Enriched and depleted microbes at phyla level in humic acid (HA) fertilizer associated with delayed drought responses in maize

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Abstract

Background: Humic acid (HA) fertilizer was previously considered to be able to enlarge the effective absorption area of roots and promote plant root growth. While recent evidences suggested that certain root-associated microbes might be able to mitigate the negative responses of drought stress. In this study, we sought to explore abundance and diversity of root-associated bacterial communities under humic acid (HA) fertilizer and drought stress treatments. Results: We collected rhizospheres of three groups (HA, drought, and control) and microbiomes from bulk soil during flowering period in maize. We obtained the bacterial community for the V4 region of the 16S rRNA gene using Illumina Hiseq2500. By analyzing the sequencing data, we identified 40 bacteria phyla across samples. The abundance level of the 12 bacterial communities at phyla level are significantly different in the rhizosphere of the drought treated samples as compared to the controls, two of which, Actinobacteria and Bacteroidetes, were also significantly enriched (FC=1.79, adj.P=0.043) and depleted (FC=0.70, adj.P=0.086) in HA fertilizer treated samples. Conclusions: The results suggested that the delayed responses of plants to drought stress in HA fertilizer treated soils might be through supply a resource of substance to recruit drought-resistance microbiomes.

Background

Humic acid (HA) fertilizer was previously considered to be able to enlarge the effective absorption area of roots and promote plant root growth [1, 2], hence, promoting the recruitment of nutritional resources such as carbon (C) and nitrogen (N) from the fertilizer and bulk soil. Under water stress, studies showed that HA fertilization stimulates root development, i.e. increased root length, root number and root branching [3, 4], thereby delayed the drought stress responses [5]. However, it is unclear about the connections between HA and drought resistance, especially at bacterial microbiome level. Recent evidences suggested that certain root-associated microbes might be able to mitigate the negative responses of drought stress, such as *Actinobacteria* and *Firmicutes* [6-8]. In order to test the hypothesis that HA delays negative drought responses in root development through enriching or depleting certain microbes, here, we sought to conduct experiments to explore the effects of root-associated bacterial communities on several phenotypes during maize development, and to determine how HA and drought influences the composition of microbial communities. In the experiments, we collected phenotypic traits on maize hybrids during flowering period in three treatments (HA, drought, and control). Bacterial community was determined from rhizospheres and bulk soils for the V4 region of the 16S rRNA gene using Illumina Hiseq2500 sequencer (Figure 1a). A total of 18 samples (6 plants per treatment) were used for rhizospheres and 6 samples from the control were used for bulk soil (Figure 1b and 1c), resulting in ~763 million reads. Across all the samples, we have detected 40 bacterial phyla. As expected, the bulk soil microbiome and maize rhizospheres differed greatly (Figure 1d), but all of the 40 phyla were detected in these four sample types (rhizospheres from the control, HA, drought group, and bacterial microbiomes of bulk soil from the control group).

Results And Discussion

Drought stress induces shifts of abundance in the profile of rhizosphere at phyla level

To explore the effect of drought to root-associated microbial communities, we applied drought treatment to the plants (45% SWC). Plant height of the drought group was obviously shorter than that of the control group during flowering stage (Figure 1b middle left); and weights of both the fresh (185.62 vs. 37.67, P-values= 0.003) and dry roots (117.48 vs. 14.41, P-values=0.017) are significantly heavier in the control group at the same stage (Figure 1b bottom and 1c). The composition of rhizosphere at phylum level caused by drought treatment differed significantly from that of the control (Figure 1d “Control Rhizo & Drought Rhizo”; Figure 1e “Control *vs.* Drought”). Consistent with previous reports in sorghum [9], the relative abundance of *Actinobacterial* (Drought, foldchange (FC)=2.31, adj.P-value=0.022), *Firmicutes* (Drought, FC=2.00, adj.P-value=0.022) were significantly enriched and the relative abundance of the *Bacteroidetes* (Drought, FC=0.75, P-value=0.025) and *Proteobacterial* (Drought, FC=0.62, adj.P-value=0.022) were significantly depleted in rhizosphere of drought group as compared to the controls (Figure 1e “Control *vs.* Drought”). In addition, compared with the control group, there were still some other microbials at phyla level significantly over-represented in the rhizosphere of the drought group, such as *Euryarchaeota* (FC=4.67; adj.P-value=0.022), *Parvachaeota* (FC=7.71; adj.P-value=0.088), *Chlorobi* (FC=1.63; adj.P-value=0.022), while some microbials at phyla level were significantly depleted, such as *Fibrobacteres* (FC=0.29; adj.P-value=0.022) and *Tenericutes* (FC=0.20; adj.P-value=0.022) (Figure 1e “Control *vs.* Drought”).

