**(干湿稻)**

**Microbial load as a key feature of the microbiome alterations associated with drought stress (Drought increase the total microbial load relative to plant host)**

The drought effect has been observed across some plant species, including grass [2017.ISME. Drought and host selection influence bacterial community dynamics in the grass root microbiome], rice [2017.mBio. Drought Stress Results in a Compartment-Specific Restructuring of the Rice Root-Associated Microbiomes] and sorghum [2018.PNAS. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria], as well as soil types [文献]. These studies have shown that drought significantly altered the bacterial or fungal communities. Consistent with these published studies, the results of Principal coordinate analyses (PCoA) of Bray Curtis distances from Hainan filed showed that drought treatment impacted the rice root-associated bacterial communities rather than bulk soil communities (**Fig. 5a**). The first two axes explained 58.03 % of the variance, with the primary axis (37.4%) primarily distinguishing samples by compartment and the secondary axis (20.63%) by treatment. Similar effect of drought treatment on the bacterial community were also observed in Anhui filed (**Supplementary Fig. 9**). Higher levels of moisture in bulk soil is likely to weaken the drought stress on bacterial communities [2017. mBio. Drought Stress Results in a Compartment-Specific Restructuring of the Rice Root-Associated Microbiomes].

The total microbial load effect has been observed in the gut microbiome [2017.Nature. Quantitative microbiome profiling links gut community variation to microbial load; 2018. Keeping tally in the microbiome]. Vandeputte et al. found absolute microbial counts were three times lower in individuals with Crohn’s disease through quantifying microbial abundances in faecal material [2017.Nature. Quantitative microbiome profiling links gut community variation to microbial load]. We hypothesized that the root microbial load might be sensitive to drought stress. To test this, we calculated the total microbial load and compared them between drought treatment and control group. We observed that the total bacterial load relative to host plant showed approximately 1.6~4.3 fold change (*P* < 0.05) in abundance within drought-treated roots compared to control roots, with an exception on cultivar WYJ in Anhui filed (WYJ-AH, *P* > 0.05) (**Fig. 5b; Supplementary Fig. 10**). However, the major load trends for WYJ-AH were still consistent: the bacterial load is more abundant in drought-treated roots than in control samples. Contract to root samples, no significant variation within microbial loads were shown for bulk soil samples. In general, total bacterial biomass has been observed to go down under drought【Hueso S, García C, Hernández T (2012) Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biol Biochem 50:167–173； 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland.. 2017. Drought Stress and Root-Associated Bacterial Communities】, only few studies have observed bacterial biomass remains stable (Hartmann M, et al. (2017) A decade of irrigation transforms the soil microbiome of a semi-arid pine forest. Mol Ecol 26:1190–1206) or increase (Bouskill NJ, et al. (2016) Belowground response to drought in a tropical forest soil. I. Changes in microbial functional potential and metabolism. Front Microbiol 7:525). However, these cases are soil samples which usually used phospholipid fatty acid (PLFA) content to assess the bacterial biomass. In contrast to prior published results, root samples from our field experiment exhibited an absolute increase in bacterial abundance following drought treatment in both cultivars and both filed sites. Detection methods、compartments and intensity of drought treatment may be responsible for the inconsistence.

**Compare the drought-induced shift in root microbiome using RA and AA method**

To assess the influence of detection methods on the patterns of differential taxa abundance between drought and control treatments, we compare the enrichment and depletion profiles based on the RA and AA datasets at the phylum and OTUs levels.

