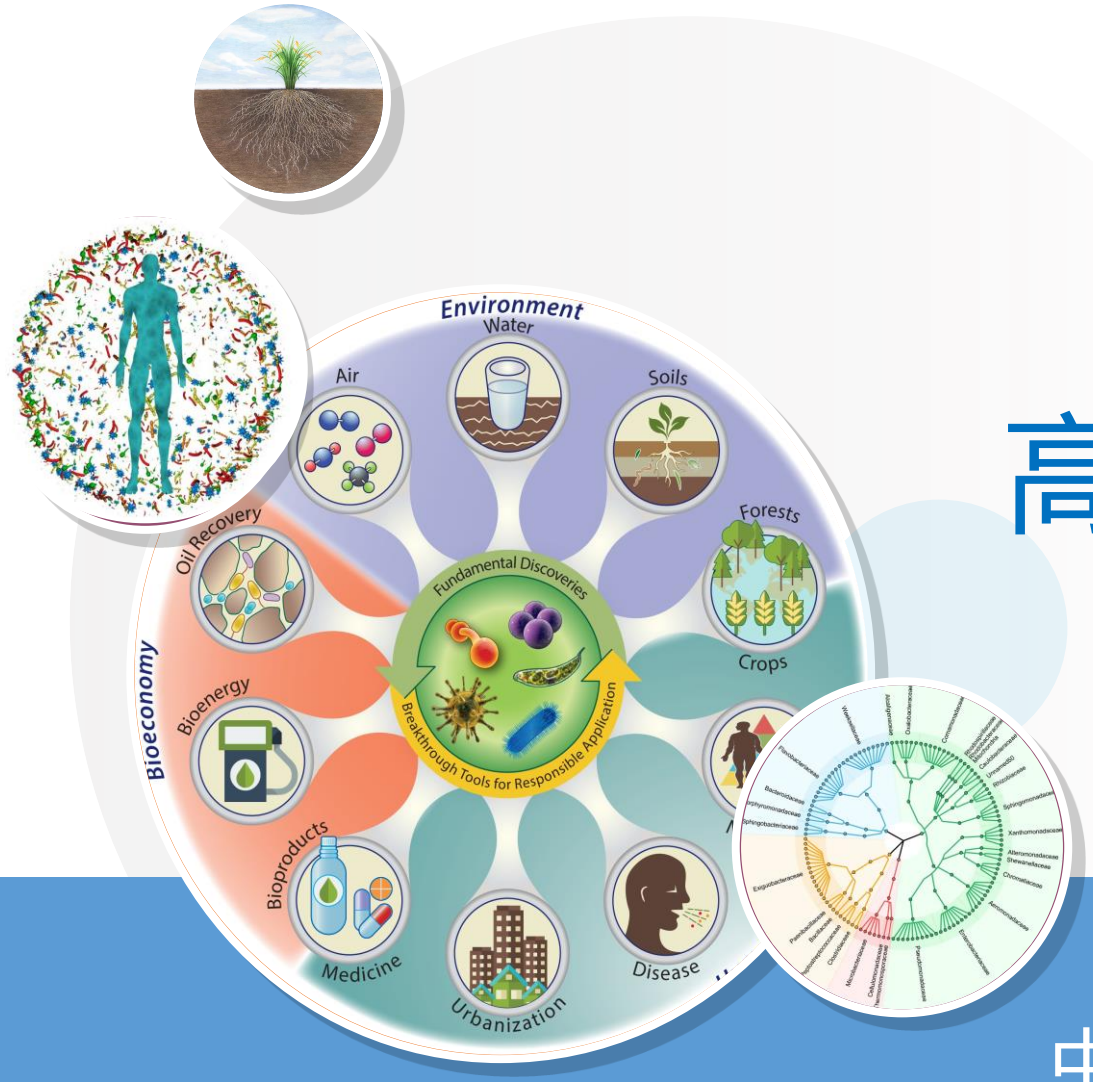
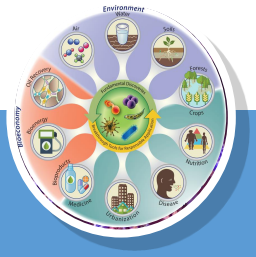


TOPEDIT 投必得

宏基因组
meta-genome

高水平组学文章的分析和可视化套路

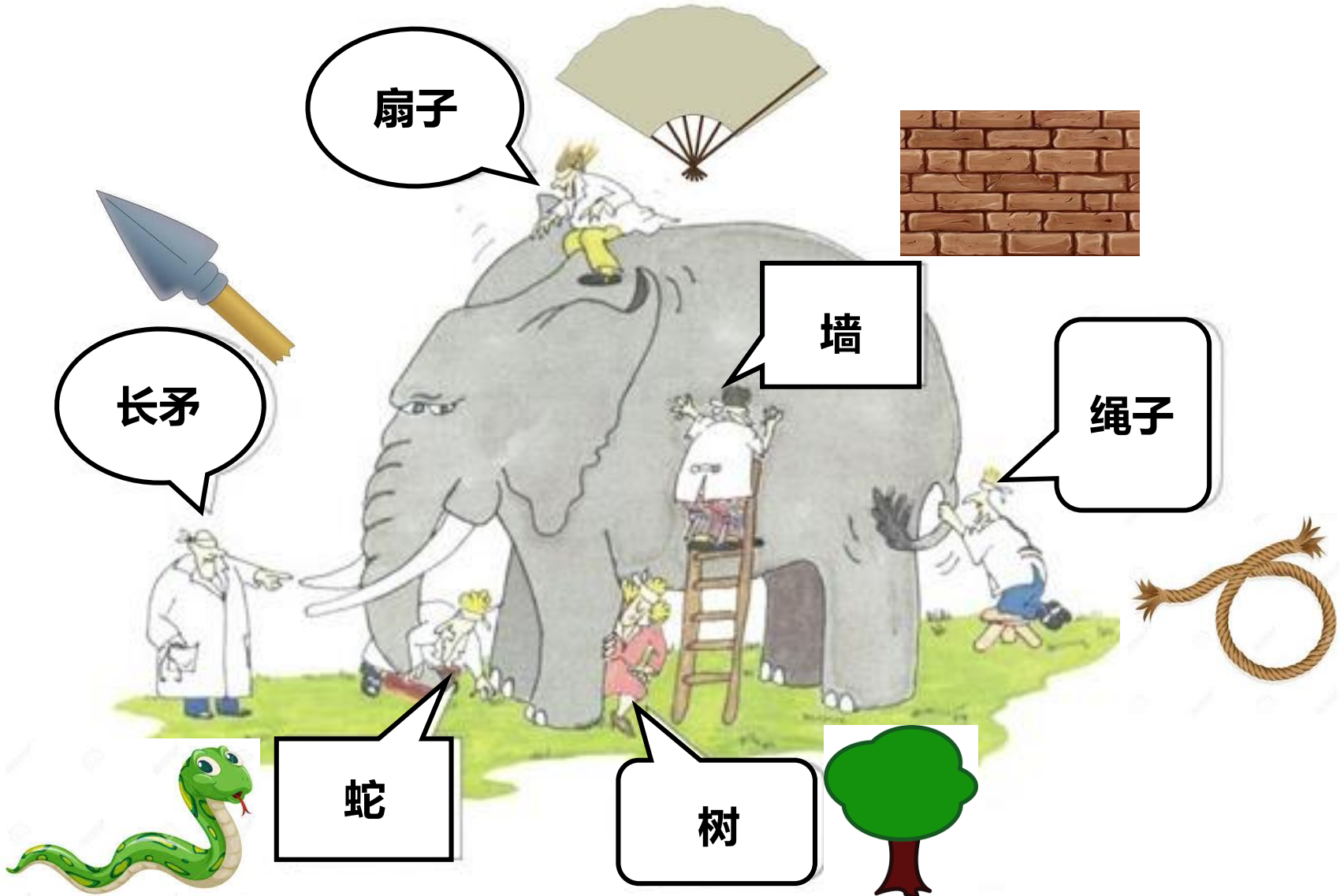
刘永鑫
中国科学院遗传与发育生物学研究所
2019年6月30日

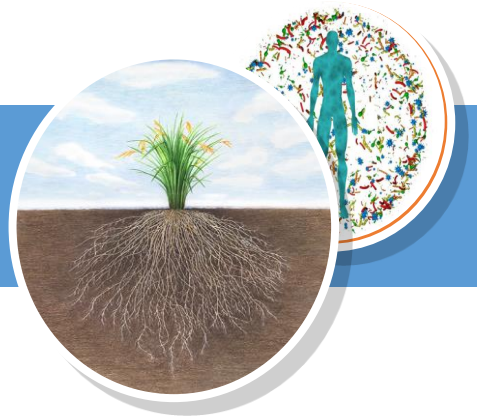


什么是组学？

- 组学(Omics)主要包括**基因组学（Genomics），转录组学(transcriptomics），蛋白组学（Proteinomics），代谢组学（Metabolomics）**，脂类组学（lipidomics）， 免疫组学（Immunomics），糖组学（glycomics ）， RNA组学(RNomics)学，影像组学（Radiomics），超声组学（Ultrasomics）等。
- Omics是组学的英文称谓，它的词根'-ome'英译是一些种类个体的系统集合，例如**Genome（基因组）是构成生物体所有基因的组合**，基因组学（Genomics）这门学科就是研究这些基因以及这些基因间的关系。

组学的优势——更全面地看问题

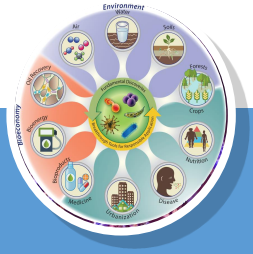


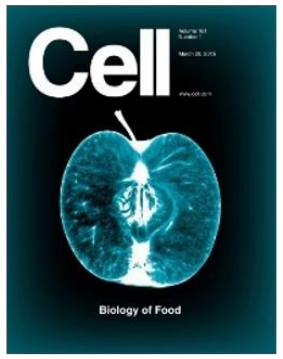


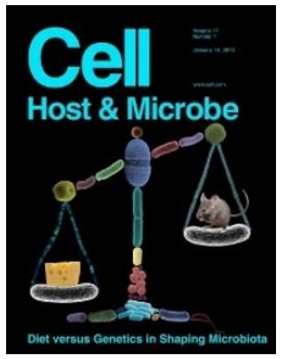
我的组学研究历程

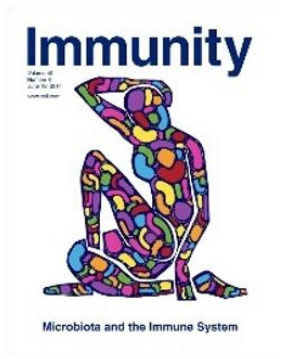
2004	东北农业大学	学士	微生物学	食用真菌
2008	东北农业大学	硕士	作物遗传育种	miRNA组
2011	中科院遗传发育所	博士	生物信息	小RNA组
2014	中科院遗传发育所	博士后	细胞生物学	转录组、表观组
2016	中科院遗传发育所	工程师	微生物组	扩增子、宏基因组

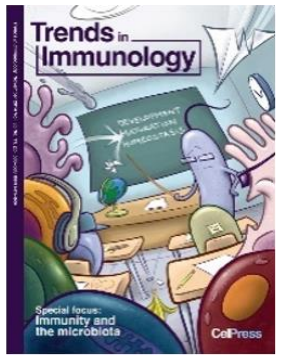
微生物组领域为什么这么热?

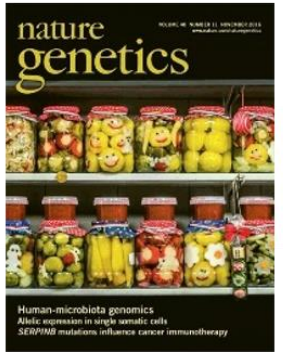


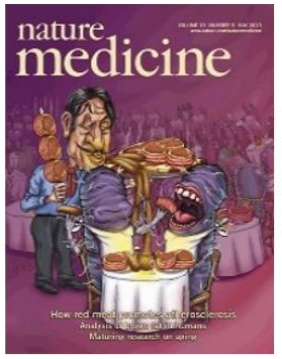


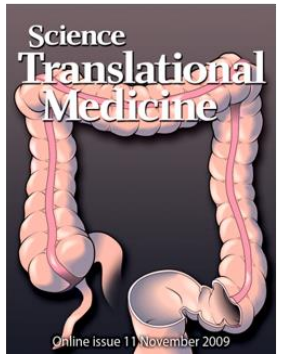


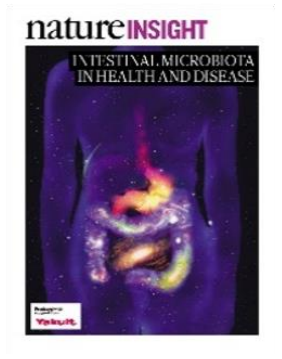


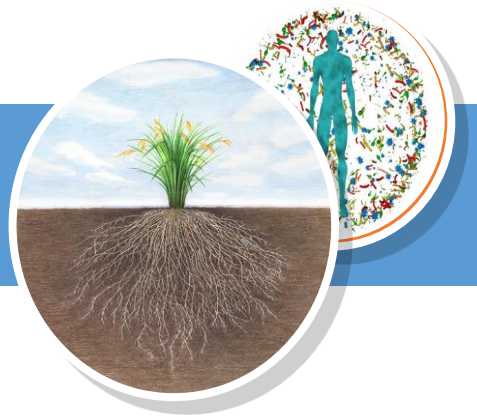




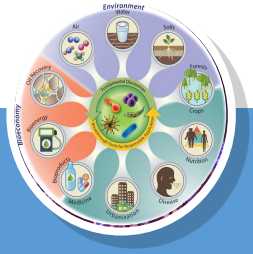


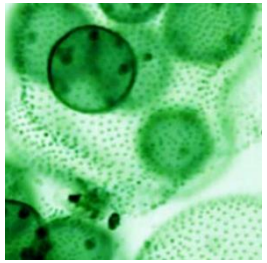


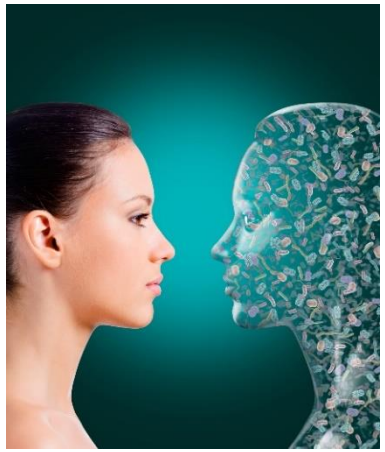


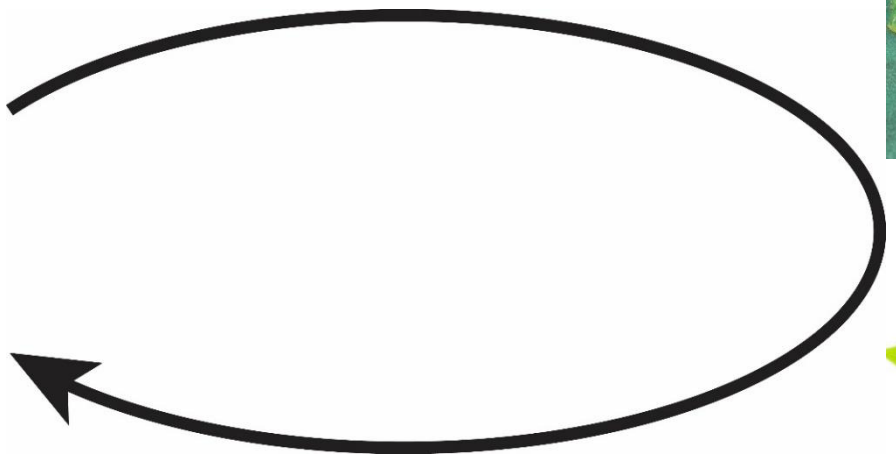


动植物生活环境中充满微生物









脱离了微生物的生物学研究是不完整的

近年我参与发表的微生物组文章

nature biotechnology

ARTICLES

https://doi.org/10.1038/s41587-019-0104-4

RESEARCH ARTICLE

NRT1.1B is associated v composition and nitro

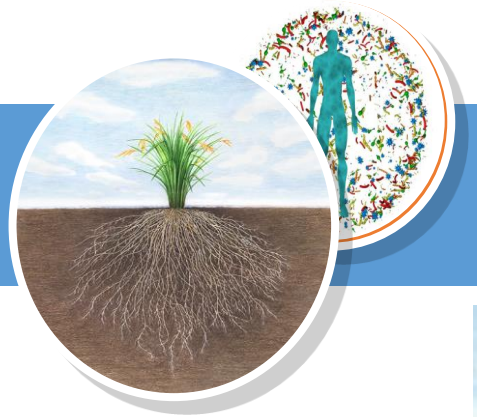
Jingying Zhang[1,2,10], Yong-Xin Liu[1,2,10], Na
Yuan Qin[1,2,3], Pengxu Yan[4,5,6], Xiaoning Zhang
Xin Wang[1,2], Chao Wang[1,2], Hui Wang[1,2,3], Bac
Ruben Garrido-Oter[8,9], Chengcai Chu[1,3*] an

PLANT SCIENCE

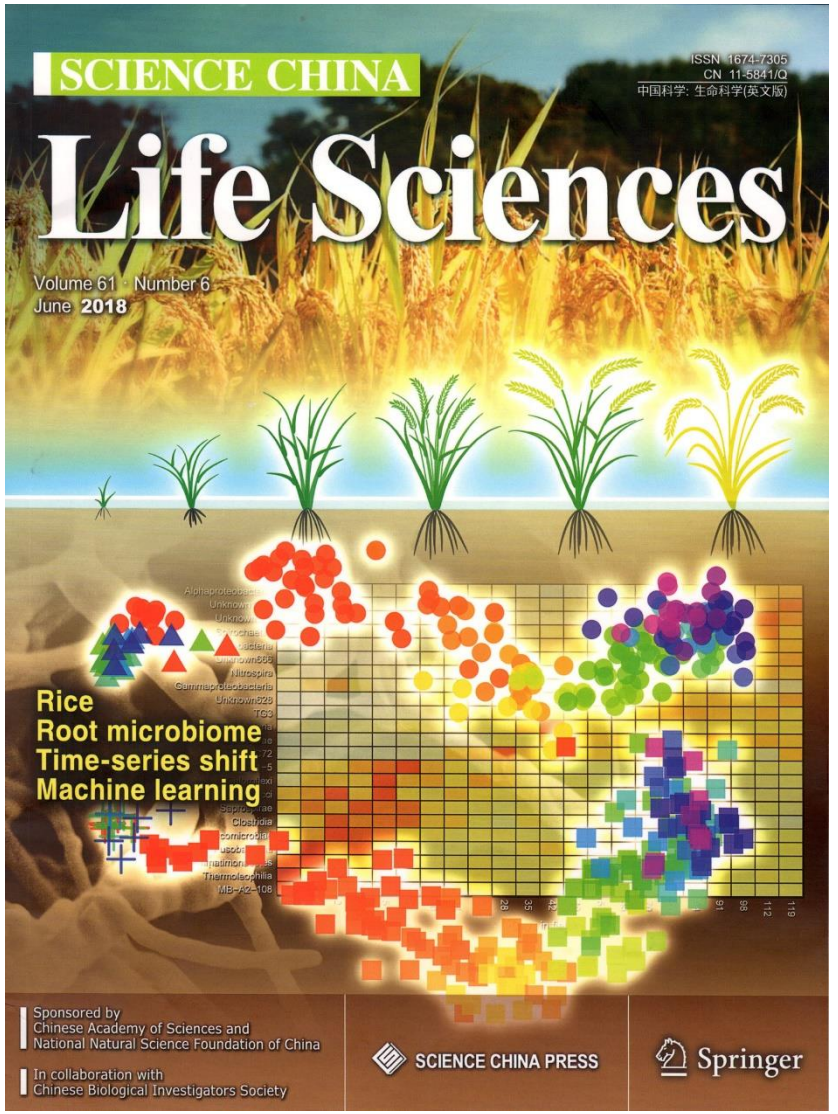
A specialized metabolic network selectively modulates *Arabidopsis* root microbiota

