Single and combined abiotic stressors affect maize rhizosphere bacterial microbiota

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Abstract

Rhizosphere microbiomes are influenced by abiotic stresses, but we know a little about their response to combinations of stresses. In this study we tested: (i) if drought and heat stress influence the maize rhizosphere microbial community; (ii) if the combination of drought and heat has a different outcome compared to a single stress; (iii) if rhizosphere microbiota clusters according to root class and root zone. We setup a microcosm system using maize as model plant. We exposed plants to drought, heat stress and their combination, and used 16S amplicon-sequencing to reconstruct bacterial communities of different root classes (crown and primary) and root zones (apical, sub-apical and basal). We found both drought and heat affect the structure of rhizosphere bacterial communities. The combination of these stressors also influenced the structure of rhizosphere microbial communities, but this effect did not differ compared to the single stresses. Interestingly, we found differences in microbial communities inhabiting the rhizosphere of crown and primary roots in the control treatment, but this difference disappeared once stresses were applied. Stress also lead to an increased abundance of beneficial organisms.

Introduction

Abiotic stressors are a major limiting factor for crop production worldwide (Mantri et al., 2012; Wien 2020). Variation in water avail- ability and increasing of global air temperature are major abiotic stresses posed by climate changes (IPCC 2019). As result, the increase in air temperature and drought events are likely to become more frequent and severe (Spinoni et al., 2018). Recent development in plant micro- biome research highlighted the potential of plant-associated microbial communities in alleviating the negative effects of changes in water availability and air temperatures (Hussain et al. 2018; Naylor and

Coleman-Derr 2018; Saikkonen et al. 2020).

Plants play an active role in selecting their own microbiota and can recruit beneficial organisms in response to stresses, especially in the rhizosphere (Berendsen et al. 2012; Turner et al. 2013; Rolfe et al. 2019). Both drought (Naylor and Coleman-Derr 2018) and high air temperatures (van der Voort et al., 2016) can produce a change in the structure of rhizosphere microbiomes. For example, the rhizosphere of plants under drought stress is enriched with plant growth promoting bacteria mainly belonging to the classes Actinobacteria and Firmicutes (Marasco et al., 2012; Edwards et al., 2018; Fitzpatrick et al., 2018; Xuet al., 2018; Simmons et al., 2020). While drought can influence the rhizosphere microbiome directly (via reduction of available water) and indirectly (via the host plant), air temperature only influences the rhizosphere microbiome indirectly (via the host plant). These two different pathways are likely to produce big differences in rhizosphere responses; however, to date, the differential response of rhizosphere microbiomes to heat and water stress has not yet been determined. Furthermore, the combination of heat and water stress commonly occurs in field conditions, and this has an additive detrimental effect on plant growth (Pandey et al., 2017). However, the combination of drought and heat on rhizosphere microbiomes has yet to be examined.

The plant root system comprises different root classes, usually classified accordingly to their ontogenesis (i.e. primary, nodal, lateral), each one characterized by distinct developmental, physiological and functional signatures (Waisel and Eshel 2002; Hodge et al., 2009; Tai et al., 2016), and different responses to environmental stresses determining a large within-root phenotypic plasticity (Vescio et al., 2021). For example, different root classes vary in their response to nutrient deficiency (Rubio et al. 2004; Sorgonà et al. 2005; Sorgonà et al., 2007), allelopathy (Abenavoli et al., 2004; Lupini et al., 2016) and drought (Romano et al., 2013; Abenavoli et al., 2016). In addition to this diversification among root classes, roots show differences in function- ality in different root zones (Rubio et al., 2004; Sorgonà et al., 2010, 2011). Along the root axis, morphological and functional differences in root architecture reflect the relationship between roots and their environment, with variation in nutrient uptake, water transport, carbon exudation, proton/hydroxyl excretion and respiration (Hodge et al., 2009). Although the physiological and morphological differences between root classes and in different root zones are widely reported, their microbiome remains currently little explored.

