三峡消落带植物应对长期淹水的生态策略—狗牙根的固氮作用

Nitrogen fixation of *Cynodon dactylon*: a possible strategy coping with long-term flooding in the Three Gorges Reservoir

李姗泽，王雨春，胡乐晨，等

Shanze Li, Yuchun Wang, Lechen Hu, et al.

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Sep 13th, 2023

蓄水河流：筑坝拦截对天然河流产生深刻影响

Impounding rivers: Damming has a profound impact on natural rivers!

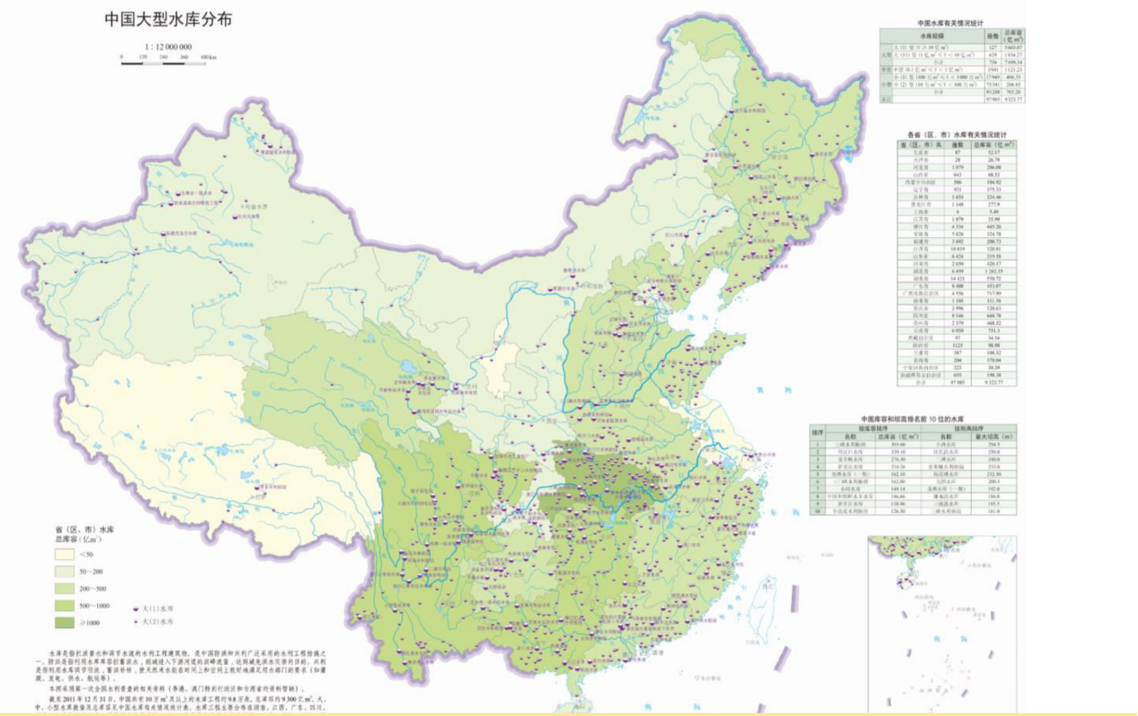
在过去二百多年间，全球大坝经历了飞速发展的建设历程

In the past 200 years, the construction of Dams around the world has experienced rapid development

Lehner B. et al. (2011) High-resolution mapping of the world's reservoirs and dams for sustainable river-flow management.

Front. Ecol. Environ. 9, 494-502.

Distribution Map of Large Reservoirs in China

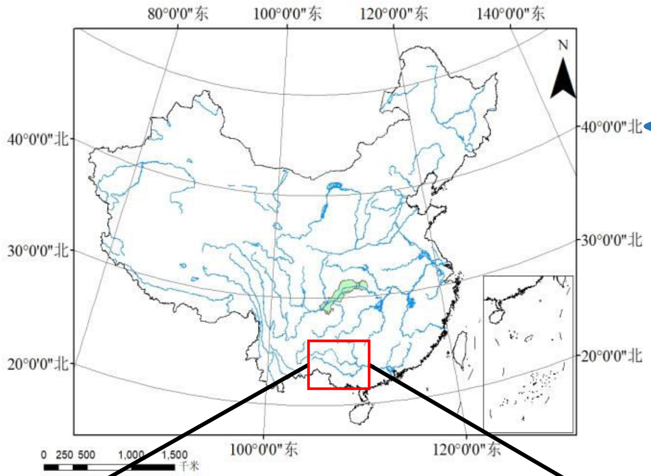


- **全国建成水库：98112座　Reservoirs in China: 98112，**
- **总库容：9323.12亿m^3 Total Capacity 9323.12billion m^3**

《全国水利普查》MWR. 2015；《中国湖泊生态环境研究报告》2022

确保三峡水库水质安全是长江大保护国家战略的重要需求

Ensuring the safety of water quality in the Three Gorges Reservoir is an important requirement of the national strategy of Yangtze River protection

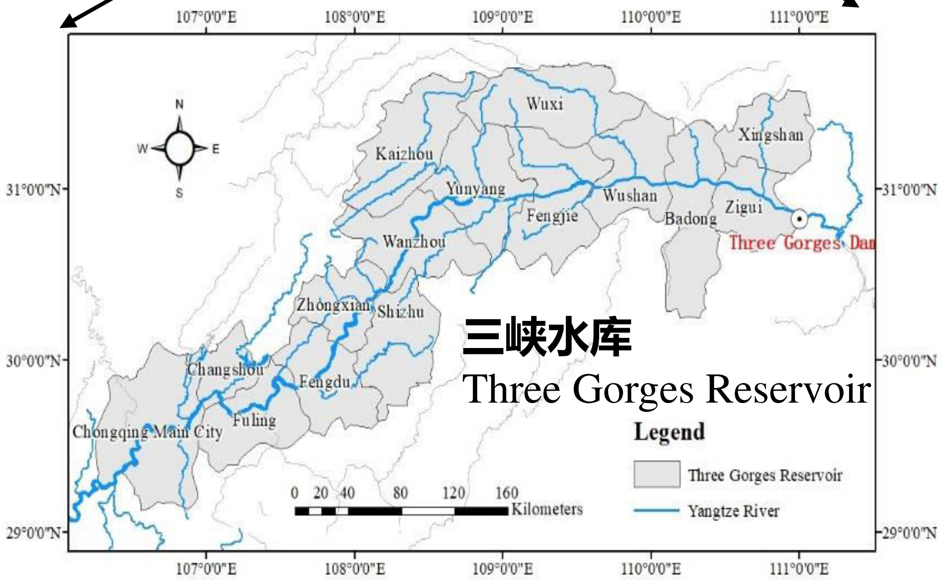


三峡工程是国之重器，地理位置关键，战略作用重要

The Three Gorges Project is an important tool of the country, the key geographical position and the important strategic role

三峡水库是调节长江生态和保障我国供水安全的关键性战略淡水资源库

The Three Gorges Reservoir is a key strategic freshwater resource for regulating the ecology of the Yangtze River and ensuring the safety of water supply in China



流域面积
100万平方公里

库区面积
7.9万平方公里

坝址流量
年均14300m³/s

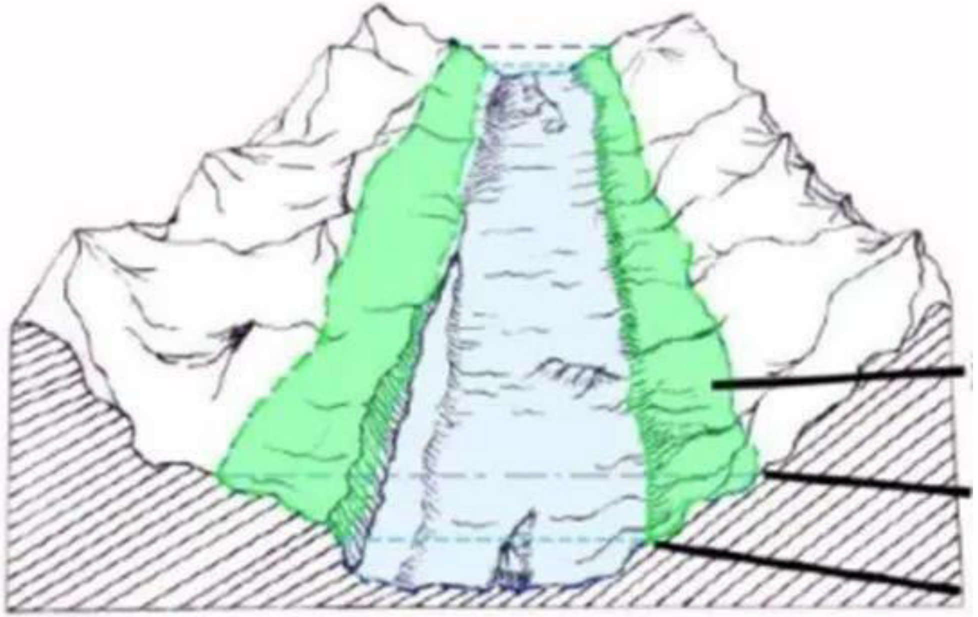
总储水量
393亿立方米

水库消落带是通过人为水位调蓄而形成的特殊区域。

Reservoir riparian zones are formed by artificial adjustment and storage of water levels.

