



读书报告

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ORIGINAL ARTICLE

Predicting taxonomic and functional structure of microbial communities in acid mine drainage

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Abstract

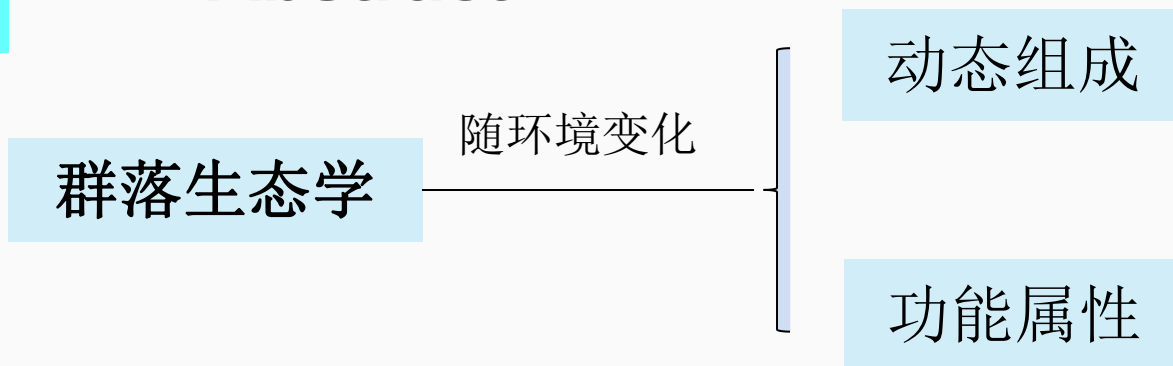
Introduction

Materials and methods

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Abstract



目的：建立一个低生物丰富度的模型系统。

对象：中国东南亚地区**40**个酸性矿水排水系统（**AMD**系统）的微生物群落的空间分布和功能结构。

方式：**16s rrna**焦磷酸测序、**GeoChip**微阵列。

功能基因水平 > 分类水平

自然选择 > 地理距离

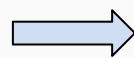
Introduction

已有研究:

描述分析

预测物种分布的统计模型的发展和应用

研究植物和动物物种分布模型



环境变化对自然群落和生态系统造成的影响和结果

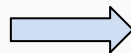


环境变异、空间隔离等塑造大型微生物生态位宽度

预测模型分析

高通量分子技术的发展

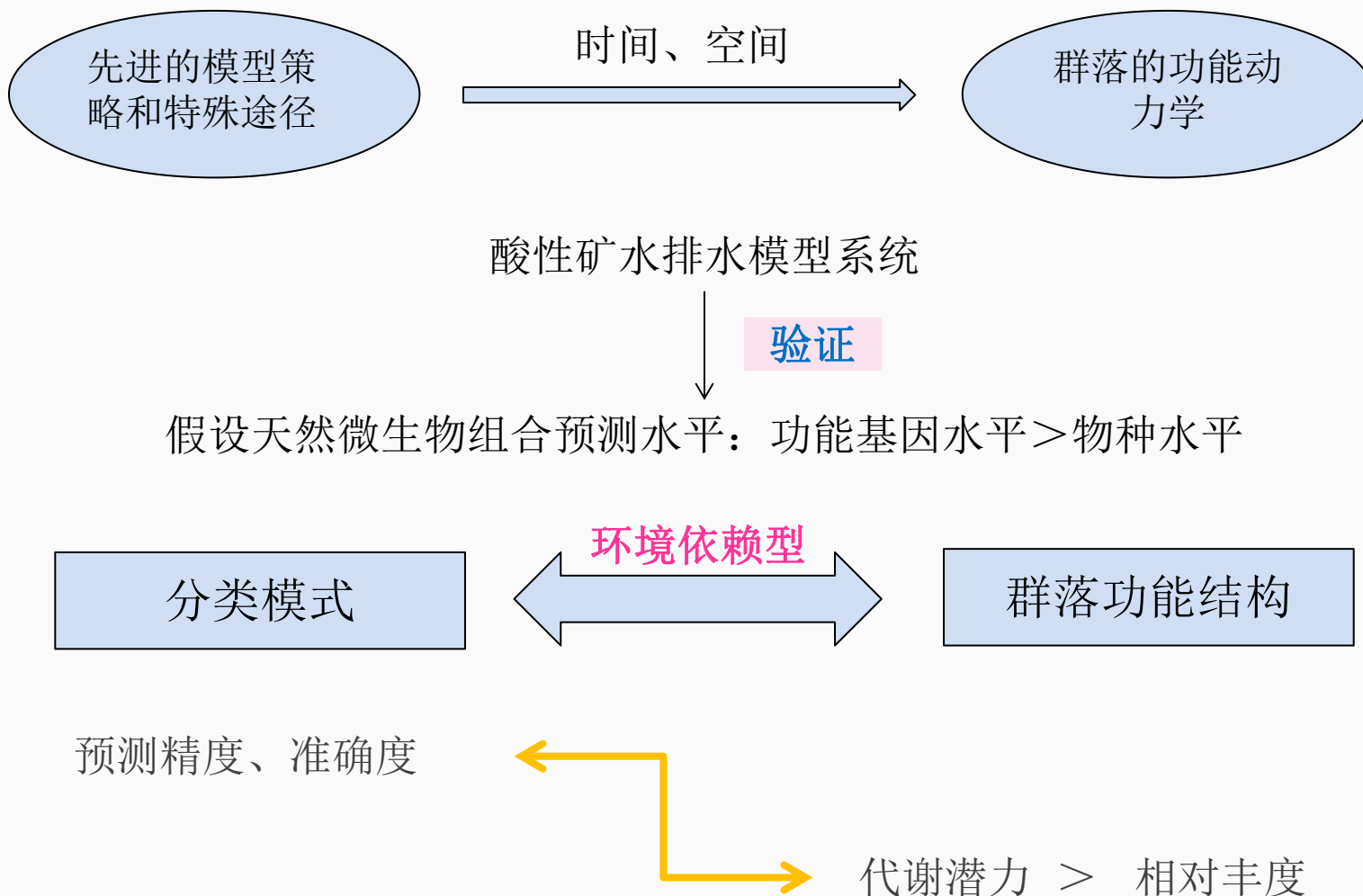
先进的生物信息学技术



预测多种多样的生态系中的微生物的生物地理分布

生态地理学

Introduction



Materials and methods

1

- 微生物组合和功能性代谢潜力的预测模型: ANN(artificial neural networks).
- 30个随机样本用于训练模型, 余下10个用于验证模型。

2

- 高通量基因芯片分析和数据处理: 功能代谢潜力(即基因丰度)是反映在检测探针的信号强度的总和
- 基因参与的关键生物地球化学和生态过程,包括C、N、S循环、P利用率、能量流程、应激反应、重金属和抗生素耐药性

3

- 微生物组合和功能性代谢潜力的预测模型: ANN(artificial neural networks).
- 30个随机样本用于训练模型, 余下10个用于验证模型。

Materials and methods

4

- 比较不同生物之间的预测精度水平
- 预测值和观察值的Bray-Curtis距离 → 预测精度
- 分析t检验和统计意义(P值)
- 微生物分类/系统发育水平: 门, 目和操作分类单位(16 s rrna相似性水平高于97%)

5

- 统计分析
- Bray-Curtis距离矩阵 → 构造不同的微生物群落组成和功能群落结构
- Euclidean距离 → 使用标准化的环境变量和地理位置
- 根据地球化学数据聚类样本进行统计学意义上的分析: 置换多变量方差分析(Adonis函数), 分析相似(ANOSIM函数)和多元响应置换过程分析(MRPP函数)

Results

1. 不同环境条件下的分类模式和功能性群落结构

Table 1 Results of significant differences of the geochemical properties, the microbial community composition and the functional community structure between the sample groups