In this study, we exposed maize plants to drought and heated air, alone and combined, and we characterized the rhizosphere bacterial community in three root zones (apical, subapical and basal) for two different root classes (primary and crown). We hypothesize

that drought and increased air temperatures influence the composition of rhizosphere bacterial microbiome differently due to the difference in direct and in- direct influences on bacterial microbiome composition, and this response will vary with root class and zone. We also hypothesize that the combination of drought and heat will produce a unique rhizosphere microbiome signature.

Material and methods

Study system

Soil was collected from the top 10 cm layer of an uncultivated field located at the Agricultural Experimental Station of the University of Reggio Calabria (38.08N, 15.68E – Tab. S1), and coarsely sieved (4 mm mesh). Pots were filled with a mix of 1 part collected field soil and 2 parts of quartz sand (Ø 1–2 mm, Croci Trading Company s.r.l., Italy, autoclaved for 3 h at 121 °C, allowed to cool overnight and then other 3 h at 121 °C). Maize seeds (genotype KXB7554, provided by KWS Italia S.p.A.) were surface sterilized with 20% bleach solution for 20 min and rinsed with deionized water 5 times. Seed germination was synchronized by soaking seeds in deionized water for 24 h and providing air flow through an air pump. We selected maize as model species because: (i) it is an economically important crop and a model species for research; (ii) it has been used as model for abiotic stress research; (iii) it has well-defined root classes and zones.

Experimental design and sample collection

We conducted an experiment testing the effects of two levels of water availability nested in two levels of air temperature on the diversity and composition of the rhizosphere microbiome. Maize plants (*Zea mays* L.) were exposed to two levels of air temperature (25 °C and 32 °C), and two levels of water availability (30% and 80% of soil field capacity, corresponding to a severe drought and no drought). The experiment was split into two blocks to account for the variability introduced by working with two climatic chambers. Each block contained 3 replicates and were temporally distinct: once the first block was harvested the second block was set up, and the air temperature treatments were inverted between the two chambers. The entire experiment yielded a total of 4 treatments (2 water

availability treatments × 2 air temperature treatments) × 6 (replicates) = 24 plants. Pairs of plants exposed to the same treatment in the two different blocks were grouped together, and for both we collected two root classes (primary and crown root) that were divided into three zones (apical, sub-apical and basal) yielding a total of 72 samples.

To start each block, after 24 h of soaking, 5 maize seeds were sown in 1 L pots filled with the soil mix. Three replicates of each treatment were then randomly distributed within one of the two climatic chambers (according to the temperature treatment) both initially set at 25 °C, 70% relative humidity and a 14:10 light:dark photoperiod and left to grow for 2 weeks. During this timeframe each pot was weighted every two days and watered to guarantee a minimum of 80% of soil field capacity. Fifteen days after sowing, plants were exposed to the 30% field capacity and 32 °C air temperature treatments (or both) for 7 days. Heat stress was applied by increasing the air temperature to 32°C (Hussain et al., 2019) in one climatic chamber. Drought stress was imposed at 30% soil field capacity (Hussain et al., 2019) by reducing water availability from 80% to 30% of the pot capacity (determined by weighing the pots) (Anderson et al. 2018) for plants in both climatic chambers. Control plant were kept at 25°C and 80% of soil field capacity. With preliminary trials, we determined the amount of water necessary to reach 30% and 80% of field capacity on the same soil used for this experiment. During the experiment, we maintained 30% or 80% of field capacity (according to the treatment) by weighting pots twice a day, calculating the difference in weight compared to our target (either 30% or 80% of field capacity), and compensating this difference with distilled water.

At the end of stress exposure, we measured photosynthetic rate and stomatal conductance to confirm that treated plants were actually stressed (see Supplementary material, Note S1). Plants were then removed from pots and gently shaken to remove bulk soil. The root system was divided into primary and crown roots. Each root class was then divided into three zones: apical (portion from the root tip to the first lateral root), subapical (following the apical portion, same length, but including lateral roots) and basal (same length as the others but excised starting from stem). To extract rhizosphere soil, root sections were put in a 2 ml tube containing 300 μl of lysis buffer (10 mM Tris, 100 mM NaCl, 10 mM EDTA, 0.5% SDS), and vortexed at maximum speed for 2 min (McPherson et al., 2018). Roots were then discarded, and rhizosphere samples were stored at —80°C before being processed using 16S rRNA metabarcoding procedures (Abdelfattah et al., 2018).