三峡建库后水面面积增大一倍

After the construction of the Three Gorges reservoir, the water surface area has doubled



干湿交替

dry and wet

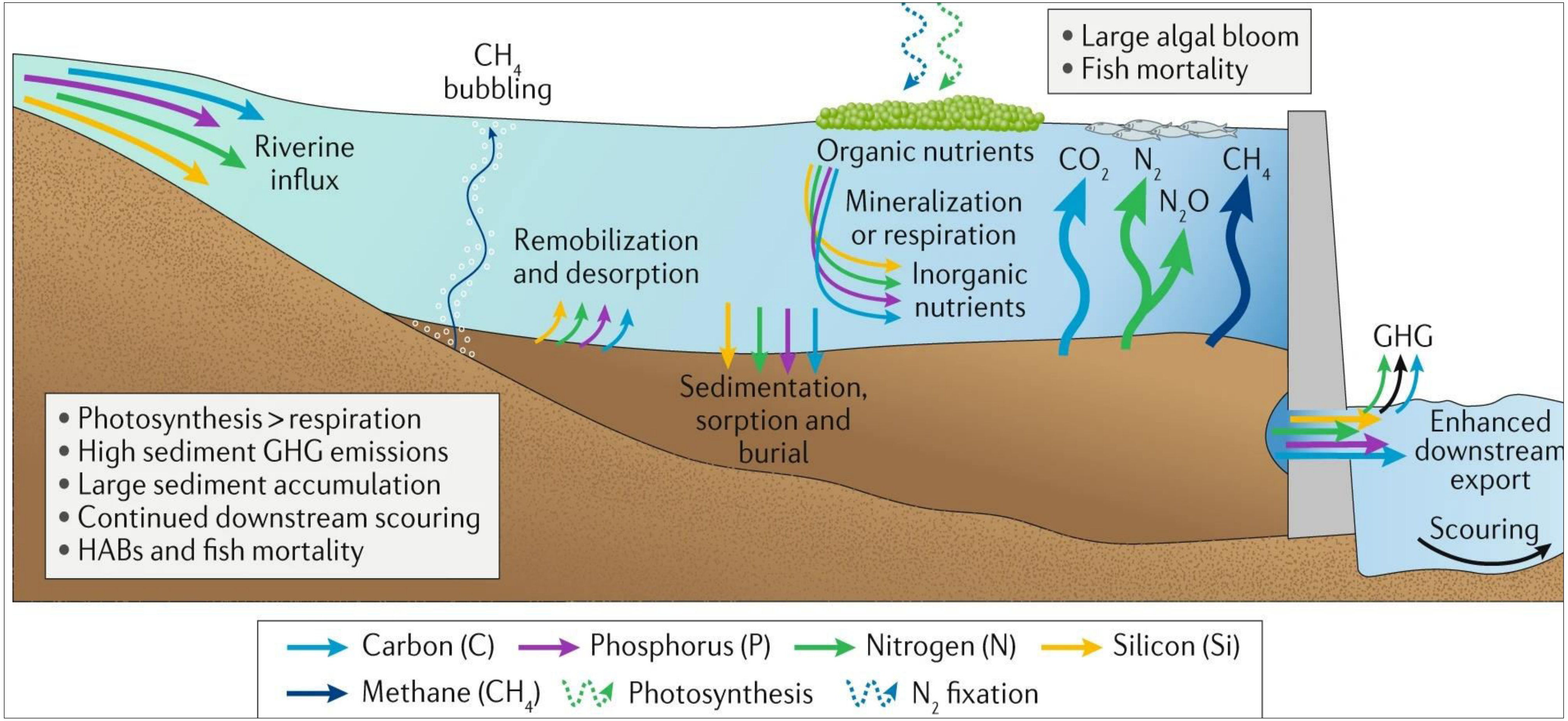
生态脆弱

fragile

（三峡工程公报，2021；李姗泽 王雨春 等，湖泊科学，2023）

水利水电工程极大地改变了流域生态环境格局和生物地球化学循环过程（氮 Nitrogen）

Water conservancy and hydropower projects have greatly altered the ecological environment patterns and biogeochemistry processes



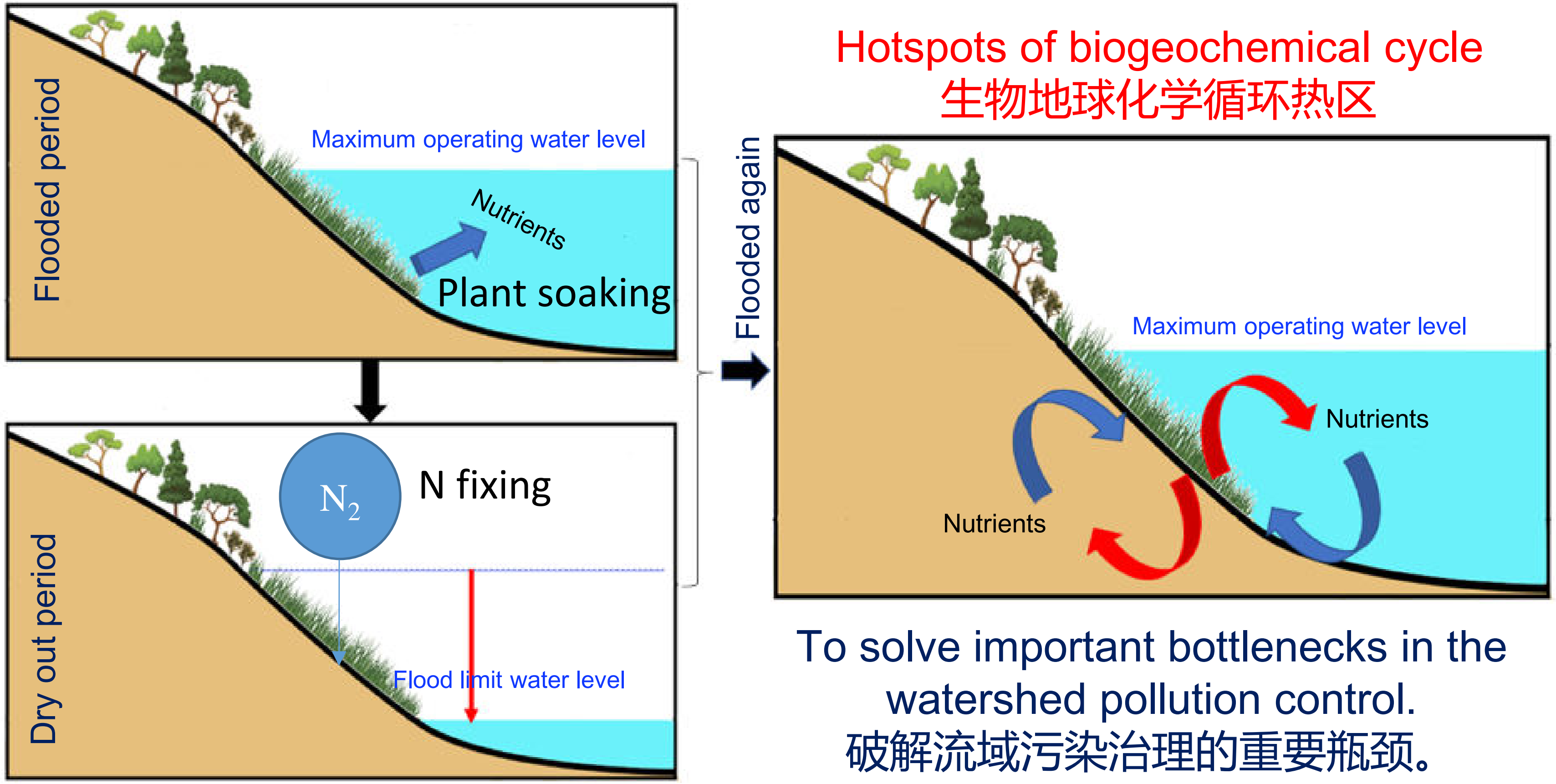
Maavara et al. Nat Rev Earth Environ 2020

关键科学问题 Key scientific issue

寻找“迷失的氮源” Search for the ‘missing nitrogen’

——理解流域氮循环和解决流域氮污染控制的重要瓶颈问题。

Understanding the watershed nitrogen cycle and solving the important bottleneck of watershed nitrogen pollution control



To solve important bottlenecks in the watershed pollution control.
破解流域污染治理的重要瓶颈。

消落带植物群落逆向演替是否会形成消落带毗邻水域新的活性氮源
Whether retrogressive succession of plant communities will form new active nitrogen sources in the waters adjacent to the riparian zone

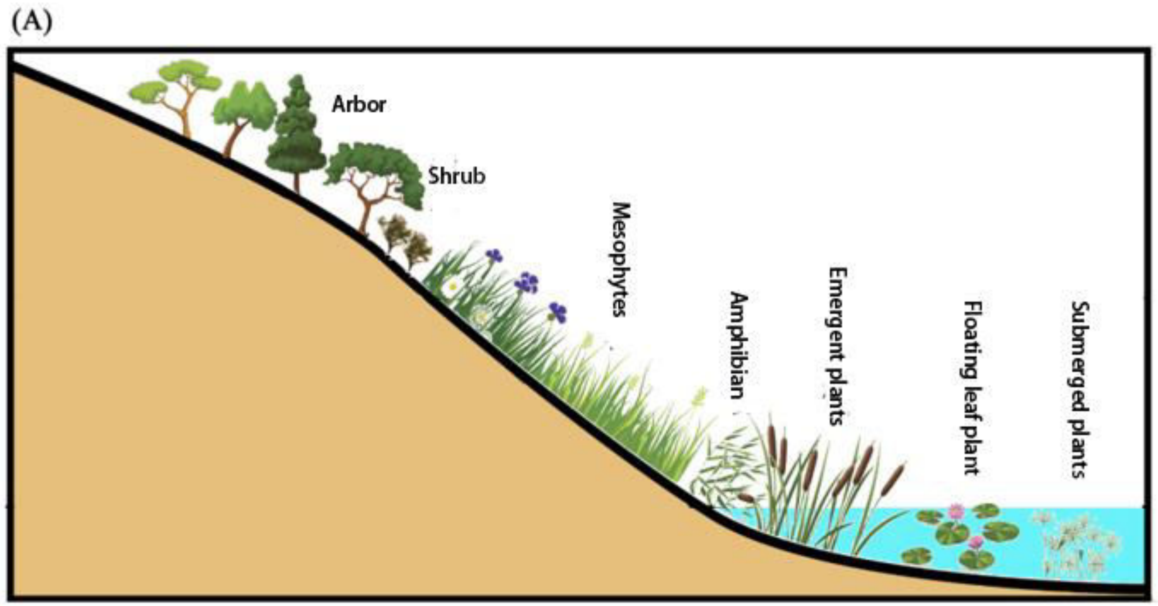
水淹胁迫是否可刺激消落带抗逆植物形成特殊的氮利用强化补偿机制
Whether waterlogging stress can stimulate the formation of nitrogen utilization enhancement and compensation mechanisms in water-resistant plants