Enrichment and depletion of rhizosphere communities in dominant phyla under HA fertilization

To explore the hypothesis that HA has greater influence on the development of root microbiome than the control during flowering time, we utilized commercial compound fertilizer and organic HA fertilizer treatments. The HA group seemed to have better developed lateral roots than control during flowering stage, although there were no statistically significant weight differences of both fresh and dry roots between the two groups (Figure 1b bottom and 1c; fresh root, P-value=0.716; dry root, P-value=0.462). To better understand the progression that HA induces in root microbiome from rhizosphere, we sought to determine the microbiome from rhizosphere under HA fertilization. Phylum-level relative abundance of *Acidobacteria*, *Acitinobacteria*, *Bacteroidetes*, *Crenarchaeota*, *Cyanobacteria*, and *Proterobacteria*, revealed that the rhizosphere microbial communities exhibit a different composition compared with control rhizosphere microbial communities (Figure 1d, “Control Rhizo” & “HA Rhizo”).

To better identify the bacterial linages recruited by HA treatment relative to the rhizosphere in control conditions, operational taxonomic unit (OTU)-level rhizosphere and bulk soil microbial community were examined using Wilcoxon rank sum test with False Discovery Rate (FDR) < 0.1 as a cutoff [10]. Of the top

most abundant phyla based on numbers of OTUs, *Actinobacteria* (FC=1.79, adj.P=0.043) and *Cyanobacteria* (FC=2.33, adj.P=0.043) were significantly enriched in HA-treated rhizosphere compared with that of control treatment; whereas, *Bacteroidetes* (FC=0.70, adj.P=0.087) and *Crenarchaeota* (FC=0.42, adj.P=0.058) exhibited the lower preference for the HA-treated environment (Figure 1e "Control *vs.* HA").

The delayed responses of plants to drought stress may occur in HA fertilizer treatment

As shown in Figure 1d, the relative enrichment of bacterial microbial communities decreased in their abundance during drought treatment. However, that *Actinobacteria* show notably greater increase to the shifts from both HA and drought group to the control rhizosphere, compared with *Bacteroidetes* with decrease shifts (Figure 1d). Although the HA group was watered with the same amount as the control, the rhizosphere from HA group displayed the potential of resistance to drought stress by significantly recruiting more *Actinobacteria* (increased by 79%; average OTU number of HA (13.66) vs. control (7.62), FC=1.79, adj.P=0.043) and less *Bacteroidetes* (decreased by 30%, average OTU number of HA (13.75) vs. control (19.55), FC=0.70, adj.P=0.086), which showed similar pattern of the drought group with 131% increase of *Actinobacteria* and 25% decrease of *Bacteroidetes*, compared to the control rhizosphere (Figure 1d, e).

Conclusions

As a conclusion, we obtained the bacterial community for the V4 region of the 16S rRNA gene using Illumina Hiseq2500. By analyzing the sequencing data, we identified 40 bacteria phyla across samples. Strikingly, we found the diversity of the 12 bacterial communities at phyla level were significantly different in resistance to drought stress, two of which, *Actinobacteria* and *Bacteroidetes*,were also significantly enriched and depleted in HA fertilizer treated samples, respectively. The results suggested that the delayed responses of plants to drought stress in HA fertilizer treated soils might be through supply a resource of substance to recruit drought resistance microbiomes.