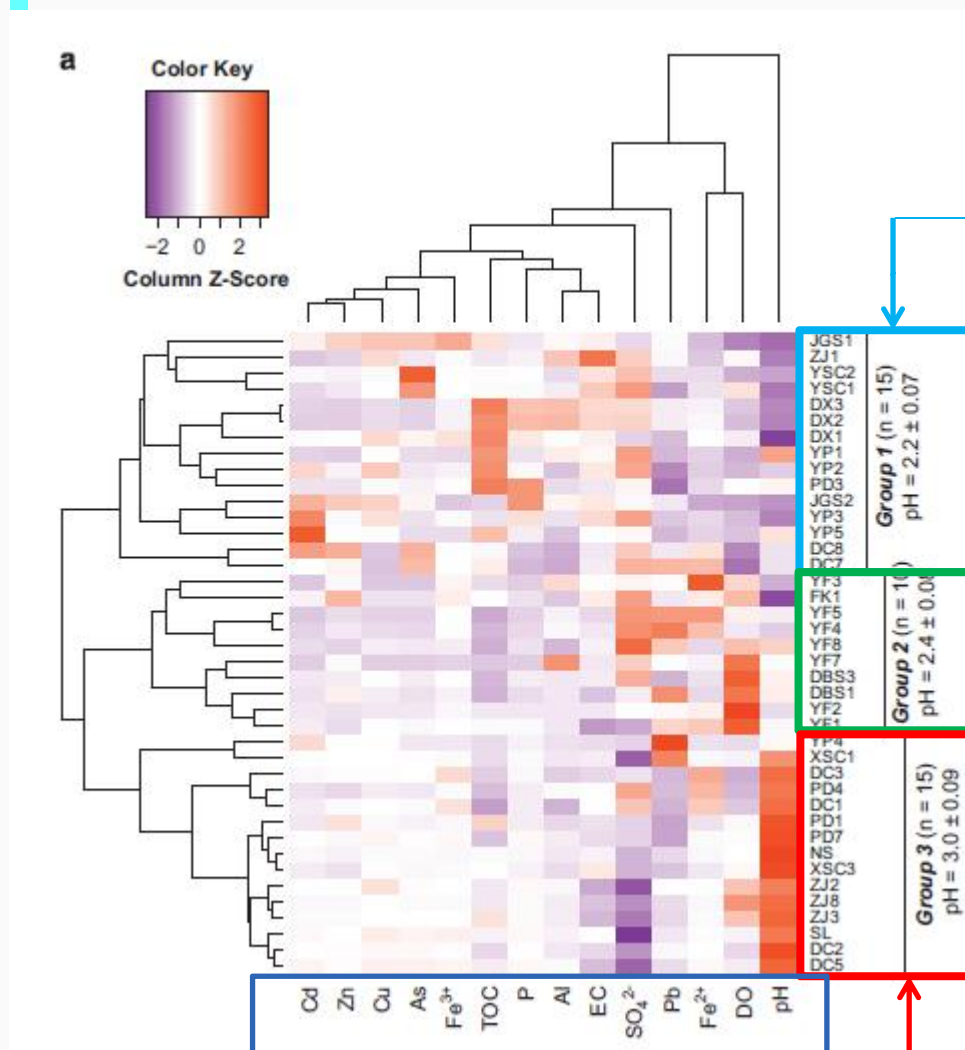
<i>Dissimilarity method</i>	<i>Geochemical properties (Euclidean distance)</i>		<i>Microbial community composition (Bray–Curtis)^a</i>		<i>Functional community structure (Log, Bray–Curtis)^b</i>	
	<i>Statistic</i>	<i>P-value</i>	<i>Statistic</i>	<i>P-value</i>	<i>Statistic</i>	<i>P-value</i>
Adonis ^c	0.029	0.001	0.098	0.018	0.207	0.001
ANOSIM ^d	0.244	0.001	0.041	0.015	0.238	0.001
MRPP ^e	0.082	0.001	0.032	0.031	0.106	0.001

^aBray–Curtis distance of microbial community composition is calculated based on the OTUs defined at the 97% similarity level. ^bThe signal intensity of each probe was log-transformed before Bray–Curtis distance calculation. ^cPermutational multivariate analysis of variance. ^dAnalysis of similarity. ^eMulti-response permutation procedure analysis.

置换多元方差分析、相似性分析、多响应置换进程分析

Results

分层聚类分析

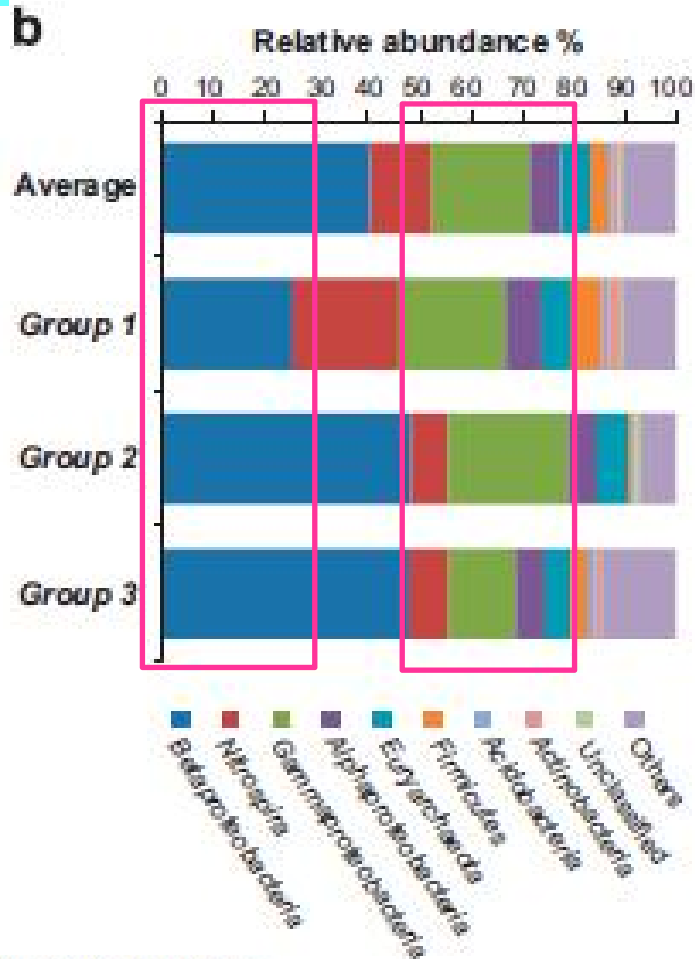


Group1 (n=15) : pH = 2.2±0.07;
JGS1、JGS2、YSC1、YSC2、ZJ1、
DC7、DC8、PD3、DX1、DX2、
DX3、YP1、YP2、YP3、YP5

Group2 (n=10) : pH = 2.4±0.08;
DBS1、DBS3、FK1、YF1、YF2、
YF3、YF4、YF5、YF7、YF8

Group3 (n=15) : pH = 3.0±0.09;
NS、XSC1、XSC3、ZJ2、ZJ3、ZJ8、
DC1、DC2、DC3、DC5、DC7、
DC8、PD3、PD4、PD7、SL、YP4

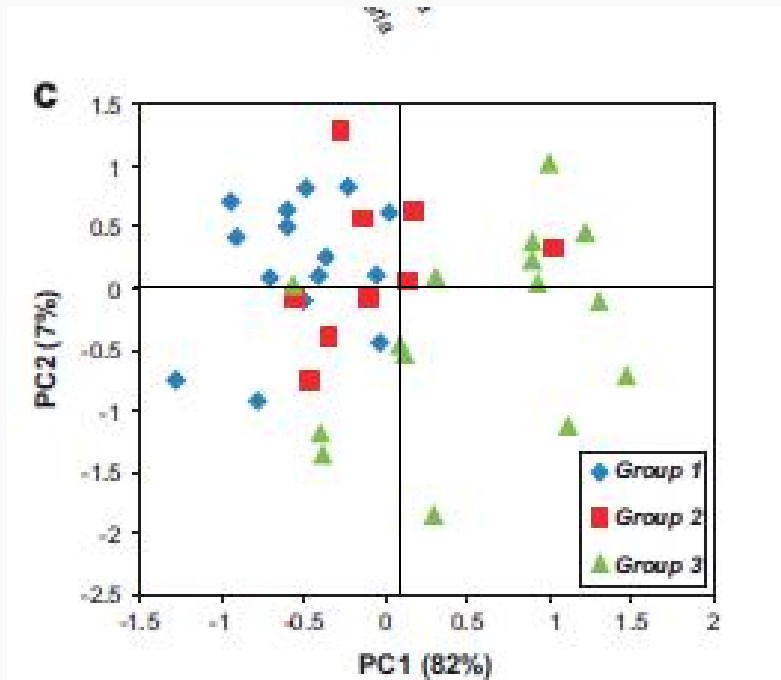
Results



the distribution patterns of microbial community composition (b) among distinct environmental conditions.

变形菌门: β -变形菌
 γ -变形菌

Results



functional community structure (c) among distinct environmental conditions.