植物群落逆向演替

Reverse succession of plant community

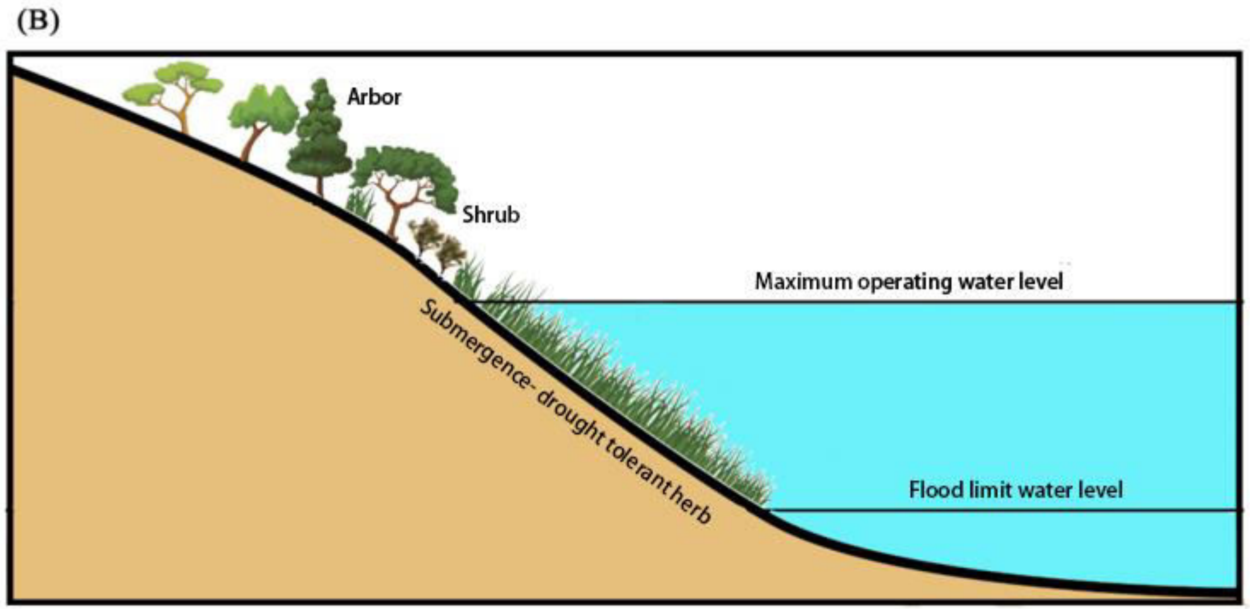
消落带的形成改变了原本的陆生环境，形成了具有特殊结构和适应性的生物群落。

The formation of the riparian zone changed the original terrestrial environment, and formed the biological community with special structure and adaptability.



自然河湖水陆交错带植被分布格局

(A) Vegetation distribution pattern in the riparian zone of natural rivers/lakes.



三峡水库消落带植被分布格局

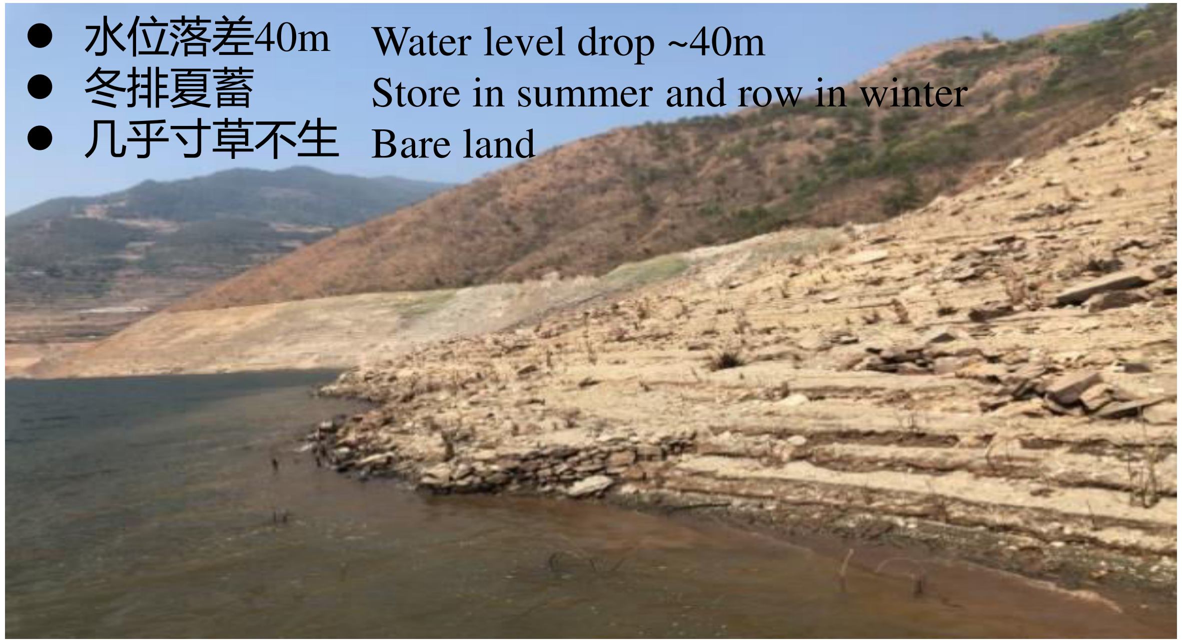
(B) Vegetation distribution pattern in riparian zone of the Three Gorges Reservoir.

水库消落带植物群落结构随调蓄方式的不同而异。

The plant community structure in riparian zones varied due to the different operation mode of reservoirs.

