

读书报告

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环境科学与生态学：1区

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BRIEF COMMUNICATION



SPRINGER NATURE

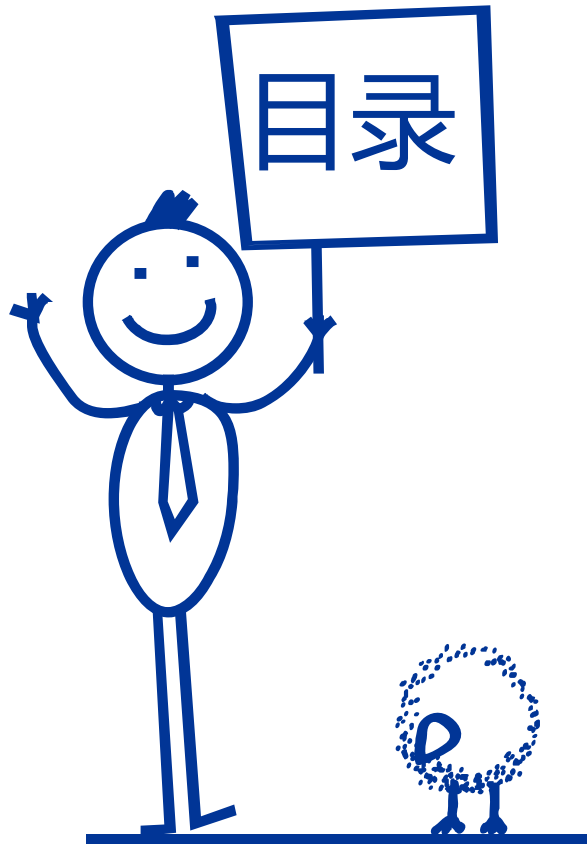


Resuscitation of anammox bacteria after >10,000 years of dormancy

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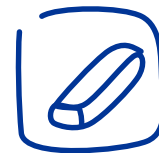




1. Introduction



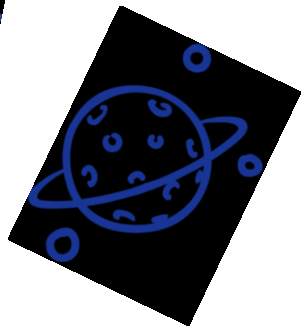
2. Materials and methods



3. Results



4. Discussion



PART 01

Introduction



Introduction



厌氧氨氧化菌 (anaerobic ammonium oxidation, **Anammox**) 广泛存在于缺氧条件下的海洋, 淡水, 湿地, 土壤和干燥的陆地生态系统中。在全球氮循环中起着重要作用, 可将污水中所含有的氨氮转化为氮气去除, 是污水处理中重要的细菌。

Introduction

微生物学报

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DOI: 10.13343/j.cnki.wsxb.20170582



Microbiome in the Hydrosphere and Related Elemental Geochemical Cycling

水圈微生物-元素地球化学循环

热泉微生物驱动的氮循环研究进展及展望

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Introduction

驱动氮循环的功能微生物：如固氮菌、氨氧化菌、**厌氧氨氧化菌**、反硝化菌、异化硝酸盐还原菌。

厌氧氨氧化：指在厌氧条件下以 NO_2^- 作为氧化剂，将 NH_4^+ 氧化成 N_2 的过程，这一过程由厌氧氨氧化菌(Anammox)催化完成。

Anammox 生长缓慢且难培养，至今无纯培养物，只能以富集产物的形式生长于序批式反应器 (SBR) 或生物膜反应器(MBR)中。主要分布在浮霉状菌目 (Planctomycetales) 的 5 个属 *Candidatus Brocadia*, *Candidatus Kuenenia*, *Candidatus Scalindua*, *Candidatus Jettenia* 和 *Candidatus Anammoxoglobus*。

Introduction

在厌氧氨氧化过程中，**羟胺**和**胛**作为代谢过程的中间体。Anammox也具有细胞内膜结构，其中进行氨厌氧氧化的囊称作**厌氧氨氧化体**(anammoxosome)，小分子且有毒的胛在此内生成。厌氧氨氧化体的膜具有特殊的**脂质核心结构-阶梯烷** (Ladderane core lipids) 结构，可阻止胛外泄，从而充分利用化学能，且避免毒害。



长期的干燥缺水环境迫使厌氧氨氧化菌进入休眠状态。使其在干燥或寒冷的环境中生存。

Introduction

迄今为止，没有关于休眠体或厌氧氨氧化体的报道。前期研究表明，静置6个月后，SBR反应器的厌氧氨化菌重新复苏，但未充分研究休眠的上限时间。环境气候变化可能导致土壤湿度增加，休眠多长时间的Anammox可以复苏？

本研究目的：

- (i) 探索长期休眠后厌氧氨氧化菌是否可以从干燥的陆地环境中复苏出来。
- (ii) 使用同位素示踪技术，功能基因表达和高通量测序来阐明潜在的代谢机制。

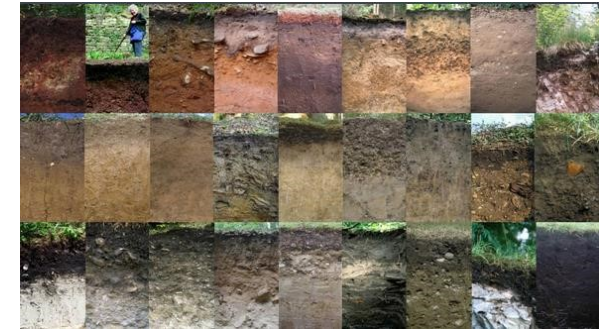


PART 02

Materials and methods



Field description



研究地点: Goldfields Esperance region, Western Australia

澳大利亚西部的地质历史悠久，已有44亿年，大部分沉积物形成于浅海环境中。年平均降雨量为260毫米，属干旱气候。选择典型的旱地土壤/碎屑岩演替层（深度为63m）作为采样点（6617774N, 330210E）。



- 0-A层 (腐殖质-淋溶层)**：厚度在10厘米左右，含有丰富的有机物和腐殖质；
- B层 (沉积层)**：由上层土淋滤出来的有机物、盐类、以及粘土颗粒组成；
- C层 (母质层)**：风化的成土母岩组成；
- R层 (原始层)**：为未风化的基岩。

腐殖质-淋溶层

淀积层

腐泥岩

母质

基岩

From (m)	To (m)	Soil horizon Regolith	Lithology	Description
0	3	Eluvium soil cover	Transported clays	light cream, kaolinitic
3	6	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
6	9	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
9	12	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
12	15	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
15	18	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
18	21	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
21	24	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
24	27	Illuvium	Transported clays	medium brown transported alluvium, clay, gravels
27	30	Illuvium	Transported clays	light red transported alluvium, clay, gravels
30	33	Illuvium	Transported clays	light brown transported alluvium, clay, gravels
33	36	Leached zone	Upper Saprolite	leached zone, kaolinitic, fabric destroyed
36	39	Soil parent material	Regolith	clay>20% - primary fabric preserved, with few aggregates
39	42	Soil parent material	Regolith	clay>20% - primary fabric preserved, with few aggregates
42	45	Soil parent material	Regolith	clay>20% - primary fabric preserved, with few aggregates
45	48	Soil parent material	Regolith	pale yellow, <20% weathered minerals with abundant aggregates
48	51	Soil parent material	Regolith	pale yellow, <20% weathered minerals with abundant aggregates
51	54	Soil parent material	Regolith	pale yellow, <20% weathered minerals with abundant aggregates
54	57	Soil parent material	Regolith	pale yellow, <20% weathered minerals with abundant aggregates
57	60	Soil parent material	Regolith	pale yellow, <20% weathered minerals with abundant aggregates
60	63	Bedrock	Fresh Porphyritic Basalt	medium green porphyritic, strong chlorite alteration, large plagioclase phenocrysts