功能性群落结构

分类模式和群落功能结构可能受到不同地球化学性质，丰生物和生物结构之间的差异以及三个在样品中明显不同的非参数多变量的影响。

Results

2. 对于环境动态属性和微生物分类模型的代谢潜力的响应

Table S5. Environmental and taxonomic variable loadings on the PCs across the AMD samples.

Environmental properties (Abbr.)	PC _{Env} 1		PC _{Env} 2		Microbial taxa (Abbr.)	PC _{Taxa} 1		PC _{Taxa} 2		PC _{Taxa} 3		PC _{Taxa} 4	
	exp.*	(E1)	(E2)			exp.	(T1)	(T2)	(T3)	(T4)			
pH		0.636	-0.001		<i>Euryarchaeota</i> (Eury)		-1.050	0.251	-0.691	0.062			
Dissolved Oxygen (DO)		0.103	0.148		<i>Acidobacteria</i> (Acido)		-0.088	-0.769	0.368	-0.647			
Total Organic Carbon (TOC)		-0.397	-0.175		<i>Actinobacteria</i> (Actino)		-0.842	-0.812	0.019	0.081			
Electrical Conductivity (EC)		-0.503	-0.040		<i>Firmicutes</i> (Firm)		-0.808	-0.690	0.180	0.184			
Sulfate (SO ₄ ²⁻)		-0.592	0.118		<i>Nitrospira</i> (Nitro)		-0.712	0.711	0.133	-0.420			
Ferric ion (Fe ³⁺)		-1.519	0.387		<i>Alphaproteobacteria</i> (Alpha)		0.262	-0.074	-0.083	-1.214			
Ferrous ion (Fe ²⁺)		-1.174	0.983		<i>Betaproteobacteria</i> (Beta)		1.151	-0.370	-0.620	0.298			
Aluminum (Al)		-1.047	-0.152		<i>Gammaproteobacteria</i> (Gamma)		-0.222	0.708	0.980	0.230			
Copper (Cu)		-0.669	-1.246										
Zinc (Zn)		-1.032	-0.342										
Arsenic (As)		-0.615	-0.348										
Cadmium (Cd)		-0.194	-0.205										
Lead (Pb)		-0.376	-0.278										
Phosphorus (P)		-0.100	-0.024										

Variables in bold show the dominant influence (top-50%) on each PC.

* Proportion explained.

Results

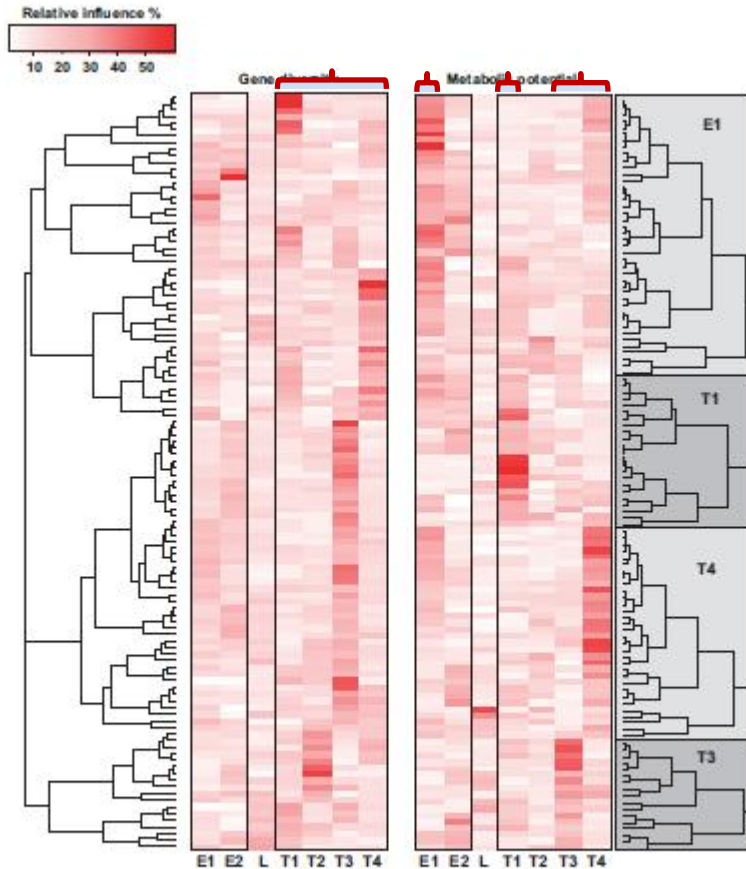


Figure 2 Relative influence (%) of environmental properties (PC_{Env} , E1, E2), spatial distribution ($PC_{Location}$, L) and microbial community composition (PC_{Taxa} , T1–T4) for gene diversity (Simpson index) and functional metabolic potential that evaluated by ABT models. Data profiles (that is, the relative influence of different PCs for each gene) were clustered with average clustering method based on the Pearson correlation. The 'boxes' qualitatively reveal the major factors (including E1, T1, T3 and T4) for the metabolic potentials of different functional genes according to the results of hierarchical clustering.

Gene diversity:
 PC_{Taxa}

Metabolic potential:
 PC_{Env} E1
 PC_{Taxa} T1、T3、T4

$PC_{Location} < PC_{Env} PC_{Taxa}$

Results

进一步了解代谢潜力对环境变化和微生物分类变化的响应

Table S6. Multiple linear regression (MLR) of environmental variables and relative abundance of dominant microbial lineages on metabolic potential of functional genes.

Category	Subcategory	Gene	PC ^a	AIC	Best model ^b	Environmental properties							Microbial taxa					
						pH	Fe ²⁺	Fe ³⁺	Al	Cu	Zn	Eury	Acido	Nitro	Alpha	Beta	Gamma	
Nitrogen cycling	Denitrification	<i>narG</i>	E1	112.51	pH + Al + Cu	-0.470			-0.452									
Nitrogen cycling	Denitrification	<i>narK</i>	E1	112.06	Cu					0.386								
Nitrogen cycling	Assimilatory N reduction	<i>nirB</i>	E1	112.46	Fe ²⁺ + Fe ³⁺		0.666	-0.571										
Sulfur cycling	Sulfite reductase	<i>dsrB</i>	E1	107.26	pH + Cu	-0.589												
Energy process	Electron transport	Fe-S cluster binding protein	E1	114.04	pH	-0.325												
Energy process	Electron transport	ferredoxin	E1	114.04	Fe ²⁺ + pH	-0.365		0.447										
Energy process	Electron transport	NADH ubiquinone oxidoreductase	E1	113.82	pH	-0.331												
Energy process	Electron transport	terminal quinol oxidase	E1	114.34	pH	-0.314												
Energy process	Hydrogenase	hydrogenase	E1	113.57	pH	-0.340												
Metal resistance	As	<i>arsB</i>	E1	113.17	pH	-0.333												
Metal resistance	As	<i>arsM</i>	E1	113.80	pH	-0.333												
Metal resistance	Cd	<i>cadA</i>	E1	112.78	pH	-0.365												
Metal resistance	Cr	<i>chrA</i>	E1	112.66	pH	-0.369												
Metal resistance	Cu	<i>copA</i>	E1	114.03	pH + Al	-0.529												
Metal resistance	Te	<i>terC</i>	E1	114.33	Cu					0.315								
Metal resistance	Te	<i>terD</i>	E1	110.58	Zn + Fe ²⁺ + pH + Al	-0.553		-0.368			0.326							
Stress response	Heat	<i>dnaK</i>	E1	113.66	Cu					0.338								
Stress response	Nitrogen limitation	<i>ghsA</i>	E1	113.37	pH + Fe ²⁺ + Zn	-0.398												
Stress response	Oxygen stress	<i>fur</i>	E1	107.60	pH	-0.488												
Stress response	Oxygen stress	<i>oxyR</i>	E1	114.20	pH	-0.320												
Stress response	Protein stress	<i>clpC</i>	E1	112.74	Al + pH	0.415		0.577										
Antibiotic resistance	Transporter	MAR antibiotics	E1	115.38	pH + Zn	-0.352												
Antibiotic resistance	Transporter	SMR antibiotics	E1	113.91	pH	-0.329												
Nitrogen cycling	Denitrification	<i>norB</i>	T1	112.60	Eury + Firm							0.316						
Nitrogen cycling	Dissimilatory N reduction	<i>oxyA</i>	T1	113.90	Eury							0.330						
Stress response	Heat	<i>hrcA</i>	T1	114.24	Eury							-0.318						
Stress response	Nitrogen limitation	<i>ghsR</i>	T1	111.51	Eury							0.401						
Stress response	Phosphate limitation	<i>pstC</i>	T1	102.82	Eury							0.570						
Antibiotic resistance	Transporter	ABC antibiotic transporter	T1	113.35	Beta												-0.348	
Nitrogen cycling	Assimilatory N reduction	<i>nirA</i>	T3	113.81	Gamma													-0.333
Phosphorus	Phosphorus utilization	<i>ppk</i>	T3	112.60	Gamma + Eury													-0.303
Sulfur cycling	Sulfur oxidation	<i>sor</i>	T3	113.18	Gamma													0.353
Metal resistance	Ag	<i>atp</i>	T3	109.03	Eury + Beta							0.552					0.326	
Metal resistance	Cd	<i>cadB/D</i>	T3	111.11	Gamma + Beta												0.366	0.530
Metal resistance	Ni	<i>nrkB</i>	T3	111.07	Gamma													-0.412
Stress response	Heat	<i>groES</i>	T3	106.08	Gamma + Acido + Eury								-0.309					-0.405
Stress response	Glucose limitation	<i>ghgH</i>	T3	113.75	Gamma + Beta													0.464
Antibiotic resistance	Transporter	<i>hlyE</i>	T3	112.56	Gamma + Eury													0.318
Nitrogen cycling	Ammonification	<i>gdh</i>	T4	112.59	Nitro + Acido								0.297	0.333				
Energy process	Electron transport	ferredoxin oxidoreductase	T4	114.35	Nitro								0.314					
Metal resistance	Hg	<i>mer</i>	T4	113.13	Alpha													0.155
Stress response	Oxygen limitation	<i>oxyB</i>	T4	114.32	Alpha													0.315
Antibiotic resistance	other category	<i>Fan</i>	T4	111.90	Beta + Alpha													-0.304 0.307