（A）2019年4月三峡库区消落带实景

Real scene of riparian zone in the Three Gorges Reservoir in April 2019



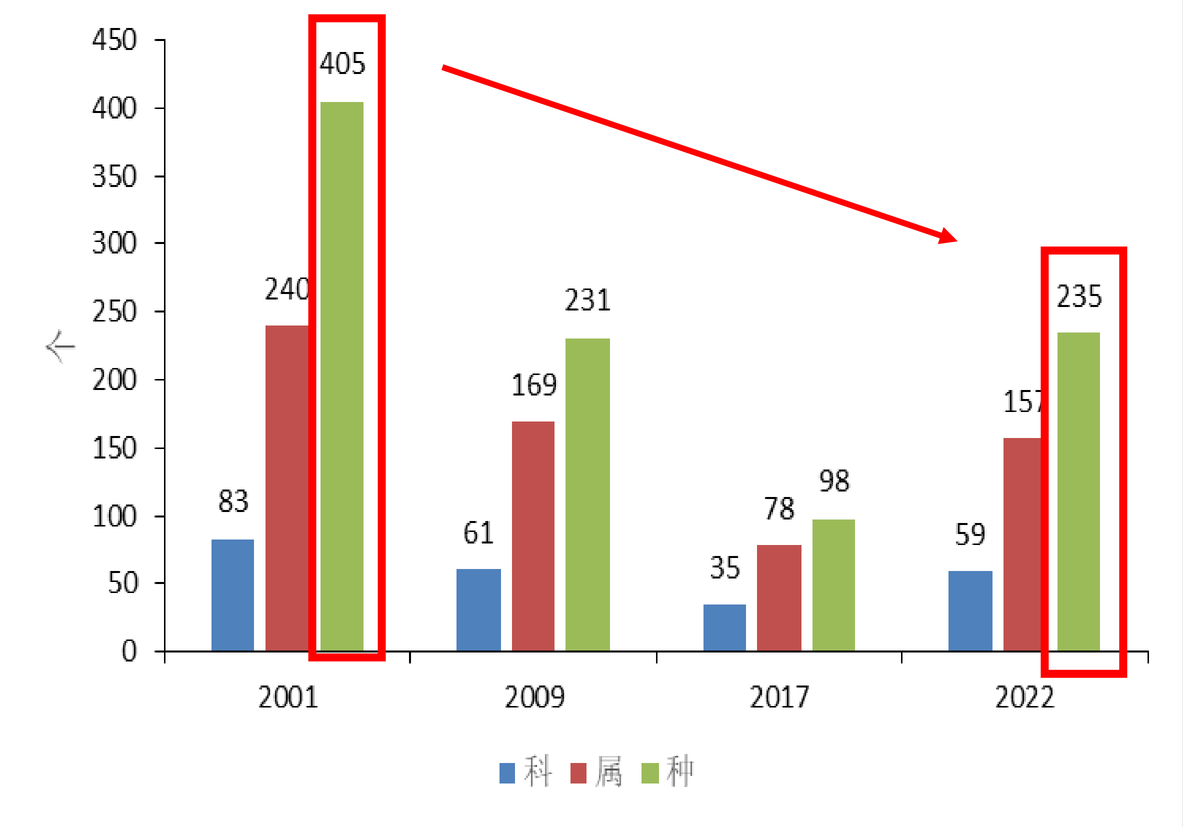
（B）2019年4月澜沧江消落带实景

Real scene of riparian zone in the Lancang Reservoir in April 2019

李姗泽 王雨春等，湿地科学，2019

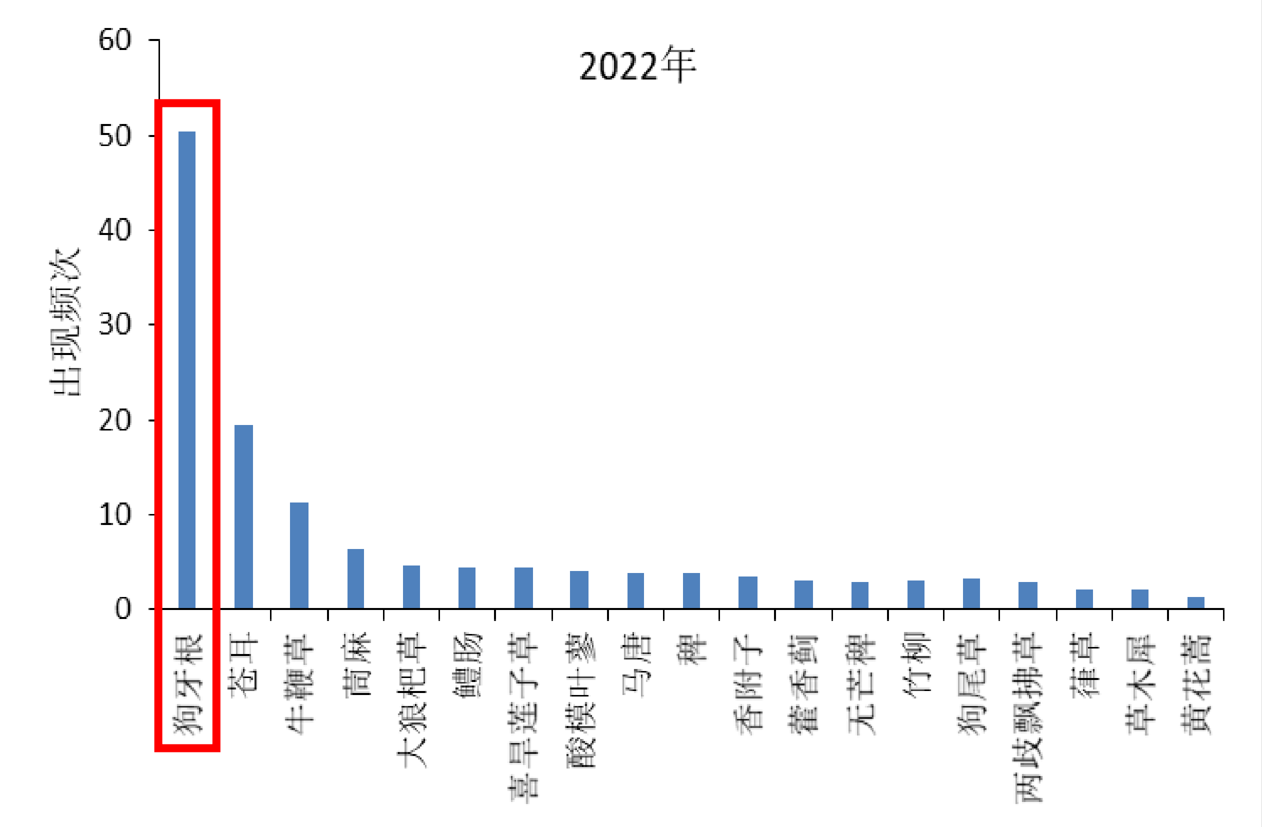
三峡消落带植物多样性变化

Plant diversity in riparian zone of TGR



草本植物物种数明显下降

The number of herbaceous plant species decreased significantly



优势物种：狗牙根

Dominant species: *Cynodon dactylon*

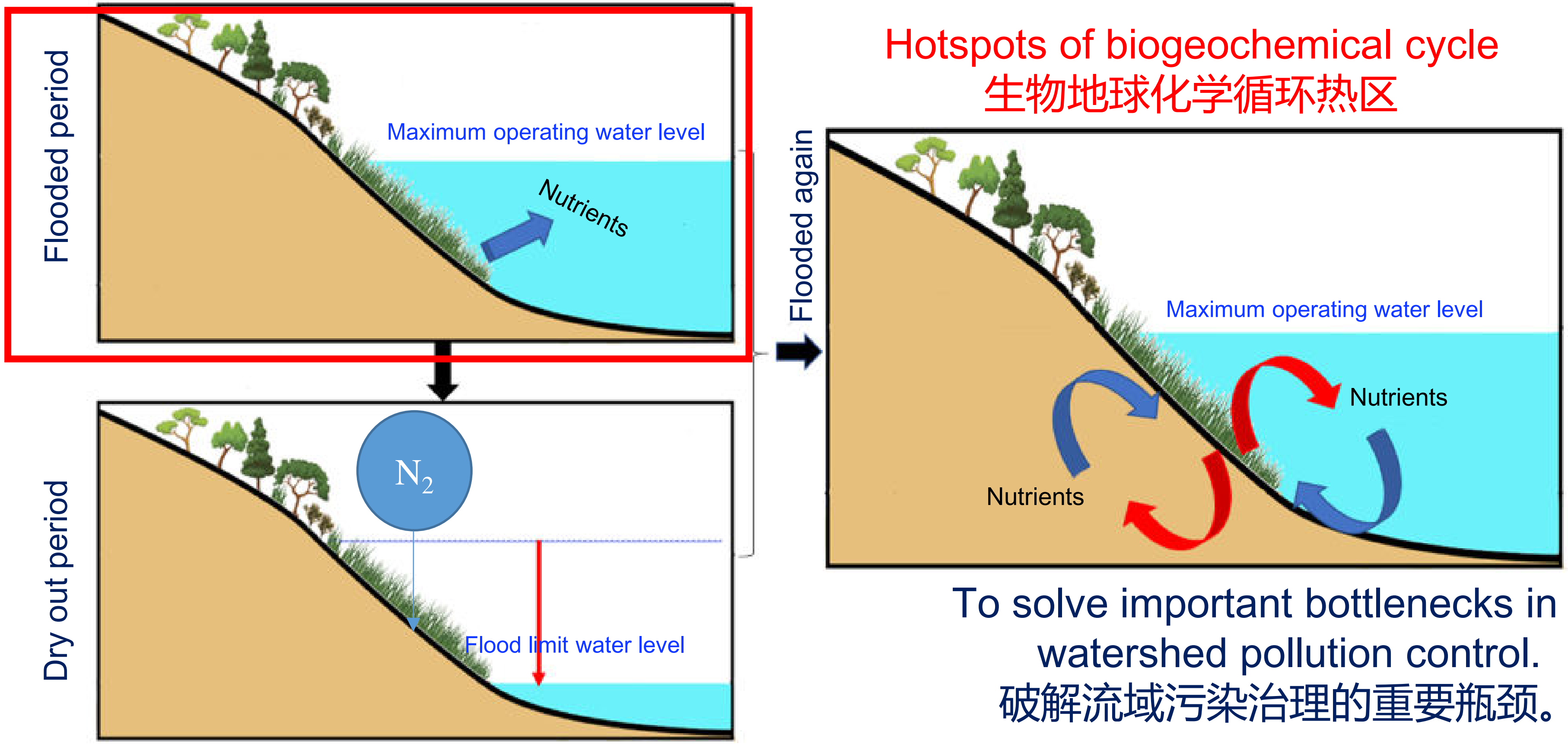
《三峡水库消落区生态环境状况报告（2021-2023年）》

水库调度运行下消落带的潜在生态效应

Three ecological effects of riparian zone during reservoir operation

●淹水期植物分解向水体释放营养元素造成二次污染?

Secondary pollution caused by plant decomposition releasing nutrient elements to water during flooding period?



Hotspots of biogeochemical cycle
生物地球化学循环热区

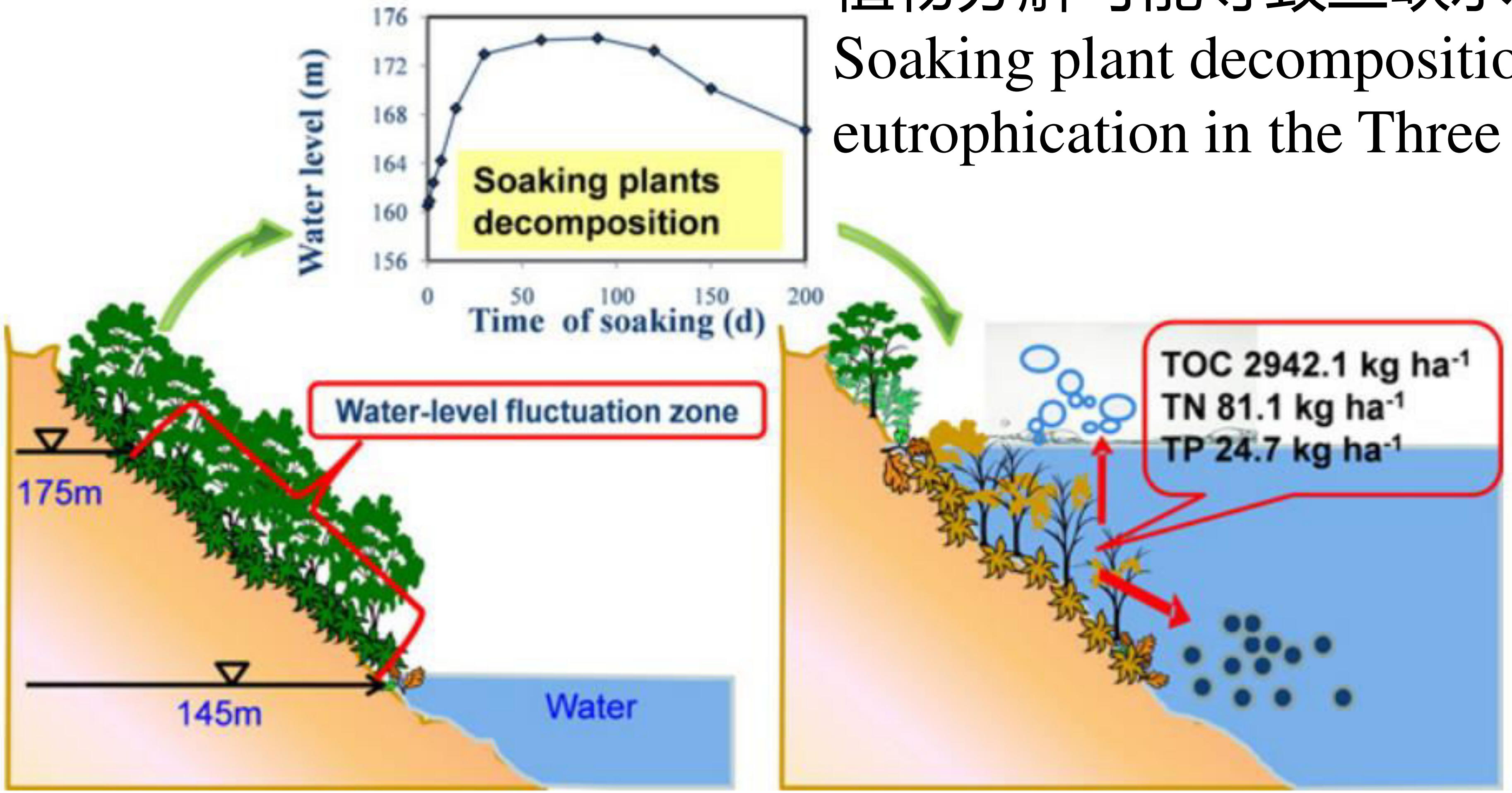
To solve important bottlenecks in the watershed pollution control.
破解流域污染治理的重要瓶颈。

淹水期消落带植物分解导致水体营养负荷

Large quantities of nutrient loadings resulted from soaking decomposition.