Ancheng C. Huang[1*], Ting Jiang[2,3,4*], Yong-Xin Liu[2,3], Yue-Chen Bai[5,6], James Reed[1], Baoyuan Qu[2,3], Alain Goossens[5,6], Hans-Wilhelm Nützmann[1†], Yang Bai[2,3,4‡], Anne Osbourn[1‡]

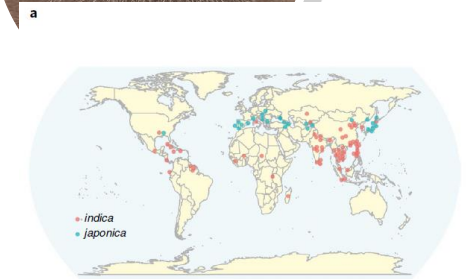
Jingying Zhang, **Yong-Xin Liu**, Na Zhang, et. al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. ***Nature Biotechnology***. 2019. doi:10.1038/s41587-019-0104-4
Ancheng C. Huang, Ting Jiang, **Yong-Xin Liu,** et. al. A specialized metabolic network selectively modulates Arabidopsis root microbiota. ***Science***. 2019, 364: eaau6389. doi:10.1126/science.aau6389

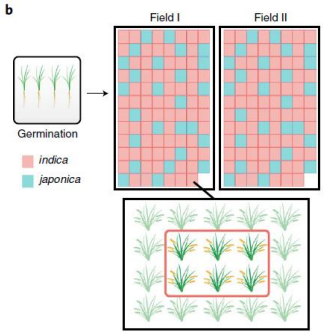


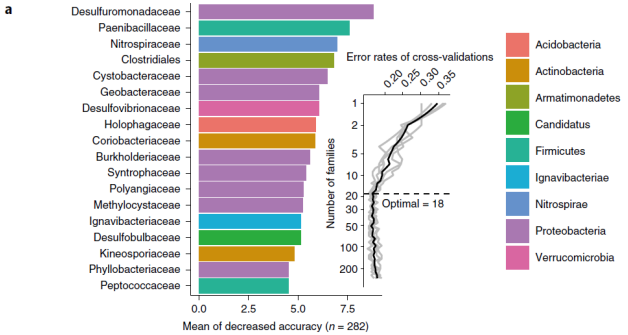
其中一部分也被入为封面文章

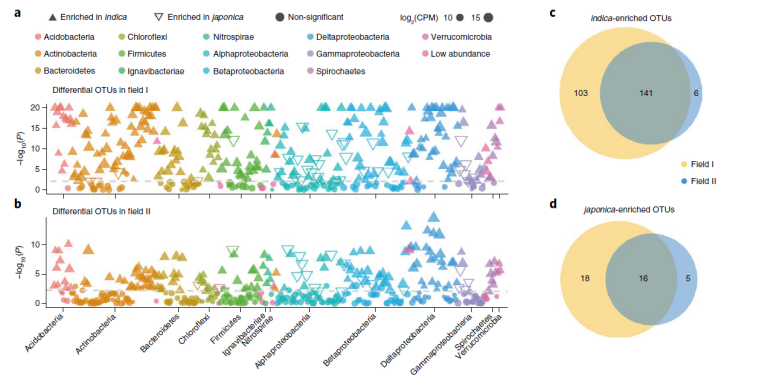


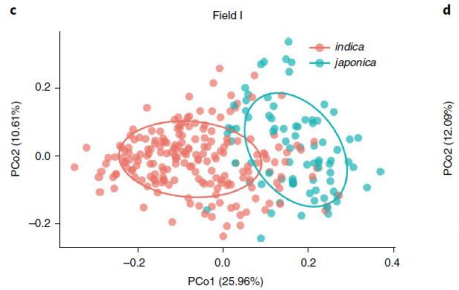
组学文章可视化结果常用图表

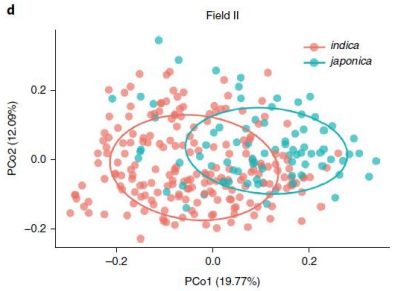


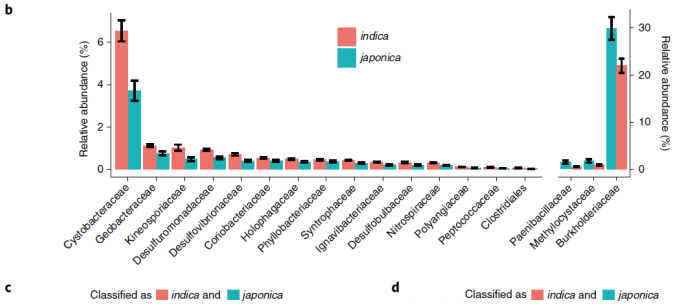


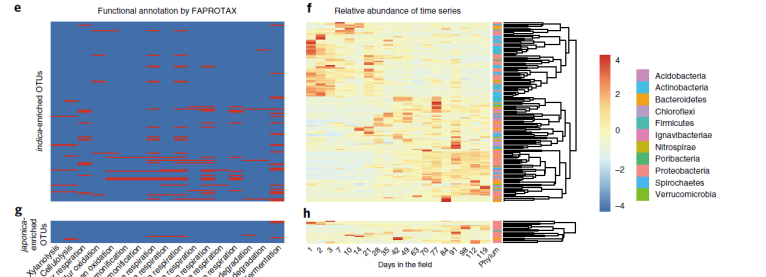


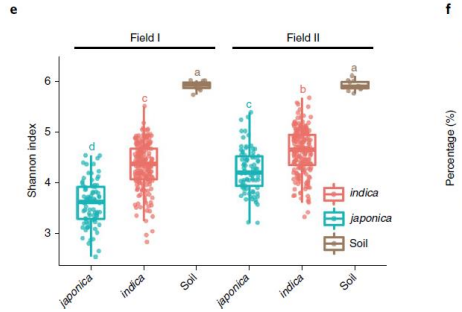


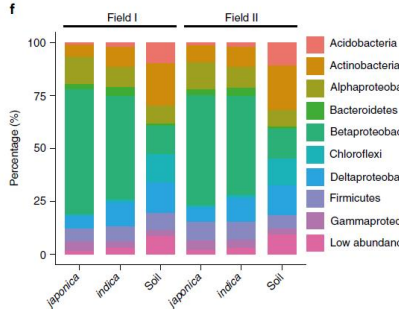


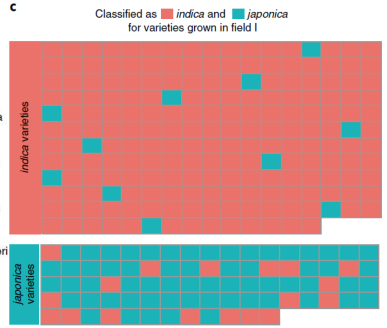


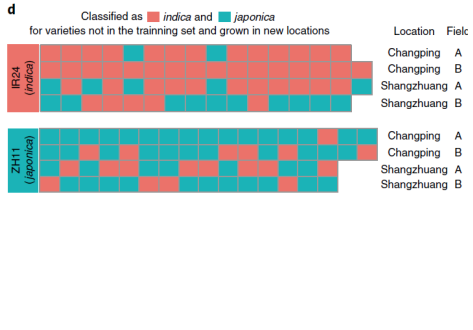


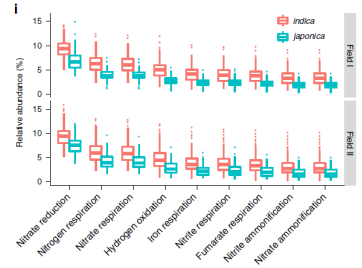


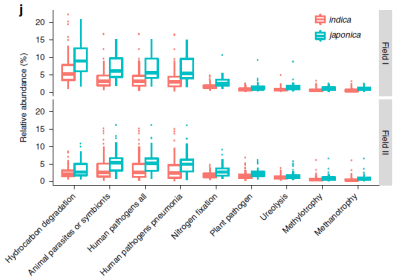




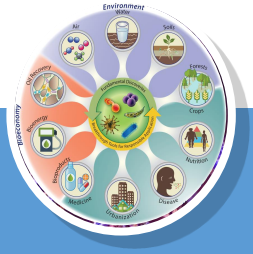








Jingying Zhang, **Yong-Xin Liu**, Na Zhang, et. al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. ***Nature Biotechnology***. 2019. doi:10.1038/s41587-019-0104-4



目录

- 整体概述
  - 材料地图和/或实验设计
  - 样本概述：α多样性箱线图、β多样性PCoA散点图、物种组成柱状图
- 细节展示
  - 物种组间差异：曼哈顿图、韦恩图
  - 功能注释或差异：热图有/无或时间序列、箱线图
- 应用场景——机器学习挖掘生物标记
  - 随机森林分类——区分不同组，如疾病诊断、来源或品种鉴定
  - 随机森林回归——时间序列，如年龄、死亡时间预测

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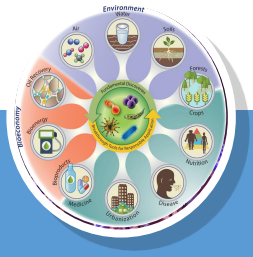

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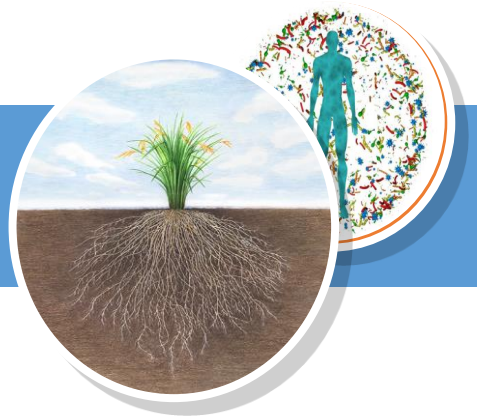
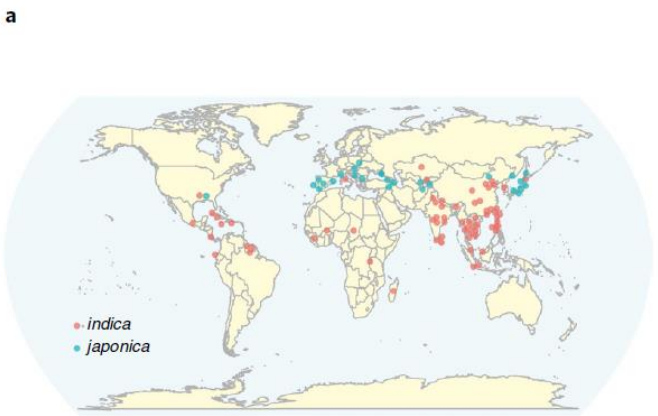
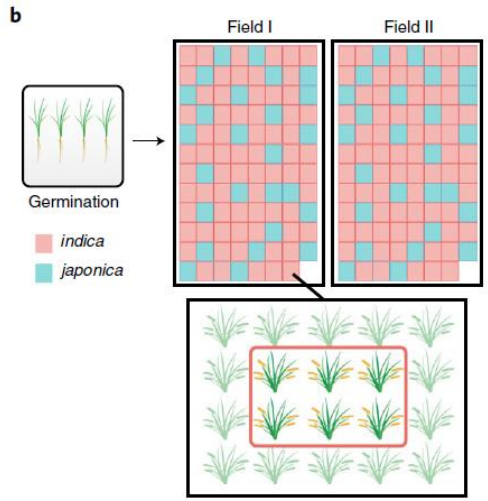
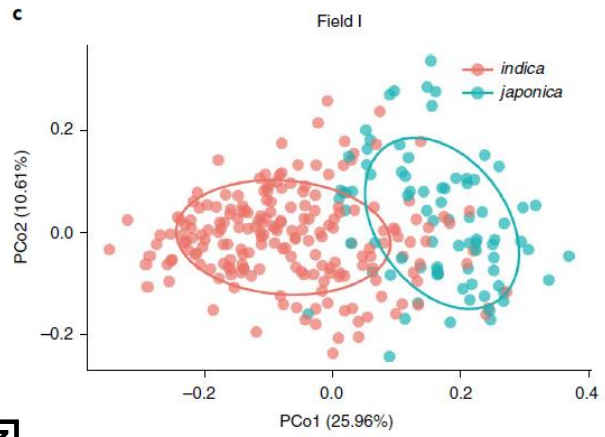


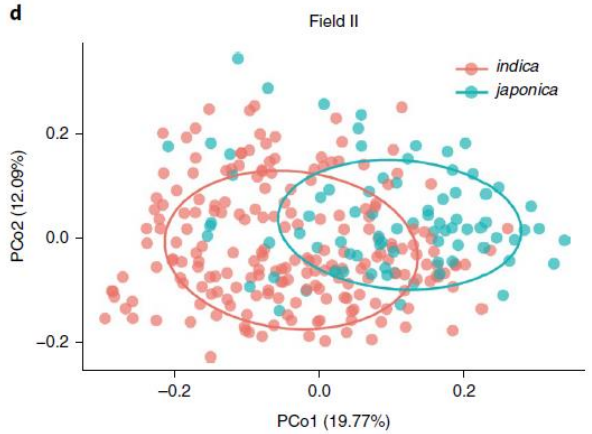
整体概述

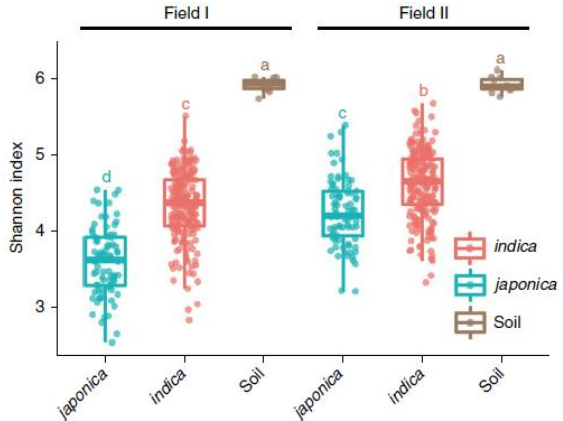
1. 材料地图
2. 实验设计
3. 样本概述
   - α多样性箱线图、
   - β多样性PCoA散点图、
   - 物种组成柱状图