For bulk soil samples, no significant abundance shifts of dominant phyla or proteobacterial classes were detected between drought stress and control samples using either RA or AA methods, which was in consistent with previous reports [2018.mBio.Drought Stress Results in a Compartment-Specific Restructuring of the Rice Root-Associated Microbiomes]. For root-associated communities, soil type, rice cultivar and quantitative methods seemed to affect the outcome of differential phyla (**Fig. 5c; Supplementary Fig. 11**). Considering the results from RA methods, increase or decrease in relative abundance after the drought treatment were roughly similar regardless of cultivar or soil type.In contrast to prior published results [2017. Drought and host selection influence bacterial community dynamics in the grass root microbiome; 2017. mBio. Drought Stress Results in a Compartment-Specific Restructuring of the Rice Root-Associated Microbiomes], root samples showed no significant increase in relative abundanceof *Actinobacteria* or *Chloroflexi*. As expected, method changes affected the detections of differential phyla. Taking MH63 as illustrated examples, most of indistinctive phylum (eg., *Actinobacteria*) and dominant classes (eg., *Alphaproteobacteria* and *Betaproteobacteria*) were detected as significantly enriched groups under drought in both filed sites. On the other hand, *Chloroflexi*, *Gammaproteobacteria* and *Verrucomicrobia* identifiedas depleted groups under drought using RA was found indistinctive in Hainan filed, or even enriched in abundance relative to host in Anhui filed when using AA method. To a great extent, this alternation signal is mainly driven by an overall increasement in total bacterial load. However, some differential phyla identified by RA and AA also existed overlaps within the same filed or cultivar, eg., *Nitrospirae* and *Spirochaetes*, both significantly depleted under drought in Hainan filed regardless of cultivar.

Similar trends were also observed in WYJ. Notably, we even observed the inconsistent directions of the responsed, potentially resulting from the supreme compositionality effects. Phylum *Actinobacteria* was detected as depleted group in root samples of WYJ in both fields when applying RA method, which may be a result of an absolute decrease in their abundance, or an absolute increase in other taxa. After calibrating the data through AA methods, the abundance of *Actinobacteria* did not significantly decrease; Conversely, *Actinobacteria* in drought samples significantly enriched (*P*<0.05) at the phylum level in Hainan filed. More than that, specific classes and families within *Actinobacteria* also responded in the opposite direction when comparing two testing results. This observation highlights the influence of the total microbial load, which have been further responsed at the OTU level.

To demonstrate the potential of AA approach on higher taxonomic rank, we compare the differential taxa confirmed by RA and AA at the OTUs level. Detailed comparison of drought-associated microbiome showed that the major taxonomic trends were roughly consistent in both methods: *Deltaproteobacteria* and *Betaproteobacteria* were depleted under drought stress, whereas *Actinobacteria* and *Alphaproteobacteria* were enriched (**Supplemental Fig. 12**). The similar trend resulted from a considerable overlap between differential OTUs identified by RA and AA methods. According to the venn results (**Fig. 5d; Supplemental Fig. 13**), more significantly depleted OTUs were detected using RA or more enriched OTUs were detected using AA, indicating a potentially misjudgment of the drought-responsive OTUs numbers without considering microbial load. Although RA method did therefore permit discrimination between drought treatment and controls, AA method still detected noteworthy discrepancies which may be associated to the total microbial load relative to host. As shown by three illustrative examples inMH63 from Hainan filed **(Fig. 5e),** OTU11 is significantly depleted in dry root samples only using RA method, which is missing when using AA. Conversely, OTU16 is detected as enriched group responsive to drought only using AA. We even observed the opposite response using two methods: OTU13 is significantly enriched in drought root samples using RA but depleted using AA. Considering the discrepancy resulted from RA and AA method, we should cautiously interpret the microbial community dynamics or design further experiments based on solely the differential taxa produced by relative abundance, as it could be misleading due to compositionality 【文献.2017.microbiome; 2018. Nature; 2017, NAR】.

**真菌数据和细菌数据合表，计算真菌和细菌比 （细菌：真菌= 3:1）**

We amplified the internal transcribed spacer 1 (ITS1) to characterize the influence of drought stress on the fungal communities associated with rice roots. Due to poor taxonomic annotation for ITS sequences, we restricted our analyses to exploit the effect of drought treatment on the fungal community structure and the associations between bacteria and fungi.