1、 Soil/regolith samples collection and incubation

在6-9、18-21、45-48和51-54m深处选择四个土壤样本进行实验。
一式三份均匀混合后分为两组，设**阳性实验组（加水）**和阴性对照组。
每组平均7份，对应7个复苏时间点，即从起点到第6个月。

使用**高纯度He** (99.9%) 去除样品、实验用水、培养瓶中的氧气。将培养瓶放**高纯度Ar**入 (99.999%) 的黑暗厌氧系统中，**150 rpm**，**25°C**振荡培养。每个月六个平行瓶子进行气体样品测定 (CO_2 , CH_4 和 N_2O)，是用等体积的高纯度He代替气体样品。

- 1、理化性质分析；
- 2、进行培养以确定微生物参与的N循环过程；
- 3、-80°C冷冻进行DNA提取及测序分析。

Materials and methods

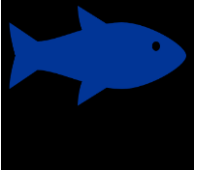
- 2、DNA extraction, (q) PCR and the related growth rate calculation
(hzsB 基因: 联氨合成酶关键基因)
- 3、Illumina sequencing and network analysis
- 4、The AMS method of direct ^{14}C isotope counting for soil dating
加速器质谱分析 (accelerator mass spectrometry analysis, AM
- 5、Analyzing physicochemical properties of the incubation slurry
- 6、Rate measurements of anammox, denitrification, NH_4^+ oxidation ($\text{NH}_4^+ \rightarrow \text{NO}_2^-$), NO_2^- oxidation ($\text{NO}_2^- \rightarrow \text{NO}_3^-$), NO_3^- reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), and NO_2^- reduction ($\text{NO}_2^- \rightarrow \text{NH}_4^+$) by ^{15}N -tracer technique
- 7、The measurements of carbon dioxide (CO_2) , methane (CH_4) , and nitrous oxide (N_2O) gas emission
- 8、Statistical analysis