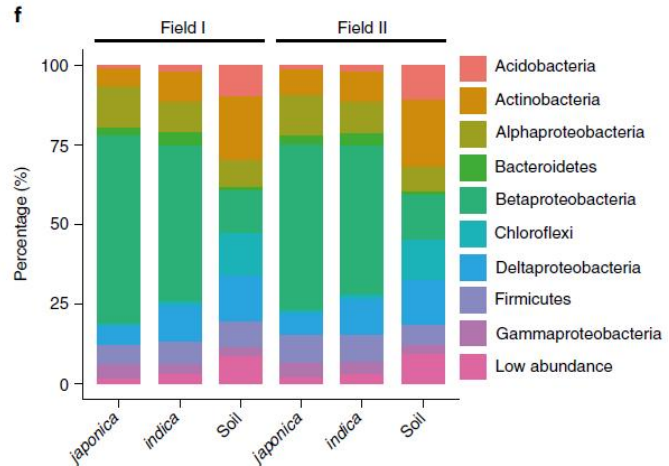




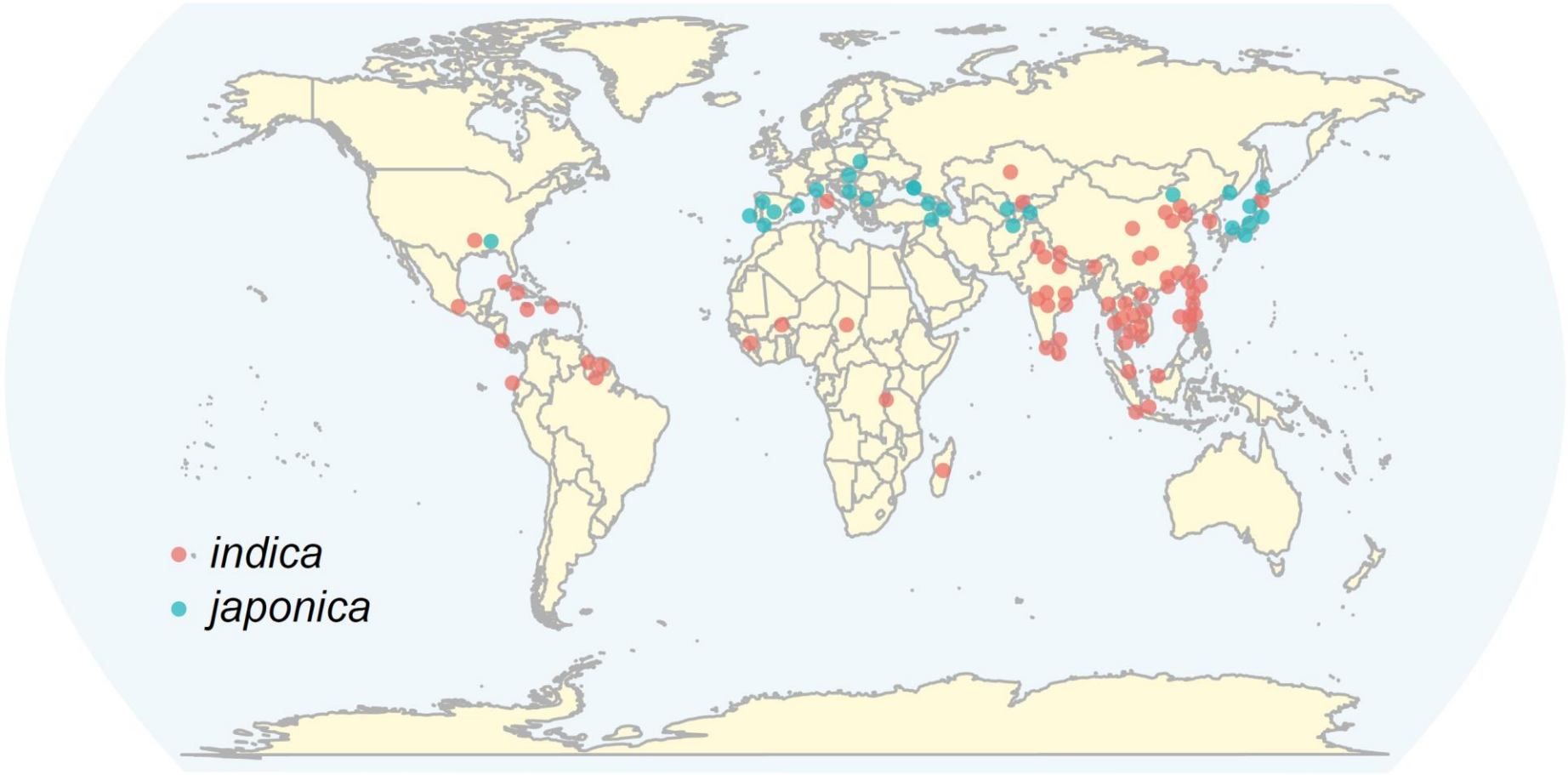








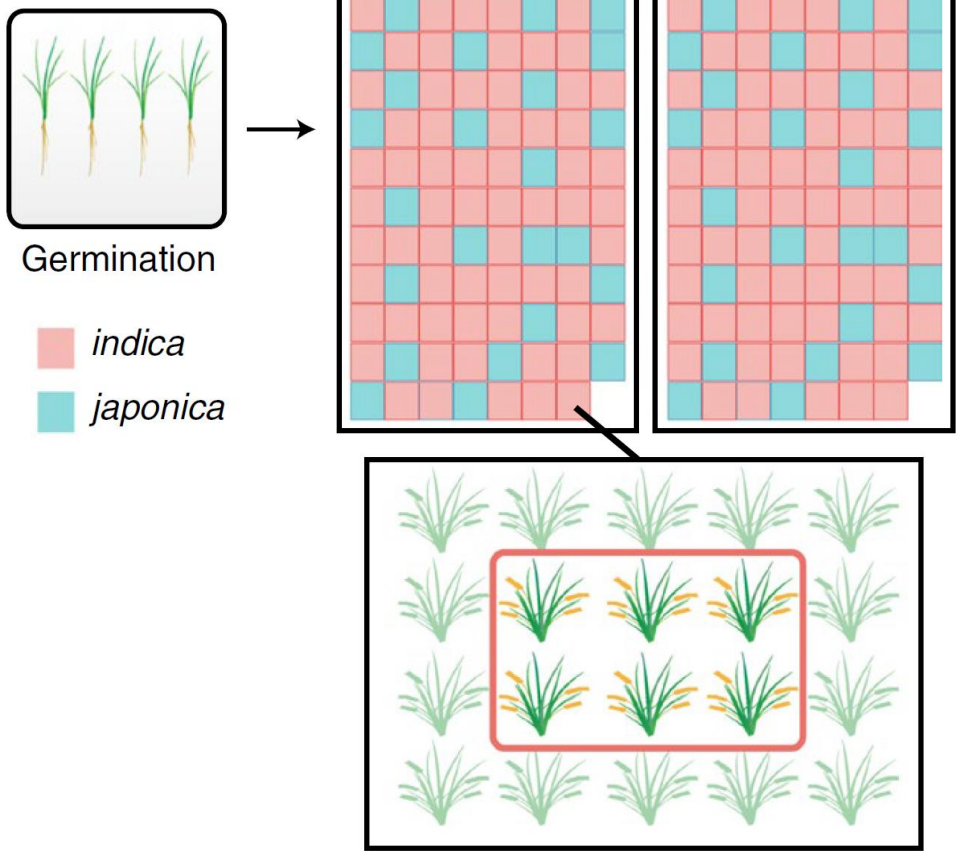
材料地图

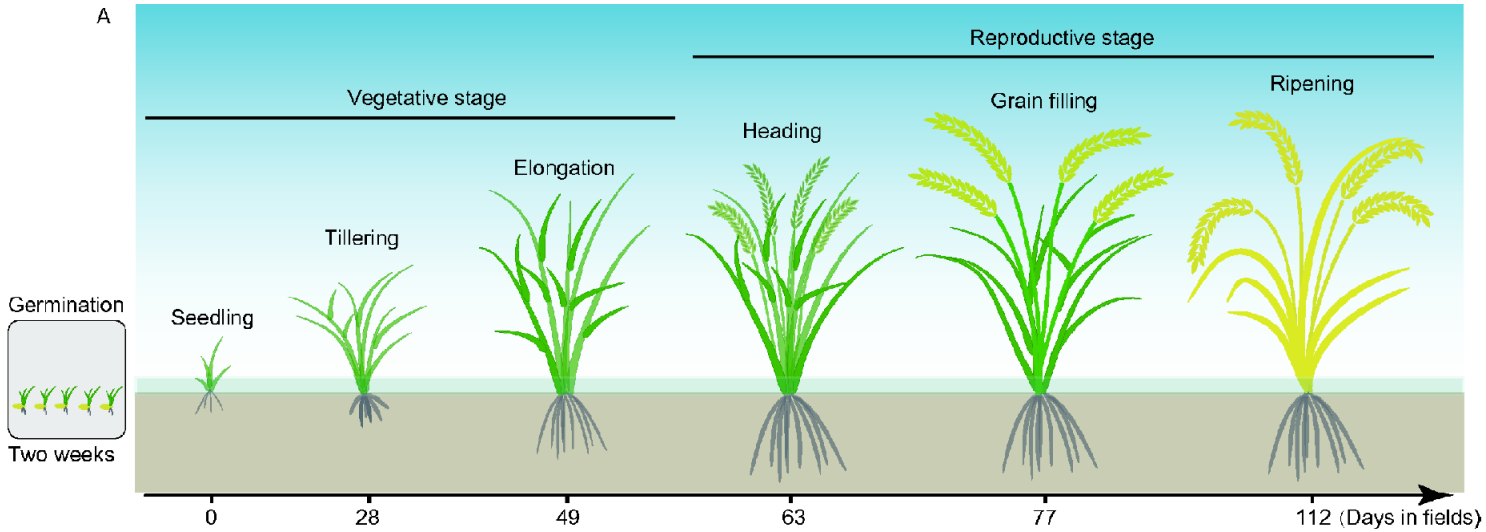


R语言maps结合ggplot2包，基于样本来源经纬度绘制
展示来源44个国家的95个品种的分布

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 1a

实验设计





Root microbiota shift in rice correlates with resident time in the field and developmental stage

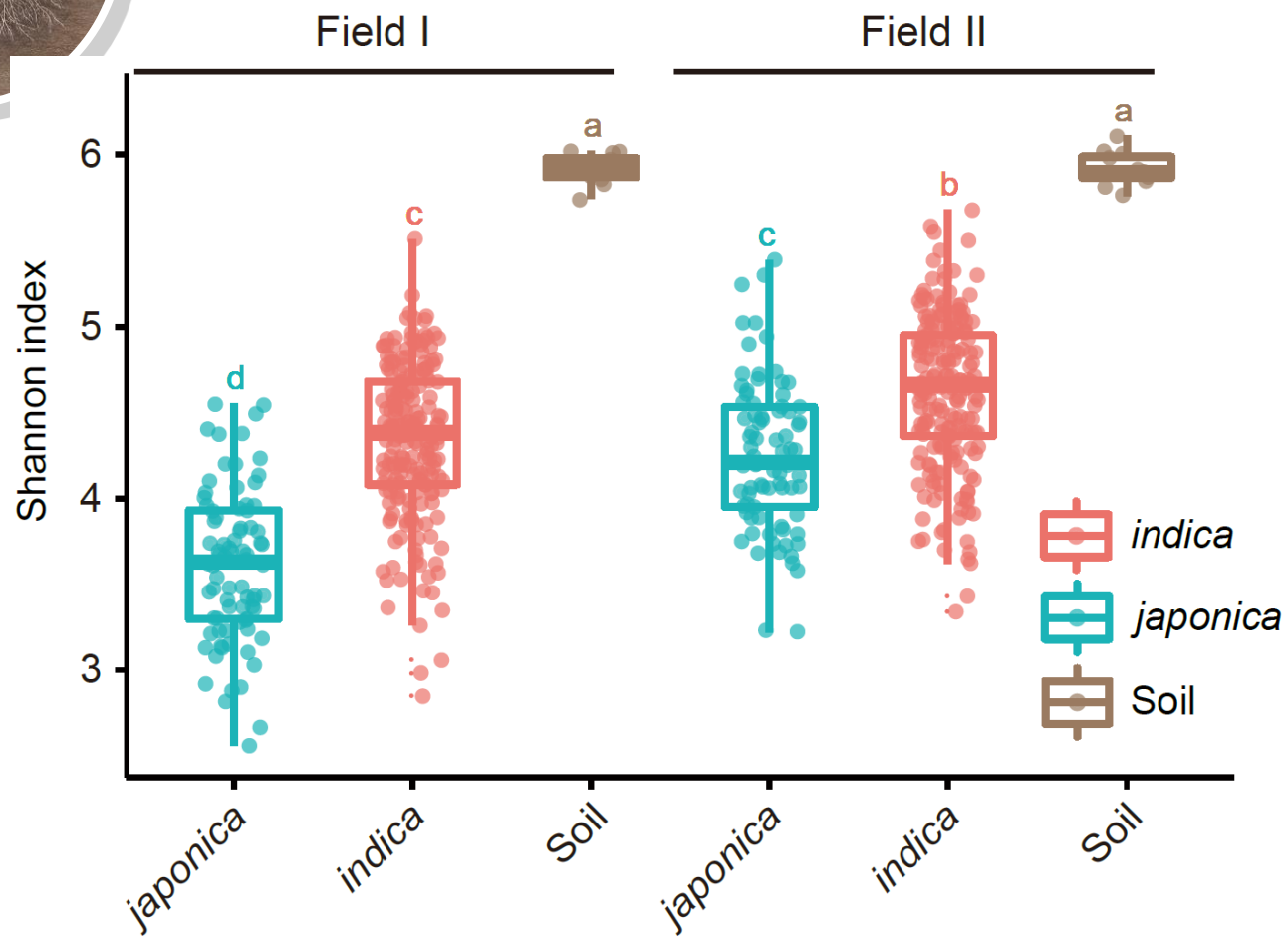
J Zhang, N Zhang, YX Liu, X Zhang, B Hu, Y Qin… - Science China Life …, 2018 - Springer

Land plants in natural soil form intimate relationships with the diverse root bacterial microbiota. A growing body of evidence shows that these microbes are important for plant growth and health. Root microbiota composition has been widely studied in several model plants and crops; however, little is known about how root microbiota vary throughout the plant's life cycle under field conditions. We performed longitudinal dense sampling in field trials to track the time-series shift of the root microbiota from two representative rice cultivars .

☆ 99 Cited by 10 Related articles All 8 versions

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 1b

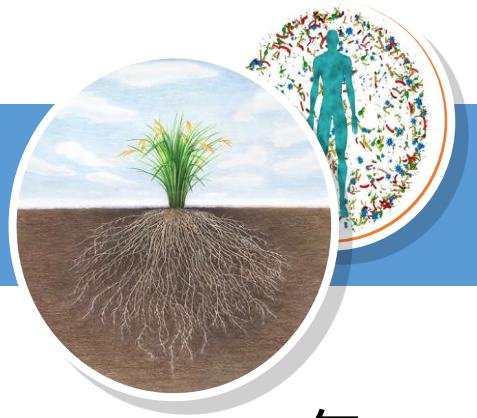
α多样性箱线图



agricolae或multcompView包
　　如LSD.test 统计组间显著性
ggplot2包
　　geom_boxplot 绘制箱线图
　　geom_jitter 添加抖动样本点
　　geom_text 添加显著性分组

结果表明不同地点组间存在物种丰富度和均匀度的一致显著差异

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 1e
Hadley Wickham. *ggplot2: elegant graphics for data analysis*. Springer, 2016. Cited > 13k 19/5/21

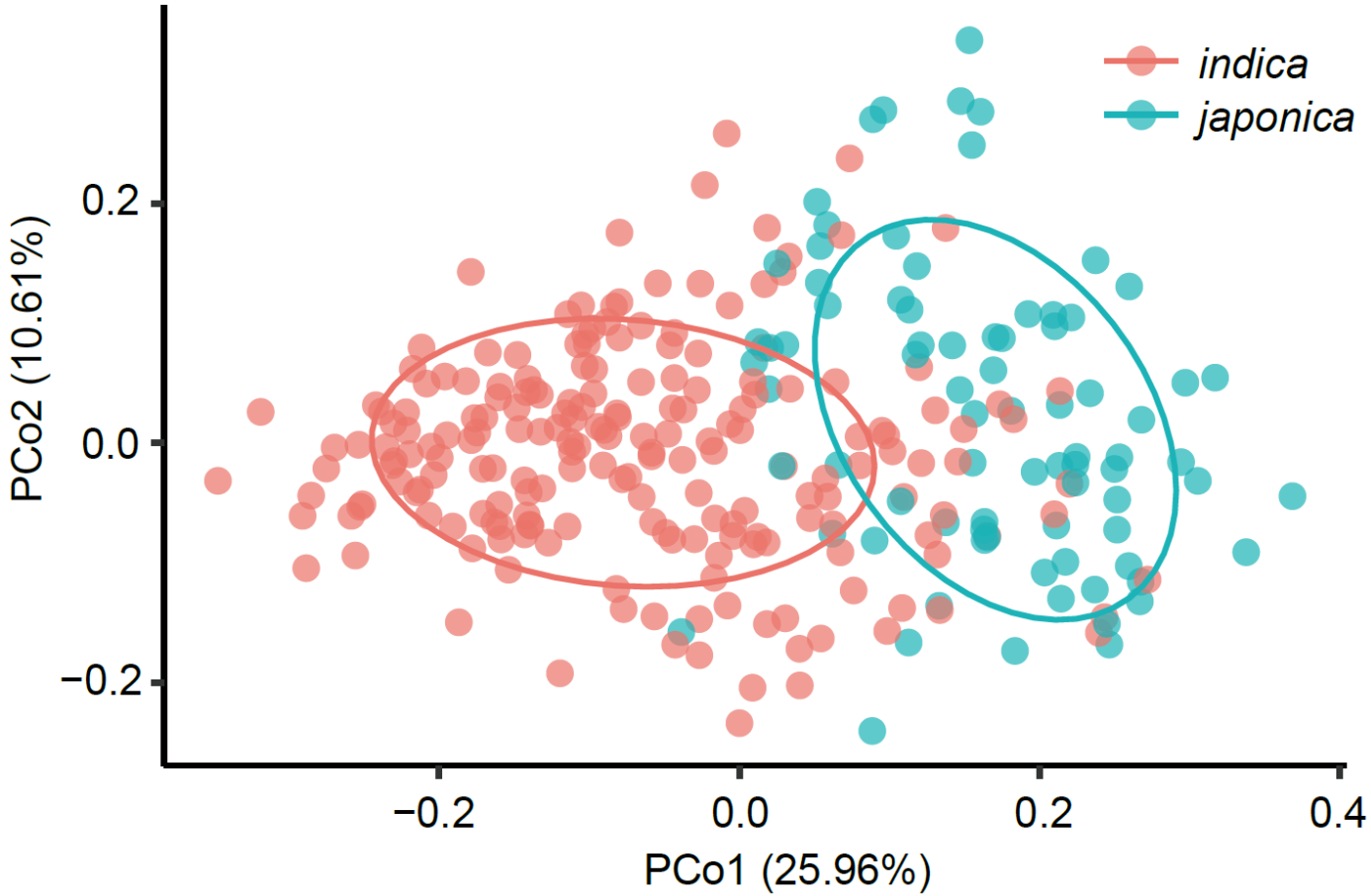


β多样性PCoA散点图

vegan包
- vegdist 计算样本间距离
- capscale 排序分析

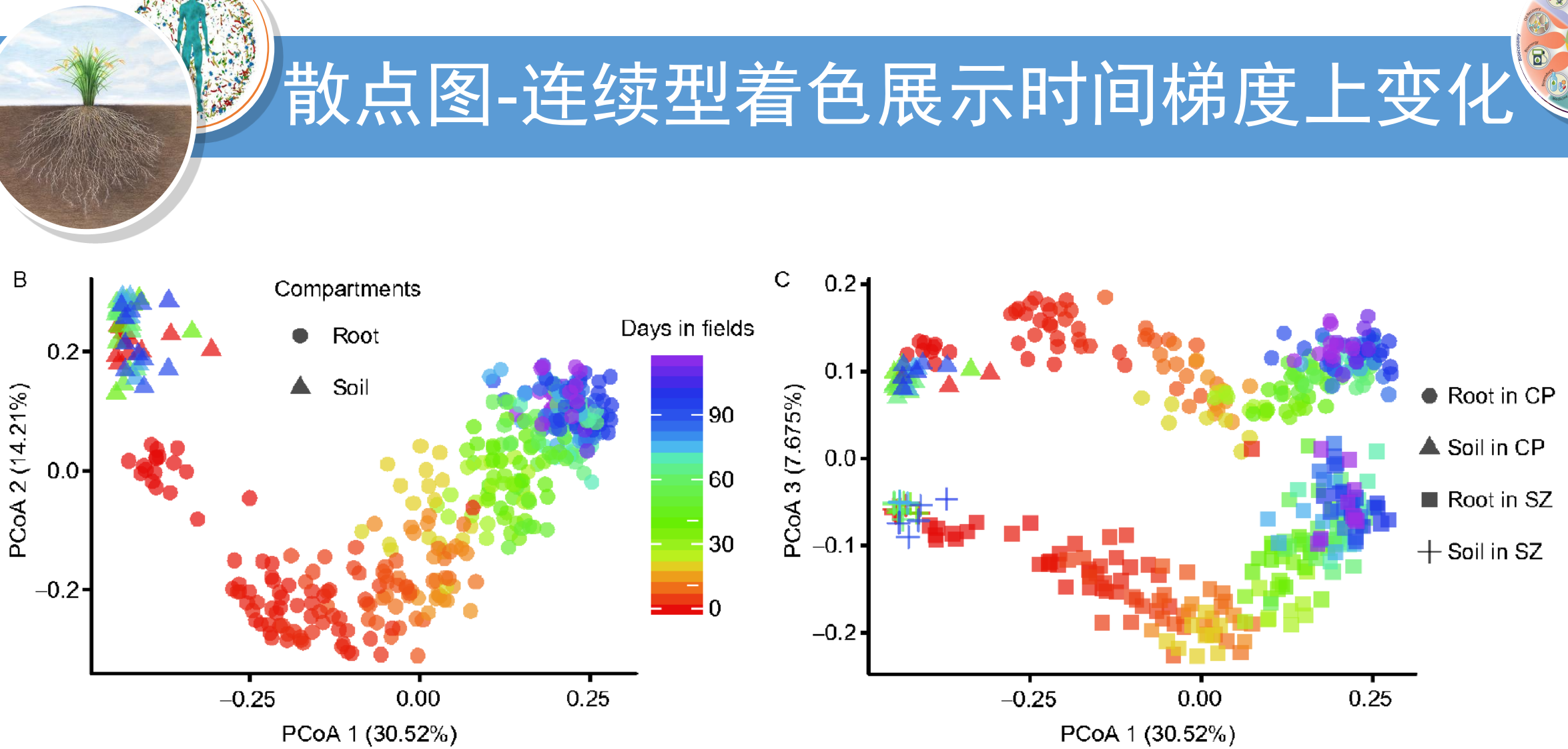
ggplot2包
- geom_point 绘制散点图
- stat_ellipse 添加统计椭圆
- labs 添加差异解释率

结果表明亚种间微生物组群落结构在第一主轴明显分开
第一主轴可解释25.96%的差异



Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 1c/d
Oksanen, Jari, et al. "The vegan package." *Community ecology package* 10 (2007): 631-637. cited > 15K，19/5/21