植物分解可能导致三峡水体富营养化

Soaking plant decomposition may lead to eutrophication in the Three Gorges Reservoir.



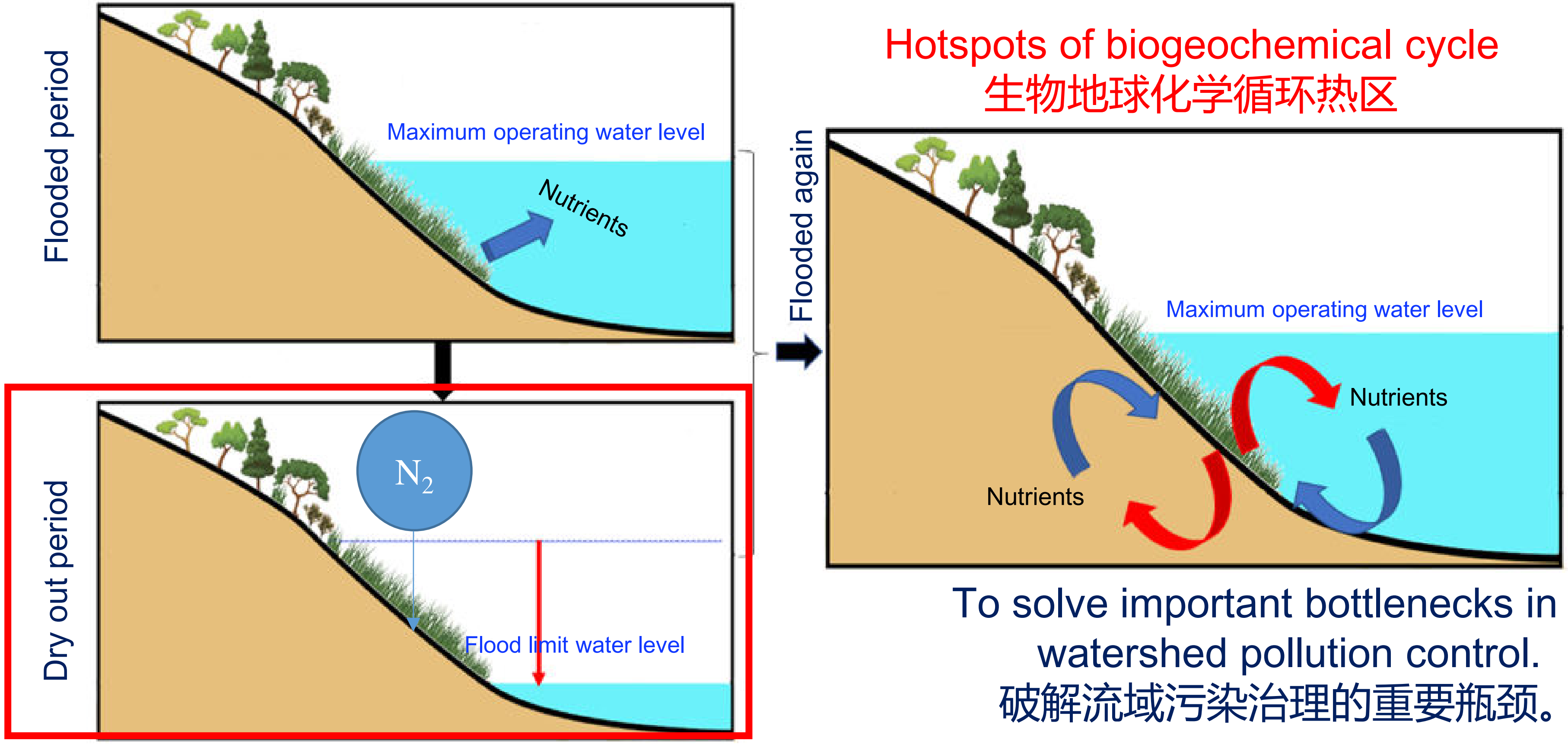
Xiao et al., 2017, Science of The Total Environment

水库调度运行下消落带的潜在生态效应

Three ecological effects of riparian zone during reservoir operation

●落干期植物是否具有额外的氮利用强化机制?

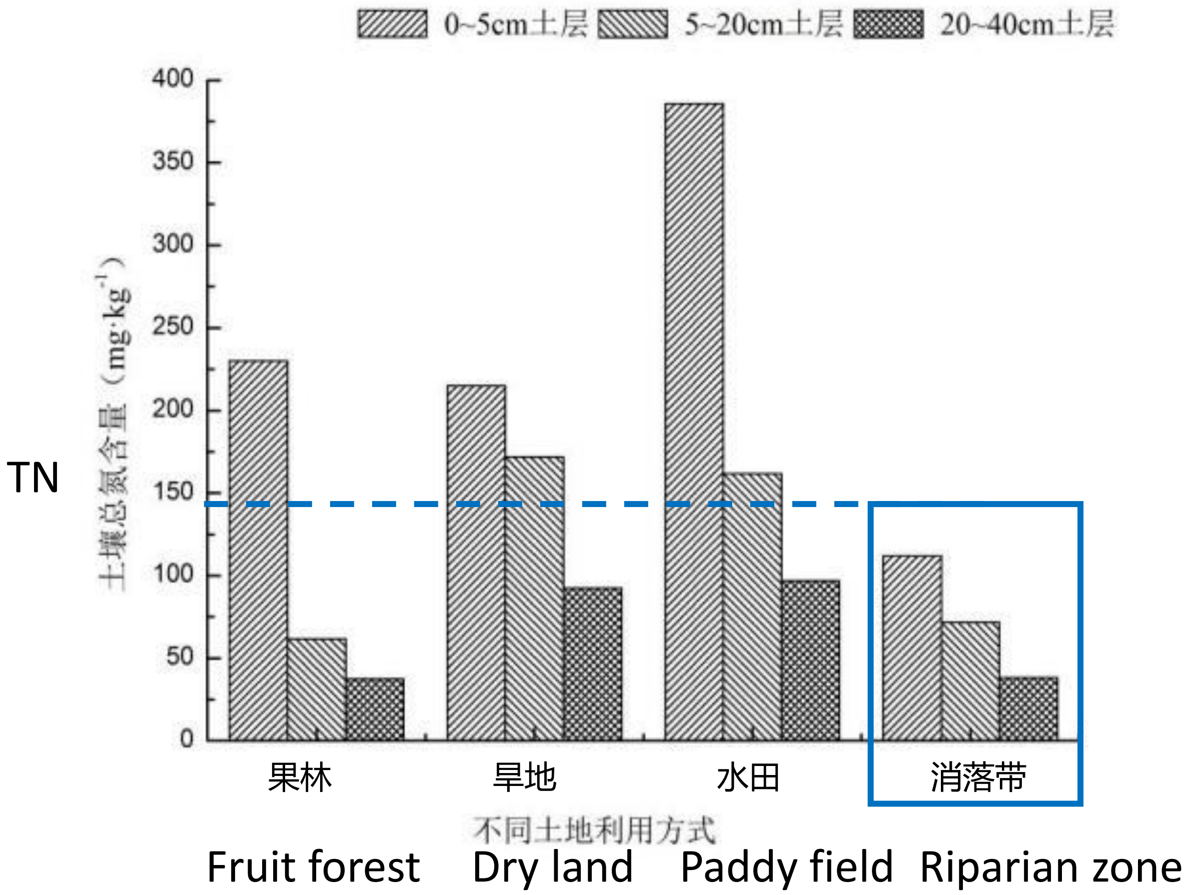
Do plants have additional nitrogen utilization enhancement mechanisms during drying period?



To solve important bottlenecks in the watershed pollution control.
破解流域污染治理的重要瓶颈。

长时间的水淹造成严重的消落带土壤氮淋失

The long-term flooding caused serious soil nitrogen leaching in the reservoir riparian zone



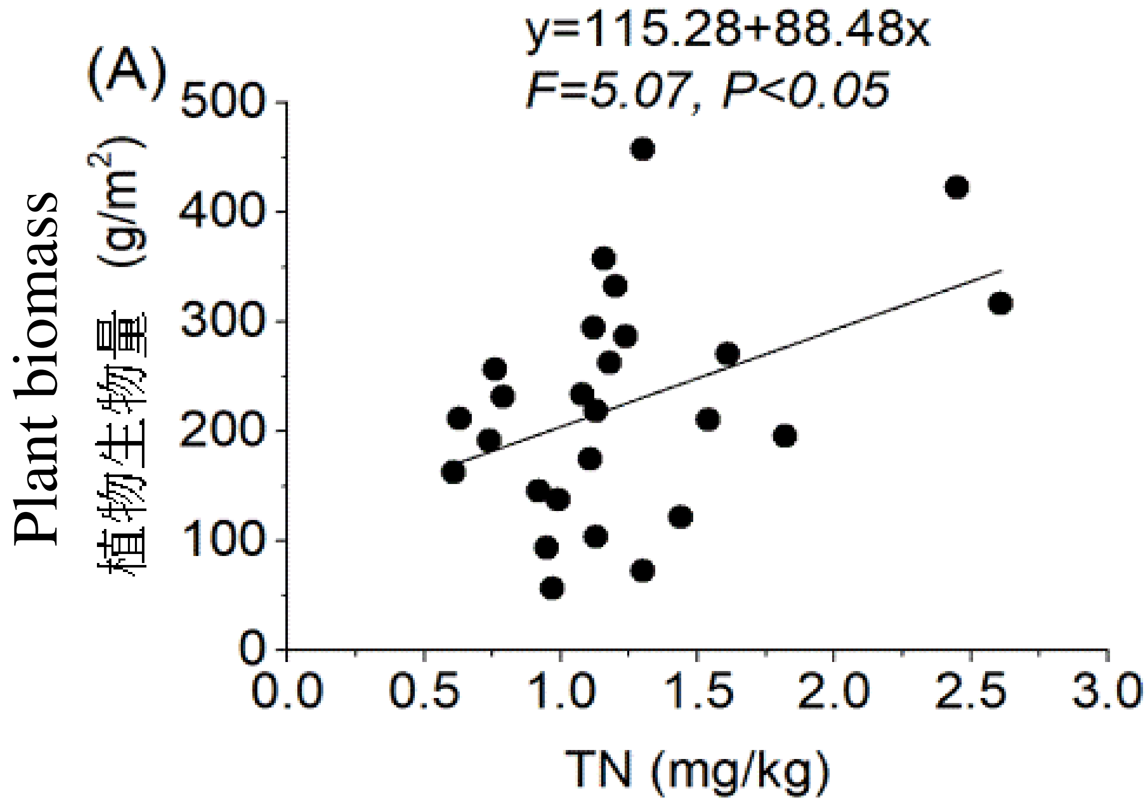
The average amount of nitrate nitrogen loss under different land use modes was in the order of riparian zone > fruit forest > dry land > paddy field.The TDN leaching loss in the riparian zone ranges from 1.17 to 15.87 kg/hm^2, with an average of 8.14 kg/hm^2.

