003; Caravaca et al., 2005; Armada et al., 2016; Rashid et al., 2016; Ghorchiani et al., 2018). In May 2019, we collected prairie soil from the top 15 cm of soil after aboveground vegetation was removed with a hoe. The prairie soil is from a restored prairie at the Kellogg Bird Sanctuary in Kalamazoo County, MI.

We made our whole soil inoculum using bacterial growth media by adding sieved restored prairie soil to a high and low nutrient media to grow a more diverse microbial community for inoculation (Kaminsky et al., 2019). To grow the microbes for inoculation, we added 2g of sieved soil to 200 ml of either R2A (low nutrient) or LB (high nutrient) liquid media in a 1L bottle. After 72 hours of room temperature incubation, we calculated colony forming units and found that the concentration of colony forming units was similar for both types of media. We therefore combined equal parts R2A and LB culture and diluted the culture to a final concentration of 8.3x109 cfus/mL using R2A media. We inoculated using growth media because the substrate that the microbes are added in is importance for decreasing desiccation and providing an energy source for microbes to establish (Malusá et al., 2012). We stored the inoculum in the fridge until use. We then applied 20ml of inocula to each inoculation plot (a standard amount of cells used in field experiments: (Bai et al., 2003; Zhang et al., 2003; Berger et al., 2018)).

To make root inoculum, we separated roots from soil during sieving. we then weighed 15g (+/-.25g) of wet root mass for each microplot. After drying the root inoculum weighed 3.04 +/- 0.5 g.

We created heat-treated inocula to control for the effect of added nutrients, carbon, and dead cells on crop growth and soil microbial communities. We killed the microbes in the whole soil liquid inoculum by heat-treating half of the inoculum in an oven >90° C for three hours. We found no colonies after 24 hours of growth in LB media, verifying that our heat treatment had killed the microbes in the inoculum. Similarly for the root inoculum, we heat-treated half of the inoculum in an oven >90° C for 5 hours to create a dead root inoculum.

We applied the inoculum to subplots one week post emergence of each crop (corn: June 5th, soy: June 25th). To explore two mechanisms of inoculation success (via plant-microbe interactions or changes in bulk soil communities), we used two different methods of application for each inoculum: a plant-focused application and a whole plot application. For the plant-focused application, we cut a shallow trench ~1 cm deep approximately 3 cm to the east of the seedlings, to minimize damage to the roots of the plants. Either whole soil or root inoculum was then dripped or placed in the trench and covered with the dirt that was displaced during the digging of the trench. For the whole inoculation plot treatments, the whole soil inoculum was dripped across the 0.5m2 area using a 5ml pipette, by adding 5ml to each quarter of the plot, and root inoculum was placed evenly across the plot on the soil surface.

**Soil moisture monitoring**

A network of soil moisture and temperature probes was installed in two transects for each shelter. Each transect contained 5TM soil moisture/temperature sensors (Decagon, Pullman,WA) connected to several EM50- series data loggers (Decagon, Pullman, WA). The west to east transect was installed 3.66m (12 ft) from the southern plot edge. Sensors were installed at -0.91m (3 ft outside shelter), 0.15m (0.5ft), 0.91m (3ft), 1.83m (6ft), 2.74m (9ft), 4.88m (16ft), and 6.10m (20ft) from the west footprint edge. The south to north transect was installed 3.66m (12ft) from the western edge. Sensors were installed at -0.15m (0.5ft outside shelter), 0.91m (3ft), 1.83m (6ft), 2.74m (9ft), 4.88m (16ft), 6.40m (21ft), and 7.92m (26ft, also 0.61m outside of northern footprint edge) from the southern footprint edge (Supplementary Figure S10). Data was logged on a per hour basis from 7/2/19 to 8/13/19. The sensors were removed early, because we had collected enough data for our primary purpose of measuring rainfall intrusion and so that they wouldn’t be in the way of soil sampling. Three of the data loggers in the corn field were removed from the analyses because of either damage or inconsistent sensor function.

**DNA extractions and sequence processing**

We extracted DNA from 0.15cm3 of soil from each sample and from whole soil inoculum (20ul) and root inoculum (0.15 cm3). We extracted the samples using the MagAttract PowerSoil DNA kit for Kingfisher Flex using the manufacturer’s protocol, with an added two-step binding process. During the binding step, we added 400ul of supernatant to two different kingfisher plates (step 11 in manufacturer’s protocol). We then added 418 μl of the ClearMag Beads/ClearMag Binding Solution to each well (step 13 of manufacturer’s protocol) and added 400ul of ClearMag Wash solution to three clean kingfisher plates (step 15 of manufacturer’s protocol). We then ran the plates on a Kingfisher Flex (Rev1.2, Thermo Scientific, Waltham, MA). We then sent DNA extractions to the Research Technology Support Facility Genomics Core at Michigan State University. The RTSF Genomics Core to complete library preparation of bacterial 16S v4 region using dual-indexing (Kozich et al., 2013), and sequence the amplicons using two lanes on Illumina MiSeq v2 Standard 500 cycle to output 2x250bp reads.