PCoA with Bray-Curtis dissimilarities revealed that the fungal community in Hainan was significantly affected by drought treatment (P值？), but not evidently in Anhui (**Supplemental Fig 14**), which is probably due to high spike-in coverage in Anhui fungal library. For spike-in reads accounted for more than 80% of total reads in bulk soil and MH63 root samples from Anhui filed and possibly introduced wrong assessment, we had to remove the related data from our analysis and only restricted our further analyses to samples from Hainan field and WYJ root samples from Anhui, in which the coverage of spike-in was below 70%.

Similar to the calculation of the bacterial load, we quantified the fungal load for each cultivar in Hainan and for WYJ in Anhui. The fungal load is significantly more abundant in drought-treated roots than in control samples in both cultivars in Hainan filed (*P* < 0.05), as well as WYJ in Anhui (**Supplemental Fig 15)**. The ratio of bacterial to fungal ribosomal content was determined thought merging both calibrated bacterial and fungal OTUs tables using AA approach. As shown in **Fig. 5f**, two cultivars MH63 and WYJ in Hainan filed have similar ratio of bacteria to fungi in root regardless of drought treatment: Bacteria contributed to 75.8% (max. %, min %) and fungi to 24.2 % (max. %, min %) of the root-associated microbiota without considering archaeal and other non- fungal eukaryotes. This ratio is far below those detected in Hainan filed soil, which is approximately 28:1 (control) and 44:1 (drought treatment), respectively (**see Supplemental Table ?**). The soil value can be compared with published results, which showed that the ratio of bacteria to fungi ranged from 12:1 [2018. Microbiome. Absolute quantitation of microbiota abundance in environmental samples] to 32:1 [Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants.]. The elevated ratio of bacteria to fungi in root 说明了什么问题? 如果样品中存在很多低丰度的序列，提高OTUs聚类标准（本研究用的是30条序列聚成一个OTUs）可能会过滤掉很多序列，无形中降低细菌/真菌的比例。

**(根腐病)**

**Quantitative microbial profiling method reveals absolute increase of total microbial load in the wheat roots infected by *Bipolaris sorokiniana***

【小麦根腐病简介及研究目的】

Common root rot is a disease of winter wheat mainly attacked by *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*)*,* which caused a series of symptoms include dark brown to black lesions on roots, subcrown internodes and stem bases, finally resulting in yield losses [2018. Spatial Distribution of Root and Crown Rot Fungi Associated With Winter Wheat in the North China Plain and Its Relationship With Climate Variables; 1998. Common root rot and Fusarium food rot of wheat]. To demonstrate the utility of the spike-in for quantitative assessment of microbiota variation which is associated with diseased or healthy host plant, we performed spike-in based quantitative method on wheat root samples including ten healthy samples and ten infected samples with Common root rot (see Supplemental Fig ?).

【传统PcoA结果 & AA定量microbial load 结果】

According to traditional PCoA on the basis of microbiome，we found both bacterial and fungal communities associated with root rot disease significantly different from healthy communities (**Fig 6a，6b)**. For bacterial communities, a clear separation of root samples distinguished by healthy state were observed on the PC2 coordinate, explaining 13.5% of the variance. For fungal communities, healthy samples and infected samples were obviously separated on the first two ordination axes, totally explaining 44.6% of the variance. These results indicated fungal community were more affected by root rot disease than bacterial community. Although PCoA could permit distinguish samples based on the RA dataset, it might omitted the microbial abundance as potential feature of the microbiome alterations associated with disease, which has been identified in Crohn’s disease【2018.Nature 文献】. Quantitative microbial profiling method reveals bacterial load were approximate two times higher in the infected root samples. Compared to bacterial community, fungal load increased four times higher in samples with root rot disease (**Fig 6c**, *P* < 0.05), resulting in decreased ratio of bacteria to fungi. Notably, the disease-associated pathogen *Bipolaris sorokiniana* (OTU45) abundance increased two-fold when applying RA, but increased almost seven fold relative to plant when using AA based calibration. Taken together, these observations…….

【如果有网络数据就可以比较得病和健康，RA和AA的数据 4个网络图4个结果】