灵敏度高, 需样量少,
用于植物微化石测年,
土壤、矿石定年。

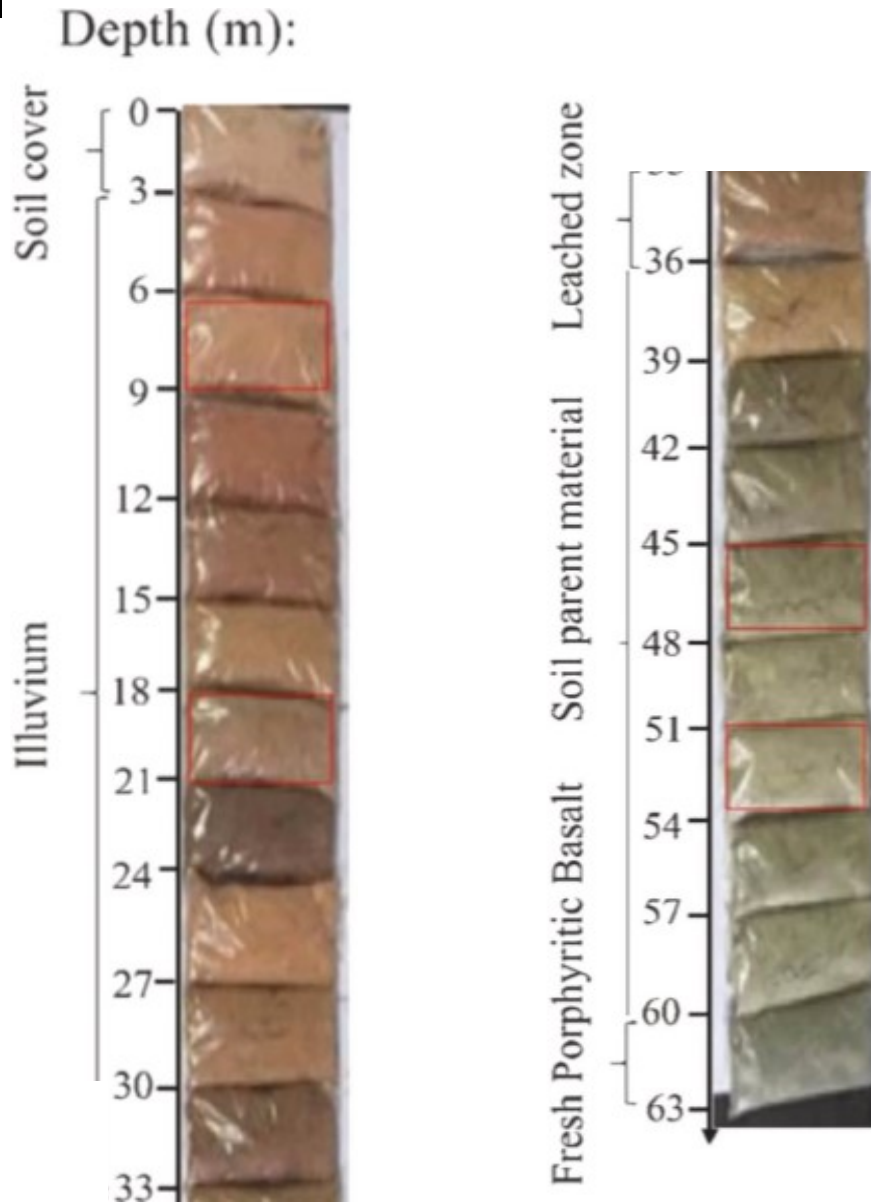


PART 03

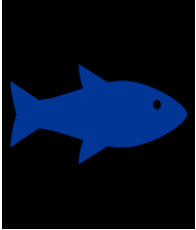
Results



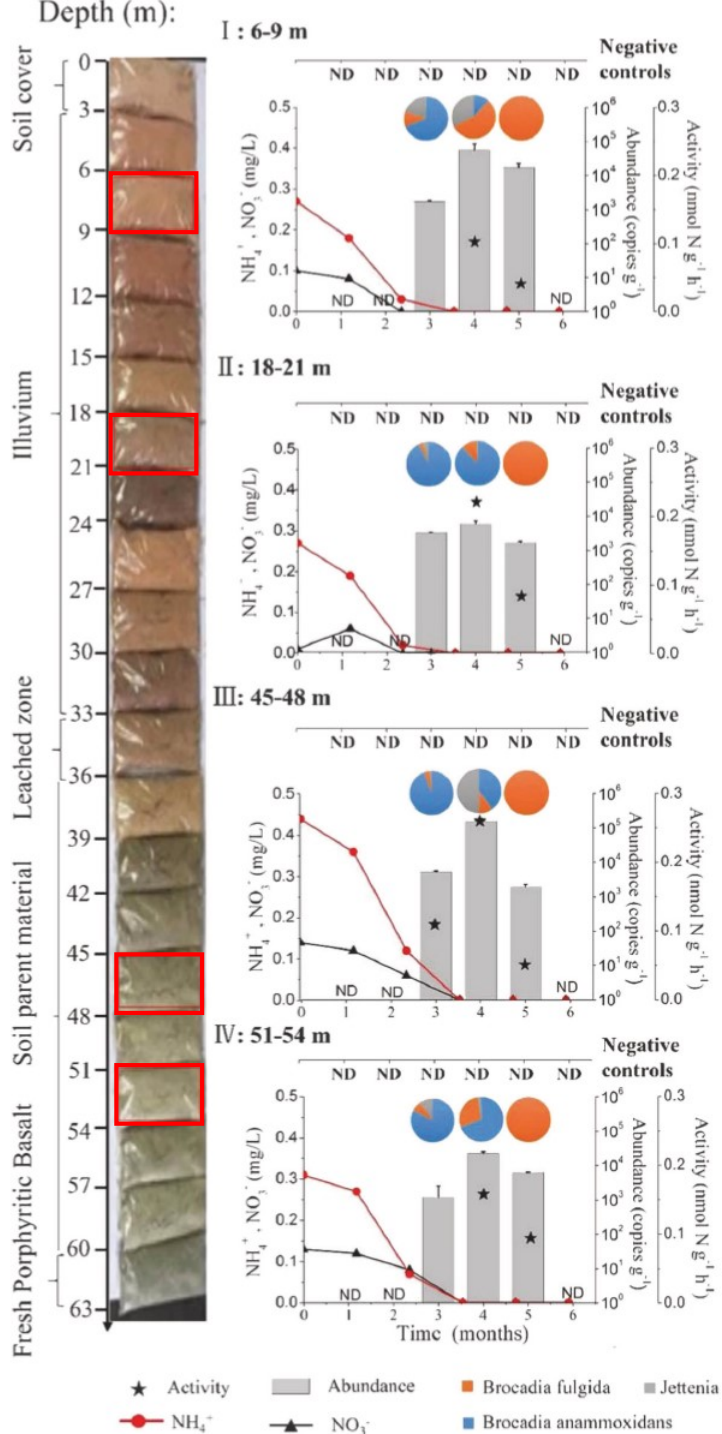
1、Age of sampled soils



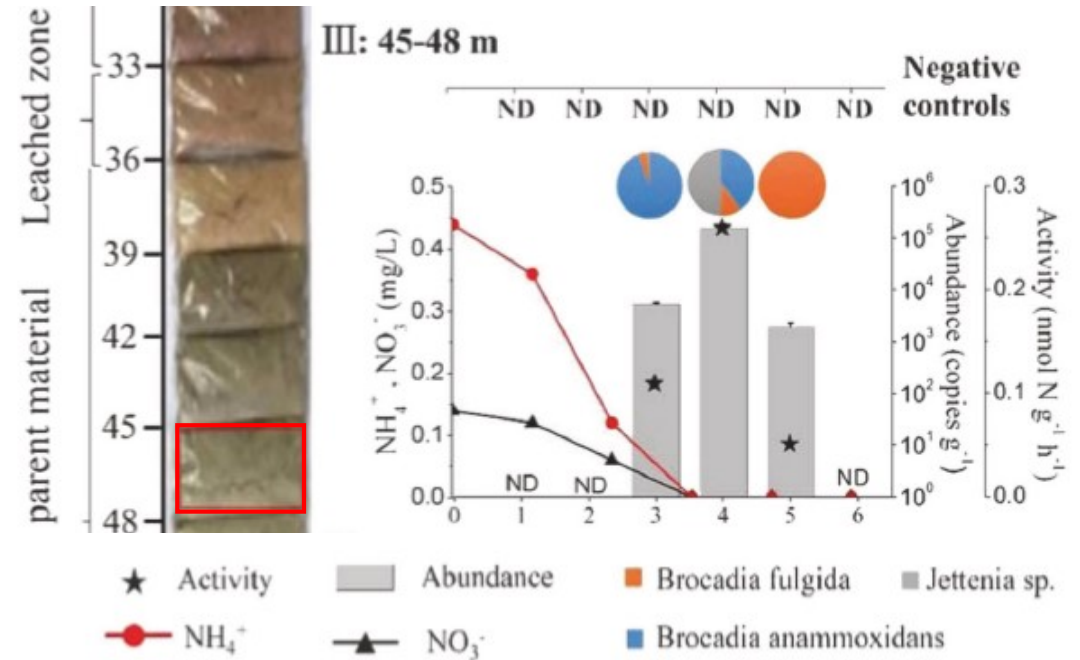
The five AMS ^{14}C radiocarbon dates determined on the soil/regolith samples ranged between **15,675** and **10,245** Cal. yr B.P. (Cal yr B.P. 为距公元1950年前的年龄)



2

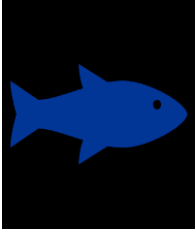


ty of anammox bacteria



复苏的Anammox菌丰度（灰色柱），Anammox菌活性（星状），群落组成（饼图）和基质浓度（黑色或红色线）。

“ND”表示厌氧氨氧化物丰度低于检测限，通过¹⁵N示踪技术没有检测到复苏过程中厌氧氨氧化菌的活性。

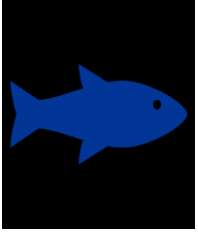


2、 Abundance and activity of anammox bacteria

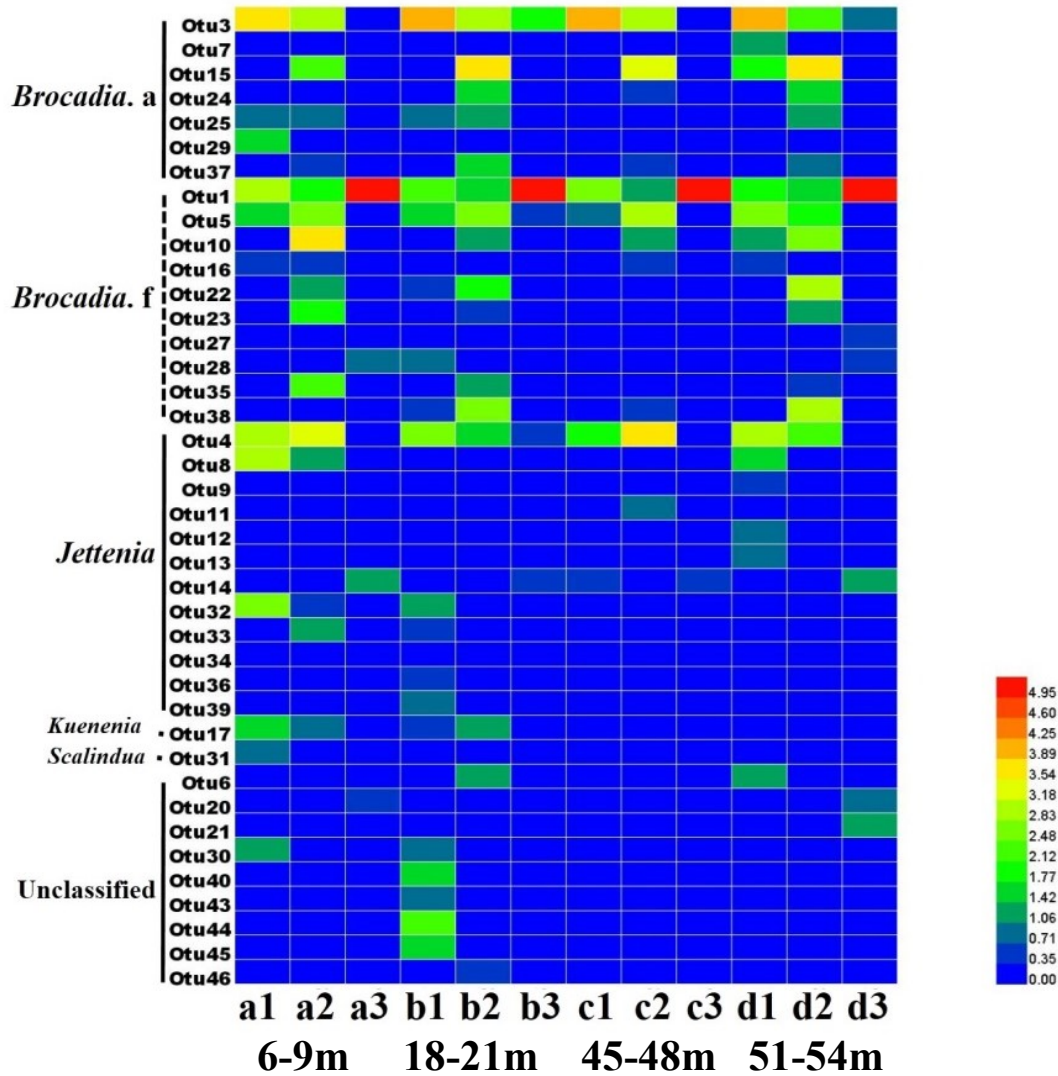
Table S2 Gas concentrations during the incubation period