We used USEARCH v11 for sequence processing (Edgar, 2010). We merged paired reads, with an pair success rate of 70%. We trimmed reads to 250 bp and screened quality with a maxEE score of 1 (which retained 99% of reads). We clustered sequences into OTUs at 97% similarity using default settings of UPARSE (*cluster\_otus* command, (Edgar, 2013)). This command used de novo filtering to remove chimeras. We then classified OTUs using the Silva v.123 database (Quast et al., 2013) and SINTAX classifier using USEARCH (Edgar, 2016). OTUs with single reads were removed for a final sequence read count of 11,488,657 across 25,434 OTUs and 440 samples (8150-43179 reads per sample). Analysis on bacterial community composition was done on relative abundance data of raw reads.

**Greenhouse experiment**

To ask whether microbial inoculations in the field persisted or responded to drought imposed during the field season to affect subsequent plant growth, we manipulated water availability on greenhouse grown plants (drought or control) and inoculated plants with field soils collected from the above experiments in a factorial design (2 greenhouse watering treatments x 2 field rain treatments x 3 field inoculation treatments). In the fall of 2019, we planted 144 corn and soybean seeds from the same commercial seed source used in the field experiment in individual 25-cm deep, 6.4-cm wide pots (Stuewe and Sons, Oregon, USA) in the Indiana University Greenhouse. All seeds were planted into steam sterilized potting soil and each deep pot rack contained a maximum of 6 plants of the same species to minimize contamination between pots and were randomly assigned to inoculation and drought treatments.

*Inoculation treatments*

We inoculated greenhouse plants using soils collected from the field experiment in September 2019. Soil was collected from field inoculation treatments (whole soil live plant focused, root live plant focused, and control) within both the ambient and drought plots (field precipitation treatment). We collected soils using a 2.5cm soil corer to 10 cm depth. Soil collections from 4 field blocks were treated as unique inocula. We created each inoculum by mixing 10 mL of soil with 40 mL of water. We shook the soil samples to combine and then allowed them to rest for about 20 minutes, allowing soil to settle and bacteria to percolate into the water/mixture. We then applied each inoculum to the base of each assigned seedling ~6 days post germination and again ~13 days post-germination.

*Drought treatments*

To simulate drought, we watered ambient plants with approximately 250 mL of water every 3 days, and we watered drought plants once per 6-10 days or when there were signs of severe drought stress (i.e., wilting, dropping leaves, etc.). Four individuals in the soy experiment died prior to collecting trait data.

*Response variables*

We recorded leaf count, chlorophyll content, and height 17-23 days post-germination and again 30-42 days post-germination. We used a chlorophyll meter (SPAD‐502, Konika Minolta, Osaka, Japan) to measure the amount of chlorophyll of the tallest leaf of each individual plant. For the corn plants, 3 separate measurements were taken from the widest point on the tallest leaf and averaged to prevent biases from the venation. We measured height from the base of the plant (without touching the soil) to the topmost node. We also measured specific leaf area (SLA) near the end of the experiment, but due to thrips and the conditions of the leaves, we only harvested a subset of leaves for the soybean plants. To measure SLA, we first harvested the third leaf from the bottom of each plant, pressing the leaf between wet paper towels and placing in a Ziploc bag, and then estimating leaf area with a LI-3100C leaf area meter (LI-COR Biosciences, Nebraska, USA). Leaves were weighed after drying at 65℃ for at least 72 hours.

We harvested aboveground and belowground biomass after ~7 weeks for corn and ~9 weeks for soy, when some plants showed signs of becoming root bound. For the soy plants, we counted the number of nodules. All above- and belowground biomass was weighed after drying at 65℃ for at least 72 hours.

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**Supplementary Figure Legend**

**Figure S1: Plot layout**

A diagram of the inoculation subplots under the rainout shelters and in ambient rainfall plots labeled with inoculation treatment.

**Figure S2: Wind Direction**

Average windspeed (average meters per second for each day) and average direction (degree) for July and August at Kellogg Biological Station from 1992-2021. Data from: <https://lter.kbs.msu.edu/datatables/155>

**Figure S3:** **Volumetric water content under the shelters after rain event**

Change in median volumetric water content (initial VWC – final VWC) from initial measurement on July 2nd to final measurement on July 29th, 2019. For location of the rainout shelter relative to the location of the VWC censors, see Supplementary Figure 8.