Methods

Plant materials and growth conditions

The experiment was conducted in Shandong Agricultural University farm. Before planting, the soil was treated with commercial compound fertilizer (normal fertilization) and commercial organic humic-acid fertilizer (HA fertilization). Then, Xianyu335, a commercial maize hybrid, was planted with three treatments, 1) control group with normal fertilizer and conventional growth condition, 2) humic acid group (HA) with HA fertilizer and conventional growth condition, and 3) drought group with the normal fertilizer as that in the control group and moderately drought growth condition (45% soil water content,

SWC). Each treatment has six replications. The soil was collected manually with sterile shovel from the farm and was allocated equally into pots of the three treatments before fertilization. Additional fertilization was performed at nine to ten leaves with one-fold stage until sample collection.

Sample collection and DNA extraction

Root samples were collected manually by extracting whole plants with root using sterile shovel to a depth of approximately 20cm ~ 30cm following the method described in Xu, et al., 2018 [9]. Sample collection was conducted during flowering stage. Roots were vortexed in sterile phosphate-buffered saline (PBS) buffer (Catalog No. E607008; Sangon Biotech, Shanghai, China) for 10 min at 4000 g and centrifuged to obtain rhizosphere soil pellet after removing the root tissue. DNA extraction was performed using the FastDNA™ SPIN Kit for Soil (Catalog No. 116560200; FastDNA™, Solon, OH, USA) following the manufacture's protocol.

Library construction and sequencing

After quality checking of the DNA sample, all the qualified DNA was used to construct 16S library. Briefly, the qualified DNA samples were amplified using dual indexed primer specific to the V4 region (515F (5'-GTGCCAGCMGCCGCGGTAA -3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3')), and the PCR product was converted into blunt ends with T4 DNA polymerase, klenow fragment, and T4 polynucleotide kinase. Then, after A tailing of 3' end of each fragment, adapters were added. Then, AMPureXP beads (Beckman-Coulter, West Sacramento, CA) was used to remove fragments that are too short. Finally, qualified libraries were sequenced with paired-end 250-bp reads using Illumina Hiseq2500.

Data processing

After sequencing, the data were cleaned by removing low quality reads and eliminating the adapter pollution with maximal 3 bases mismatch allowed. The paired-end reads with overlap were merged into tags using FastLength Adjustment of Short reads (FLASH, v1.2.11) [11] (Magoc and Salzberg, 2011) with minimal overlap length of 15bp and mismatching ratio of overlapped region less or equal to 0.1. Tags were then clustered into operational taxonomic unit (OTU) using USEARCH (v7.0.1.1090) [12]. In details, the tags were clustered into OTU with a threshold of 97% using UPARSE, and then obtained the OTU unique representative sequences, which were taxonomically classified using Ribosomal Database Project (RDP) Classifier (v2.2) [13]. Unassigned OTU and those not assigned to the target species were removed. For differentially expressed OTUs analysis, Wilcoxon rank sum test was used with False Discovery Rate (FDR < 0.1) [10] as cutoff.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Available of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XuerongY and JY designed the study. LZ, XiuweiY, MS, CK, YW, XL, YD, and XZ performed the experiments. XuerongY, YW, HL, JY analyzed the data, XuerongY, HL, and JY drafted the manuscript. All authors critically revised and provided final approval of this manuscript.