散点图-连续型着色展示时间梯度上变化

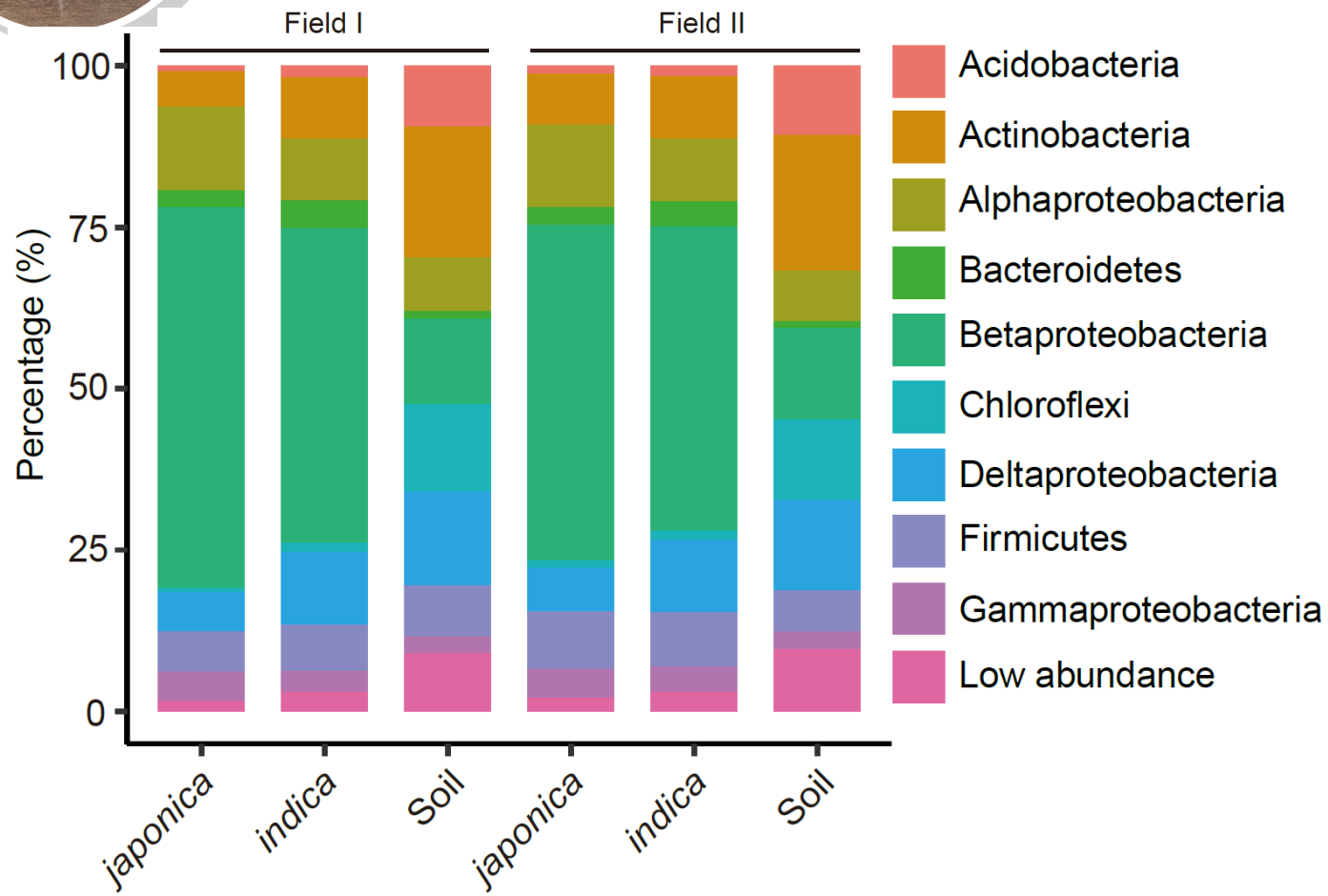


p + scale_color_gradientn(colours = rainbow(7))

https://github.com/microbiota/Zhang2018SCLS

Jingying Zhang, Na Zhang, **Yong-Xin Liu**, et. al. Root microbiota shift in rice correlates with resident time in the field and developmental stage. ***Science China Life Sciences***. 2018. doi:10.1007/s11427-018-9284-4 Fig 1B/C

物种组成堆叠柱状图



数据分析

样本标准化为百分比

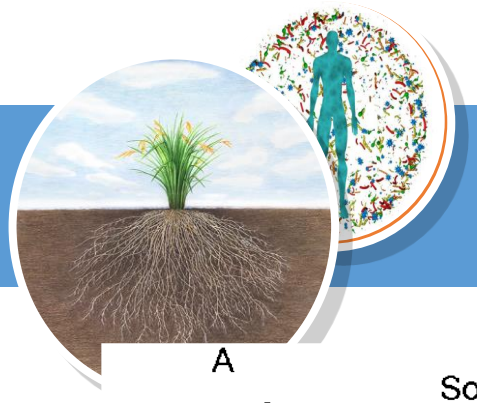
按组求均值

ggplot2包

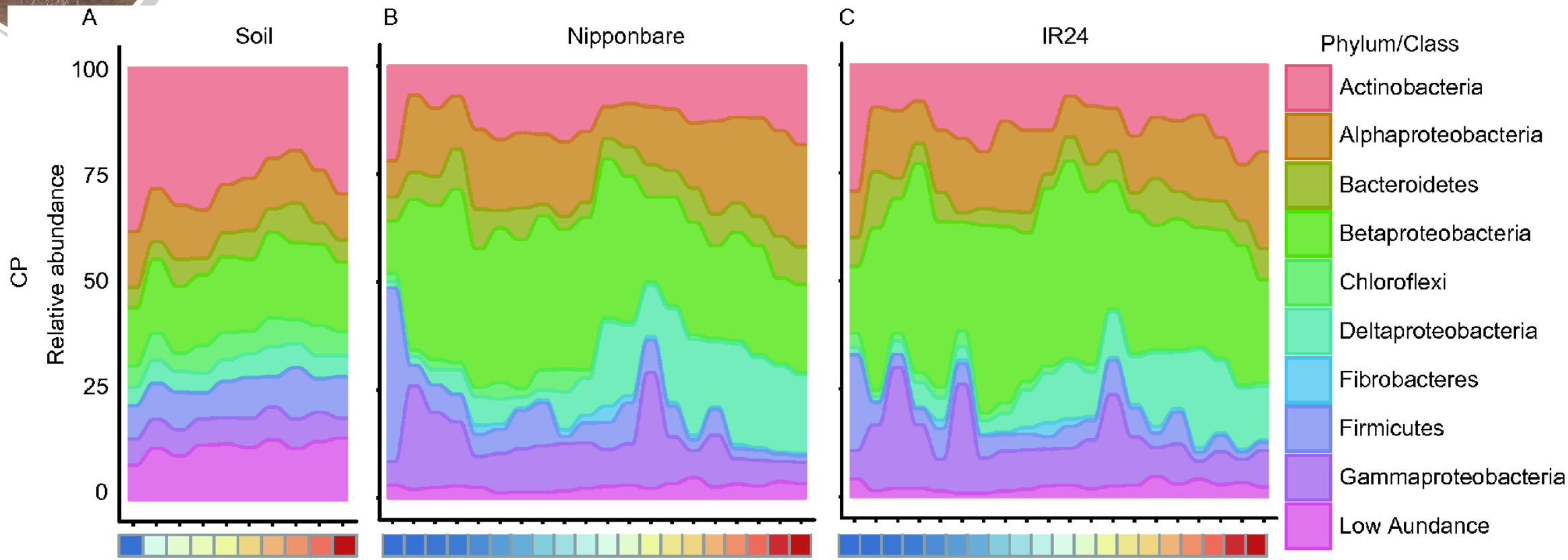
geom_bar(stat = "identity") 绘制堆叠柱状图

结果表明亚种间存在的物种组成在门水平即存在差异且不同地点可重复

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 1f



冲击图展示物种组成动态变化




```
library(ggalluvial)
p + geom_alluvium(aes(fill = phylumpro, colour = phylumpro), alpha = .75)
```

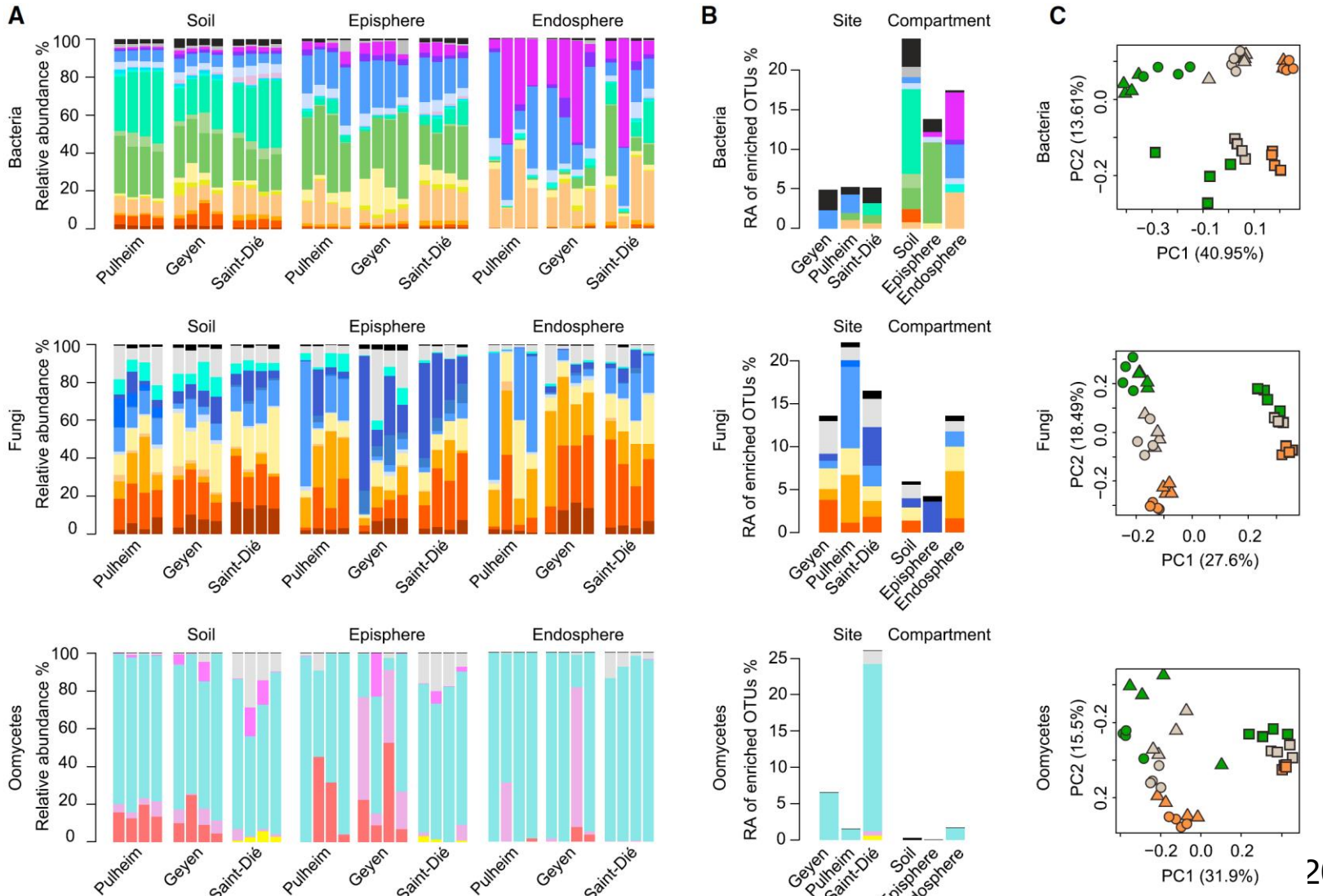

Jingying Zhang, Na Zhang, **Yong-Xin Liu**, et. al. Root microbiota shift in rice correlates with resident time in the field and developmental stage. ***Science China Life Sciences***. 2018. doi:10.1007/s11427-018-9284-4 Fig 1B/C

关于重复

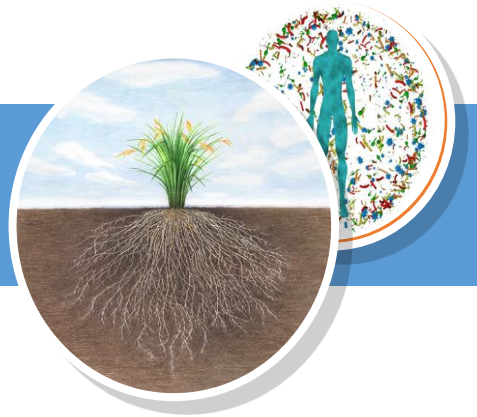
1. 多组学
   细菌、真菌、卵菌
2. 多部位
   土壤、根表、根内
3. 多地点
   德国Geyen、Pulheim、法国

样本量 = 4个生物学重复 X 3种组学 X 3个部位 X 3个地点 = 108个

[Cell：根部微生物跨界的互作促进拟南芥生存]



Durán, P. *et al.* Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. *Cell* **175**, 973-983.e914, doi:10.1016/j.cell.2018.10.020 (2018).



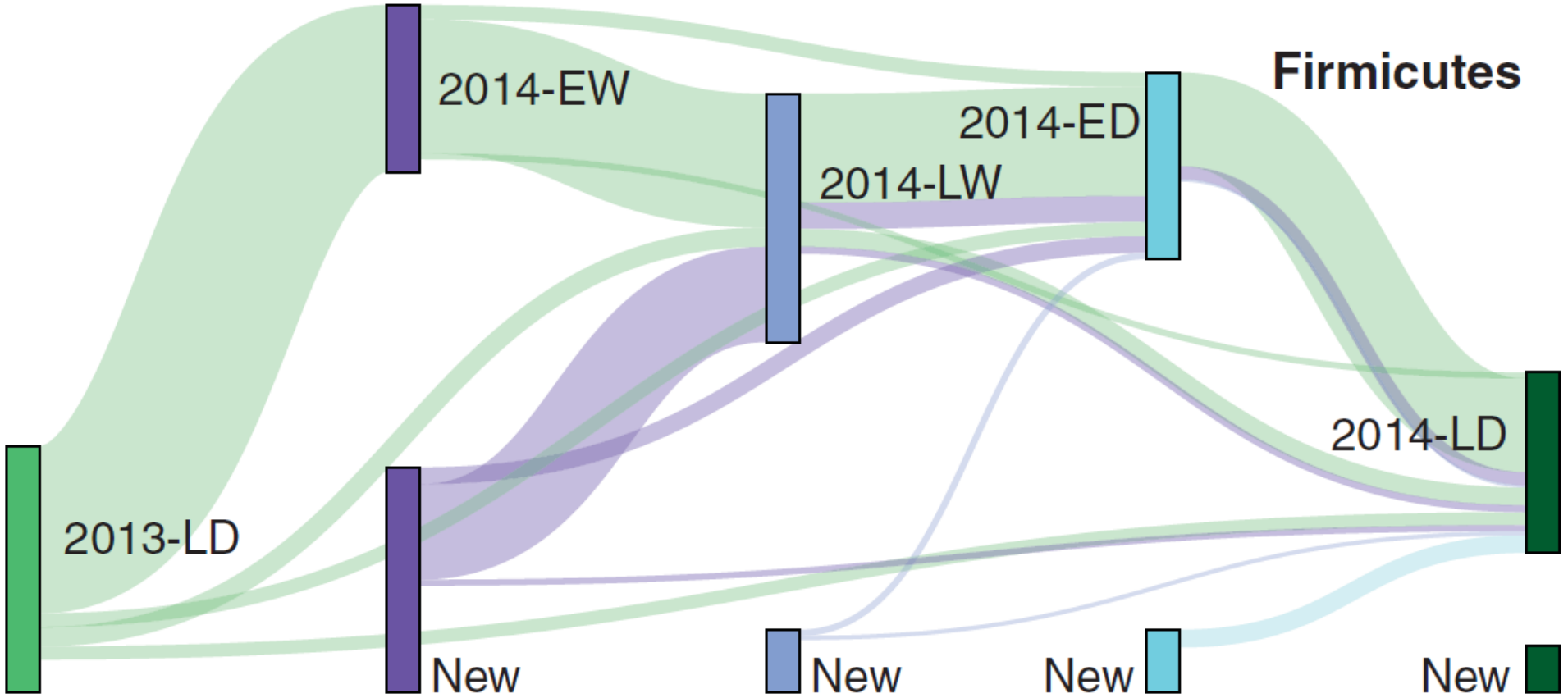
重复之时间点

一年内连续采样

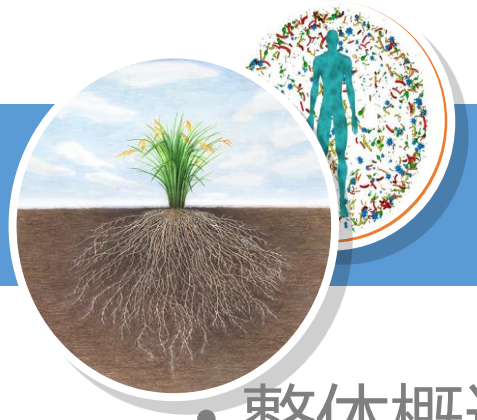
三分法：旱季、雨季、旱季(冬-夏-冬)

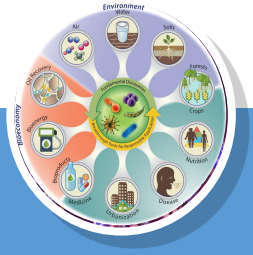
五分法：冬、春、夏、秋、冬

样本量 = 41 + 19 + 58 + 30 + 40 = 188



Smits, S. A. *et al.* Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* **357**, 802-806, doi:10.1126/science.aan4834 (2017). 3分和30分文章差距在哪里?





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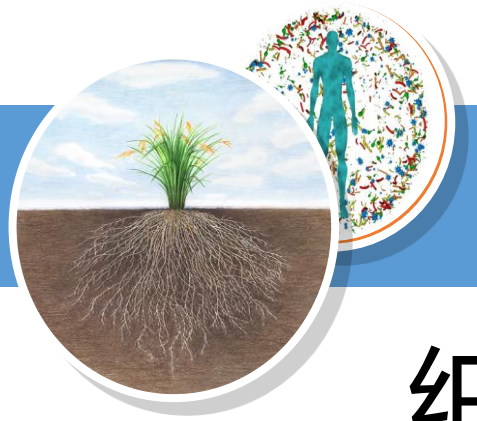
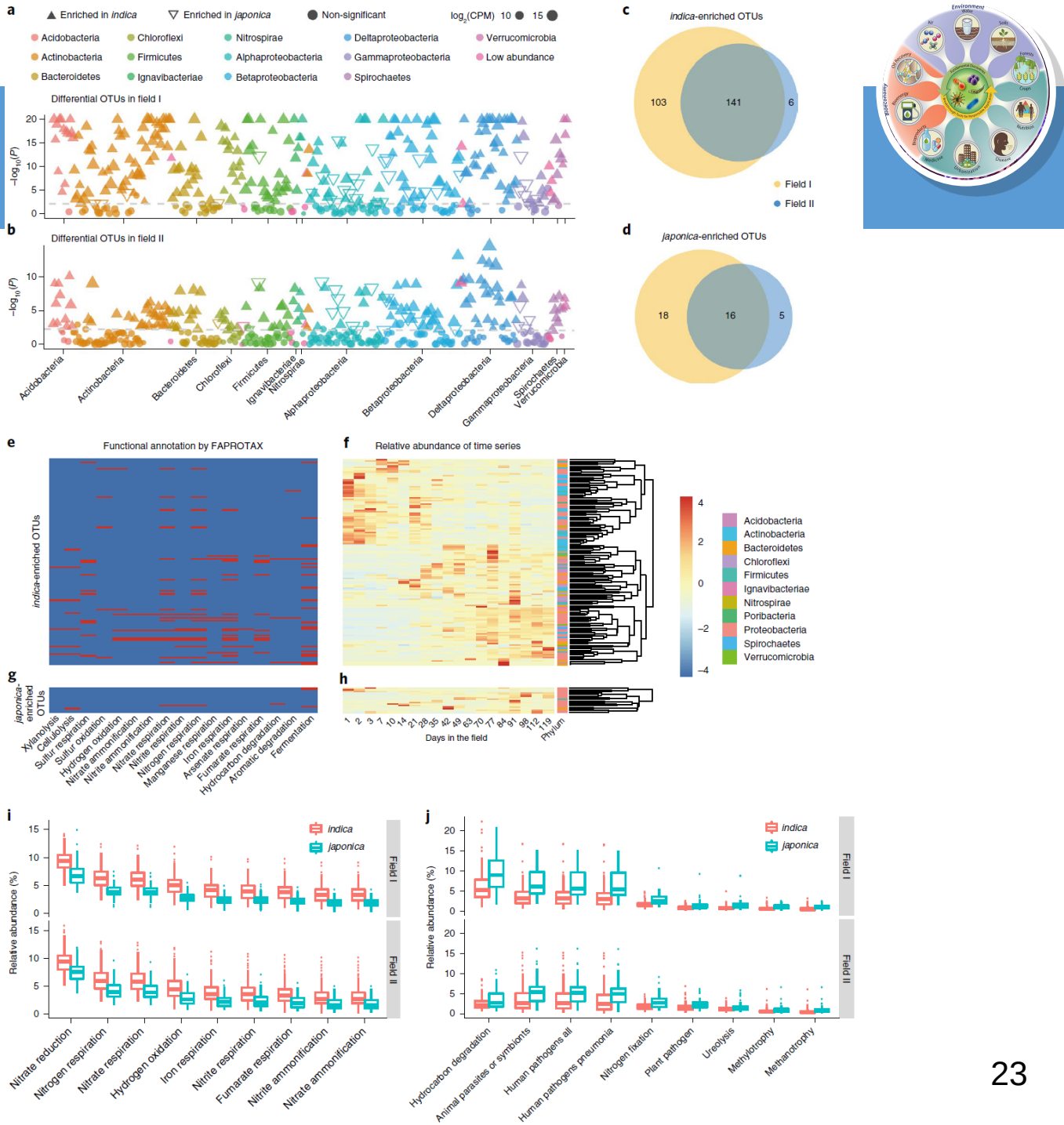
- 整体概述
  - 材料地图和/或实验设计
  - 样本概述：α多样性箱线图、β多样性PCoA散点图、物种组成柱状图
- 细节展示
  - 物种组间差异：曼哈顿图、韦恩图
  - 功能注释或差异：热图有/无或时间序列、箱线图
- 应用场景——机器学习挖掘生物标记
  - 随机森林分类——区分不同组，如疾病诊断、来源或品种鉴定
  - 随机森林回归——时间序列，如年龄、死亡时间预测