**Figure S4: Volumetric water content and rainfall over time.**

Change in median daily volumetric water content (initial VWC – final VWC) from initial measurement on July 2nd to August 10th, 2019. Sensors that were used were “W1” (0.5 m outside the shelter) and “W4” (1.8m inside the shelter). For specific location of the sensors, see Supplementary Figure 8. Rainfall data is from the Kellogg Biological Station weather station online data (https://lter.kbs.msu.edu/datatables/7).

**Figure S5:** **Bacterial phyla abundance across crop and rain treatments**

Relative abundance of phyla (median, 25 and 75 percentile) across crop and rain treatments in the field experiment.

**Figure S6: Bacteria phyla in inocula and inoculated soil**

The relative abundance of OTUs in the phyla that make up >1% of the sequence reads in inocula and soil from plots after six weeks of shelter treatment.

**Figure S7: Bacterial diversity and inoculation in corn plots.**

Percent change in bacterial Shannon’s diversity (mean ± standard error) between diversity before and one week after inoculation in corn plots. \* p<0.05, \*\* p<0.01

**Figure S8: SLA across field and greenhouse water treatments**

Mean and standard error bars of corn specific leaf area (SLA) across field rain treatment and greenhouse watering treatment. Letters indicate significant difference (p<0.05).

**Figure S9: Uncorrected microbial biomass across crop and rain treatments.**

Mean and standard error bars of microbial biomass carbon (µgC/g dry soil) uncorrected for fumigation efficiency and negative values.

**Figure S10: The placement of soil moisture sensors relative to the location of the rainout shelter.**

The orange rectangle represents the location of the rainout shelter.

**Supplementary Table S1:** Bacterial community composition and diversity before inoculation in corn and soybean fields separately explained using dbRDA (community composition) and linear mixed effects models (diversity) with rain treatment (rain) and inoculation treatment (inoculation) as fixed effects and plot x and y coordinates as conditional variables in dbRDA and block as a random effect in linear mixed effects models. Dashes indicate variables were removed during model selection. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001

|  |  |  |
| --- | --- | --- |
|  | **Community composition analysis** | **Change in diversity analysis** |
|  | **Corn** | **Soybean** | **Corn** | **Soybean** |
|  | **Sum of Sqs** | **F** | **Sum of Sqs** | **F** | **F** | **F** |
| **rain** | 0.33 | 2.81\*\*\* | 0.86 | 8.34\*\*\* | 3.44 | 26.13\*\*\* |
| **inoculation** | 0.97 | 1.02 | 0.80 | 0.97 | - | - |
| **rain x inoc.**  | 0.78 | 0.83 | 0.73 | 0.89 | - | - |

**Supplementary Table S2:** Bacterial community composition of the most abundant 10 phyla and unclassified bacteria after drought treatment applied analyzed using partial dbRDA analysis with inoculation treatment (inoc.), crop, and rain treatment (rain) as fixed effects and x and y plot coordinates as conditional variables. F values presented. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Inoc.** | **rain** | **crop** | **Inoc. \* rain** | **Inoc. \* crop** | **Rain \* crop** | **Inoc. \* rain \* crop** |
| **Acidobacteria** | 0.99 | 1.83\* | 2.4\*\*\* | 1.03 | 0.99 | 6.33\*\*\* | 0.99 |
| **Actinobacteria** | 0.98 | 1.62\* | 3.07\*\*\* | 1.06 | 0.93 | 7.53\*\*\* | 0.97 |
| **Bacteroidetes** | 1.02 | 1.73\*\* | 2.35\*\*\* | 1.05 | 1.03 | 5.5\*\*\* | 0.96 |
| **Chloroflexi** | 1.04 | 1.66\*\* | 2.07\*\*\* | 1.01 | 0.95 | 6.14\*\*\* | 0.92 |
| **Cyanobacteria** | 0.96 | 1.56\*\* | 1.69\*\* | 1.01 | 0.97 | 2.31\*\*\* | 1.02 |
| **Firmicutes** | 0.99 | 1.26 | 1.65\* | 0.98 | 0.95 | 1.79\*\* | 0.9 |
| **Gemmatminoadetes** | 0.96 | 1.94\*\*\* | 2.61\*\*\* | 1.06 | 0.98 | 3.49\*\*\* | 1.01 |
| **Planctomycetes** | 1.02 | 1.77\*\* | 2.41\*\*\* | 1.06 | 1.02 | 7.24\*\*\* | 0.99 |
| **Proteobacteria** | 1.01 | 1.9\*\* | 3.03\*\*\* | 1.03 | 0.97 | 8.5\*\*\* | 0.92 |
| **unclassified** | 1.06 | 1.63\*\* | 1.57\*\* | 1.04 | 1.03 | 3.00\*\*\* | 1.02 |
| **Verrucomicrobia** | 0.93 | 1.31 | 1.92\*\* | 1.06 | 0.96 | 3.88\*\*\* | 0.95 |