Sample layer (m)	Incubation period (month)	CO ₂ (ppm)	CH ₄ (ppm)	N ₂ O (ppm)
6-9	1	89.2	n.a.	n.a.
	2	453	n.a.	n.a.
	3	681	1.13	0.02
	4	1784	1.96	0.10
	5	2431	4.83	0.07
18-21	1	134	n.a.	n.a.
	2	540	n.a.	n.a.
	3	860	0.71	0.05
	4	1898	0.92	0.42
	5	2089	2.05	0.07
45-48	1	99.2	n.a.	n.a.
	2	422	n.a.	n.a.
	3	716	2.15	0.02
	4	1261	2.03	0.01
	5	1619	2.47	0.10
51-54	1	108	n.a.	n.a.
	2	488	n.a.	n.a.
	3	744	1.31	0.04
	4	1300	1.37	0.00*
	5	1711	1.69	0.06

n.a.: No data

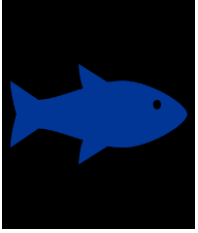


3、Anammox bacterial community

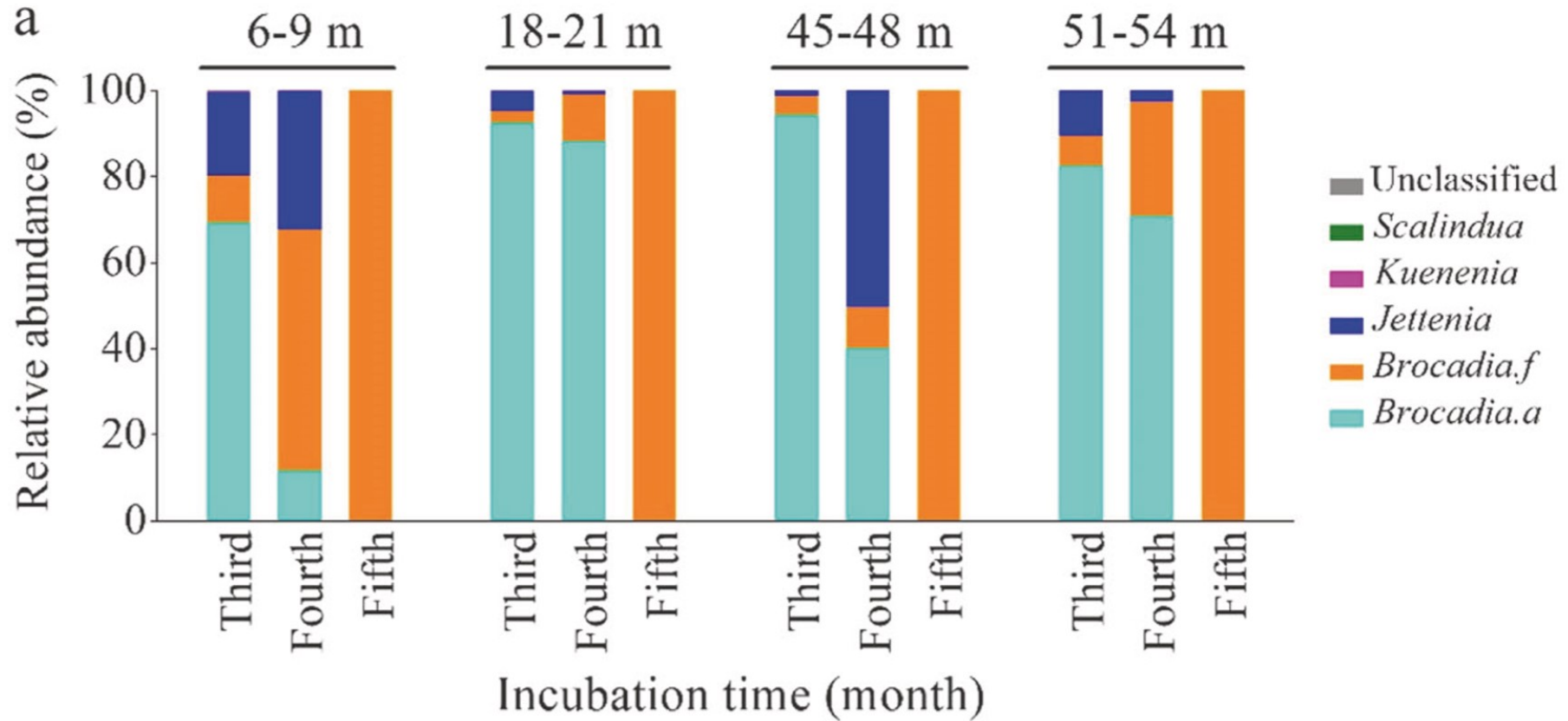


Heatmap of anammox bacterial community compositions on OTUs level in soil/regolith samples during the incubation process. (a1, b1, c1, d1), (a2, b2, c2, d2) and (a3, b3, c3, d3) represent soil/regolith samples (6-9m, 18-21m, 45-48m, 51-54m) in the third month, fourth month and fifth month, respectively.

Diversity of anammox bacterial decreased with time.