DNA extraction and library preparation

Samples were homogenized with the lysis buffer using two 1 mm ∅ stainless steel beads per tube, with the aid of a bead mill homogenizer set at 30 Hz for 2 min (TissueLyzer II, Qiagen, USA). Total DNA was extracted using a phenol-chloroform protocol. DNA quality and quantity were checked with a Nanodrop 2000 instrument (Thermo Fisher Scientific Inc., USA). DNA was extracted also from non-template control samples, where experimental samples were replaced by 100 μl of nuclease-free water, in order to account for contamination of reagents or instruments. PCR amplifications were performed in a reaction mixture containing ~20 ng of template DNA, 1X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, USA), 0.5 μM of 515 F and 806 R primers (Apprill et al., 2015; Parada et al. 2016), and nuclease free water was added to create a final volume of 12.5 μL. Amplifications were performed in a Master- cycler Ep Gradient S (Eppendorf, Germany) with an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, 50 °C for 60 s, 72 °C for 90 s and a final extension step at 72 °C for 10 min. All PCR reactions included three non-template control wells, where DNA was replaced with nuclease-free water to check for contamination of PCR reagents. PCR products were inspected for correct amplification on 1% agarose gel. PCR products were then purified with Agencourt AMPure XP kit (Beckman and Coulter, Brea, CA, USA), following the manufacturer's instruction. A short-run PCR was performed on purified samples in order to include the Illumina i7 and i5 indices using the producer's protocol (Nextera XT, Illumina, San Diego, CA, USA). Amplicons were purified again with Agencourt AMPure XP kit as reported above and their concentration was quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific Inc., USA). Samples were pooled together at equimolar rations and sequenced with an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using the 300 PE chemistry. No non-template control sample yielded a band after PCR, and the few reads retrieved from sequencing did not pass quality filtering.

Raw reads processing

De-multiplexed forward and reverse reads were merged using the PEAR 0.9.1 algorithm using default parameters (Zhang et al., 2014). Data handling was carried out using QIIME

1.9.1 (Caporaso et al., 2012), and we quality-filtered reads using default parameters, discarded chimeric sequences and binned OTUs with VSEARCH 2.14.2 (Rognes et al., 2016). OTUs coming from amplification of chloroplast DNA were discarded from the downstream analyses. Taxonomy was assigned to each OTU through the BLAST method by querying the SILVA database (v. 132) (Quast et al., 2012).

Data analysis

Data analysis was performed using R statistical software 3.5 (R Core Team, 2020) with the package *phyloseq* (McMurdie and Holmes 2013). Shannon index was calculated using the package *vegan* (Dixon 2003), and comparison among groups was performed by fitting a linear mixed-effect model to account for the nested design, specifying the formula ~ treatment * root_class * root_zone * (1|root_class/root_zone) + (1|temperature/water). Models were fit using the *lmer* () function under the *lme4* package (Bates et al., 2015) and the package *emmeans* was used to infer pairwise contrasts (corrected using False Discovery Rate, FDR).

Furthermore, we studied the effects of the same factors on the structure of the microbial communities using a multivariate approach. Distances between pairs of samples, in terms of community composition, were calculated using a Bray-Curtis matrix, and then visualized using Canonical Analysis of Principal Coordinates (CAP) procedure. Differences between groups were inferred through PERMANOVA multivariate analysis (999 permutations, stratified using the factors "root_class/ root_zone" and "temperature/water"). Pairwise comparisons were calculated using a custom script, correcting *P* values using the FDR method. We used the R package *DESeq2* (Love et al. 2014) to search for OTUs differentially abundant between each treatment groups and the control. First, we built a model using *treatment*, *root class* and *root zone* as factors, and then we extracted the appropriate contrasts (Stress/Control) for each treatment group. OTUs significantly more abundant in the stressed group were identified by filtering the contrast table by $\log_2$FoldChange>1 and $P_{adj} < 0.05$.