^a The most important PCs to the metabolic potential of functional genes that determined by APT model, and the variables with dominant influence based on PC loadings were selected as input in the multiple linear regression (MLR) models.

^b The best model is based on the AIC value.

Only significant estimates ($P < 0.05$) for the best model with stepwise method were reported to show the most important environmental properties and dominant taxa to the metabolic potential of functional gene.

E1: PH

Relative microbial abundances:
Euryarchaeota
Gammaproteobacteria

pH和微生物相对丰度是
AMD生态系统中决定功能
代谢潜力的主要因素

Results

3 预测微生物群落组成和功能性代谢潜力

根据预测值和观察值的Bray-Curtis距离对不同生物水平的微生物群落组成和功能性代谢潜力进行比较

功能性代谢潜力比微生物群落组成更易预测

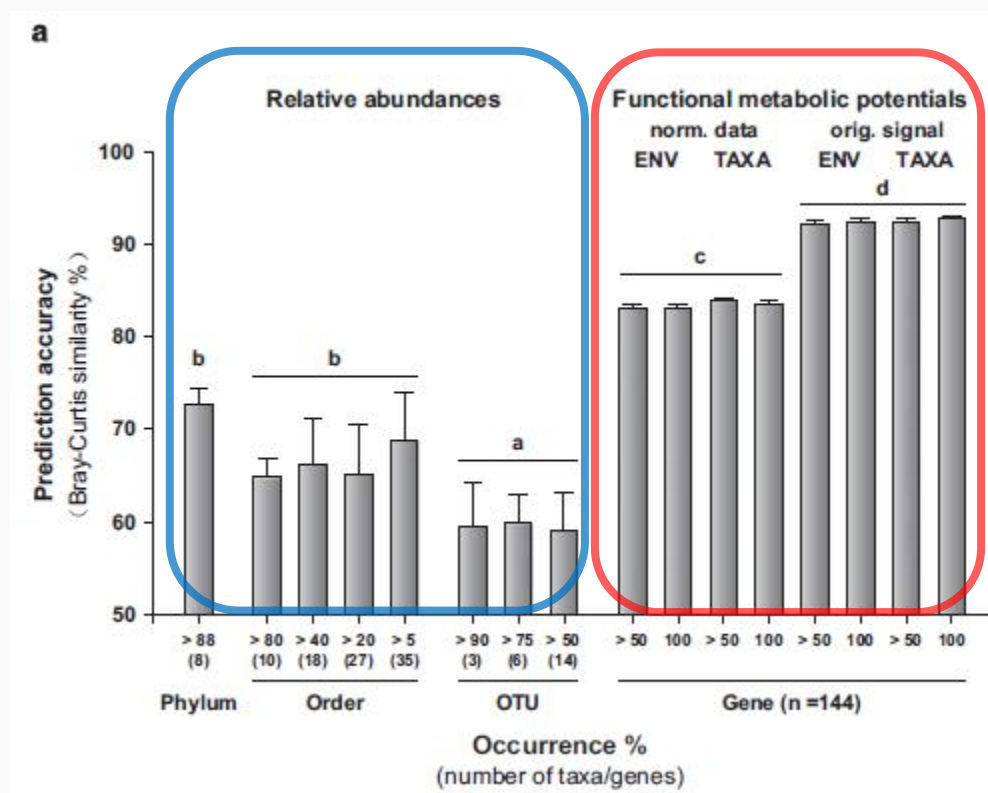
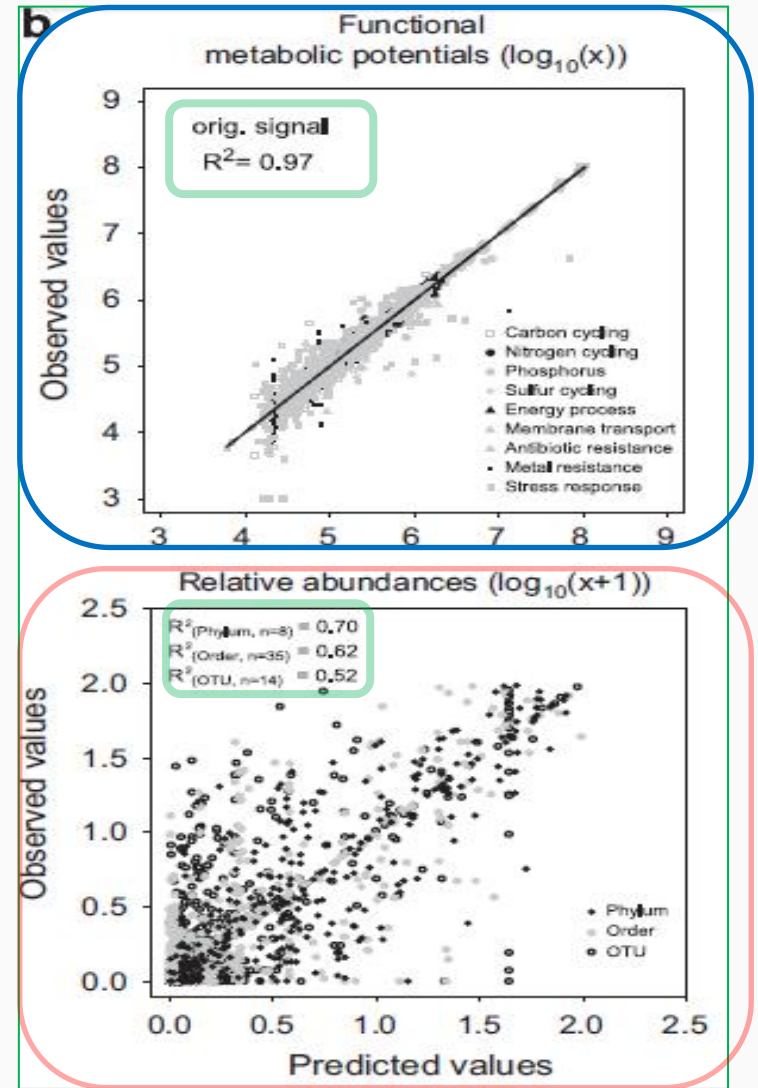
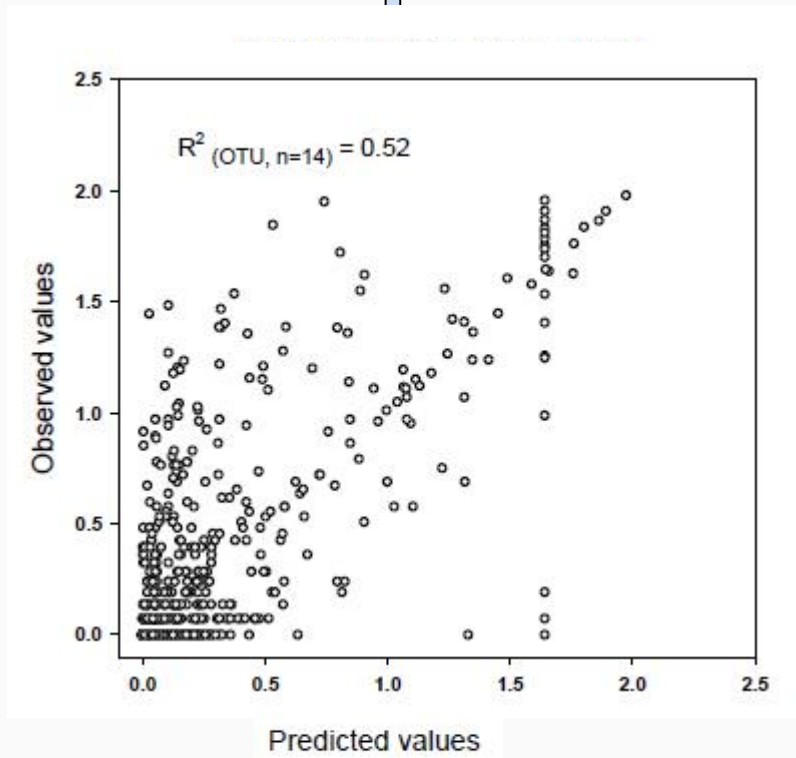