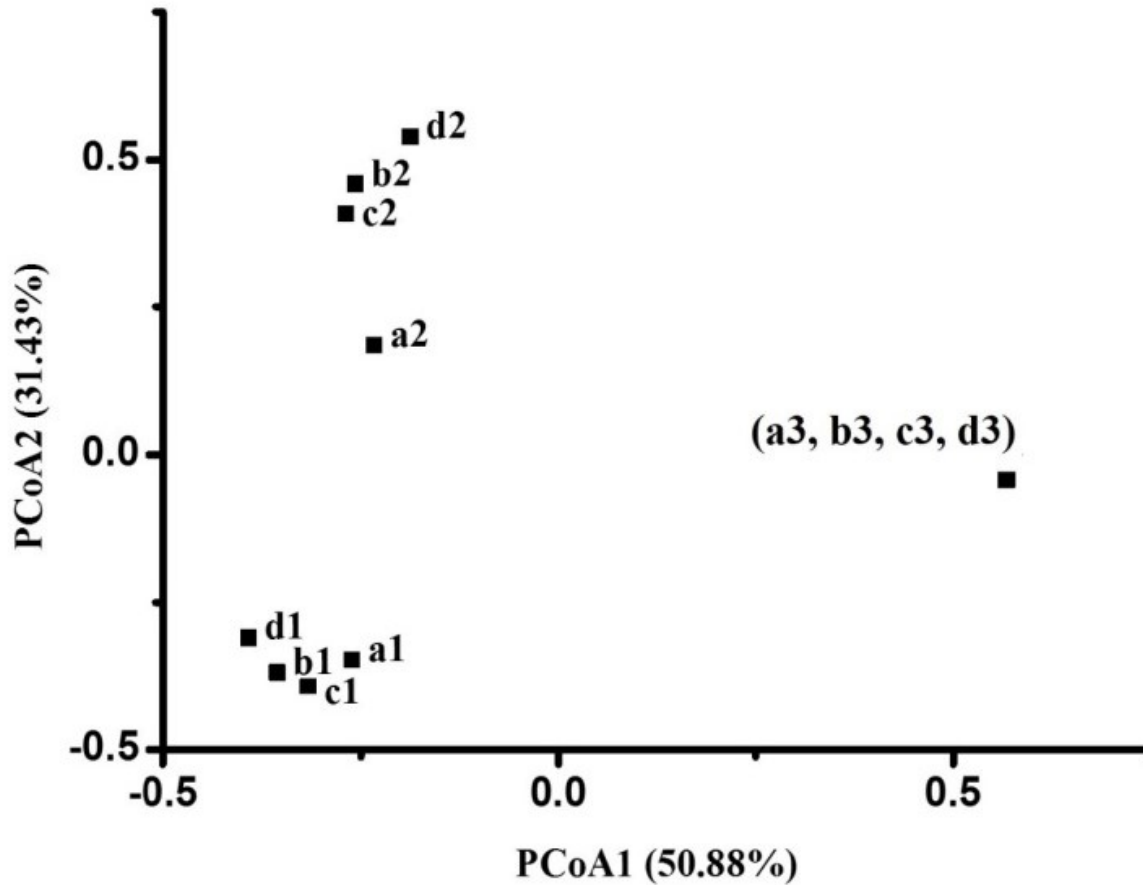
3、Anammox bacterial community



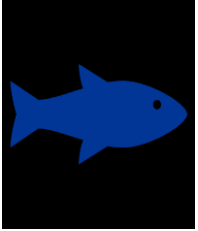
The anammox community shifted from multiple *Candidatus Jettenia*, *Candidatus Brocadia*, and *Candidatus Anammoxoglobus* to a **single species of *Candidatus Brocadia fulgida***.



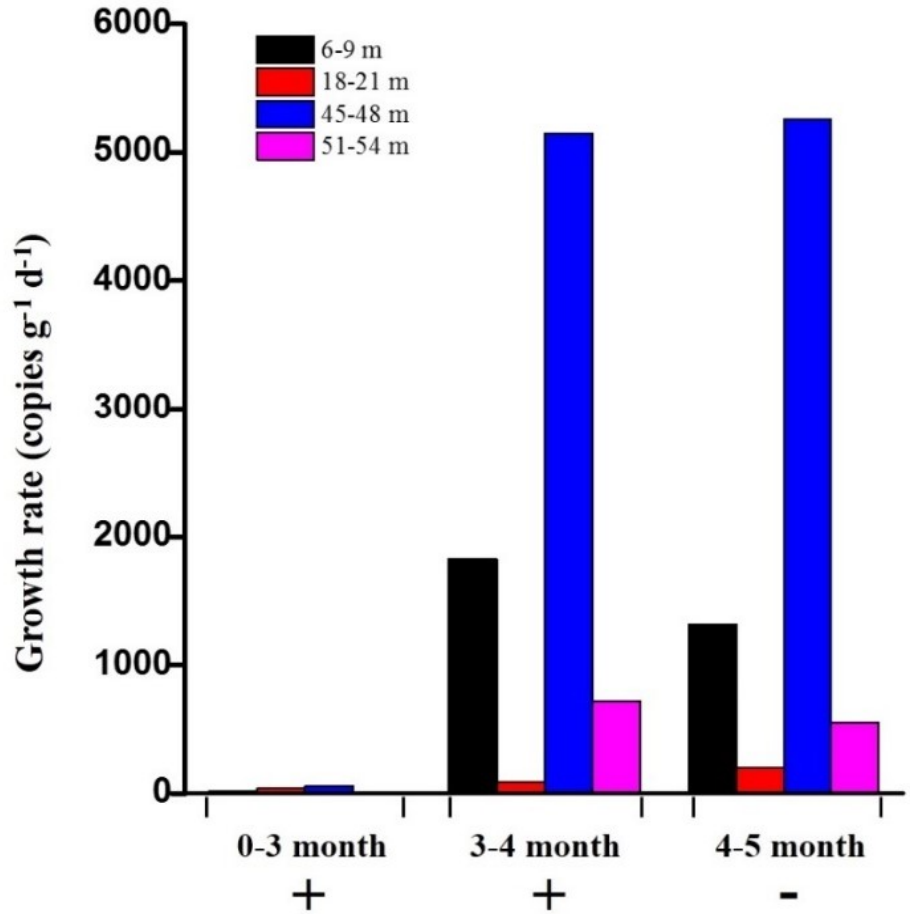
3、Anammox bacterial community



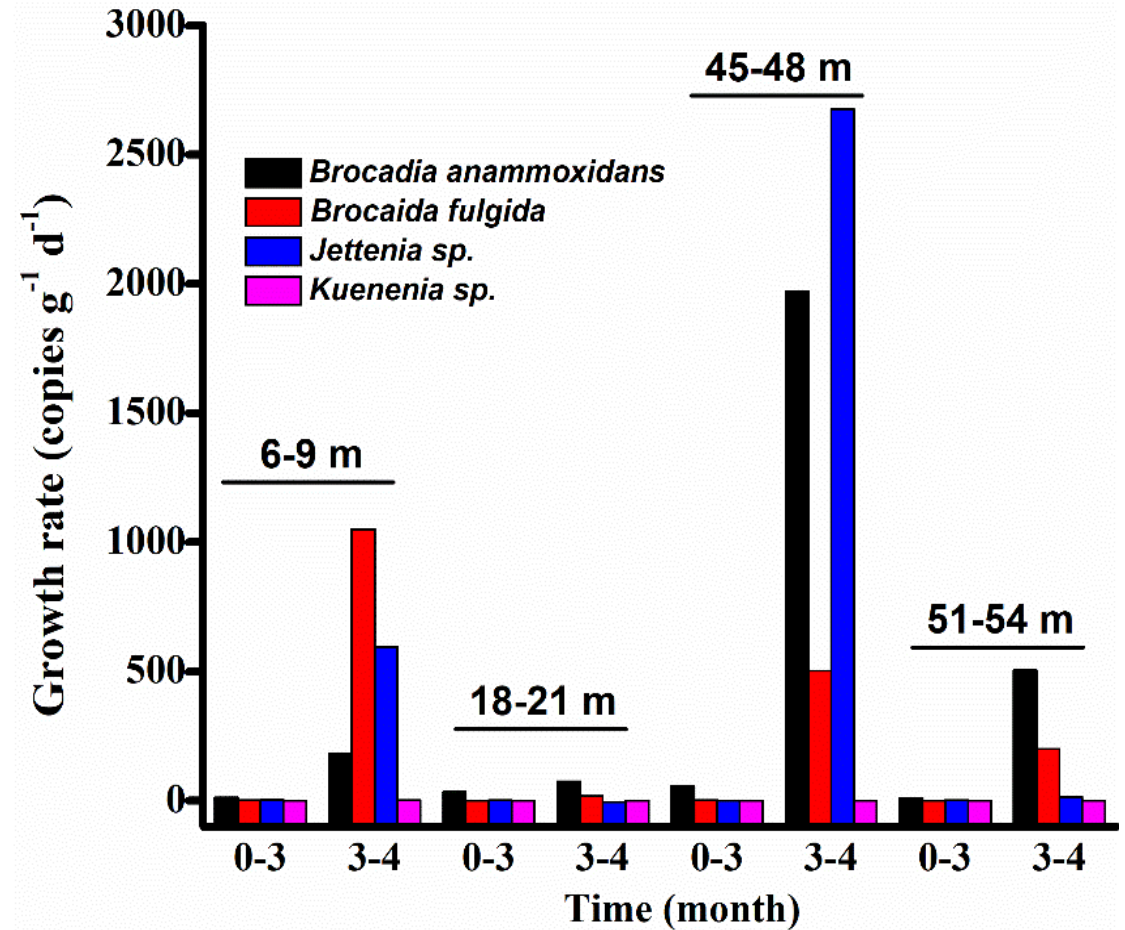
Principal component analysis of soil/regolith samples during the incubation period displayed with Bray-Curtis distances. (a1, b1, c1, d1), (a2, b2, c2, d2) and (a3, b3, c3, d3) represent soil/regolith samples (6-9m, 18-21m, 45-48m, 51-54m) in the third month, fourth month and fifth month, respectively.



