**HOME 页---------------------------------------------------------------------------------------------------------------**

CCDL&Weather

**Brief Introduction**

Large scale campus is sociologically and biologically defined as a semi-open community, consisting of school gate, teaching buildings, school service buildings, living quarters, roads and other facilities of varying sizes similar to society. Stressors such as temperature and the population density differ among the surface of functional partitions in campus, having potential associations with mirco-environment. However, there still lacks ystematic and comprehensive researches in campus microbiome. Our website provides models and pipelines to reveal the myth of campus mircobiome, bridging the blank space between macro-environment to micro-environment students exposed to. We were especially interested in and tried to address the following questions: (i) Does campus microbiome exert seasonal alteration and how? (ii) Does the density of population influence the microbial communities? (iii) Does campus microbiome feature robustness at the interface with the outside?

**Our values**

Microbiome makes a difference Data tell story Insights worth sharing

**Results（做成滚轮可切换的模式）**

**Seasonality:**

Seasons.gif

Campus microbial composition showed seasonality, with typical biomarkers presenting marked cyclicity during six seasons. Species-species network was applied to reconstruct the inter-species interactions within microbial community. The integral pattern of campus microbiome did not show detectable difference among seasons, while the relative abundance of some components varied markedly.

**Composition versus Function:**

Comfuncompare.gif

Campus microbiome performed considerable robustness against outside disturbances, serving as an independent functional unit that would closely interact with environment. However, the player may change, but the game remains: the compositional pattern of campus microbiome varies in different sites within campus, however, the congruent functional similarity indicates the relative robustness of the integral functions microbiome exert.

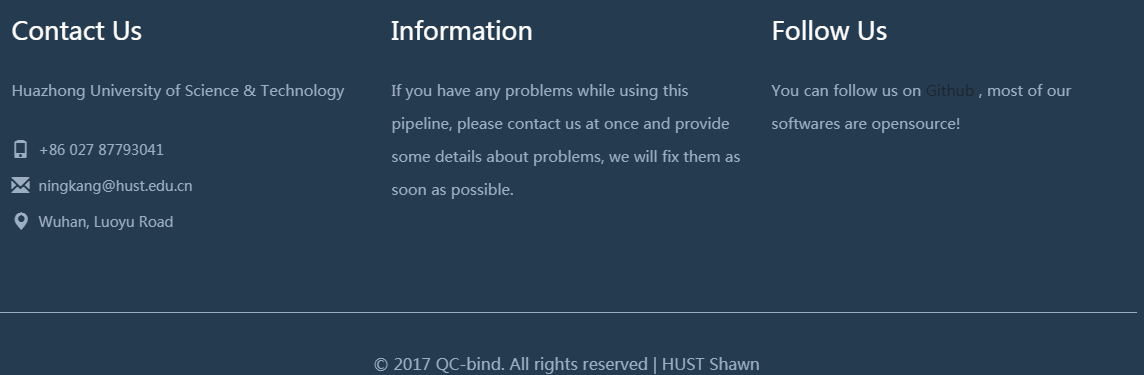
Effectors.gif

**Multiple effectors:**

We examined the ESs (effect size) of seasons, human effects and temperature on the microbial structure and function. Human effects (ES=0.167) and temperature (ES= 0.176) contributed approximately to microbial structure, while season exerted less contribution (ES= 0.097). As for microbial functions, similarly, the influences of human effects (ES=0.139) and temperature (ES=0.130) were stronger than that of season (ES=0.096).

**Our service的部分先去掉吧，暂时没有内容**

每一个页面都有：



只保留Contact us（可以重新拍一下版）然后最下面一行的文字改成：

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Project 页-------------------------------------------------------------------------------------------------------