Figure 3 The comparison of prediction accuracies (a)

Results

交叉验证

功能性代谢潜力比微生物群落组成更易预测



Results

微生物群落组成和功能代谢潜力对pH变化的响应

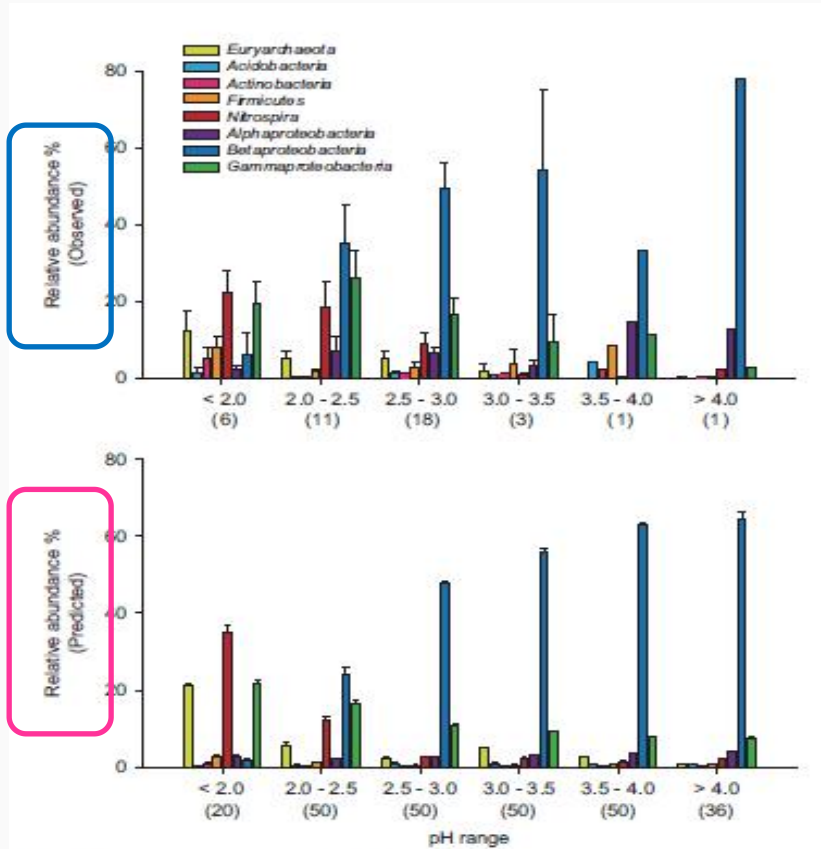


Figure 4 The comparison of predicted and observed relative abundances (%) of dominant lineages along the gradient of pH levels. Values were mean \pm s.e. and the numbers in bracket indicate the number of samples that considered in each pH group.

Euryarchaeota

Nitrospira

Gammaproteobacteria

高度准确性

Res

微生物群落组

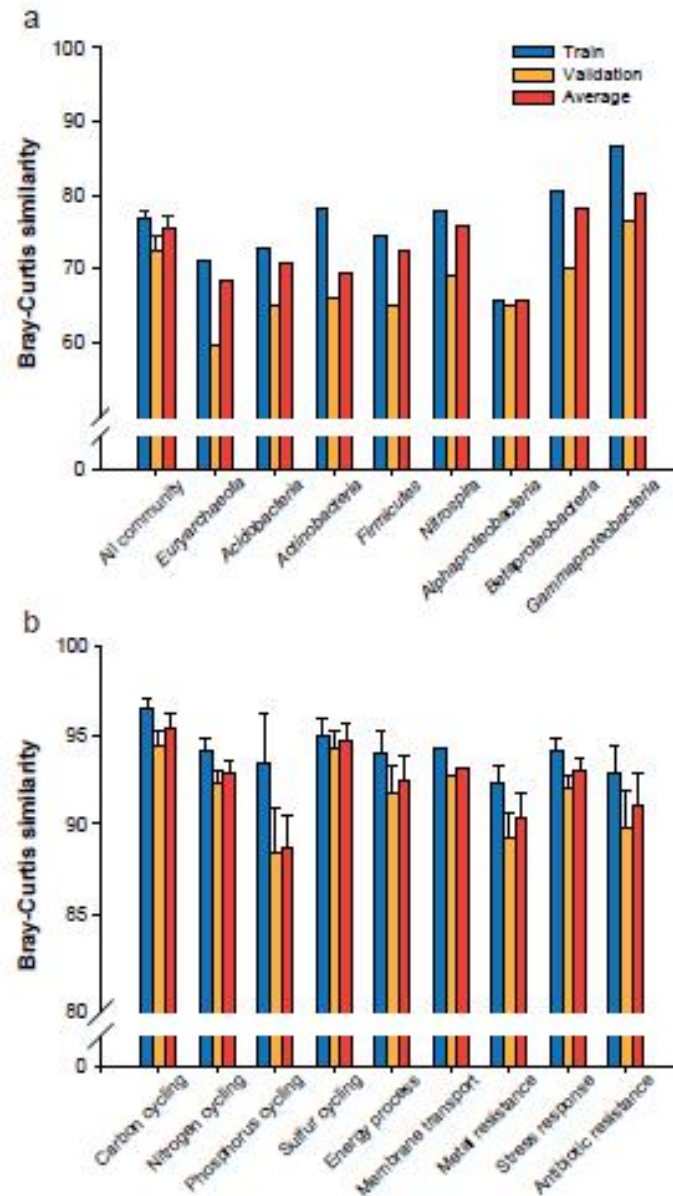


Figure S4. Bray-Curtis similarity between predicted and observed values of relative microbial abundances (phylum level, a) and gene metabolic potentials of different functional categories (with relative abundance information of microbial phyla, b). The similarity of the overall microbial community composition was calculated based on these eight microbial phyla. Average includes the data sets for training and validation. Values are mean \pm SE and the significances of the similarity were listed in supplementary tables.