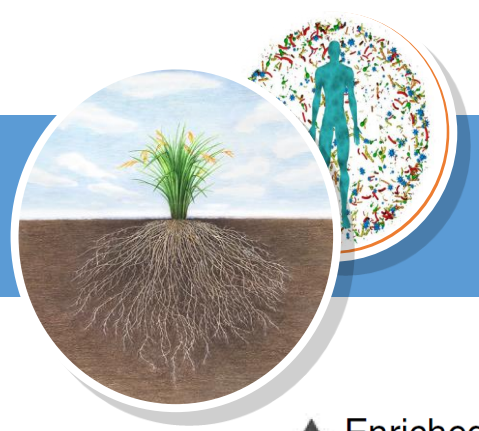
细节展示

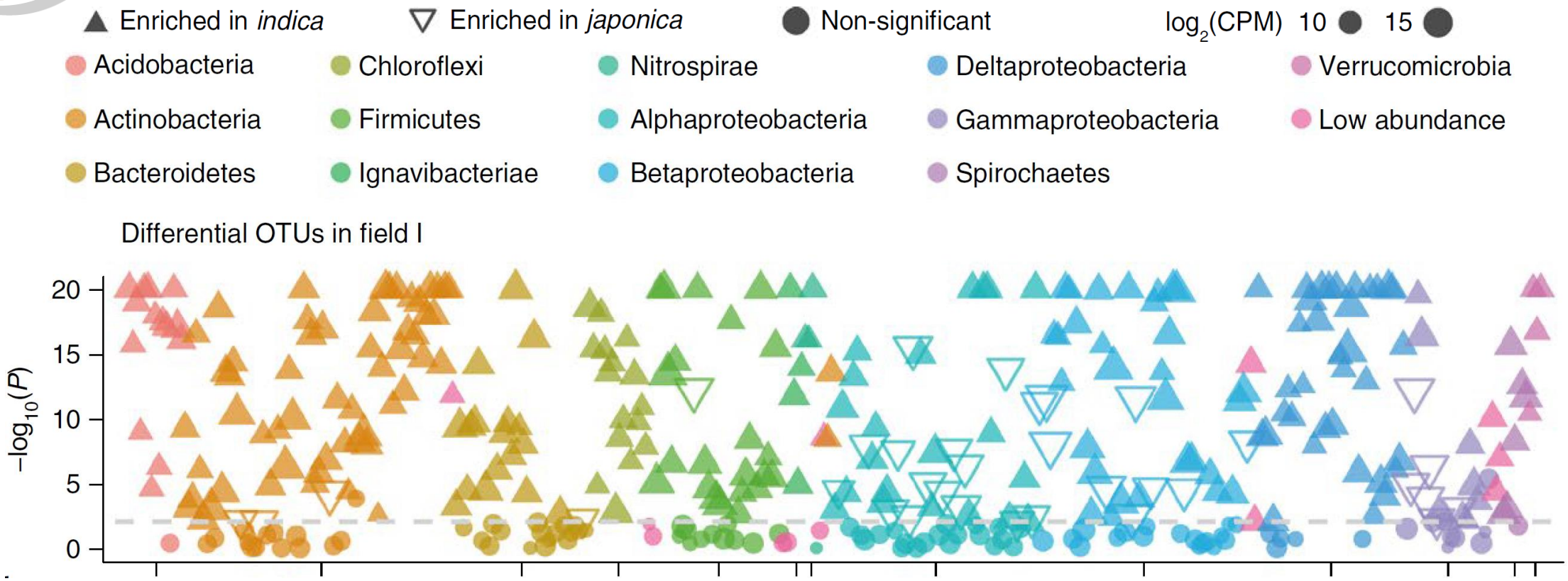
1. 物种组间差异：
   - 曼哈顿图
   - 韦恩图
2. 功能注释或差异：
   - 热图有/无
   - 热图时间序列
   - 箱线图



Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 3

曼哈顿图展示差异



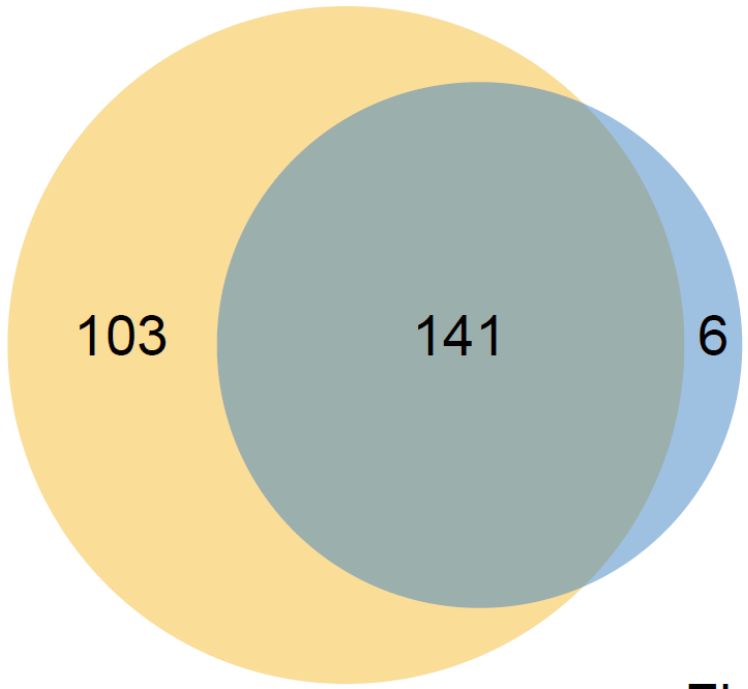


点代表物种，上三角为上调，下三角为下调，大小为丰度，颜色为物种分类

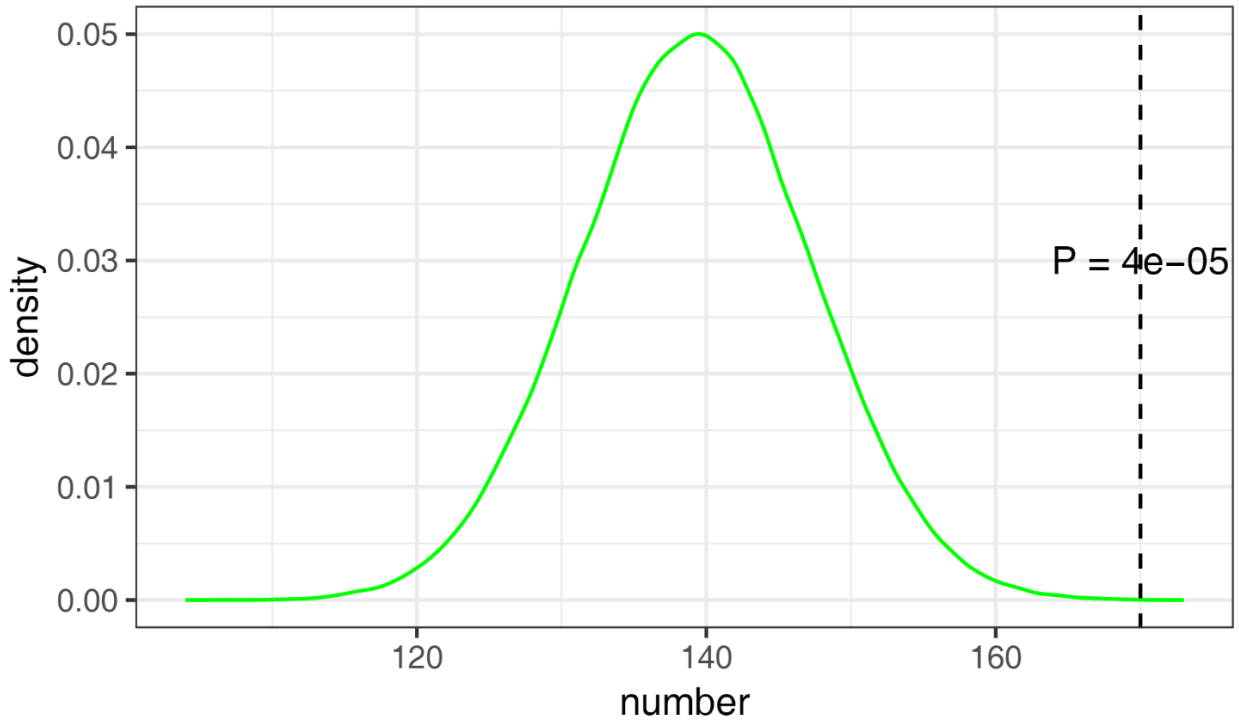
ggplot + geom_point + geom_hline + scale_shape_manual + scale_size

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 3a/b

韦恩图展示一致性



VennDiagram包绘制



置换检验统计重叠区域是否显著

https://github.com/microbiota/Huang2019SCIENCE

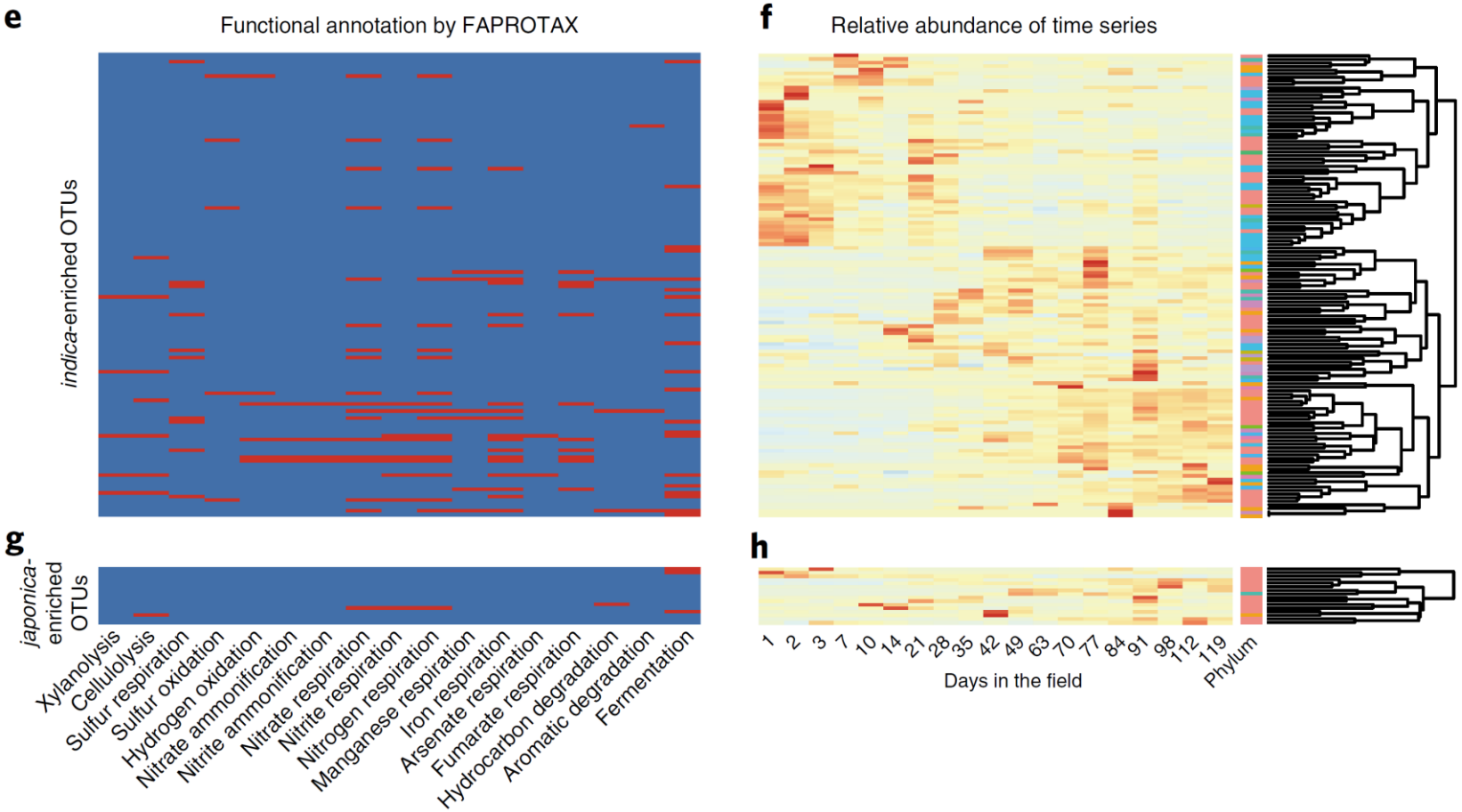
参考 https://github.com/genomicsclass/labs

Jingying Zhang, **Yong-Xin Liu**, et. al.
Nature Biotechnology. 2019. Fig 3c/d

热图展示有无或梯度

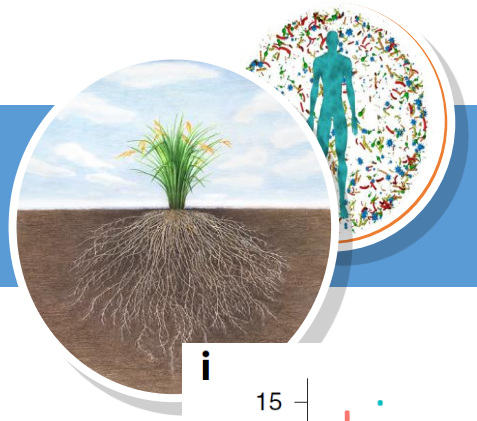
pheatmap包绘制

双色热图展示每个OTUs中是否存在某种功能

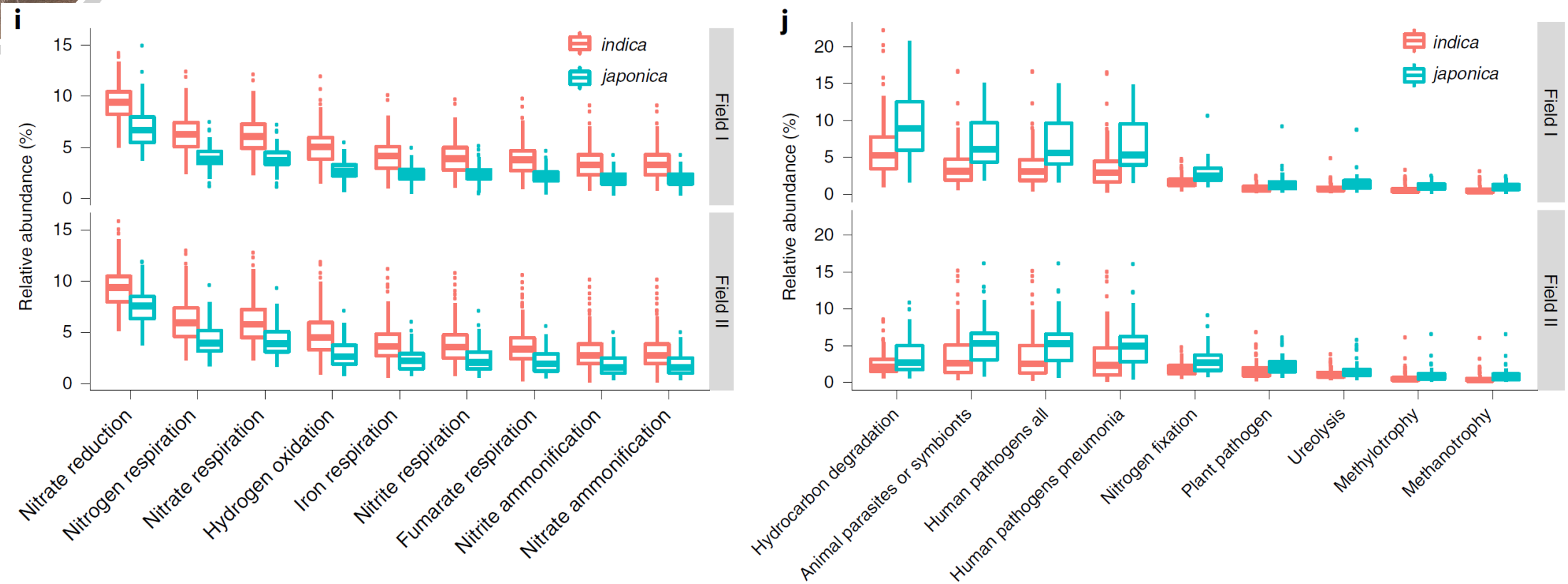


热图Z-Score展示每个OTUs在时间序列中的动态变化，方便观察丰度富集的时期

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 3e/f/g/h

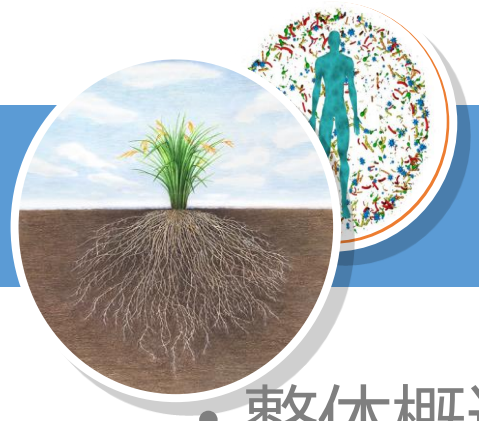


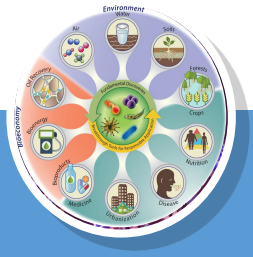
箱线图展示功能通路组间差异



ggplot2绘制默认格式箱线图，左右分别为两组特异富含的功能通路的相对丰度，上下为两块地的重复facet_grid(soiltype ~ .)