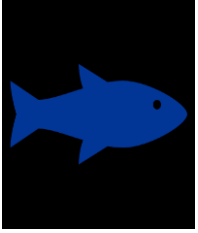
3、Anammox bacterial community



Net growth rates of anammox bacterial abundance in different soil/regolith samples during 0-3 months, 3-4 months and 4-5 months period. “+” means positive growth, “-” means negative growth.

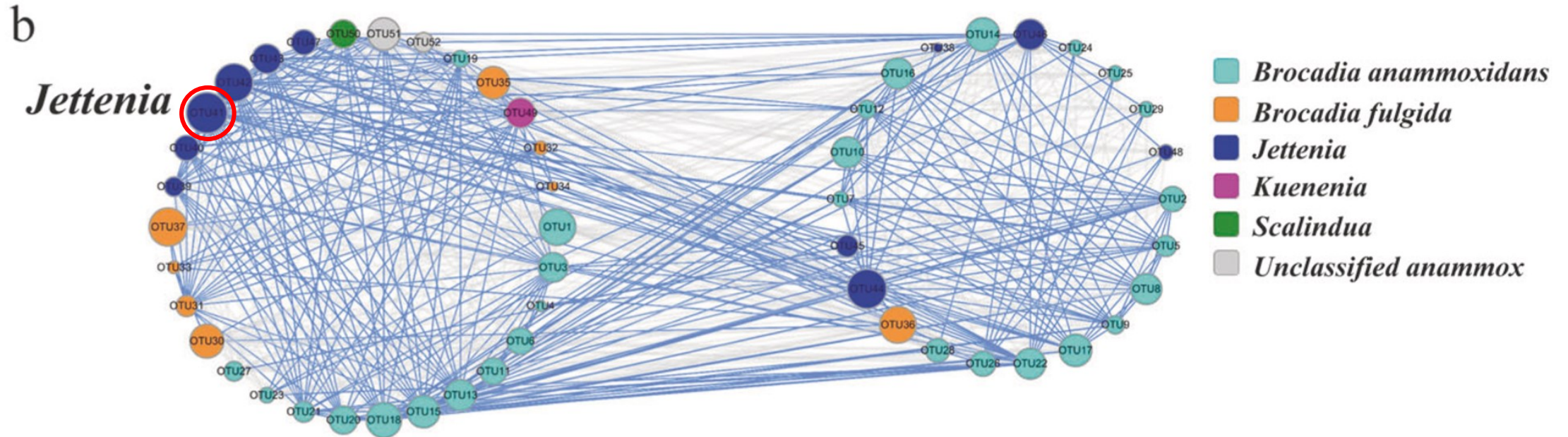


Growth rate of specific anammox species in different soil/regolith samples during 0-3 months and 3-4 months period.



3、Anammox bacterial community

The co-occurrence network analysis of the anammox community

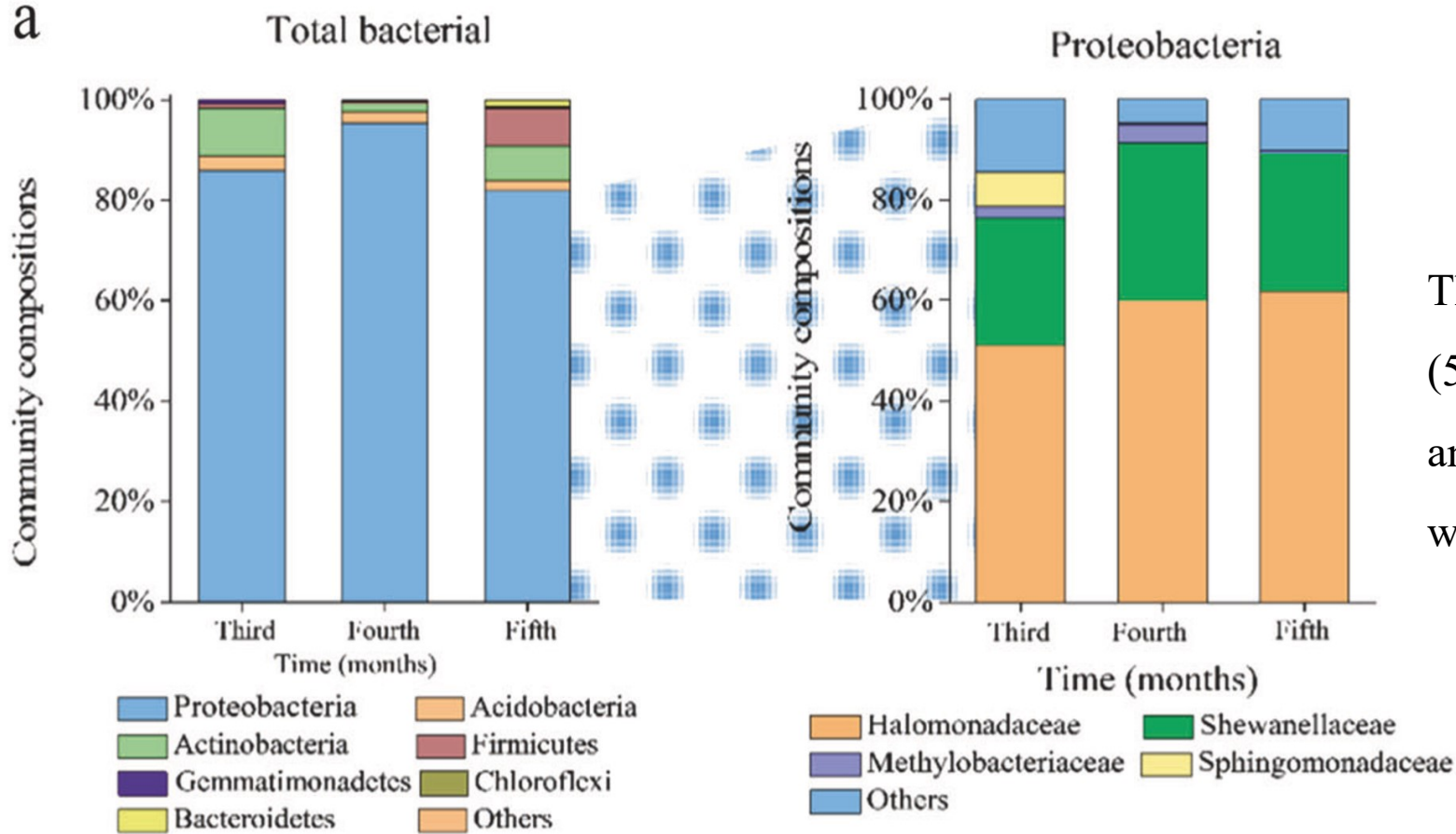


The network of anammox bacterial community with avgCC of 0.639. Nodes are colored and shaped for phylogenetic taxa with the size proportional to node connectivity. The blue link indicates a positive interaction and the gray link negative interaction.