Project下面的下拉菜单共分为4个部分：Introduction Results Methods Reference

点击每个部分都可以切换到相应的内容

**Introduction >> Map of HUST (麻烦找一个Google地图,华中科技大学的链接？)**

Microbial ecology plays a significant role in biogeochemical cycle[[1](#_ENREF_1)], as well as regional nutrient cycle such as microbes in soil[[2-4](#_ENREF_2)], marine[[5](#_ENREF_5), [6](#_ENREF_6)], lake[[7-9](#_ENREF_7)] environment and etc., the composition of which varies under marked pressure of urban stress[[10](#_ENREF_10), [11](#_ENREF_11)], climate[[12](#_ENREF_12)] and geomorphology [[6](#_ENREF_6), [13-15](#_ENREF_13)]. With the development of sequencing technology[[16](#_ENREF_16)], the detection and identification of massive microbes in diverse biological niches have been possible. General profiles of and the interactions between microbiome and its living conditions have been widely reported, suggesting its potential to influence human health[[17](#_ENREF_17), [18](#_ENREF_18)]. A longitudinal analysis of microbial interaction between humans and the indoor environment indicated that human effects can largely shape the microbial pattern in house surface of occupants[[19](#_ENREF_19)]. Similary, microbial pattern influenced by population density within boroughs [[11](#_ENREF_11)].

Large scale campus is sociologically and biologically defined as a semi-open community, consisting of school gate, teaching buildings, school service buildings, living quarters, roads and other facilities of varying sizes similar to society. Stressors such as temperature and the population density differ among the surface of functional partitions in campus, having potential associations with mirco-environment. However, there still lacks ystematic and comprehensive researches in campus microbiome.

In this study, HUST (Huazhong Univ of Sci & Tech, along with several campuses in Wuhan) served as a model, offering us a unique landscape of campus microbiome and its interactions with surrounding ecosystems. We were especially interested in and tried to address the following questions: (i) Does campus microbiome exert seasonal alteration and how? Seasonal factor and other climatic factors were considered to investigate the chronological dynamics of microbiome. (ii) Does the density of population influence the microbial communities? Samples were collected according to different partition types in HUST. Classroom, Canteen, Dorm and Library were classified “CCDL” which routinely accommodates a densely populated flow from the east to the west of the campus. Bus stations, sports fields, clinics, gates and hotels, which are typical of non-routine areas for students in campus. Samples from hills and lakes were categorized in another group featuring sparse population in most of times. (iii) Does campus microbiome feature robustness at the interface with the outside? Samples from school gates, clinics, bus stations and other campus in Wuhan were also collected examine characteristic consistency of campus microbiome.

Map

Results

这一部分一共有 个副标题，希望可以 采取折叠菜单的形式，点击副标题再出现正文的内容

***General profile of campus microbiome***

The RA (relative abundance) profile for campus microbial composition of each samples was generated according to seasons (from the winter of 2015 to the spring of 2016). 110,998 unique high quality reads per sample (31,523,358 reads in total for 284 samples) were obtained after quality control. Total 541,981 OTUs were then assigned at family level. The pattern of microbial RA within each season showed comparable homogeneity. Notably, the relative abundance of some families characterized dominance in a certain season, such as Acetobacteraceae (Average RA=0.093, Autumn) generally known as acetic acid bacteria.

General

***Seasonality of campus microbiome***

Marker

Campus microbial composition showed seasonality, with typical biomarkers presenting marked cyclicity during six seasons. For example, *Erwinia* (LDA= 3.964, p=2.07E-19, plant pathogenic species) was the biomarker of spring, *Flavobacterium* (LDA= 4.681, p=3.46E-24, freshwater fish pathogen) and *Acinetobacter* (LDA= 4.364, p=7.80E-07, soil mineralization) were the two for summer, while *Chryseobacterium* (LDA=4.062, p=9.65E-14, cold tolerance) was found to indicate the duration of winter.

Seasons.gif

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Species-species network was applied to reconstruct the inter-species interactions within microbial community. The integral pattern of campus microbiome did not show detectable difference among seasons.

The sample similarities (Euclidean Distance) of the microbial structure among six seasons performed seasonal alteration with fitted curve of SIN function. Interestingly, average temperature among different sampling sites also featured cyclical variation (R2=0.8236).

SINtemperature

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To further understand the potential drivers of the campus microbial seasonality, we examined the correlations between sample similarities and other climatic factors including humidity, UV intensity and barometric pressure. Similarly, average UV intensity at Wuhan area showed seasonality, but comparable positive correlation to sample similarities (R2=0.115) owing to the phase shift.