Results

The linear mixed-effects model analysis revealed that microbial rhizosphere Shannon

diversity only varied significantly with the interaction between treatment and root class (Table 1). Specifically, primary roots had a higher diversity than crown roots ($P = 0.003$, Fig. 1B), but this difference disappeared with reduced water availability and increased temperature ($P > 0.05$, Fig. 1B).

We used a multivariate approach (PERMANOVA) to test the influence of treatments, root class, and root zone on the structure of microbial communities, and we found similar results to microbial diversity. We found a significant effect of treatment ($P = 0.005$) and the interaction between treatment and root class ($P < 0.001$) on the structure of rhizosphere microbiome in our experiment (Table 2). In both primary and crown roots, we consistently found that the microbial community of stressed plants was different from control ones and, also, between drought and heat plants (Tab. S2 and S3). However, the rhizosphere microbial community of plants exposed to both single stressors (drought and heat) was not different from the one of plant exposed to their combination (Tab. S2 and S3). Furthermore, for each treatment group, we tested for differences between root classes. We found that the microbiota of primary and crown roots was different in control ($P = 0.001$), but not in any treatment ($P > 0.05$, Tab. S4).

Given the differential response of rhizosphere microbiome to plant stress, we took a closer look to the bacterial taxa that were significantly more abundant in stressed plant compared to the control group (Fig. 2). As general response among the three stresses, we found an increase of three bacterial groups: *Solirubrobacter*, *Massilia*, *Agrobacterium*. While we did not found a specific response to the drought treatment, the treatment with heated air increased the abundance of: *Blastococcus*, *Bosea* (2 OTUs), *Burkholderia*, *Caulobacter* (3 OTUs), *Conexibacter*, *Dactylospor- angium*, *Flavisolibacter*, *Leptothrix*, *Massilia* (5 OTUs), *Mesorhizobium*, *Micromonospora* (3 OTUs), *Niastella*, *Phenoylbacterium*, *Pseudomonas*, *Segetibacterium*, *Solirubrobacter* and 5 unidentified OTUs. On the other hand, when comparing the plant exposed to combined stress to the control, we found a higher abundance of *Rhizobacter*, and 2 unidentified OTUs. Furthermore, 11 OTUs were more abundant in both heat and combined stress treatments: *Acidovorax*, *Bryobacter*, *Massilia*, *Para- burkholderia*, *Pelomonas*, *Rubrobacter*, *Sphingomonas* (2 OTUs), and 3 unidentified OTUs. Two OTUs had a higher abundance in both heat and drought treatments: *Pelomonas* and *Parabulkholderia*.

Discussion

Here, we show that drought and heat stresses induce changes on maize rhizosphere bacterial microbiome. Previous studies, indeed, revealed the effect of various environmental stress on rhizosphere microbiome: drought (Marschner et al. 2005; Cherif et al., 2015; Nuccio et al., 2016; Naylor et al., 2017; Santos-Medellín et al., 2017; Fitzpatrick et al., 2018; Timm et al., 2018), metal-deficiency (Timm et al., 2018), shading (Timm et al., 2018), and nitrogen-deficiency (Allison and Martiny 2008; Roesch et al., 2008; Zhu et al. 2016). Our study supports the effects of plant stressors on rhizosphere microbial assemblages. Furthermore, we tested, for the first time, whether the combination of drought and heat stress produces a different outcome on the rhizosphere microbiome compared to the single stressors. While stress combination was different when compared to the control group, it was not different from the effects of single stressors.