Results

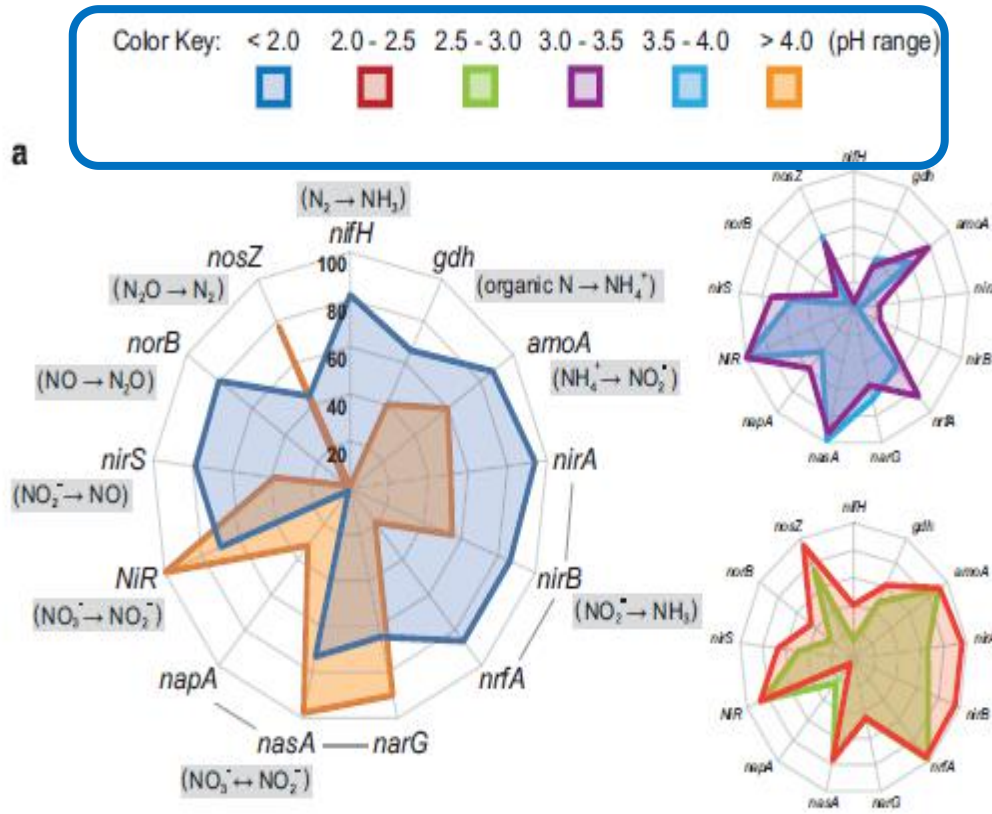
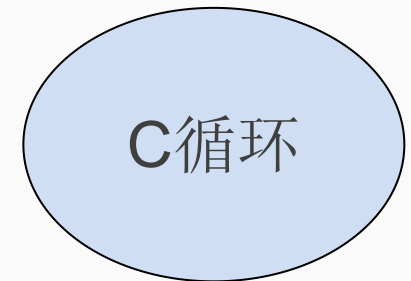


Figure 5 The predictive changes of relative metabolic potential of functional genes in nitrogen cycling (a) along the gradient of pH levels.

参考之前报道过的世界各地大地区的AMD系统的大部分的pH值(1.8-4.1, 单位0.01)



Results

b

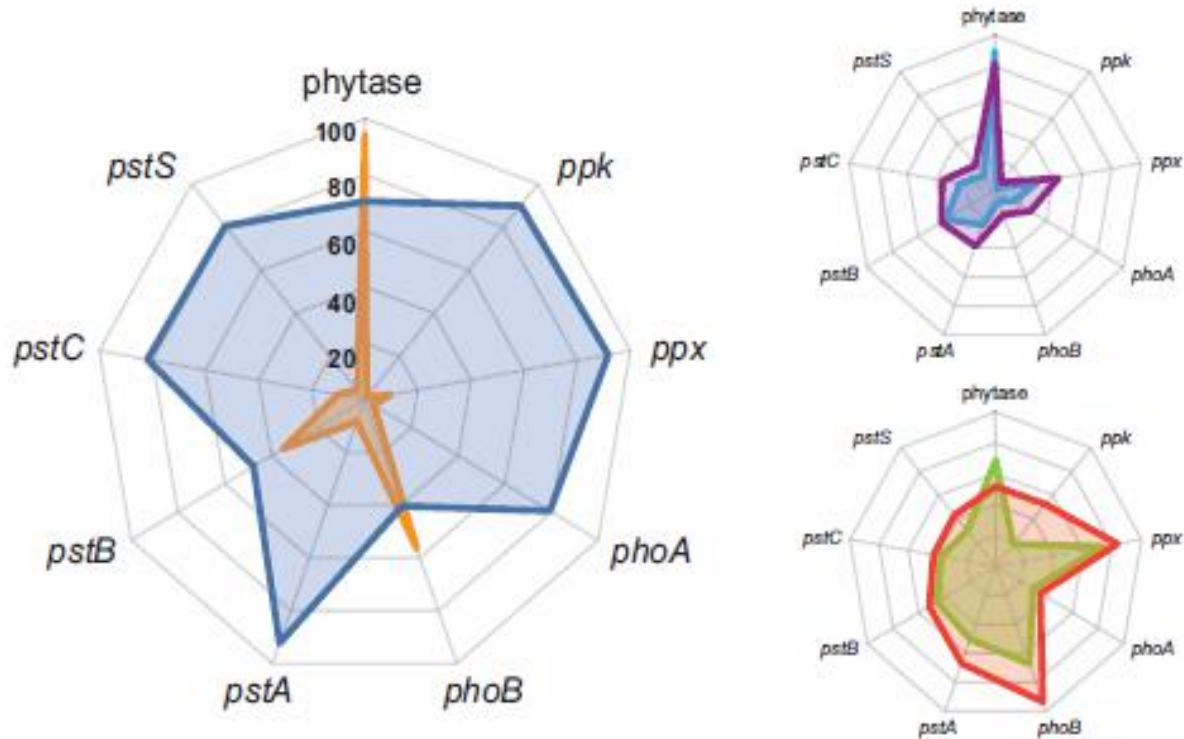


Figure 5 The predictive changes of relative metabolic potential of functional genes in phosphorus cycling (b) along the gradient of pH levels.

P循环

Results

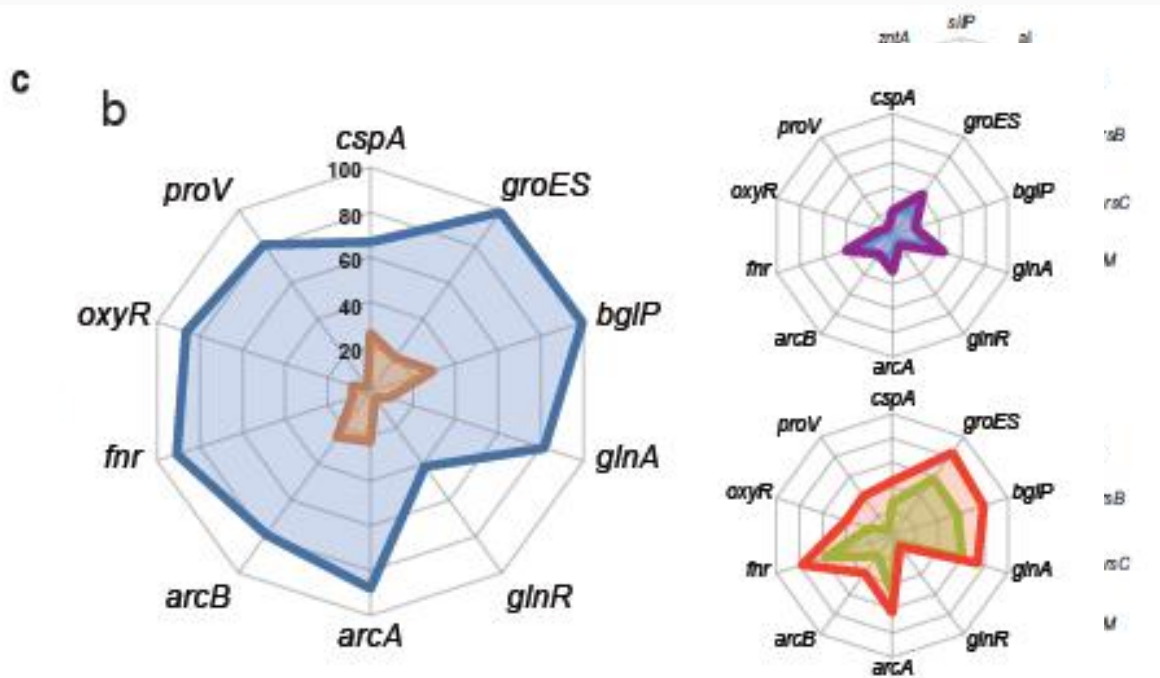


Figure S5. The changes of relative metabolic potential of functional genes in stress response (b) along the gradient of pH levels.