UVco

And barometric pressure presented obvious cyclicality through seasons, negatively correlated to sample similarities (R2=0.666 ).

Pressureco

However, there was no obvious cyclicality behavior of average humidity in the area, and the correlation between humidity and sample distance over seasons was weak.

Humidityco

***Campus microbiome exerts independent stability***

The dispersal influence of human activities on microbial community in residences has been reported at city-scale[[21](#_ENREF_21)], and the effect at campus scale was explored in this study. In HUST, roads connecting CCDL (Classroom, Canteen, Dorm and Library) accommodates a routinely large flux of students, while other roads feature lower (Roads contacting hills and lakes ) or non-routine (Roads contacting Clinics, Gates, Hotel, Bus Station, Sports Fields) human density which offered us a unique model for analysis.

CCDLHL

The PCA analysis of the microbial composition did not show integral distinction among CCDL, hill and lake (R2=0.129 , p=0.001 ). However, the compositional difference was detectable between lakes and hills, the principle compositions of which overlapped with partial distribution of CCDL’s respectively. The gradual shift of microbial composition seemingly explained the dispersal influence of human activities on campus mirobiome, since lakes were in the vicinity of CCDL comparing to hills.

***Human activities influence campus microbiome***

Campus microbiome performed stability against outer stress. School gates and hotels were regarded as interface between inside and outside of campus, harboring disturbances from the exchanges of people, vehicles and goods. PCA analysis demonstrated no taxonomic difference between school hotel or gate and others, as the inner taxonomic difference of ‘others’ was more distinguished. As to functional composition, the primary composition of the two comparisons showed coherent convergence.

HGcompare

Then we focused on contrasting the taxonomic and functional compositions of CCDL to those of gate or hotel. After curtailing sample range from all other sites (except for school gate and hotels) to CCDL, we noticed a more compressed and congruent distribution of microbial between CCDL and hotel or gate. The functional analysis of the two comparisons still showed no significant difference, which reconfirmed the robustness of campus microbiome. Moreover, these results corroborated our former statement in a cogent way that intensive human activities had dispersal influence on campus microbiome structure, since the common feature among CCDL, school gates and hotels was high human density and populational mobility.

CCDLHGcompare

A concordant pattern of microbial compositions among Samples from CCNU (Central China Normal University), HAU (Huazhong Agriculture University), WHU (Wuhan University) and HUST was also revealed, except for samples from HUST, which featured more sporadic distribution taxonomic composition (**Figure 4(E)**). And the consistence of functional composition among the four universities was even higher (**Figure 4(J)**), comparing to the one of taxonomic composition. The consistence tended to go with the endemic feature of campus in Wuhan. Located in Wuhan, intersected by a network of rivers and hills, the four universities had the similar landscape distribution within campus: artificial or nature lakes, small hills and extended vegetation coverage as well as the arrangement of typical campus buildings (**Figure X(A)**). Besides the above geological and social characters, concurrent seasonal climate factors (eg: temperature and humidity) and routine fluxes of population added up to the taxonomic and functional consistence among the four universities.

Sch4

***Multiple effectors of the functional microbial structure***

Campus microbiome is exposed to time dimensions, human effects and environmental stresses (eg. temperature), as a relatively enclosed community. We examined the ESs (effect size) of these potential drivers (**Figure 5(A)**) on the microbial structure and function. Human effects (ES=0.167) and temperature (ES= 0.176) contributed approximately to microbial structure, while season exerted less contribution (ES= 0.097, **Figure 5(B)**). As for microbial functions, similarly, the influences of human effects (ES=0.139) and temperature (ES=0.130) were stronger than that of season (ES=0.096, **Figure 5(C)**).

multieffectors

Methods

***Sample processes and sampling sites***

For investigating the bacterial community feature differences among variable seasons and sampling sites within campus, samples were collected from December 20th 2015 to \*\* 2017 in HUST(Huazhong University of Science and Technology) campus across six seasons. Sample collections were implemented every other day for three times seasonally during consecutive sunny days to eliminate unexpected deviation. To ensure sufficient replicates and plenty of bacterial quantity acquired from the habitat areas, 5 sterile swabs pre-moistened with 0.15M saline solution was applied to scratch against the surface of each sampling site about 3x10-9 km2in similarly serpentine way. Then 5 swabs from the same sites were put together into a sterile tube and stored under -80 oC for subsequent treatment.