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 3i/j





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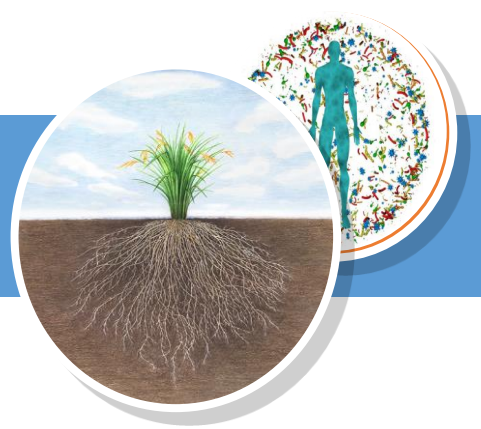
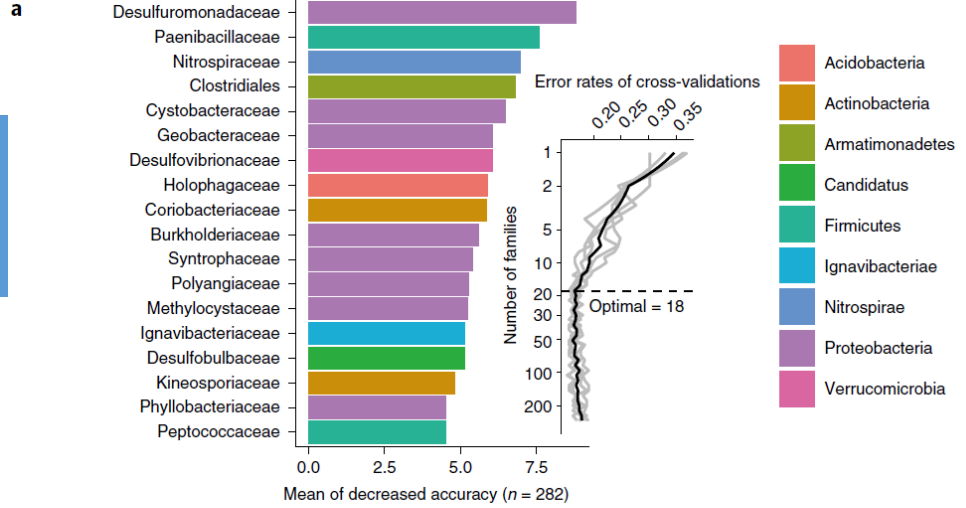
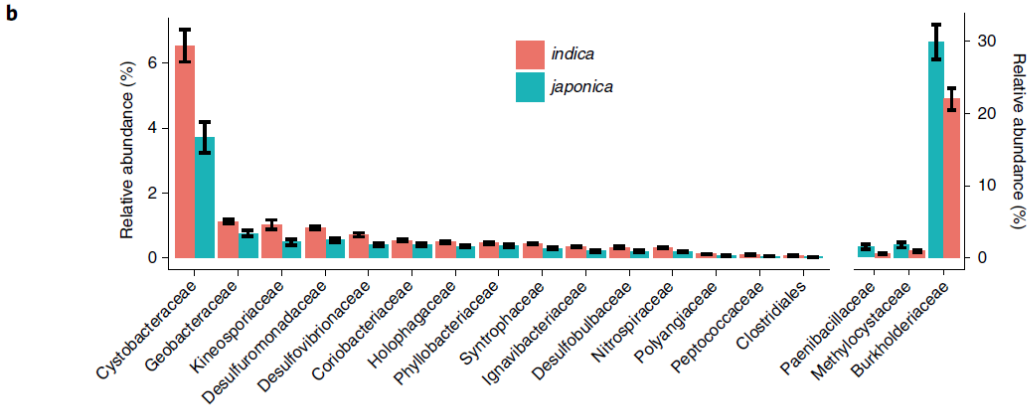
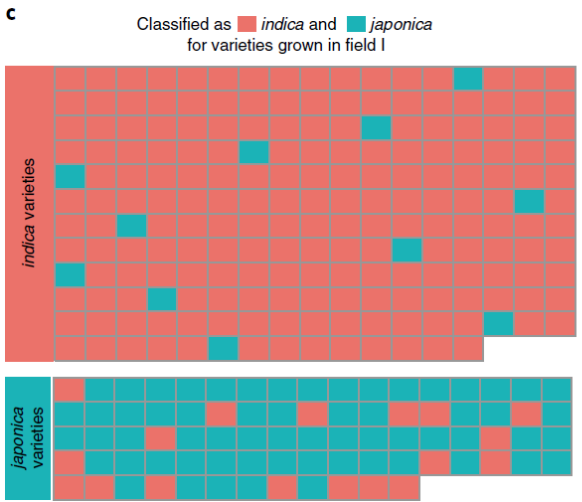


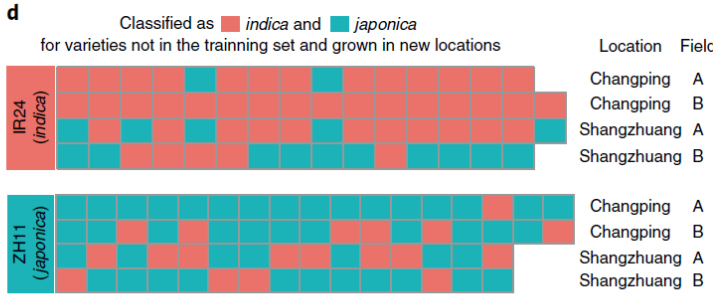
随机森林分类

1. 鉴定特征的重要性
2. 交叉验证选择合适的特征组合
3. 特征在组间相对丰度
4. 同地和异地的验证







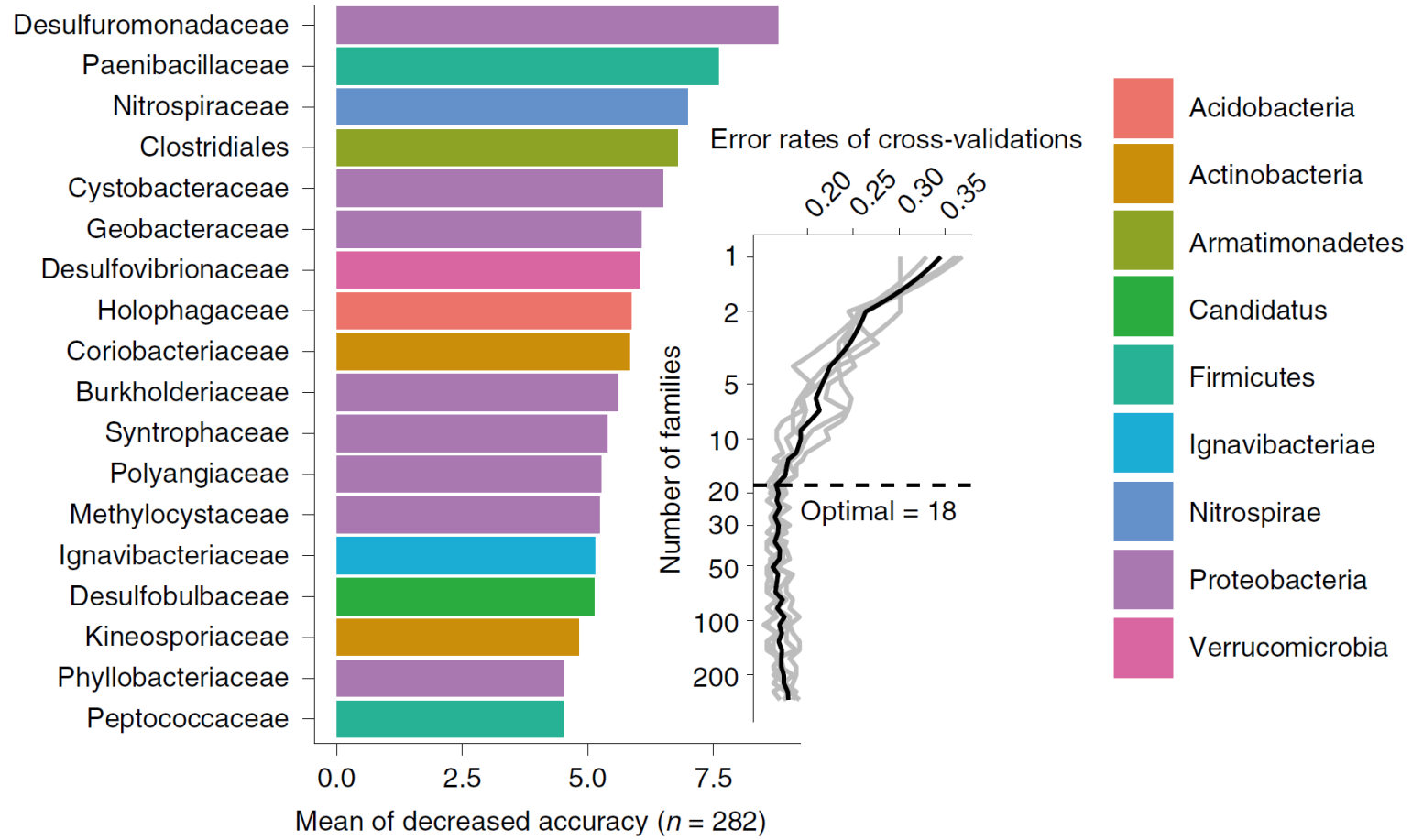


Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 2

随机森林挖掘重要分类特征

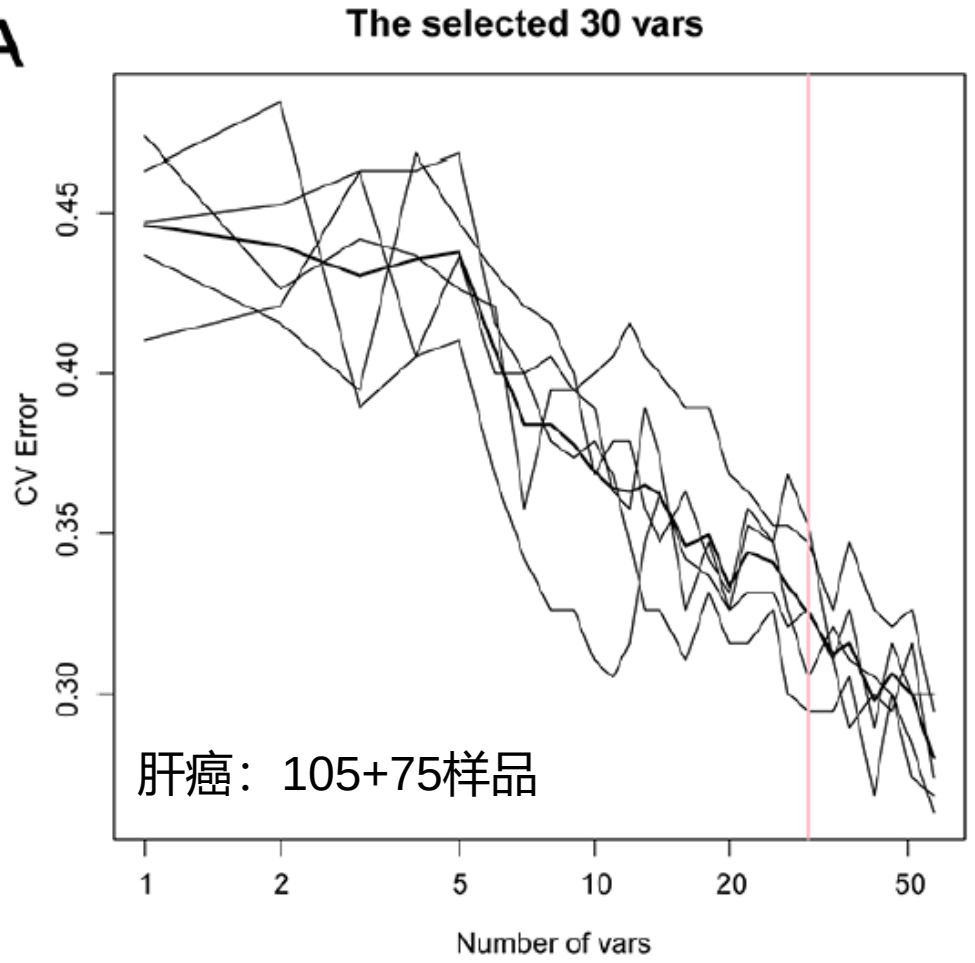
randomForest包对样本离散型进行分类，连续型进行回归

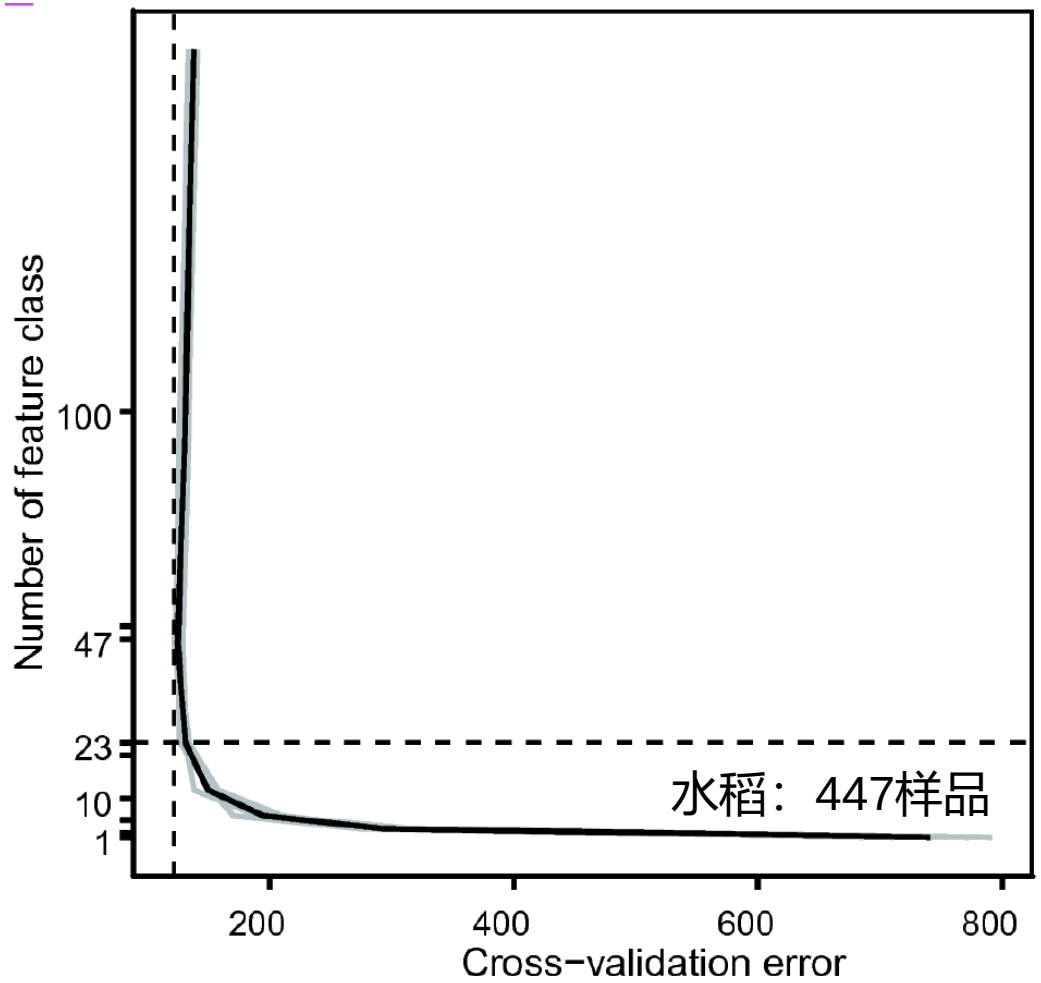
1. randomForest建模和导出特征贡献度importance，ggplot2柱状图可视化
2. 多次交叉验证，人为选择合适的特征组合，ggplot2折线图可视化



Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 2a

寻找生物标志物：交叉验证确定较优数量



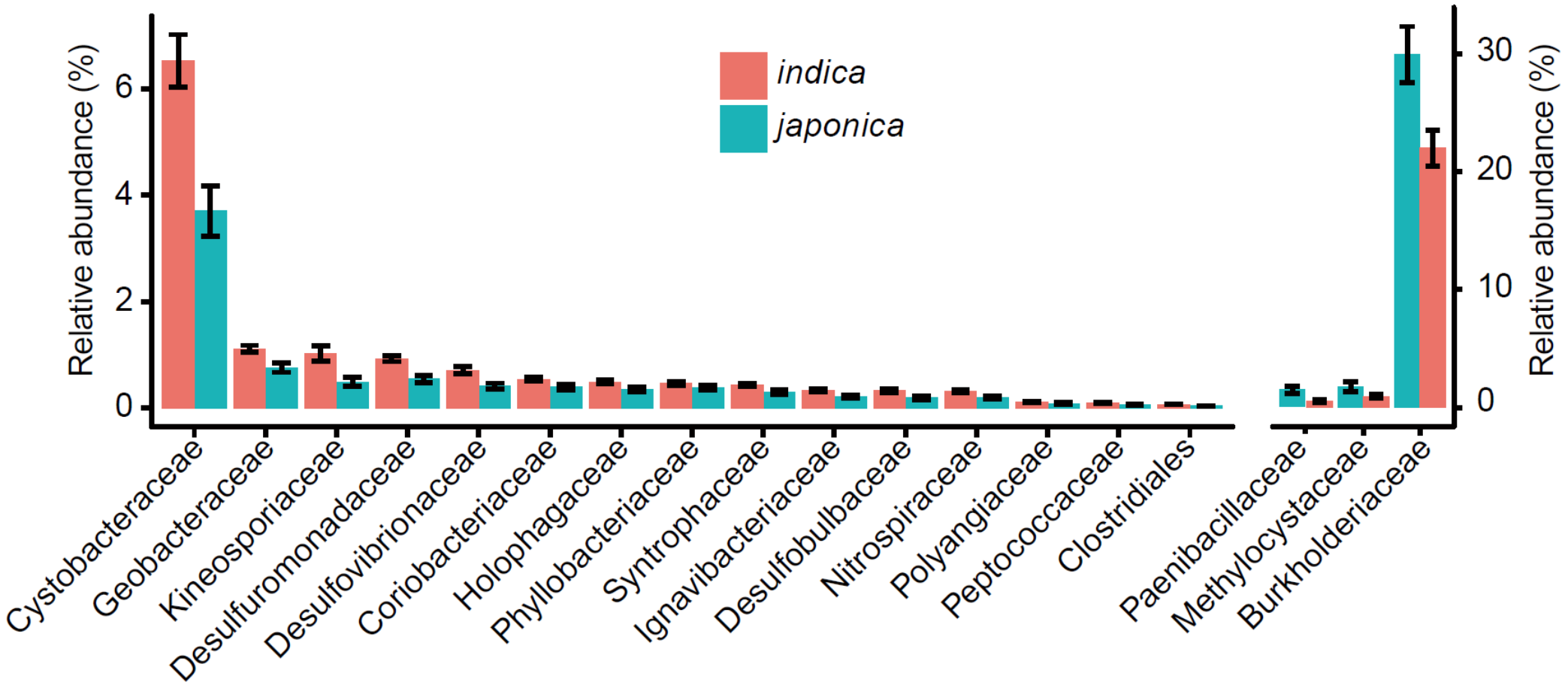


宏基因组

Ren Z, Li A, Jiang J, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma[J]. Gut, 2018: gutjnl-2017-315084.

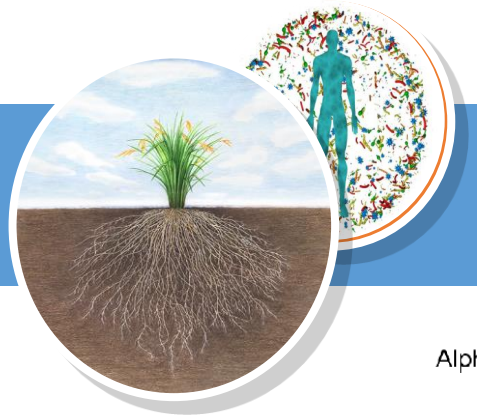
Jingying, Z., et al. (2018). "Root microbiota shift in rice correlates with resident time in the field and developmental stage." Science China Life Sciences.