AMD生态系统的酸化进程

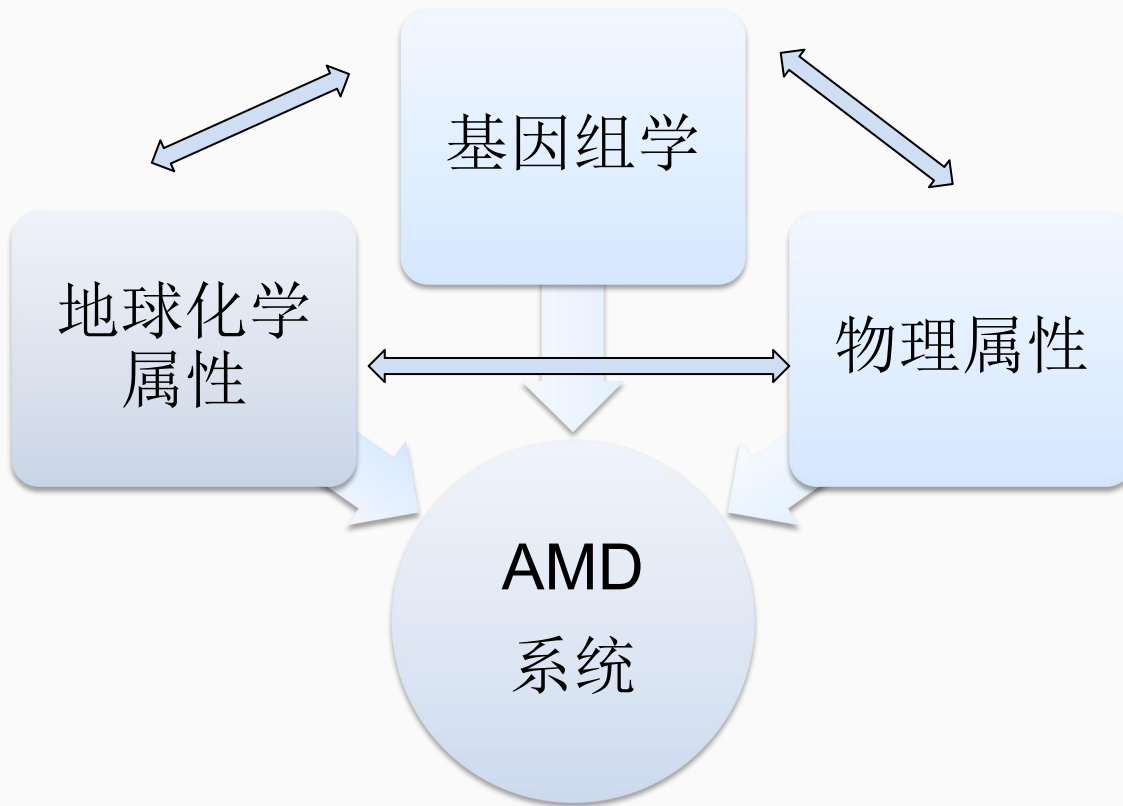
相关基因的代谢潜力的增加

重金属抗性

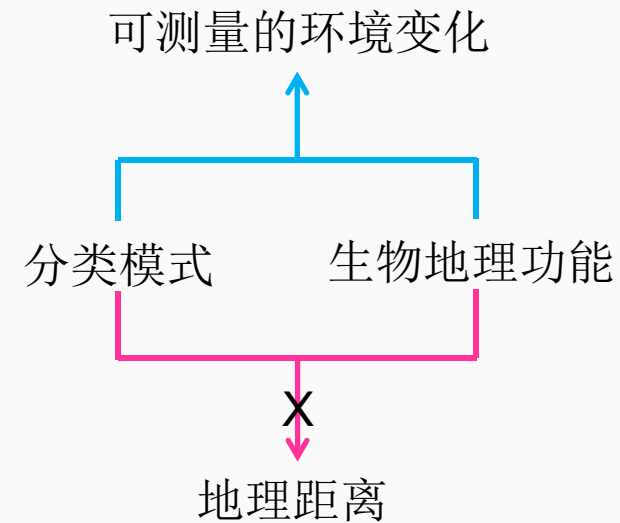
压力反应

Discussion

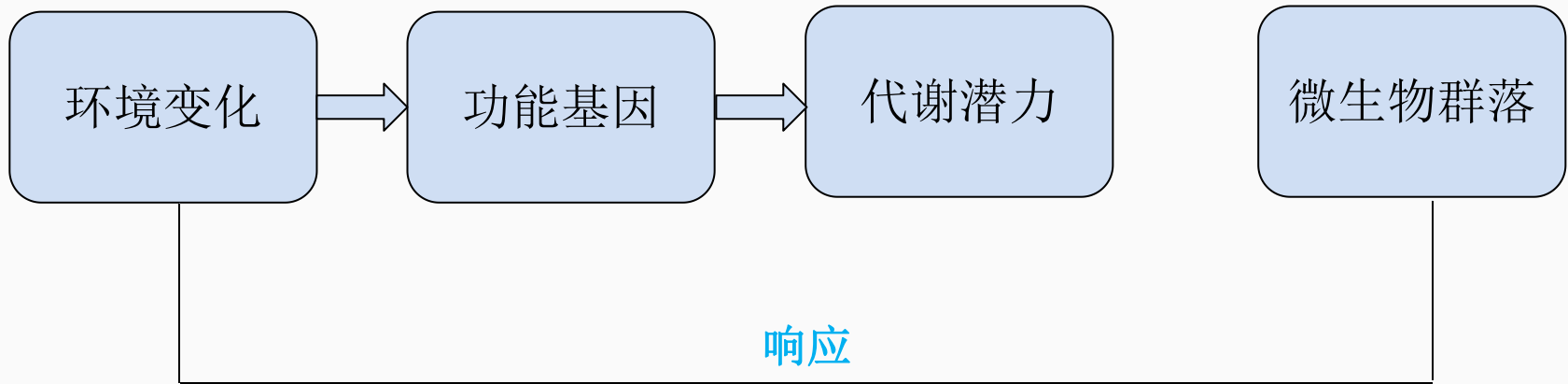
微生物分类组合模式属于显著的环境依赖型模式



极端环境中
自然选择的重要性



Discussion



获得预测代谢潜力的关键功能基因环境变化的反应
这些特定的功能可能直接影响微生物群落与环境之间的相互作用

Discussion

C循环

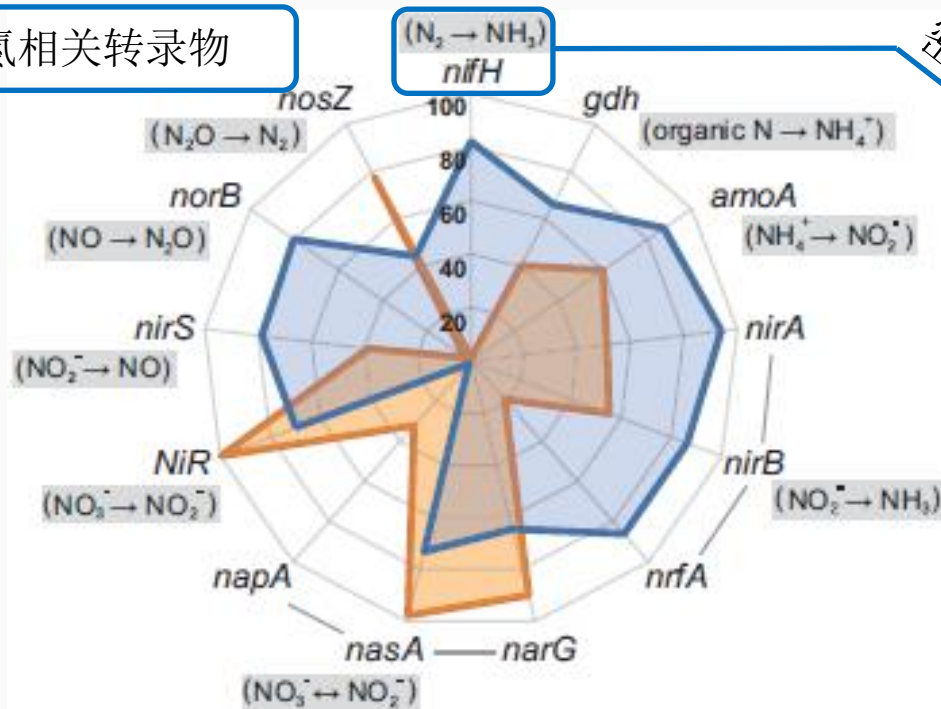
AMD系统中C资源有限

生物地球化学循环过程

嗜酸性群落对于C的生物利用度

特殊群落组合适应极端环境

固氮相关转录物



密切相关

不同的基因参与氮循环检测和预测相对代谢潜力显示清晰的模式

氧化亚铁钩端螺旋菌、嗜铁钩端螺旋菌、酸硫杆菌、硫杆菌

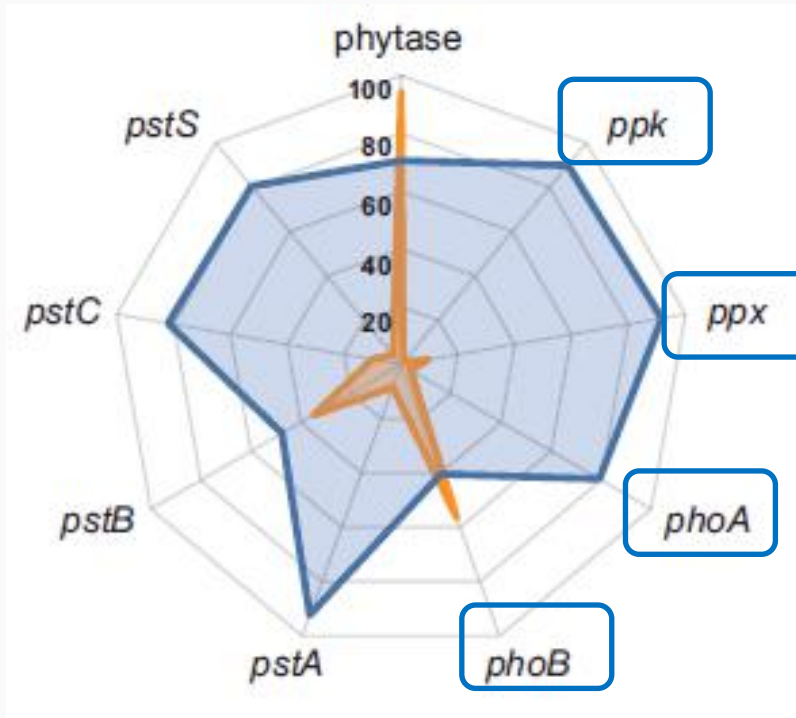