Our results also showed that, in the control group, the rhizosphere of primary and crown roots is inhabited by different bacterial communities. Few previous studies, mostly based on total count and/or trophic strategy and/or culture-dependent techniques only, suggested that different root classes are associated with a different microbiome in the rhizosphere (Gochnauer et al. 1989; De Leij, Whipps and Lynch 1994; Marschner et al. 2005) and, in particular, Sivasithamparam et al. (1979) reported that maize adventitious and seminal roots have similar diversity of bacteria, but the adventitious roots have lower fungal diversity. The only paper focused on root classes soil-based microbiomes, reported nodal roots of *Brachypodium distacum* showing a different structure of bacterial and fungal communities compared to seminal roots (Kawasaki et al., 2016). However, Kawasaki et al. (2016) focused on non-stressed plants while our study included single and combined abiotic stress. Our results supported the differentiation of rhizosphere bacterial microbiome according to root classes. Interestingly, if we extend the same analysis to the stress treatments, the difference between root classes disappears. Currently, we have a very narrow knowledge on this topic, so it is hard to outline an explanatory framework for this effect. We are confident that future studies can expand our results and provide a mechanistic explanation.

Here we also tested the hypothesis that different root zones, within each root class, would be associated to different microbial communities. In our study, we did not found differences in microbial community composition between different zones of the same root class. Following the evidence that different root zones produce different exudates (Walker et al., 2003), we would expect to observe differences in microbial assembly along the root axis. To our knowledge, a single previous study tested the hypothesis that the rhizosphere

268 microbiome associated with different root zones would respond differently to drought
269 stress (Simmons et al., 2020), and they also found no differences between root zones.
270 Previous studies showed that rhizosphere microorganisms can quickly assimilate root
271 exudates, buffering their influence on rhizosphere microbiomes (Dennis et al. 2010). This
272 mechanism might explain our results, although we should also consider a possible caveat of
273 our study. The lateral roots that stem from sub-apical and basal zones have elongation
274 regions that are morphologically and functionally similar to the apical zone. The presence
275 of these lateral roots might have confounded differences in the microbiome composition
276 between the root zones we targeted.
277 While each stressor influenced the rhizosphere microbiome in a different way, the analysis
278 of microbial community highlighted that in the control group the microbial community
279 differed between crown and primary roots, but this difference was not found in any of the
280 stressed groups. Previous research found that plants can recruit beneficial microbes in the
281 rhizosphere in an effort to alleviate stress (Lareen et al. 2016), and this can be the
282 mechanism behind our observation. To test this possibility, we focused on the taxa that
283 become significantly more abundant as consequence of plant stress. Indeed, we found that
284 several microbial taxa that were differentially more abundant in our treatment groups are
285 actually associated to plant beneficial organisms: *Massilia* (Ofek et al. 2012),
286 *Solirubrobacter* (Yang et al., 2012; Franke-Whittle et al., 2015), *Burkholderia* (Sua´rez-
287 Moreno et al., 2012), *Caulobacter* (Luo et al., 2019), *Mesorhizobium* (Laranjo et al. 2014),
288 *Micromonospora* (Martínez-Hidalgo et al., 2015), *Rhizobacter* (Lugtenberg and Kamilova
289 2009), *Paraburkholderia* (Kaur et al. 2017), *Sphingomonas* (Khan et al. 2014, 2017).
290 Furthermore, we found a higher abundance of three genera, *Agrobacterium*, *Pseudomonas*
291 and *Acidovorax*, which are widely known host both pathogenic but also beneficial bacterial
292 species.
293 Our study brings a novel view to the ecology of plant-associated microorganisms. We
294 showed that root class is an important factor in shaping the rhizosphere bacterial
295 microbiome, and that the presence of plant stressors reduces the differences between root
296 classes. Although more studies on a large set of plant species and genotypes are necessary,
297 our results can contribute in increasing the predictability of plant- microbe relationship,
298 which is an important interaction for securing the productivity of our crops. More
299 generally, our results contribute to the knowledge on the effects of climate changes on
300 crops, showing that two of the major plant stressors caused by climate change influence
301 the plant-microbiome interactions. This has potential impact on the current trend of
302 crafting agricultural practices around a holistic vision of plants- microbe-environment

interactions.