重要分类特征在组间差异

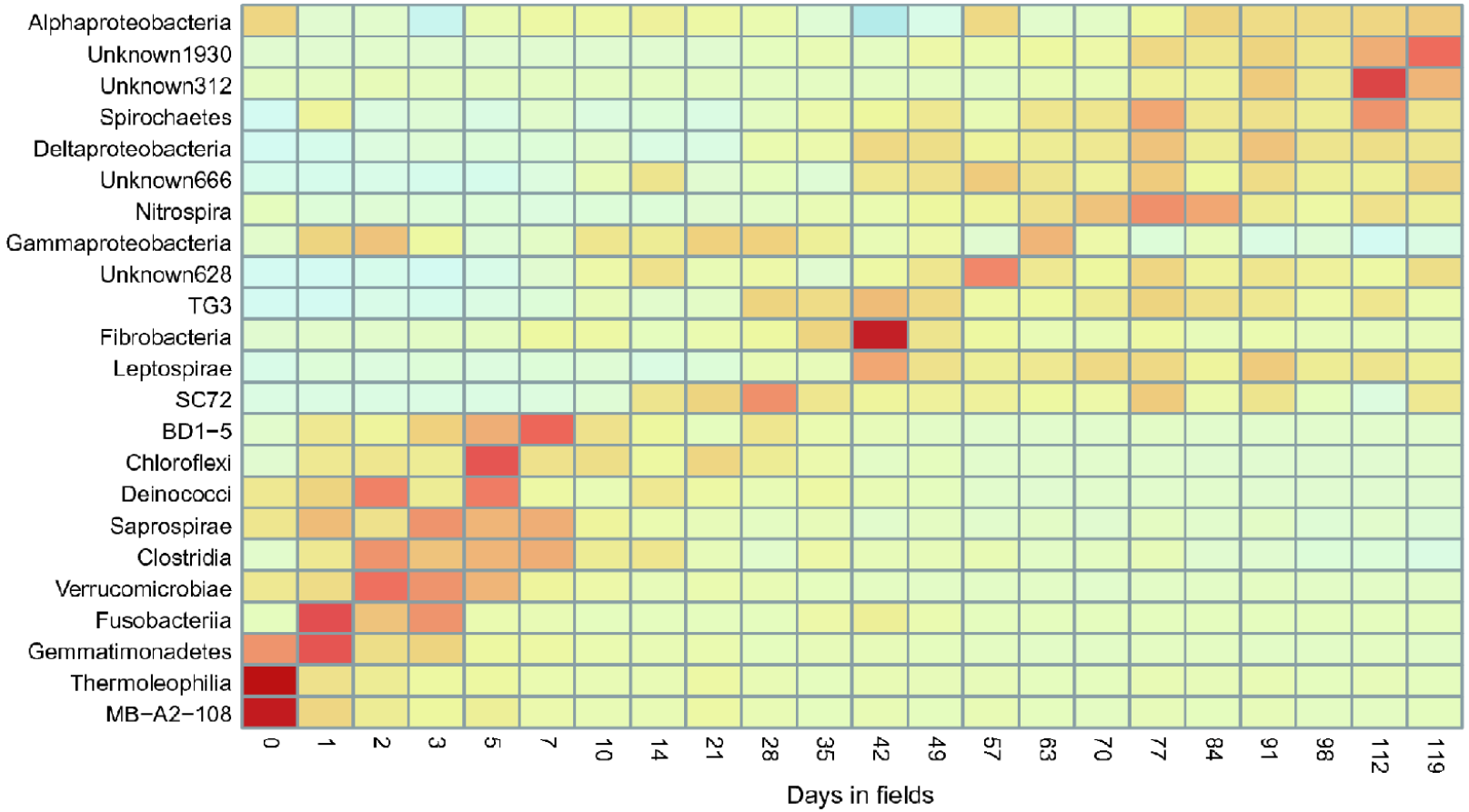


柱状图+标准误展示特征丰度的组间差异，分类再排序

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 2b



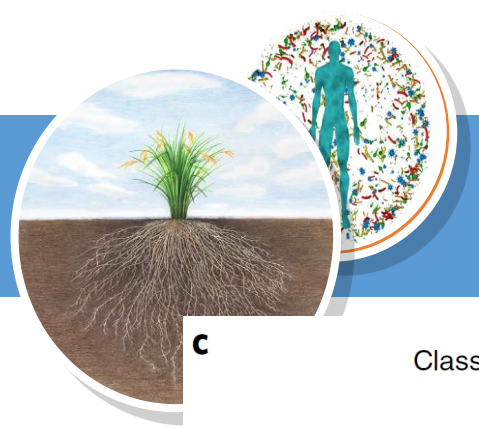
重要分类特征在时间上变化

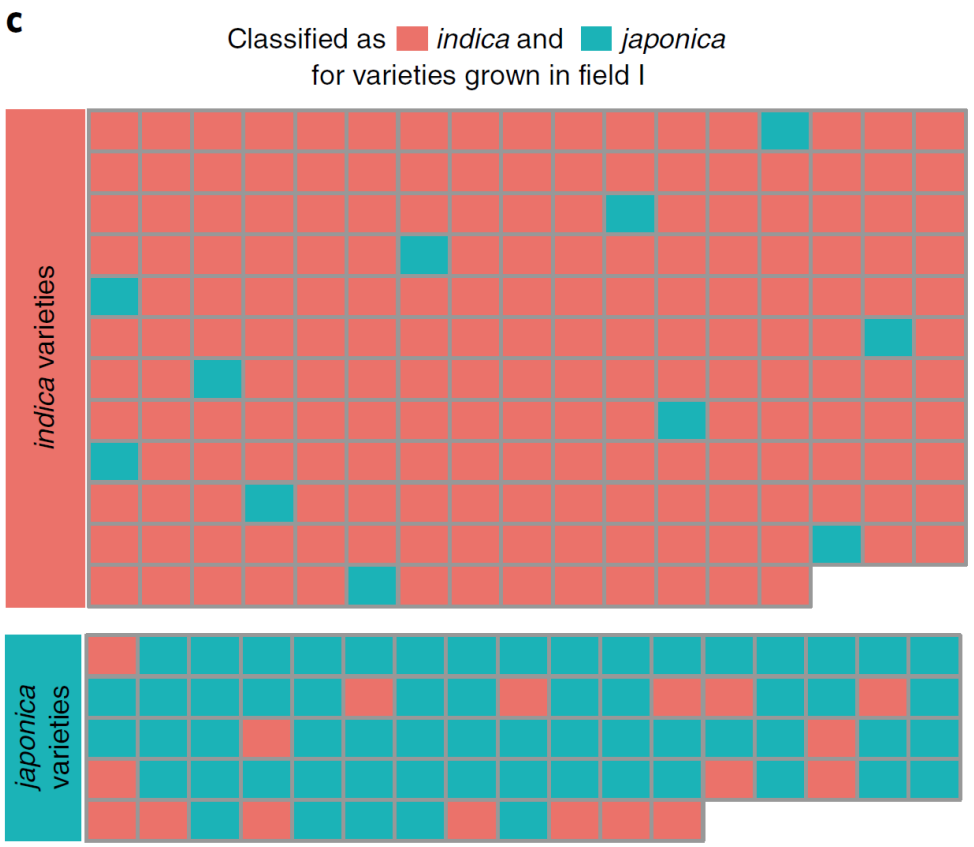


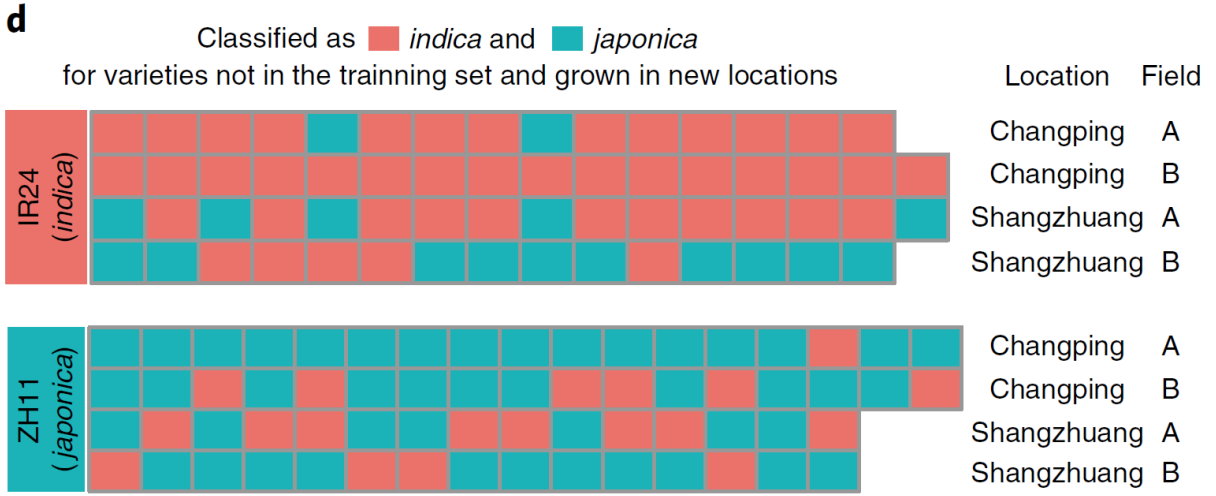
pheatmap绘制，数据行按最高丰度出现的时间排序，列按时间序列排序

Jingying Zhang, Na Zhang, **Yong-Xin Liu**, et. al. Root microbiota shift in rice correlates with resident time in the field and developmental stage. ***Science China Life Sciences***. 2018. doi:10.1007/s11427-018-9284-4 Fig 4B

模型普适性评估——预测样本分类



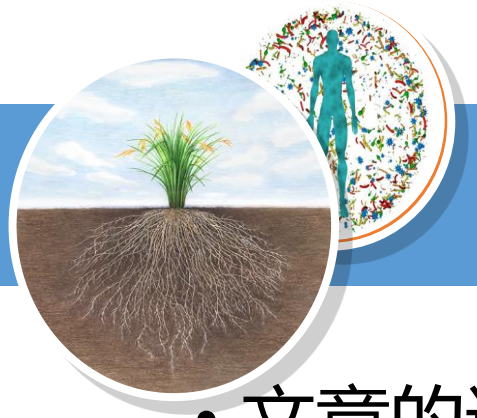


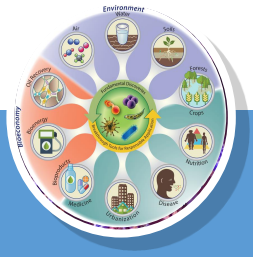


pheatmap展示预测结果，需要考虑布局换行和末行补齐

模型对另一块地相同品种(c)和不同地点不同品种(d)预测

Jingying Zhang, **Yong-Xin Liu**, et. al. ***Nature Biotechnology***. 2019. Fig 2c/d



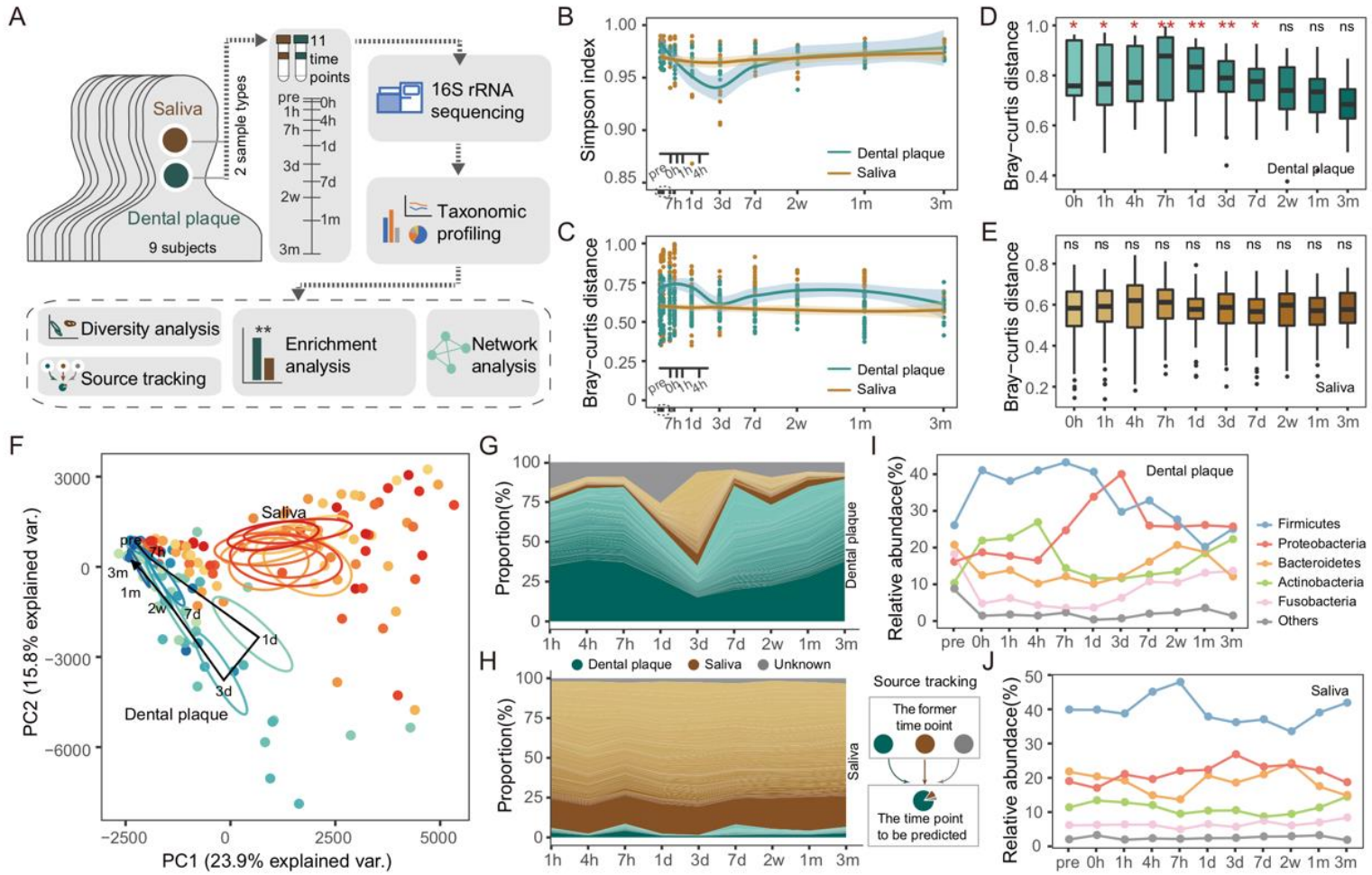


总结

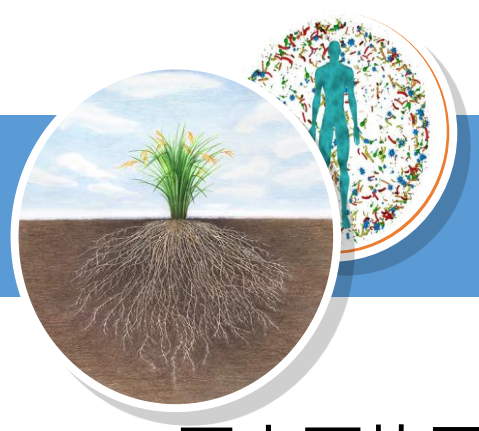
- 文章的逻辑结构：总——分——应用，3 – 4个组图，最多6个
- 图的逻辑：3-6个子图构成一个组图，说明一个主题
- 1总：地图和实验设计、α多样性箱线图、β多样性PCoA散点图、物种组成柱状图
- 2分：差异比较曼哈顿/火山图、韦恩图进一步比较重复性、热图结合有/无或丰度注释、箱线图展示差异功能
- 3应用：建模(特征数量和贡献度)、特征在组间差异、模型验证(预测不同来源数据测试准确性和普适性)
- 文章的逻辑以吸引人和可读性为主，而非实际实验或分析的顺序

规律的验证1. 整体描述

A. 实验设计
B. Alpha多样性
C. Beta多样性拟合
D. 牙菌斑Beta箱线
E. 唾液Beta箱线
F. PCA分析+置信椭圆
G. 牙菌斑来源
H. 唾液来源
I. 牙菌斑物种组成
J. 唾液物种组成

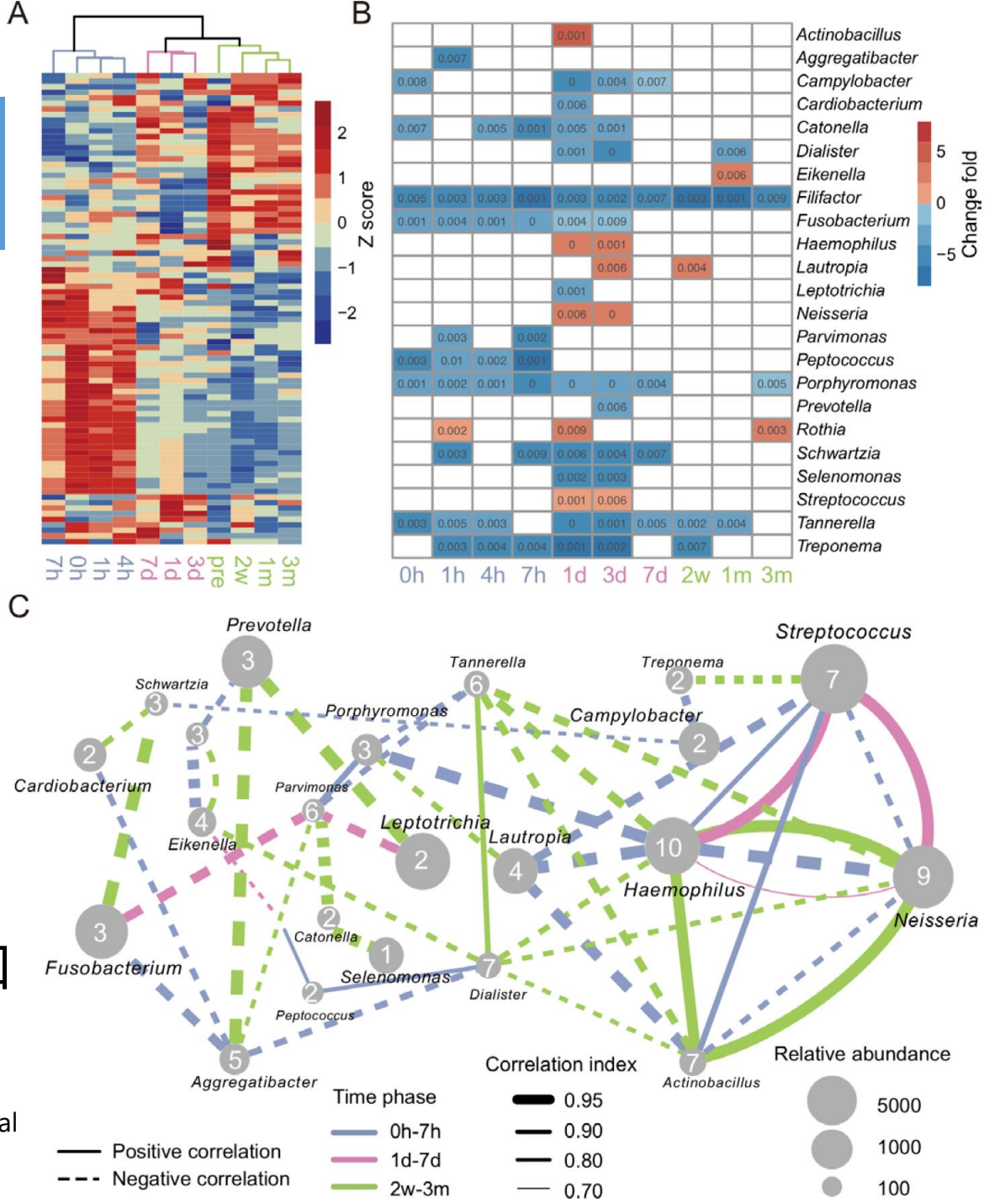


Wang, J., Jia, Z., Zhang, B., Peng, L. & Zhao, F. Tracing the accumulation of in vivo human oral microbiota elucidates microbial community dynamics at the gateway to the GI tract. *Gut*, gutjnl-2019-318977, doi:10.1136/gutjnl-2019-318977 (2019).