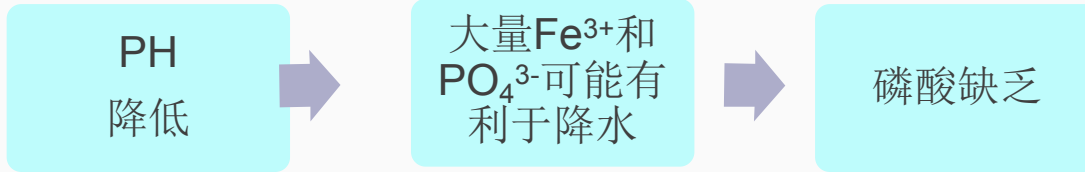
钩端螺菌属、硫杆菌属

PH \longrightarrow nifH

在酸性环境中的主导地位

Discussion

P循环

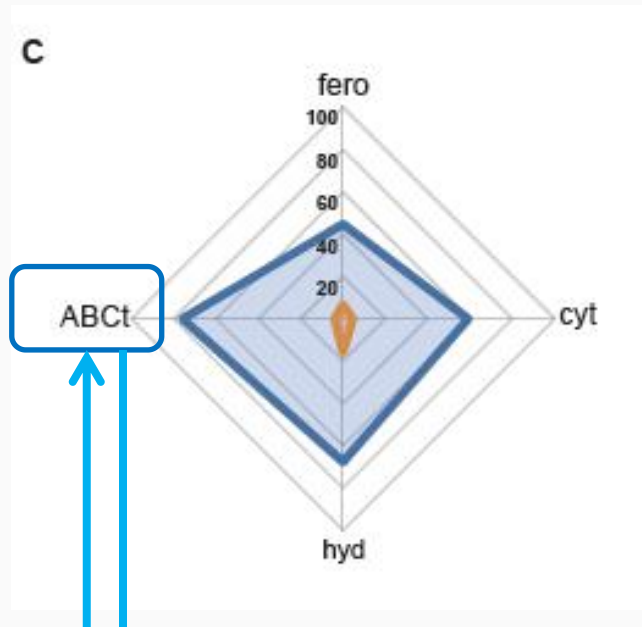
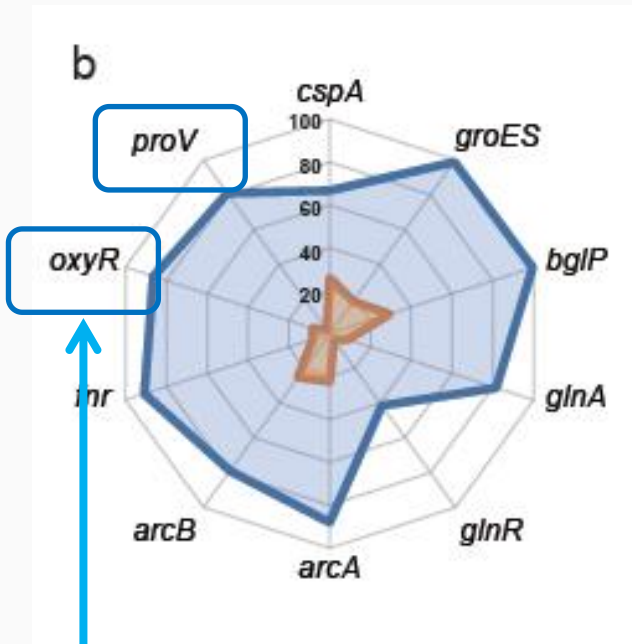


弥补极端酸度带来的有害影响



Discussion

重金属抗性



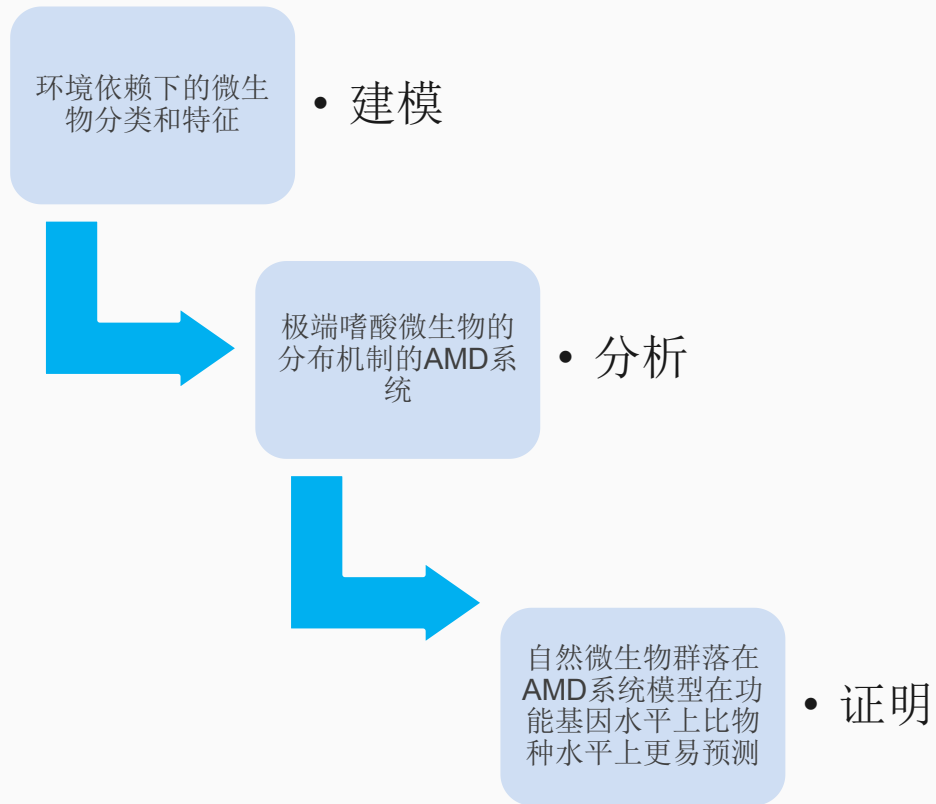
参与防御氧化和渗透压力的基因的代谢潜力预测值为高增加

有利于微生物在极端环境中生存和繁荣

平衡pH值和维持细胞渗透压的有效策略

将细胞中的毒素和药物泵出

Discussion



16S rRNA焦磷酸测序



微生物分类学成分

高通量芯片基因组技术GeoChip



微生物功能结构组合

宏基因组测序



生成功能和分类数据集的信息

大量数据处理和
结果分析验证假
设

建立预测模型并
验证成功



THANKS FOR LISTENING!