***Candidatus Jettenia* links 67.5% (27/40) revealed positive interactions, in which 88.9% were interactions with *Candidatus Brocadia anammoxidans*.**

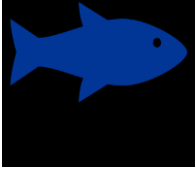


4、 Diversity of archaeal and bacterial community

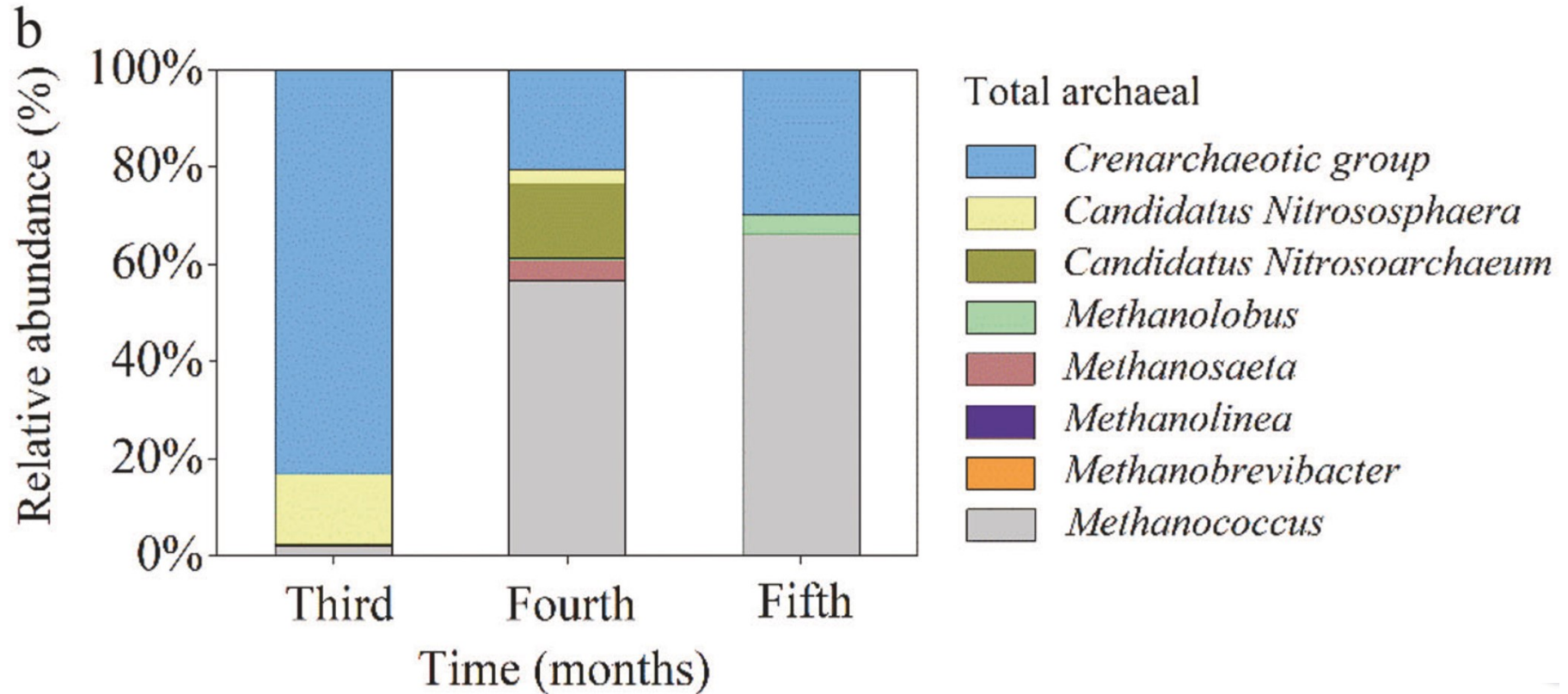


The **majority families** were *Halomonadaceae* (50.6–57.2%, affiliated with *Oceanospirillales*) and *Shewanellaceae* (22.4–29.9% affiliated with *Alteromonadales*)

Facultative anaerobic *Proteobacteria* (81.9–95.6%) dominated.



4、 Diversity of archaeal and bacterial community

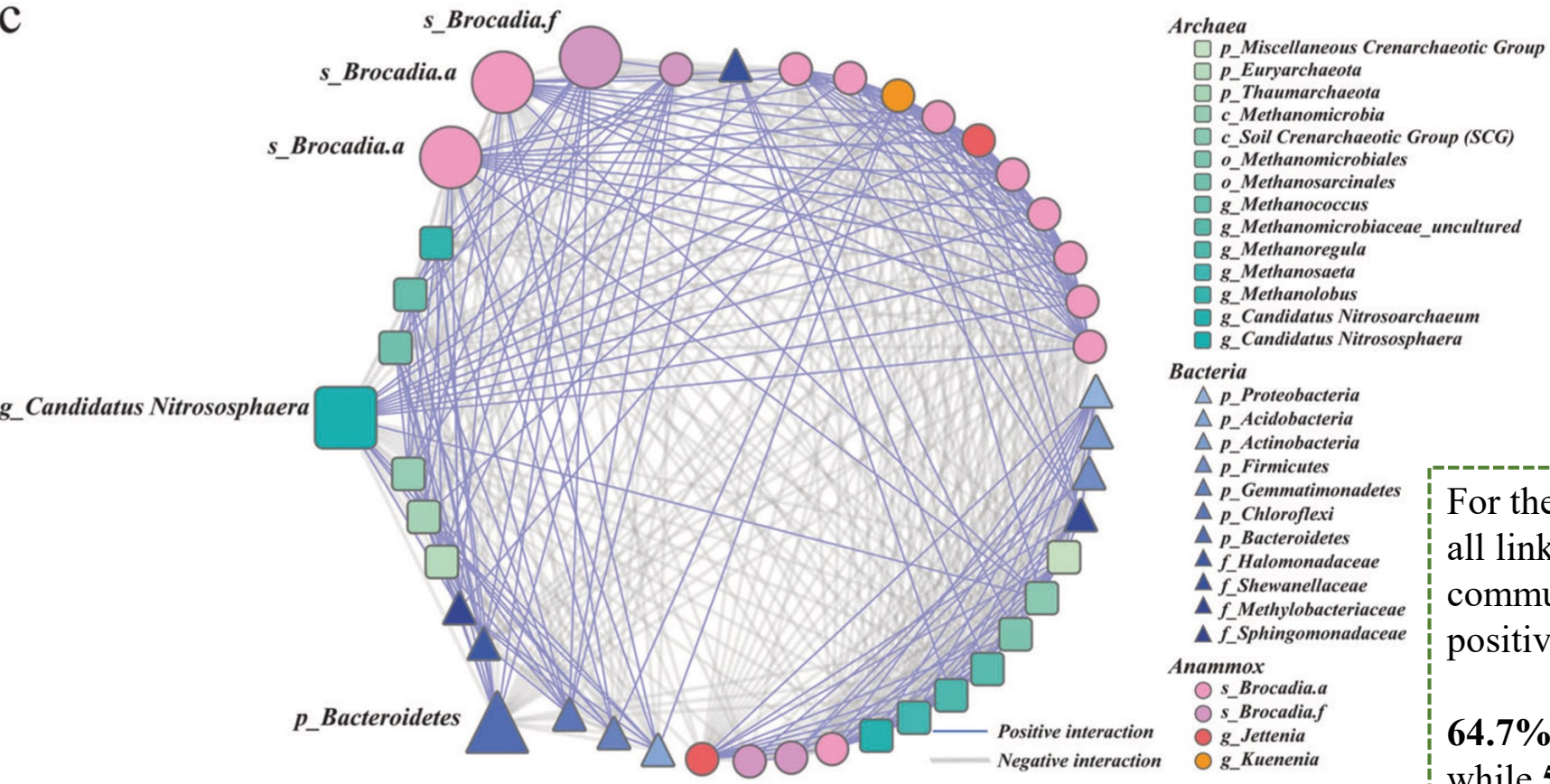


The **archaeal abundance** exhibited a community shift from the *Crenarchaeal* group (83.2%) plus *Candidatus Nitrososphaera* (14.3%) **in the third month** to multiple *Methanococcus* (61.3%), *Crenarchaeal* group (20.5%), and *Candidatus Nitrosoarchaeum* (15.3%) **in the fourth month** and finally to *Crenarchaeal* groups (29.9%) combined with typical thermophilic *Methanococcus* (66.1%) .