Sampling

For each site, 3 samples are to be collected at the different time. And for each site a specific time, a mixture including samples collected at 5 points for the same location are to be generated to represent the sample for that specific time and site.

Since there are two infirmaries only, we cannot plan three sites to gather nine infirmary samples in every season, and we plan to sample the infirmary in the middle HUST campus (FigureXXX D1) as well as the east campusonly(Figure6 D2). Scrubbing all the trail of the human beings, microbes, and other organisms, an unprecedented heavy rain lasted for a week in the summer of 2016 had stooped us from sampling, and we collected one replicate of samples before the heavy rain only. But the summer, three replicates were sampled in every season every other day for each site. Besides, the playground of the west campus has been under repairing throughout the fall (FigureXXX H1).In totally,10 samples were sampled from Wuhan University(WHU), Central China Normal University(CCNU) and Huazhong Agricultural University (HZAU) with the same method we used in HUST, in the Autumn of 2016, on 23 September. All the three campus, WHU, CCNU and HAU are in Wuhan city, and in different distance between each other as well as the HUST campus. Except the CCNU campus, there are at least one lake in the campus that we did sampled. One of the school campus which was littler closer to the CCNU campus, with a six-lane street to separate each other.

***DNA extraction and 16S rRNA sequencing***

To obtain high-molecular-weight metagenomic DNA,a modified CTAB method was chosen[[26-28](#_ENREF_26)]. Afterthe front end of the swabs were cut into small strips into small strips to 50ml centrifuge tube with sterilized surgical scissors, we add 5 ml lysis buffer (Cetyl Trimethyl Ammonium Bromide, 1% w/v; EDTA, 100mM; NaCl, 1.5mol l -1; Sodium phosphate, 100mmol l -1; Tris-Cl pH 8.0, 100mmol l -1 ). 20 µl Proteinase K was added to the reaction mixture followed by gentle shaking at 100rev min -1. SDS was added to a final concentration of 1% and the reaction was incubated at 65°C for 30min with intermittent shaking. After above steps, an equal volume of saturated Phenol, chloroform and isoamyl alcohol (25: 24:1) was added to the mixture and centrifuge at 12000 rev min -1 (12114g) for 10min to collect supernatant that free from protein, repeat again. Metagenomic DNA was precipitated with 0.6 volumes of isopropanol for 30min at -20 °C and pelleted by centrifugation at 12,000rev min -1 (12114g) for 10min. DNA was washed twice with 70% ethanol and finally dissolved into a 200µl of TE (1X), pH 8.0.

Before sequencing with Illumina Miseq PE300, DNA samples were quantified using a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and its’ quality was checked on a 0.8% agarose gel. To amplify the V4-V5 variable region of 16S rRNA genes for each individual sample with "5`-GTGYCAGCMGCCGCGGTAA-3`" as the forward primer and "5-CTTGTGCGGKCCCCCGYCAATTC-3`" the reverse[[8](#_ENREF_8)], 5-50 ng metagenomic DNA in high quality was used as the template.DNA library for sequencing was constructed using a MetaVxTM Library Preparation kit (GENEWIZ, Inc., South Plainfield, and NJ.USA). And then, indexed adapters were added to the ends of 16S rDNA amplicons by limited-cycle PCR. Verified by Agilent 2100 Bioanalyzier(Agilent Technologies, Palo Alto, CA, USA), and quantified by Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and real-time PCR(Applied Biosystems, Carlsbad, CA, USA) , the DNA libraries were normalized for sequencing. Using paired-end sequencing technology (2\*300 bp), the followed sequencing reactions were performed on the Illumina MiSeq platform.