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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Table 1

Results from the mixed-effect linear model testing the Shannon diversity index against treatment, root class, root zone and their interactions.

Factor	df	F	P
Treatment (T)	3	2.379	0.497
Root class (RC)	1	0.001	0.967
Root zone (RZ)	2	0.028	0.985
T x RC	3	16.171	**0.001**
T x RZ	6	5.118	0.528
RC x RZ	2	0.994	0.608
T x RC x RZ	6	5.874	0.437

Table 2

Results from PERMANOVA analysis testing the effects of treatment, root class, root zone and their interactions on the structure of maize rhizosphere bacterial communities.

Factor	df	R^2	F	P
Treatment (T)	3	0.065	1.616	**0.005**
Root type (RC)	1	0.012	0.953	0.491
Root zone (RZ)	2	0.026	0.987	0.451
T x RC	3	0.091	2.267	**0.001**
T x RZ	6	0.079	0.99	0.501
RC x RZ	2	0.036	1.358	0.065
T x RC x RZ	6	0.095	1.184	0.06

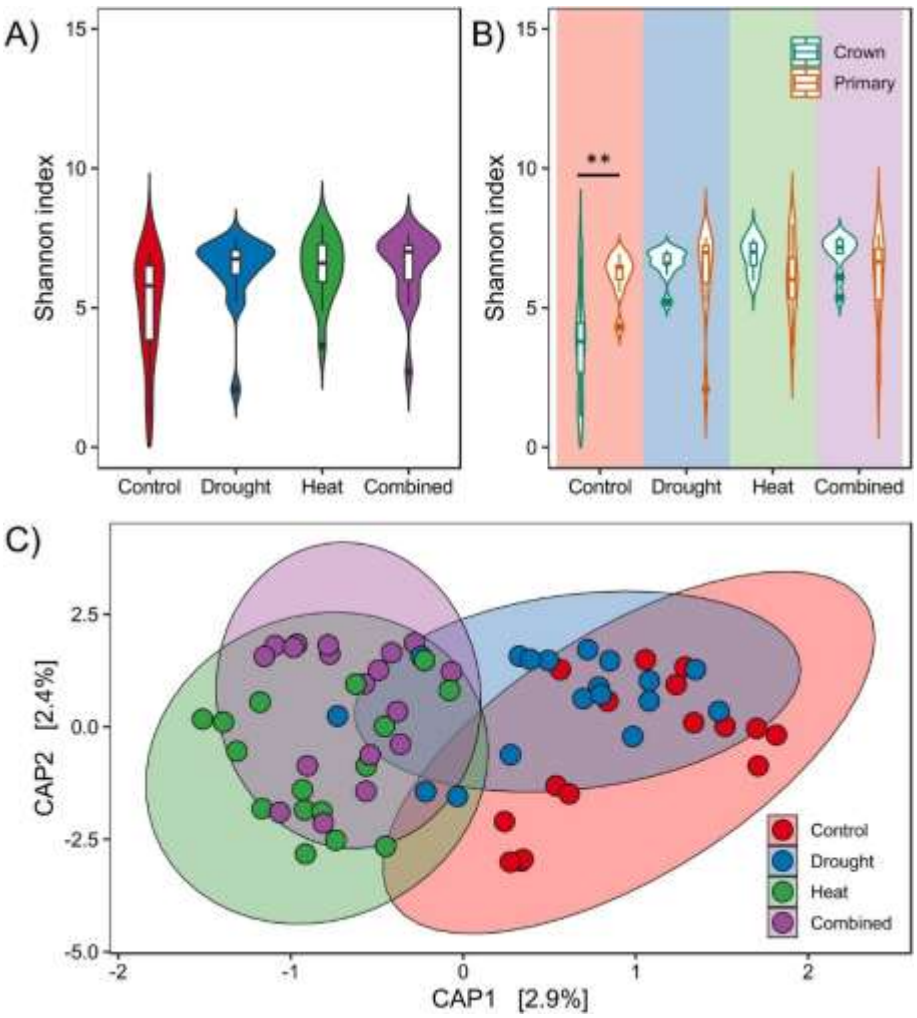

Fig. 1. Comparison of Shannon diversity index between (A) treatments and (B) root classes within treatments. (C) CAP (Canonical Analysis of Principal coordinates) ordination using a Bray-Curtis distance matrix of samples. $^{**}P = 0.003$.