4、 Diversity of archaeal and bacterial community

C



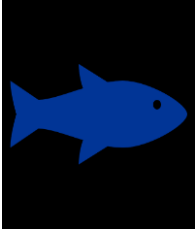
This is interpreted to indicate a **strong symbiosis or competition** within the entire microbial community.

A total of **53 nodes** gave **746 interaction links** in which there were **42.8% positive** and **57.2% negative** interactions.

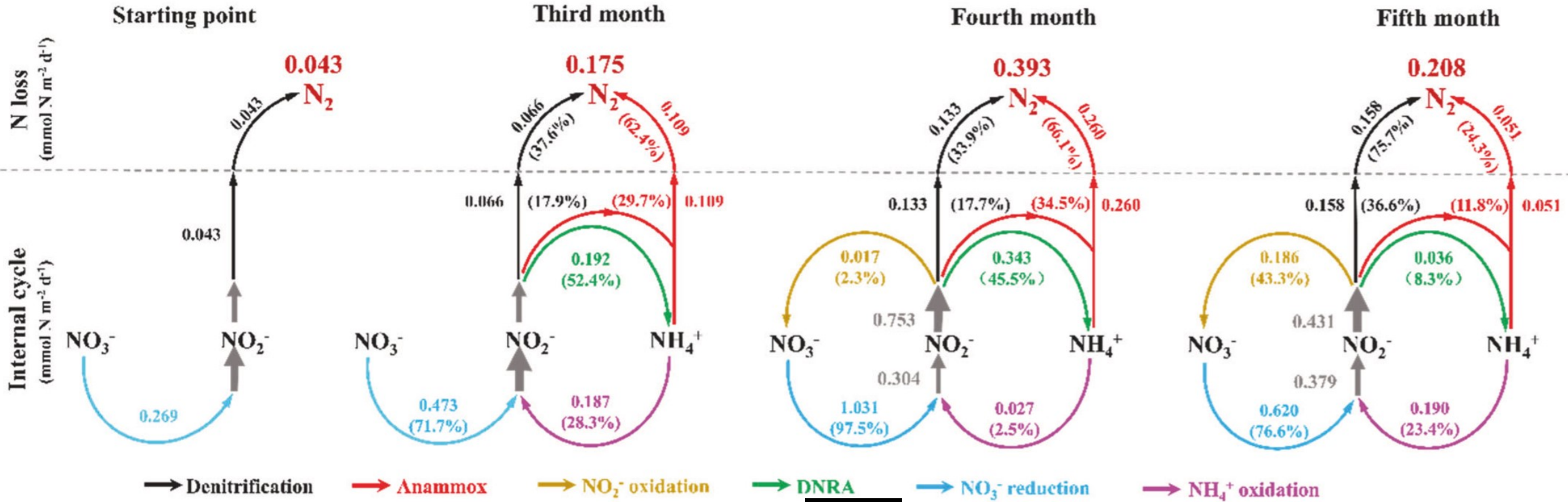
For the archaeal genus **Candidatus Nitrososphaera**, all links revealed interaction **with the anammox** community, and **68.8%** (11/16) of the links were positive interactions.

64.7% (22/34) links of **Bacteroidetes** to anammox, while **58.8%** (20/34) of links were negative interactions.

Co-occurring network of archaea, bacteria, and anammox bacteria



5、Interaction of anammox with other N cycling process



异化硝酸盐还原为铵 dissimilatory NO_3^- reduction to NH_4^+ (DNRA)



PART 04

Discussion

Discussion

水对地球上的生命至关重要，是微生物能量和新陈代谢的重要媒介。休眠是在不良环境条件下生命活动极度降低的生理状态。许多微生物在缺水转为代谢失活状态，一旦环境条件再次变得有利就会复苏。本研究通过仅添加水来探究干燥陆地生态系统中的休眠1万多年的厌氧氨氧化菌的复苏。

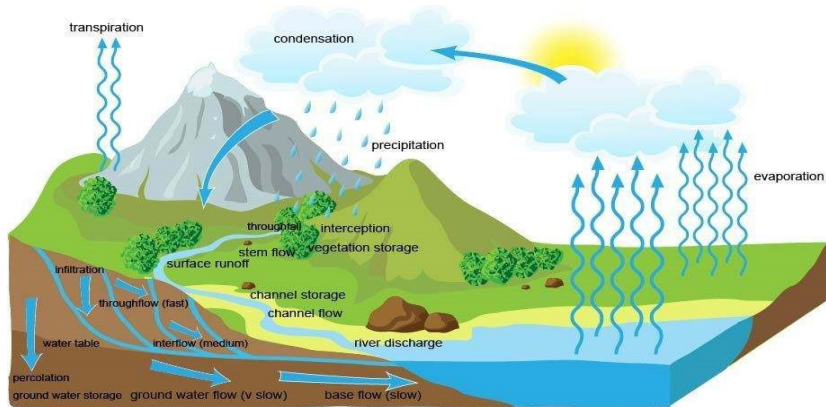
高通量测序和网络分析发现*Candidatus Brocadia* 是复苏的主要厌氧氨氧化菌，*Candidatus Jettenia* 与其他菌群存在相关性。在全球气候变化下，降水和土壤湿度的增加可能会使干旱土壤中休眠的厌氧氨氧化菌复苏，从而影响全球氮和碳循环。

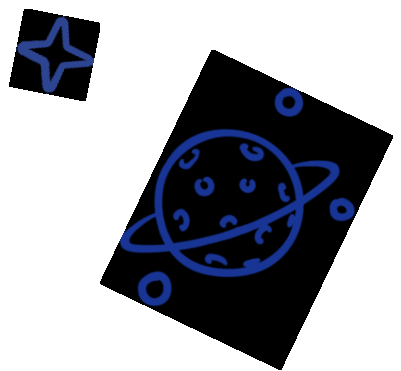
¹⁵N同位素示踪分析表明水诱导下最开始硝酸盐还原产生足够的**亚硝酸盐**作为底物使厌氧氨氧化菌得以复苏。随后，硝酸盐异化还原成铵（DNRA）为厌氧氨氧化菌提供了底物铵。形成的铵和亚硝酸盐用于生产氮气。

Conclusion

气候变化导致约占全球土壤圈40%的土壤湿度增加，目前超过10,000年的土壤，风化层和沉积物中的微生物处于休眠阶段，随着自然降雨的增加、水文循环的加剧和土壤湿度的升高，它们有可能复苏。

各种微生物唤醒后将加速生物地球化学循环过程，但是对全球范围氮和碳循环的具体影响需长时间进行探讨研究。





Thank You

敬请各位老师同学批评指正