不同土地利用方式下硝态氮流失量均值从大到小依次为：消落带>果林>旱地>水田。

消落带的 TDN 淋溶流失量为 1.17~15.87 kg/hm^2，均值为 8.14 kg/hm^2。 （孙军益, 2012）

消落带植物生物量与土壤总氮呈正相关关系

Plant biomass is positively correlated with soil total nitrogen in riparian zone



大胆假设 Bold assumption

三峡消落带优势抗逆植物狗牙根**是否具有内生固氮菌?**

Does the plant *Cynodon dactylon* have endophytic nitrogen fixing bacteria?

内生固氮菌是能与寄主植物联合固氮的一类微生物。定殖在植物体内，将大气中的氮转化为氨和其它含氮化合物，而进入到氮循环过程当中。Endophytic nitrogen fixing bacteria is a kind of microorganism which can combine with host plant to fix nitrogen. It colonizes plants and converts atmospheric nitrogen into ammonia and other nitrogen-containing compounds that enter the nitrogen cycle.

（刘天增 等，2014 ; Kuypers et al., 2018）

优势植物物种-狗牙根

Dominant plant species-*C. dactylon*

- Resistance to flooding could be over 200 days 耐淹能力可超200天

(A) Submerged plants(withered yellow) just exposed after the water level drops.
图A为水位下降后刚出露的被淹没狗牙根（枯黄）

(B) exposed yellow plants quickly turned green a few days later.
图B为水位下降数天后，出露的枯黄狗牙根迅速返青的情况

The real scene of the fluctuation zone in the typical reservoir area of the Three Gorges Reservoir
三峡水库消落带实景

李姗泽，王雨春等，湿地科学，2019

研究证实狗牙根具有内生固氮菌

It is proved that plant *C. dactylon* have endophytic nitrogen fixing bacteria

细菌分离鉴定及形态观察Isolation, identification and morphological observation of bacteria

利用CCM无氮培养基**从随机的12份不同的狗牙根植物组织叶和茎材料中分离得到54株内生细菌**，54 strains of endophytic bacteria were isolated from 12 different leaf and stem materials of *C. dactylon* using CCM nitrogen-free medium.

表 2-3 菌落形态特征

菌株	菌落特征
GY–1	浅黄色，菌落适中，形状不规则，不透明
GY–2	乳白色，菌落适中，圆形，不透明，中间凸起
GY–3	乳白色，菌落较大，圆形，透明，边缘不整齐
GY–4	浅黄色，菌落较大，形状不规则，半透明，中间凸起
GY–5	黄色，菌落较小，圆形，不透明，中间凸起
GY–6	橙色，菌落较小，圆形，不透明
GY–7	红色，菌落较小，圆形，不透明，中间凸起，边缘整齐
GY–8	红色，菌落较大，形状不规则，边缘不整齐

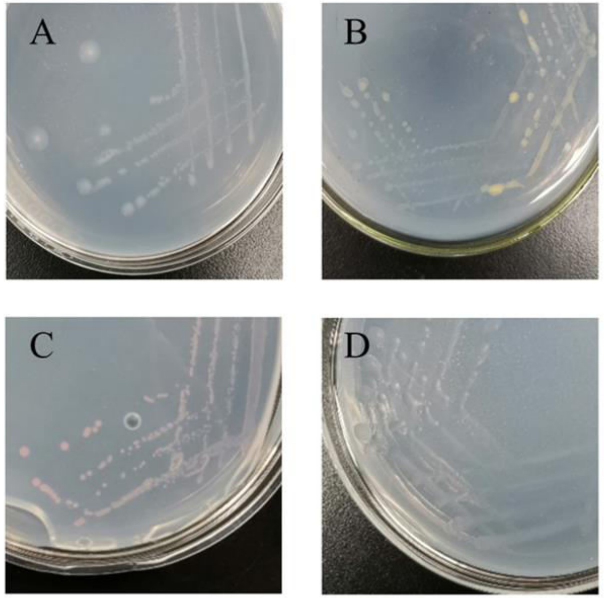


图 2-4 菌落形态

（A 表示 GY–2；B 表示 GY–4；C 表示 GY–7；D 表示 GY–3）

酶活性最大的是菌株SBL 2（4.11 nmol C_2H_4/ ml），属于菌种GY–5。固氮酶活性最低的是菌株SAH3（0.15 nmol C_2H_4/ ml），属于菌种GY–3。从狗牙根植物茎组织上分离的30株内生固氮菌中，有15株表现出固氮酶活性。

研究区域及样品组成

Study area and sample composition

时空分布规律

Spatial and temporal distribution pattern

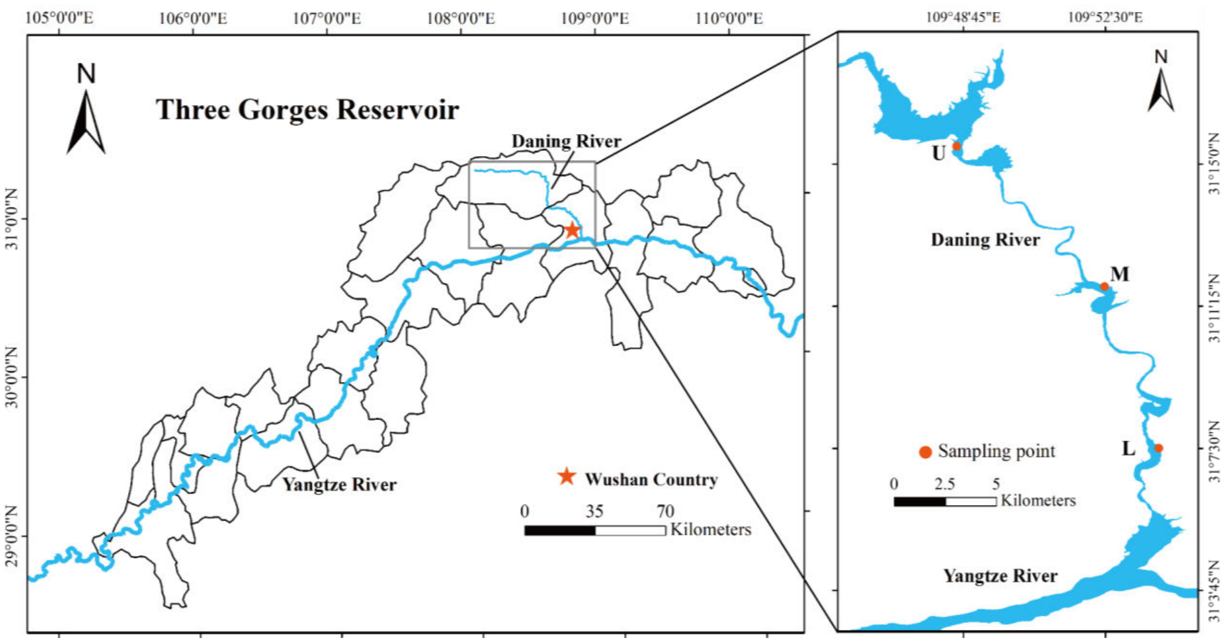
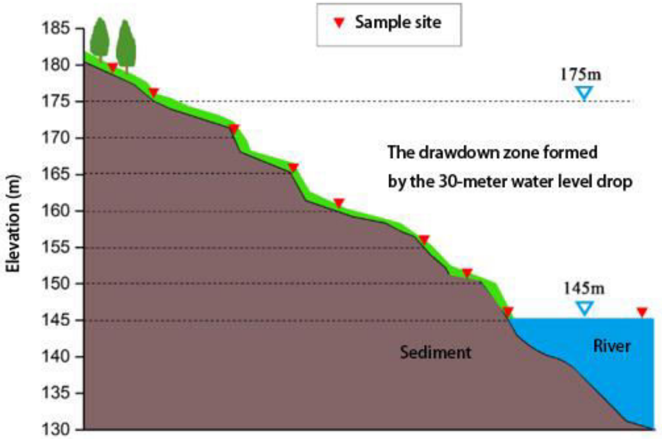


图3.1 采样区域及点位图



- Study area： Daning River
- Sites: **Upper, Middle, low**
- Elevations： 170 m， 160 m， 150 m
- Survey time： July, August

样品组成

① 狗牙根植物根、茎、叶样品54个；Plants:54

② 土壤样品108个。Soil cores:108

研究方法
Research methods

The plant samples were divided into roots, stems and foliage, and stored at −80 °C for later DNA extraction and high-throughput sequencing.