2. 细节展示

A. 属水平热图组成+聚类
B. 属水平显著差异+P值+差异倍数
C. 网络分析
   a. 线型实/虚代表正负相关
   b. 线颜色代表时间短、中、长(与A对应)
   c. 线粗细代表相关系数绝对值
   d. 点大小代表相对丰度
   e. 点数字代表节点度数
   f. 点标签为属名
   g. 有时还有点颜色，点边框色属性可添加

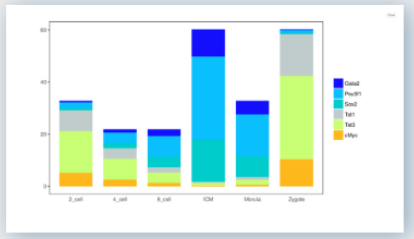
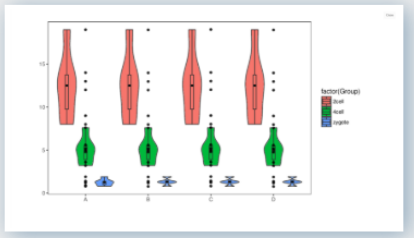
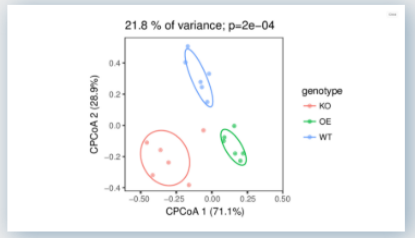


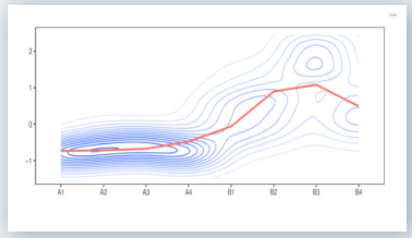
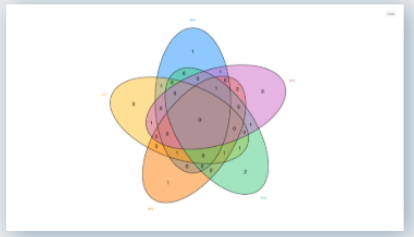
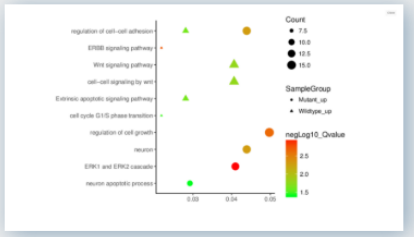
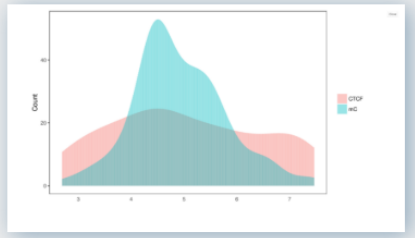
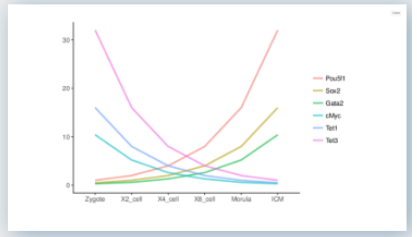
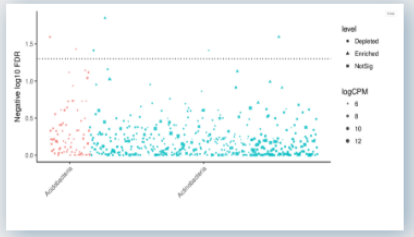
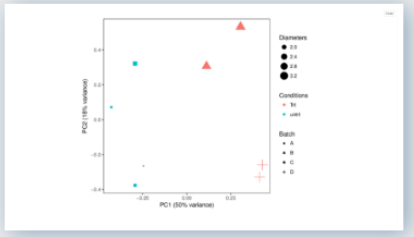
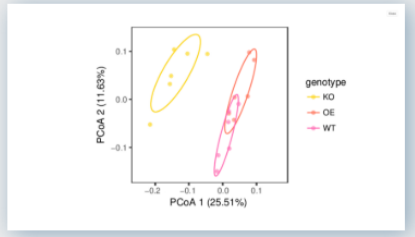
Wang, J., Jia, Z., Zhang, B., Peng, L. & Zhao, F. Tracing the accumulation of in vivo human oral microbiota elucidates microbial community dynamics at the gateway to the GI tract. *Gut*, gutjnl-2019-318977, doi:10.1136/gutjnl-2019-318977 (2019).

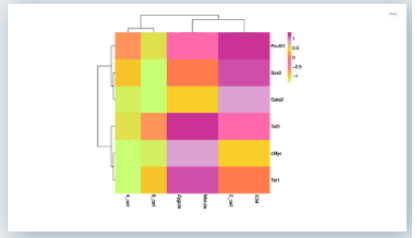
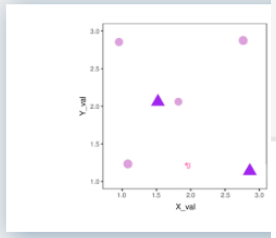
ImageGP——在线绘制20种图

- Histogram
- VennDiagram
- UpsetView
- Density plot
- PCA plot
- PCoA plot
- CPCoA plot
- Sankey
- PICRUSt
- LEFSe
- FAPROTAX
- BugBase
- Problem Feedback 反馈建议
- Manuals
- FAQ
- Code wall NEW!

Image previews http://www.ehbio.com/ImageGP/

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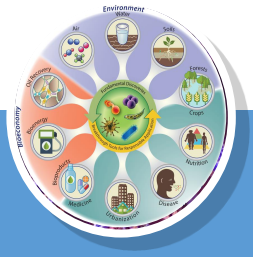
Articles About 30 results (0.08 sec)

粘贴数据 - 选择参数 - 一键出图



- Home
- Line plot
- GO Enrichment plot
- Pretty Heatmap
- Boxplot
- Scatter plot
- Bar plot
- Volcano plot
- Manhattan plot
- Histogram
- VennDiagram
- UpsetView
- Density plot
- PCA plot
- PCoA plot
- CPCoA plot

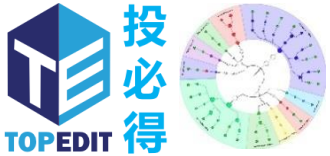
Please paste your data matrix below (tab separated text file)

Demo

Gene	Group	Expr
A	zygote	0.8
A	zygote	1.3
A	zygote	1.4
A	zygote	0.9
A	zygote	1.9
A	zygote	1.2
A	2cell	8
A	2cell	13
A	2cell	14
A	2cell	9
A	2cell	19

Essential parameters

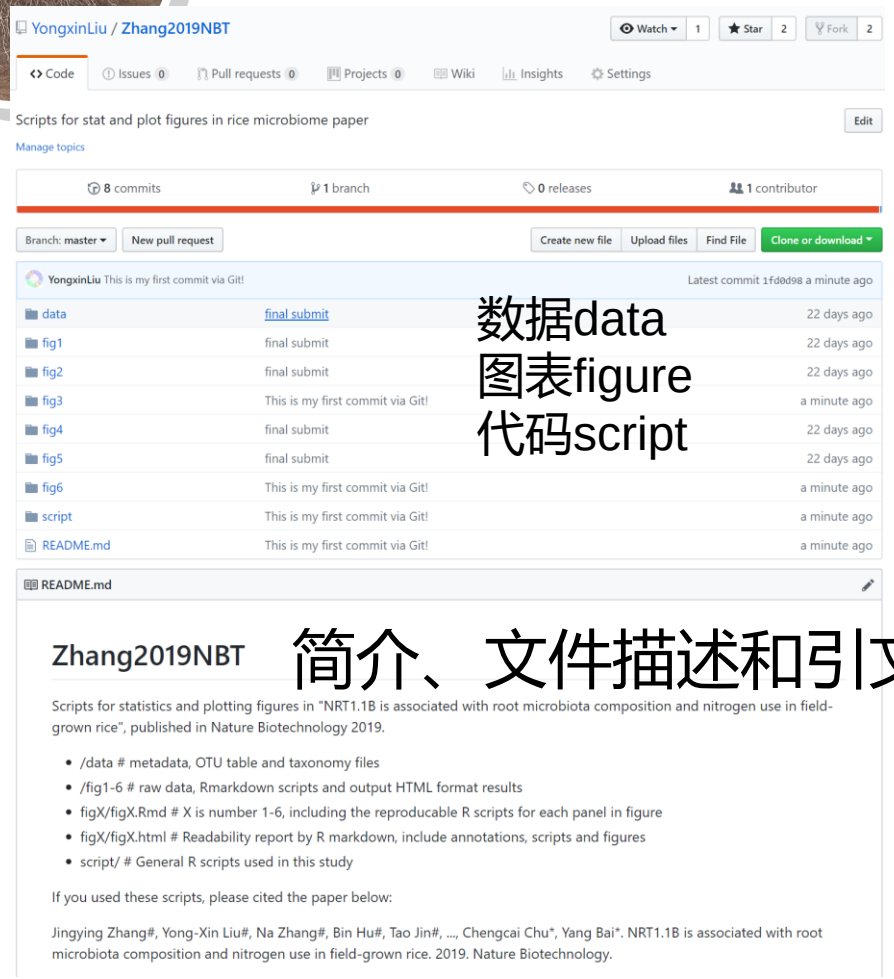
http://www.ehbio.com/ImageGP/



提供开源可重复分析的实验室

- **密歇根大学Pat Schloss http://www.schlosslab.org**
- 期坦福大学Susan Holmes http://statweb.stanford.edu/~susan/
- 德国马普Paul Schulze-Lefert https://github.com/garridoo
- 北卡教堂山Jeffery L. Dangl https://github.com/isaisg/
- EMBL-EBI Robert D. Finn https://github.com/Finn-Lab/MGS-gut
- 比利时鲁汶大学 Jeroen Raes https://github.com/raeslab
- 贝勒医学院 Christopher J. Stewart https://github.com/StewartLab
- 遗传发育所/CEPAMS白洋 https://github.com/microbiota

数据代码图表整理示例



数据data
图表figure
代码script

简介、文件描述和引文

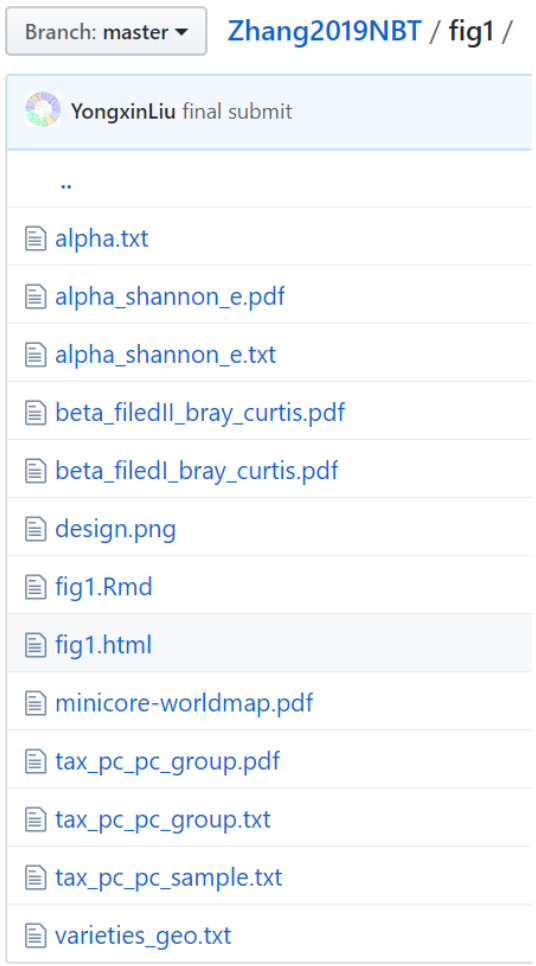


图1分析目录文件

fig1.Rmd 分析代码及注释

fig1.html 分析代码、注释和图表混排网页，方便阅读

*.txt 分析原始数据或统计结果表格

*.png 位图

*.pdf 矢量图，常用保存格式，方便查看和编辑

https://github.com/YongxinLiu/Zhang2019NBT

分析代码样式和网页预览效果

Branch: master ▾ Zhang2019NBT / fig1 / fig1.Rmd Find file Copy path

YongxinLiu final submit 94b8505 22 days ago

1 contributor

322 lines (264 sloc) 12.2 KB Raw Blame History

```
---
title: "Figure 1. Root microbiota of indica and japonica. "
author: "Yong-Xin Liu"
date: "2019/2/20"
output: html_document
---

```{r setup, include=FALSE}
knitr::opts_chunk$set(echo = TRUE)
# Clean workspace
rm(list=ls())
# Load setting and functions
source("../script/stat_plot_functions.R")
# Set output directory
output_dir="./"
---


## a. World map

(a) Diagram of original collection sites (44 countries) of indica (red) and japonica (blue) rice.

```{r geomap, echo=TRUE}
library(dplyr)
library(maptools)
library(ggplot2)
library(maps)

geotable = read.table("varieties_geo.txt", header = T, sep = "\t")
worldmap = map_data("world")

fig1 = ggplot(geotable, aes(Longitude, Latitude, color = Subspecies)) +
  geom_polygon(data = worldmap, aes(x = long, y = lat, group = group, fill = NA), color = "grey70", size = 0.25)+
  geom_point(size = 2.5, alpha = 0.5)+ scale_colour_brewer(palette = "Set1") +
  scale_fill_brewer(palette = "Set1")+
  coord_cartesian()+
  scale_y_continuous(breaks = (-3:3)*30)+
  scale_x_continuous(breaks = (-6:6)*30)+
  labs(x="Longitude", y="Latitude", colour = "Subspecies" ) +
  theme_tufte()
ggsave(paste0(output_dir, "minicore-worldmap.pdf", sep=""), fig1, width = 9, height = 5)
fig1
---
```

标题、作者、日期

设置通用参数和加载通用依赖关系

图1a，绘制材料来源地图的代码

fig1.Rmd 代码在线或Rstudio查看

Figure 1. Root microbiota of indica and japonica.

Yong-Xin Liu

2019/2/20

a. World map

a. Diagram of original collection sites (44 countries) of indica (red) and japonica (blue) rice.

```
library(dplyr)
library(maptools)
```

```
## Warning: package 'maptools' was built under R version 3.4.4
```

```
## Loading required package: sp
```

```
## Checking rgeos availability: FALSE
##      Note: when rgeos is not available, polygon geometry     computations in maptools depend on gpclib,
##      which has a restricted licence. It is disabled by default;
##      to enable gpclib, type gpclibPermit()
```

```
library(ggplot2)
library(maps)

geotable = read.table("varieties_geo.txt", header = T, sep = "\t")
worldmap = map_data("world")

fig1 = ggplot(geotable, aes(Longitude, Latitude, color = Subspecies)) +
  geom_polygon(data = worldmap, aes(x = long, y = lat, group = group, fill = NA), color = "grey70", size = 0.25)+
  geom_point(size = 2.5, alpha = 0.5)+ scale_colour_brewer(palette = "Set1") +
  scale_fill_brewer(palette = "Set1")+
  coord_cartesian()+
  scale_y_continuous(breaks = (-3:3)*30)+
  scale_x_continuous(breaks = (-6:6)*30)+
  labs(x="Longitude", y="Latitude", colour = "Subspecies" ) +
  theme_tufte()
ggsave(paste0(output_dir, "minicore-worldmap.pdf", sep=""), fig1, width = 9, height = 5)
fig1
```

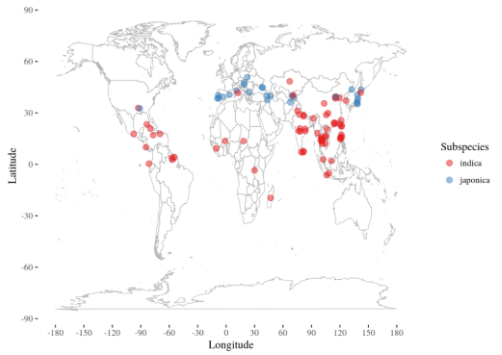


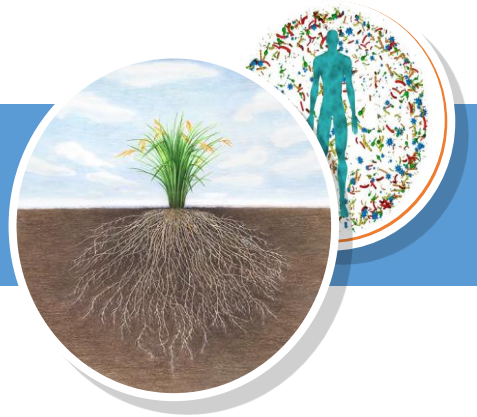
fig1.html可由Rmd生成下载用浏览器查看

标题、作者和时间带格式

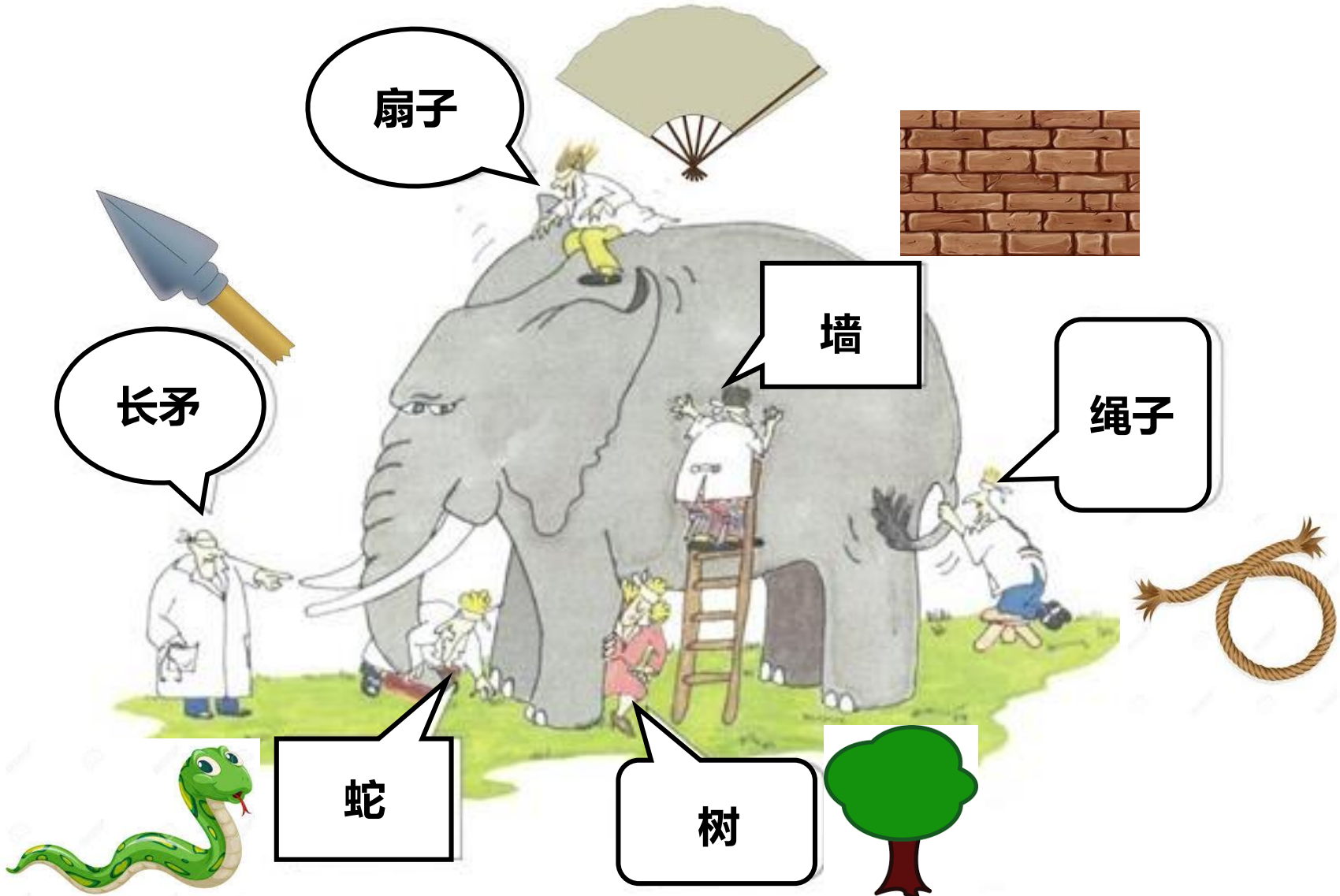
运行中的提示信息

分析代码

绘图结果



科研的过程——盲人摸象



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