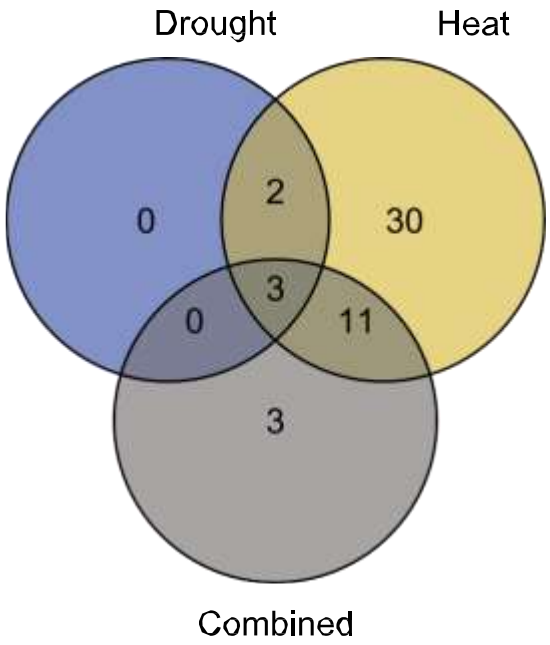

Fig. 2. Venn diagram representing the number of OTUs (Operational Taxonomic Units) differentially more abundant as response to specific stressors compared to the control group.

Single and combined abiotic stressors affect maize rhizosphere bacterial microbiota

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Supplementary material

Note S1. Supplementary results.

The photosynthetic rate and stomatal conductance was measured on intact leaves using the LI-COR LI-6400 system (LI-COR Inc.; Lincoln, NE) with a leaf temperature of 26 °C, a CO_2 concentration of 400 µmol(CO_2) mol(air)$^{-1}$ (controlled by CO_2 cylinder), an air flow rate of 500 cm^3 min^{-1}, and 1200 µmol m^{-2}s^{-1} of photosynthetically active radiation supplied by the LED light source. Each measure was taken between 120 and 200 seconds of waiting time. Between measures, the difference in the CO_2 concentration between the sample and the reference was matched to 50 µmol(CO_2) mol(air)$^{-1}$. The leaf to-air vapor pressure difference (VPD) was set to 1.5 kPa, and continuously monitored around the leaf during measurements and maintained at a constant level by manipulating the humidity of incoming air as needed. All measurements were performed inside a growth chamber. For each treatment we measured six plants, and for each plant we recorded the mean value of two measures on different leaves. Data was analysed using one-way ANOVA, and contrasts were inferred using Fisher's LSD post hoc test.