环境因子 Environmental factors

表3.1 土壤理化指标及测定方法

理化指标	测定方法
pH	电极电位法
含水率	恒重法
土壤有机质（SOM）	重铬酸钾消解法
全磷（TP）	钼蓝分光光度法
总碳（TC）	元素分析仪（Elementar Vario PYRO cube）
总氮（TN）	
氨态氮（NH_4^+-N）	流动分析仪（San++, SKALAR, Netherlands）
硝态氮（NO_3-N）	
亚硝态氮（NO_2-N）	

微生物丰度 Microbial abundance

表3.2 荧光定量PCR使用的引物及扩增条件

Target genes	Primers	Primer sequences (5′-3′)	Referen	
Comammox *amoA* clade A	CA377f	GTGGTGGTGGTCBAAYTA	(Jiang et al., 2020)	10 s at 95 °C, 20 s at 52 °C, 30 s at 72 °C.
	C576r	GAAGCCCATRTARTCNGCC		
Comammox *amoA* clade B	CB377f	GTACTGGTGGGCBAAYTT	(Jiang et al., 2020)	95 °C for 1 min; 40 cycles of 10 s at 95 °C, 20 s at 52 °C, 30 s at 72 °C.
	C576r	GAAGCCCATRTARTCNGCC		
AOA *amoA*	Arch-amoAF	STAATGGTCTGGCTTAGACG	(Francis et al., 2005)	95 °C for 1 min; 40 cycles of 10 s at 95 °C, 20 s at 56 °C, 30 s at 72 °C.
	Arch-amoAR	GCGGCCATCCATCTGTATGT		
AOB *amoA*	amoA-1Fmod	CTGGGGTTTCTACTGGTGGTC	(Meinhardt et al., 2015)	95 °C for 1 min; 40 cycles of 10 s at 95 °C, 20 s at 52 °C, 30 s at 72 °C.
	GenAOBR	GCAGTGATCATCCAGTTGCG		
NifH	Pol-F	TGCGAYCC-SAARGCBGACTC	(Poly et al., 2001)	95 °C for 1 min; 40 cycles of 10 s at 95 °C, 20 s at 52 °C, 30 s at 72 °C.
	Pol-R	ATSGCCATCATYTCRCCGGA		

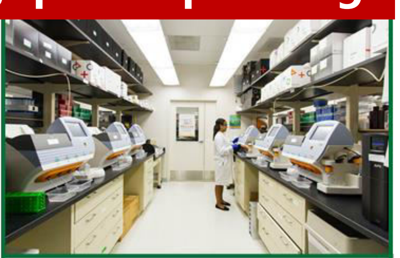
以往针对狗牙根内生固氮菌的研究，都是采用从狗牙根植物体内分离培养内生固氮菌的方法。而本论文聚焦内生细菌的DNA序列，运用高通量测序以及荧光定量PCR等技术，来探索狗牙根内生固氮菌的多样性及分布特征。

高通量测序 High-throughput sequencing

微生物多样性 Microbial diversity

步骤 1

提取DNA



步骤 2

PCR扩增，回收目的片段

步骤 3

文库构建，剪切、拼接

步骤 4

纯化与质检

步骤 5

Illumina NovaSeq 机器测序

步骤 6

以97%相似度水平聚类OTUs

研究结果

Results

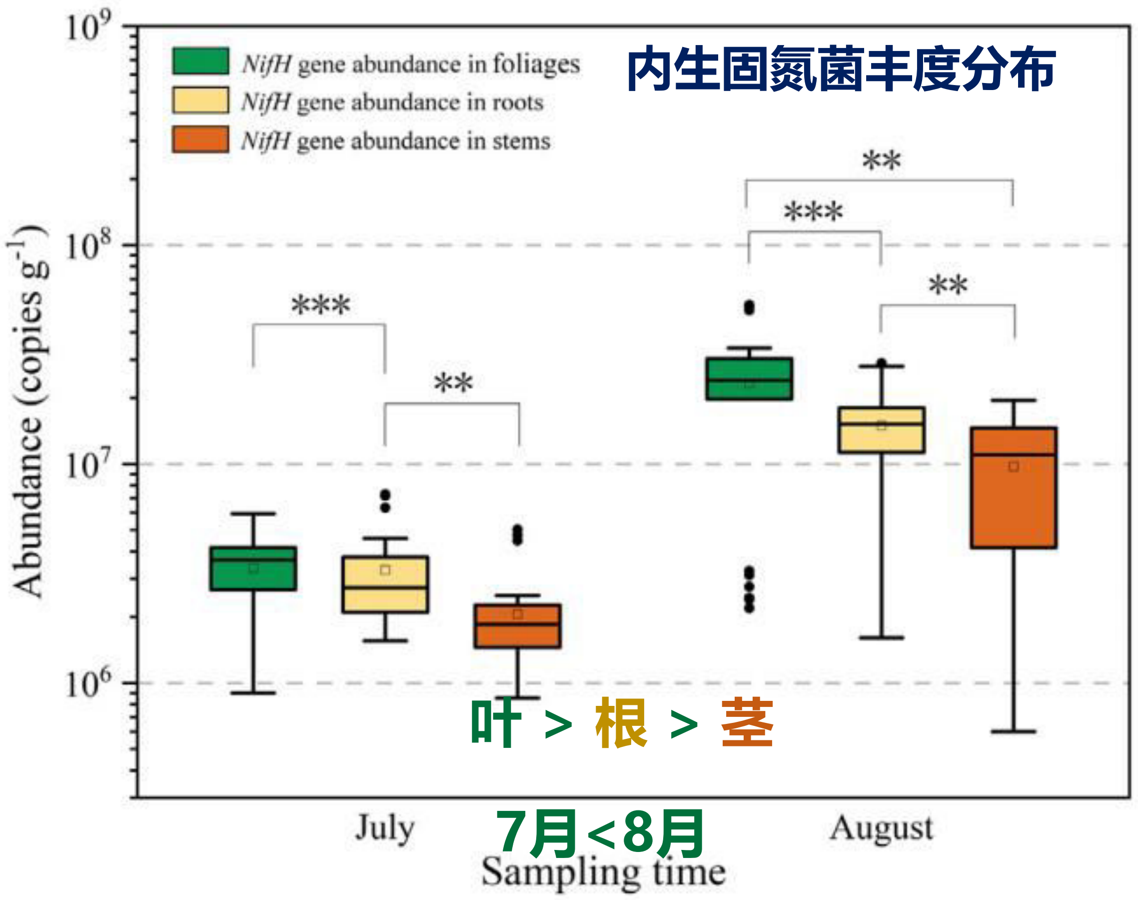
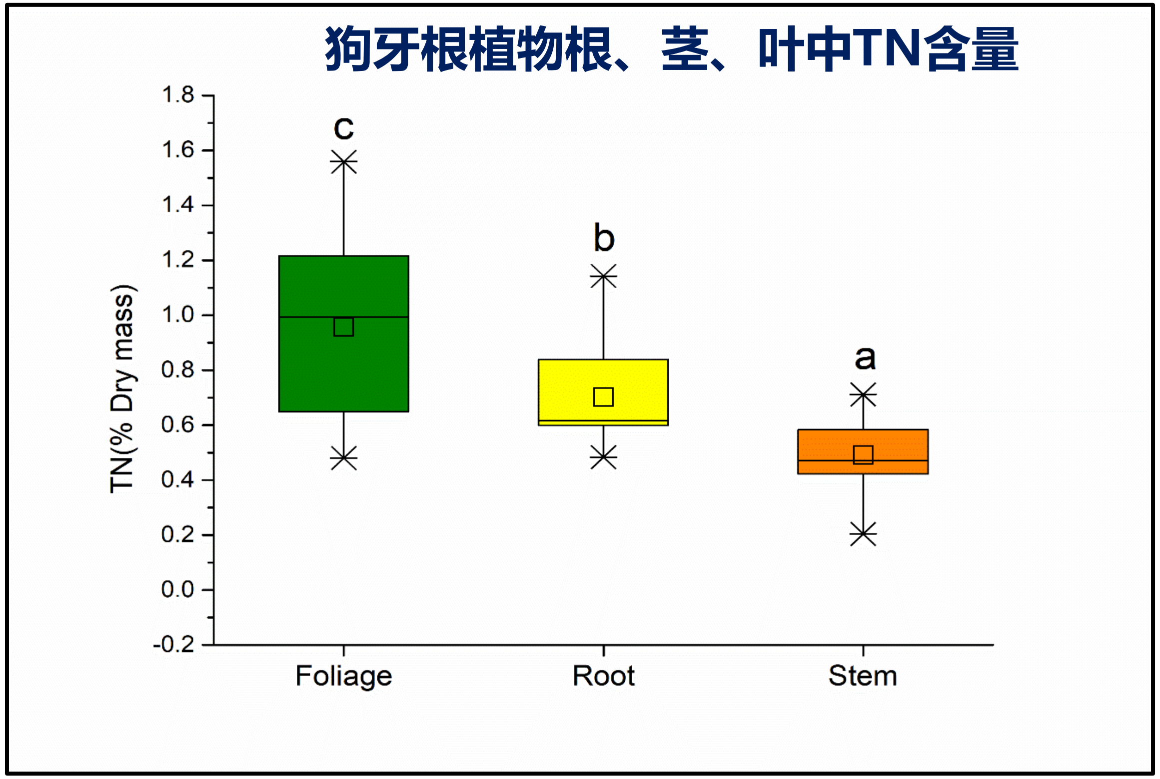


Fig. 7. Abundance of nitrogen-fixing bacteria *nifH* gene in foliage, stems and root tissues of plant *C. dactylon* in July and August (*** and ** represent $P < 0.001$ and $P < 0.01$, respectively).