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Figures

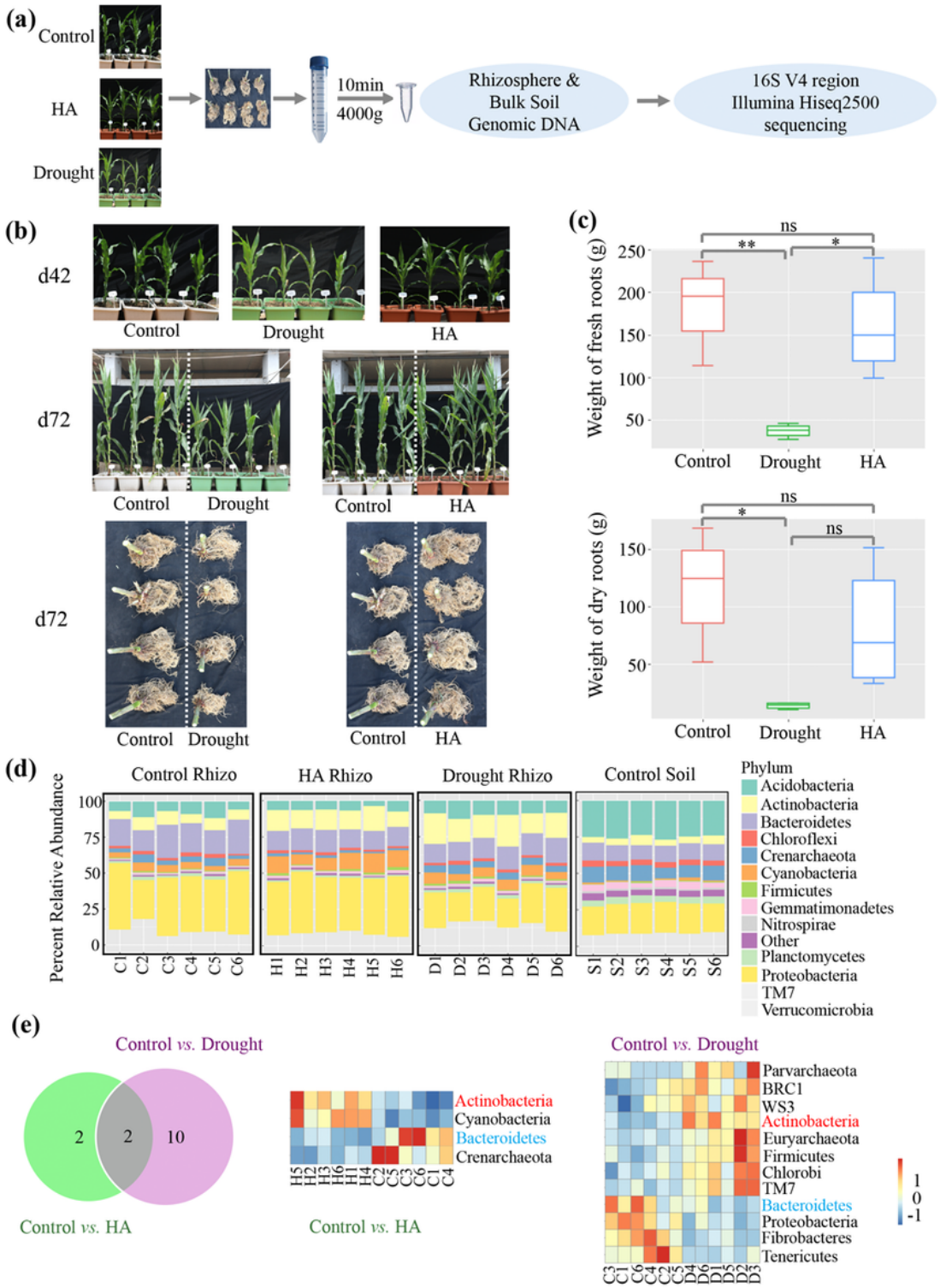

Figure 1

Humic acid (HA) is potentially resistant to drought stress in maize. (a) Flowchart of the study performed from the field work to the next generation sequencing. (b) The plant growth at days 42 (d42) and harvest at d72, as well as the roots harvest at d72 from control, HA, and drought groups. (c) Statistics of the weight of fresh and dry roots using R/Student's t-test between each two of the control, HA, and drought groups. ** indicates P < 0.01, * indicates P < 0.05, ns indicates "not significant". (d) Relative abundance of

the top 13 of the most abundant bacterial phyla. Percent relative abundance of the most abundant phyla for control, HA, drought treatments in rhizospheres and for control in bulk soils. All individuals were arranged in order within each group along the x axis. (e) Heatmap of the bacteria phyla significantly enriched in HA and drought rhizospheres, compared with the control rhizospheres. Venn diagram showed the bacterial phyla significantly represented in both HA and drought rhizospheres.