***Quality Control, OTU clustering, and taxonomy assignment***

Processed according to the following criteria, and sequences of 16S rRNA gene below the set quality were removed and be absent from the subsequent analyses. At the beginning, ‘make.contigs’ command in the mothur (version 1.38.1)[[29](#_ENREF_29)] was chosen for splicing the paired-end reads with default settings. After that, all reads that longer than 500bp and shorter than 300, or containing ambiguous base calls(N), were removed. The SILVA database[[30](#_ENREF_30)] was acted as reference when identifying putative chimeras using ‘chimrea.uchime’ command in mothur, before the putative chimeras was removed with ‘remove.seqs’ command in the mothur. After that, high-quality sequences were aligned using PyNAST[[31](#_ENREF_31)] and dereplicated using UCLUST in QIIME(Quantitative Insights Into Microbial Ecology, Bould, CO, USA, V1.9.1)[[32](#_ENREF_32)]. Then, the Greengenes database[[33](#_ENREF_33)] (version 13\_8)was used as the refrence database for operational taxonomic unit(OTU) classification(97% nucleotide identity, pick de novo OTUs). To remove singletons OTUs, the minimum reads per OTU threshold was set at 0.001%.

The functionality of the different metagenomeswas predicted using the software PICRUSt 1.1.0, which allows the prediction of functional pathways from the 16S rRNA reads. First, a collection of closed-reference OTUs was obtained by using QIIMEpick\_closed\_reference\_otus.py script in default settings. The output table was normalized based on the predicted 16S rRNA copy number by using the script normalize\_by\_copy\_number.py. Final functional predictions, inferred from the metagenomes, were created with the script predict\_metagenomes.py. When necessary, tab-delimited tables were obtained with the script convert\_biom.py

***Microbial diversity assessment***

Bacterial alpha- and beta-diversity values were determined using the QIIME[[32](#_ENREF_32)] pipeline. For alpha-diversity, rarefaction curves were draw based on the richness metric: “observe OTU”, and evenness metric: Shannon[[34](#_ENREF_34)] evenness metric. Core-OTUs were defined as a set of OTUs that were identified in all samples analyzed. For the beta-diversity analysis, Euclidean distance, weighted and unweighted UniFrac distance metrics[[35](#_ENREF_35)] were used to measure community similarity between samples. Bacterial community clustering was arrayed by Principle Coordinate Analysis(PCoA) and visualize dusing Emperor[[36](#_ENREF_36)] in QIIME. The hierarchical clustering method, was applied to cluster all samples Pheatmap was used to visualize Spearman Correlation between different samples. To fully analyse there lationship between different factors and microbiome community, Mantel test was used to calculate and Circos was used to visualize.

***Biomarker analysis***

Linear discriminate analysis(LDA) effect size (LEfSe)[[37](#_ENREF_37)] was used to find out the biomarkers that can act as microbial signature of the divided group in this research. Specifically, tables with the group information and the taxa abundance in different taxonomic levels was imported into the LEfSe pipeline and the parameters was set as follows: the alpha value for the factorial Kruskal-Wallis test between classes and the p-value for the pairwise Wilcoxon test between subclass were both 0.05. For discriminating the features, the threshold for the logarithmic LDA score was 2.0fordefault.

***Co-occurrence Network Analysis***

To reduce false-positives caused by excessivemutualexclusions, OTUs that exist in all samples, with the average relative abundances intop150were selected. Based on the relative abundance of these OTUs, Spearman Correlation similarity matrix were used and calculated as described in the literature[[3](#_ENREF_3), [7](#_ENREF_7), [22](#_ENREF_22), [38](#_ENREF_38)]. The cutoff of SpearmanCorrelation Coefficient value was set at 0.6 and the filtered co-occurrence matrix was visualized by Cytoscape[[39](#_ENREF_39)](version 3.5.1). ClusterVizapp[[40](#_ENREF_40)] with EAGLEalgorithmindefaultparameterwas used to identify the cluster(module) in the network. Network nodes and edges represent OTUs and mutual exclusion relationship between OTUs, respectively. Spearman Correlation Coefficient(SCC) values were determined to measure the strengthen of relationships among the OTUs as described in the literature[[7](#_ENREF_7), [41](#_ENREF_41)].

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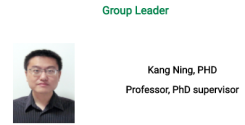
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**Outreach 页面啥也没有--------------------------------------------------------------------------------------**

**About---------------------------------------------------------------------------------------------------------------**

**成员介绍可以横向排版这个样式**

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