**Supplementary Table S3:** The relative abundance of the most abundant 10 phyla and unclassified bacteria after drought treatment applied using linear mixed effects models, with inoculation treatment (not listed), crop, and rain treatment (rain) as fixed effects and block as a random effect. We selected models via AICc using the dredge command in MuMIin package in R. Estimates are presented. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Intercept** | **Crop (soy)** | **Rain (shelter)** |
| **Acidobacteria** | 0.186\*\*\* | 0.015\* | -0.019\*\* |
| **Actinobacteria** | 0.14\*\*\* | -0.033\*\*\* | 0.019\*\* |
| **Bacteroidetes** | 0.035\*\*\* | 0.006\*\* | -0.008\*\*\* |
| **Chloroflexi** | 0.053\*\*\* | -0.006\*\* | 0.005\*\* |
| **Cyanobacteria** | 0.011\*\*\* | - | - |
| **Firmicutes** | 0.02\*\*\* | -0.007\*\* | 0.007\*\* |
| **Gemmatimonadetes** | 0.031\*\*\* | 0.011\*\*\* | -0.005\*\*\* |
| **Planctomycetes** | 0.06\*\*\* | - | -0.005\*\* |
| **Proteobacteria** | 0.319\*\*\* | -0.012\* | 0.009\* |
| **unclassified** | 0.012\*\*\* | -0.003\*\*\* | - |
| **Verrucomicrobia** | 0.092\*\*\* | 0.008\* | 0.006 |

**Supplementary Table S4:** Plant characteristics from the greenhouse study explained by linear mixed effects models with rack number as a random effect and field block nested within field block nested within inoculum. F values are reported. P values (^ p<0.1,\* p<0.05, \*\* p<0.01, \*\*\* p<0.001)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Aboveground biomass** | **Belowground biomass** | **Average leaf number** | **Average height** | **Chlorophyll content**  | **Nodule number** | **SLA** |
| **SOYBEAN** | **F** | **F** | **F** | **F** | **F** | **F** | **F** |
| greenhouse watering treatment | 2470.68\*\*\* | 162.85\*\*\* | 672.82\*\*\* | 265.96\*\*\* | 110.29\*\*\* | 120.26\*\*\* |  |
| field rain treatment | 0.63 | 0.44 | 0.85 | 0.06 | 0.31 | 2.13 |  |
| field inoculum treatment | 1.72 | 0.6 | 1.67 | 0.33 | 1.63 | 0.09 |  |
| greenhouse watering treatment x field rain treatment | 1.7 | 0.05 | 0.61 | 0.29 | 0 | 2.28 |  |
| greenhouse watering treatment x field inoculum treatment | 3.21\* | 6.98\*\* | 0.86 | 0.4 | 1.47 | 0.19 |  |
| field rain treatment x field inoculum treatment | 0.98 | 1.44 | 0.86 | 0.14 | 1.04 | 0.69 |  |
| greenhouse watering treatment x field rain treatment x field inoculum treatment | 0.51 | 1.43 | 1.01 | 0.34 | 0.58 | 0.09 |  |
|  |  |  |  |  |  |  |  |
| **CORN** |  |  |  |  |  |  |  |
| greenhouse watering treatment | 516.3\*\*\* | 156.58\*\*\* | 126.08\*\*\* | 224.07\*\*\* | 33.8\*\*\* |  | 1.81 |
| field rain treatment | 0.04 | 0.02 | 0.59 | 0.32 | 0.14 |  | 3.36^ |
| field inoculum treatment | 0.08 | 0.17 | 1.13 | 0.11 | 0.34 |  | 0.85 |
| greenhouse watering treatment x field rain treatment | 0.29 | 0.01 | 0 | 0.02 | 0.25 |  | 3.53^ |
| greenhouse watering treatment x field inoculum treatment | 0.67 | 0.87 | 0.42 | 0.93 | 0.66 |  | 2.1 |
| field rain treatment x field inoculum treatment | 0.17 | 0.4 | 1.32 | 0.42 | 0.94 |  | 0.75 |
| greenhouse watering treatment x field rain treatment x field inoculum treatment | 0.15 | 0.13 | 0.35 | 0.51 | 1.38 |  | 1.25 |

Figure S1

Figure S2



Figure S3



Figure S4

s

Figure S5



Figure S6



Figure S7



Figure S8



Figure S9



Figure S10