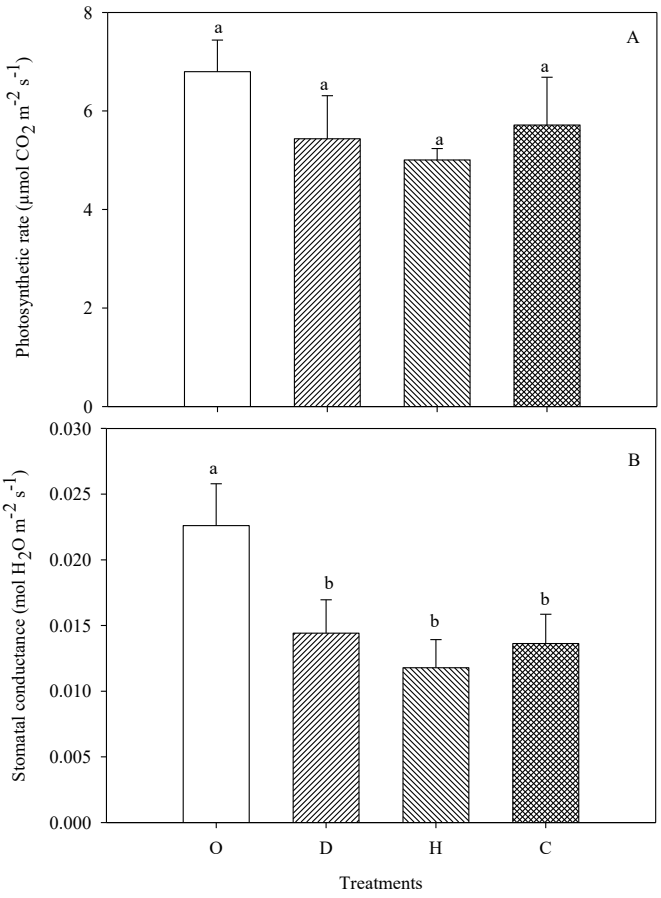

Figure S1 – Photosynthetic rate (µmol(CO_2) x m-^{2} x s^{-1}) and stomatal conductance (gs, mol H_2O x m^{-2} x s^{-1}) of maize plants exposed for seven days to drought (D), heat (H) and their combination (C). The control (O) was obtained in presence of optimal water and temperature (see Materials and Methods). Different letters indicate significant differences between treatments (Fisher's LSD test).

Results show that photosynthetic rate was not influenced by treatments (F=1.0780; df=3; P=0.38 - Fig. S1A), while we observed differences in stomatal conductance between control and all treatments (F=3.51; df=3; P=0.034 - Fig S1B). The stomatal conductance decreased by -20%, -26% and -16% in plants exposed to drought, heat and combined stress, respectively (Fig. S1B). Similar results were obtained in Hussain et al. (2019). This confirms that our treatments were successful in inducing stress to maize plants.

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Hussain, H.A., Men, S., Hussain, S. et al. (2019) Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Sci Rep 9, 3890. https://doi.org/10.1038/s41598-019-40362-7

Table S1. Soil physical and chemical characteristics

Soil texture	sand 36.0%, silt 32.0%, clay 32.0%
Bulk density	1.23 ± 0.04 kg/dm^3
pH_{water}	7.2 ± 0.2
pH_{KCl}	6.4 ± 0.1
Total organic Carbon (C)	19.3 ± 0.4 g/kg dry soil
Total Nitrogen (N)	1.8 ± 0.2 g/kg dry soil
C:N ratio	10.7
NH_4^+	17.1 ± 1.0 mg/kg dry soil
NO_3^-	13.0 ± 1.0 mg/kg dry soil
Olsen P	18.3 ± 2.3 mg/kg dry soil
Total $CaCO_3$	8.4 ± 1.0 g/kg dry soil
Active $CaCO_3$	3.9 ± 0.2 g/kg dry soil
Cation Exchange Capacity (CEC)	17.1 ± 1.7 cmol/kg dry soil
Electrical Conductivity (EC) *1:2* at 25°C	0.165 ± 0.004 dS/m

Table S2. PERMANOVA pairwise comparison between treatments in primary roots.

Pair	**FDR corrected *P* value**
Control – Drought	**0.005**
Control – Heat	**0.001**
Control – Combined	**0.002**
Drought – Heat	**0.03**
Drought – Combined	0.308
Heat – Combined	0.094

Table S3. PERMANOVA pairwise comparison between treatments in crown roots.

Pair	**FDR corrected *P* value**
Control – Drought	**0.006**
Control – Heat	**0.005**
Control – Combined	**0.004**
Drought – Heat	**0.03**
Drought – Combined	0.253
Heat – Combined	0.073

Table S4. PERMANOVA pairwise comparison between root classes (primary vs crown) within each treatment.

Pair	**FDR corrected *P* value**
Control	**0.001**
Drought	0.200
Heat	0.055
Combined	0.273