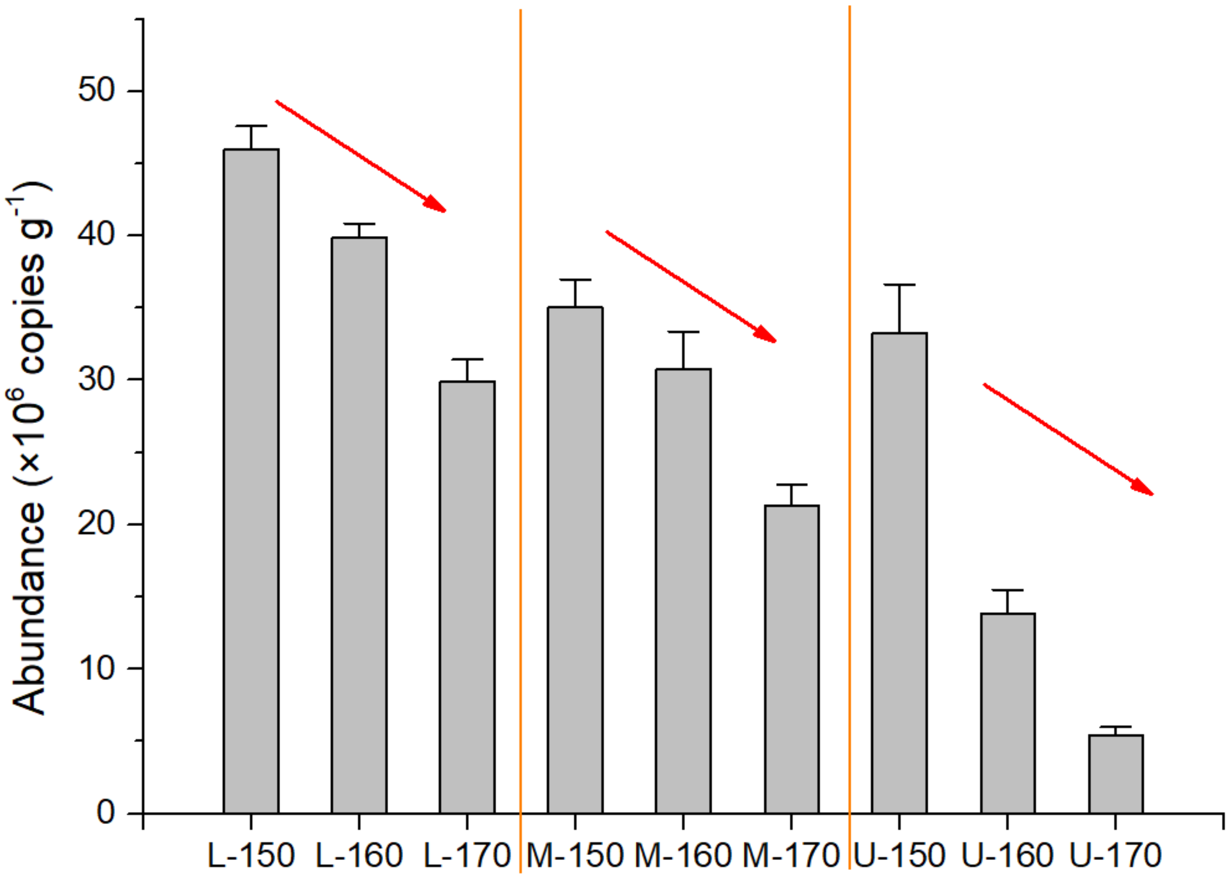
叶 > 根 > 茎

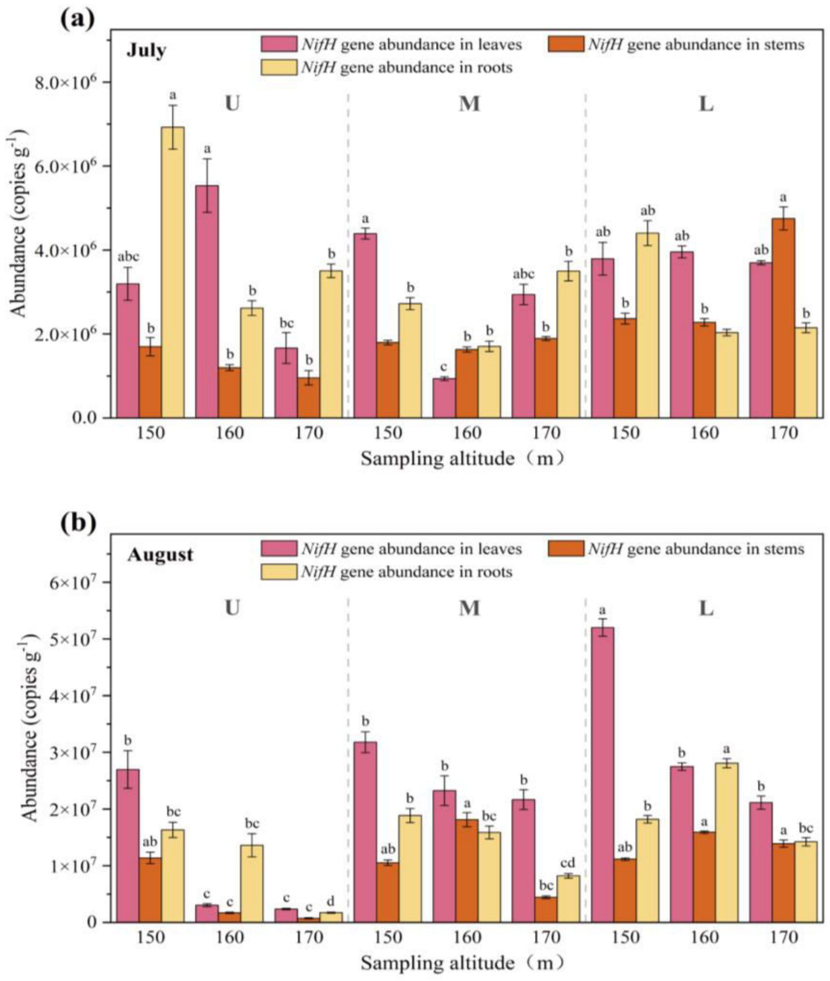
foliage>root>stem

研究结果

Results

The abundance of endophytic nitrogen fixing bacteria decreased with the elevation gradient.





不同区域（大宁河上、中、下游）的狗牙根植物内生固氮菌丰度均随着海拔梯度的上升而下降。

Foliage叶组织 5.20 × 10^7 copies g^{-1},
Stem茎组织 1.81 × 10^7 copies g^{-1},
Root根组织 2.81 × 10^7 copies g^{-1}。

研究结果

Results

The abundance of endophytic nitrogen fixing bacteria negatively correlated with ammonia nitrogen and nitrate nitrogen, and positively correlated with TP.

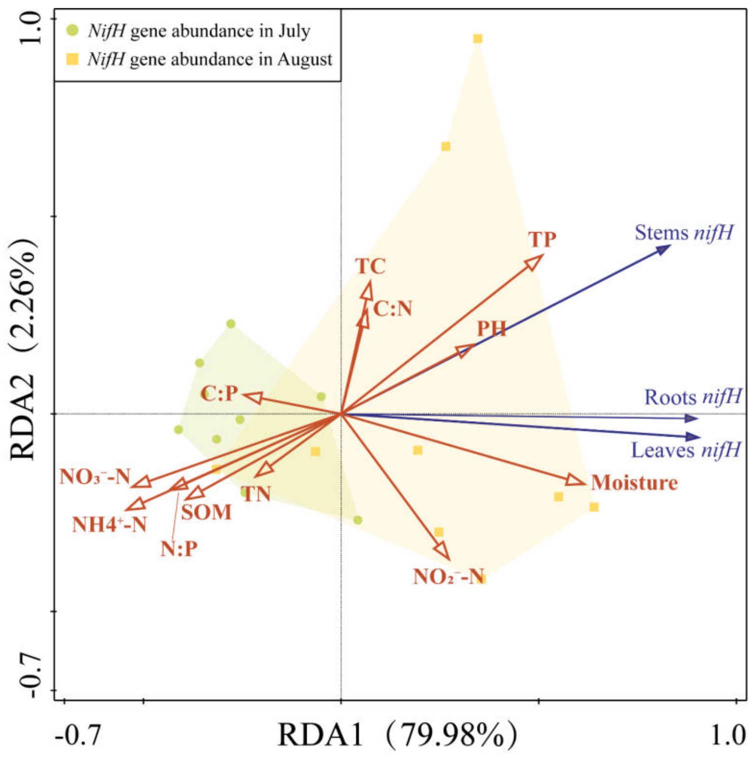


图4.3 内生固氮菌丰度与环境因子RDA分析

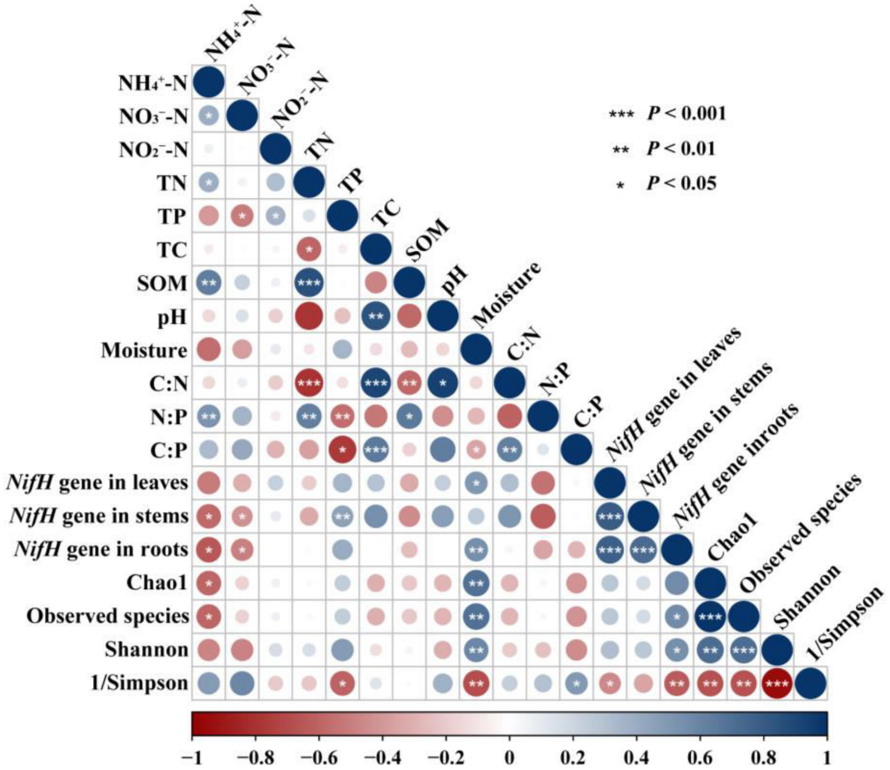


图4.4 内生固氮菌丰度与环境因子Spearman相关性分析

- RDA图两轴共解释了 82.24 % 的方差，**内生固氮菌丰度和 NH_4^+-N、NO_3^--N 呈显著负相关**。

  无机氮含量低，则内生固氮菌的固氮需求上升，进而影响内生固氮菌丰度。
- **内生固氮菌丰度和 TP呈显著的正相关。**磷（P）是细胞组成和ATP合成的重要成分，从而影响内生固氮菌丰度。

研究结果
Results

Community composition: At the phylum level, Proteobacteria was the dominant phylum in July and August. The microbial diversity of nitrogen-fixing bacteria in August was greater than that in July.

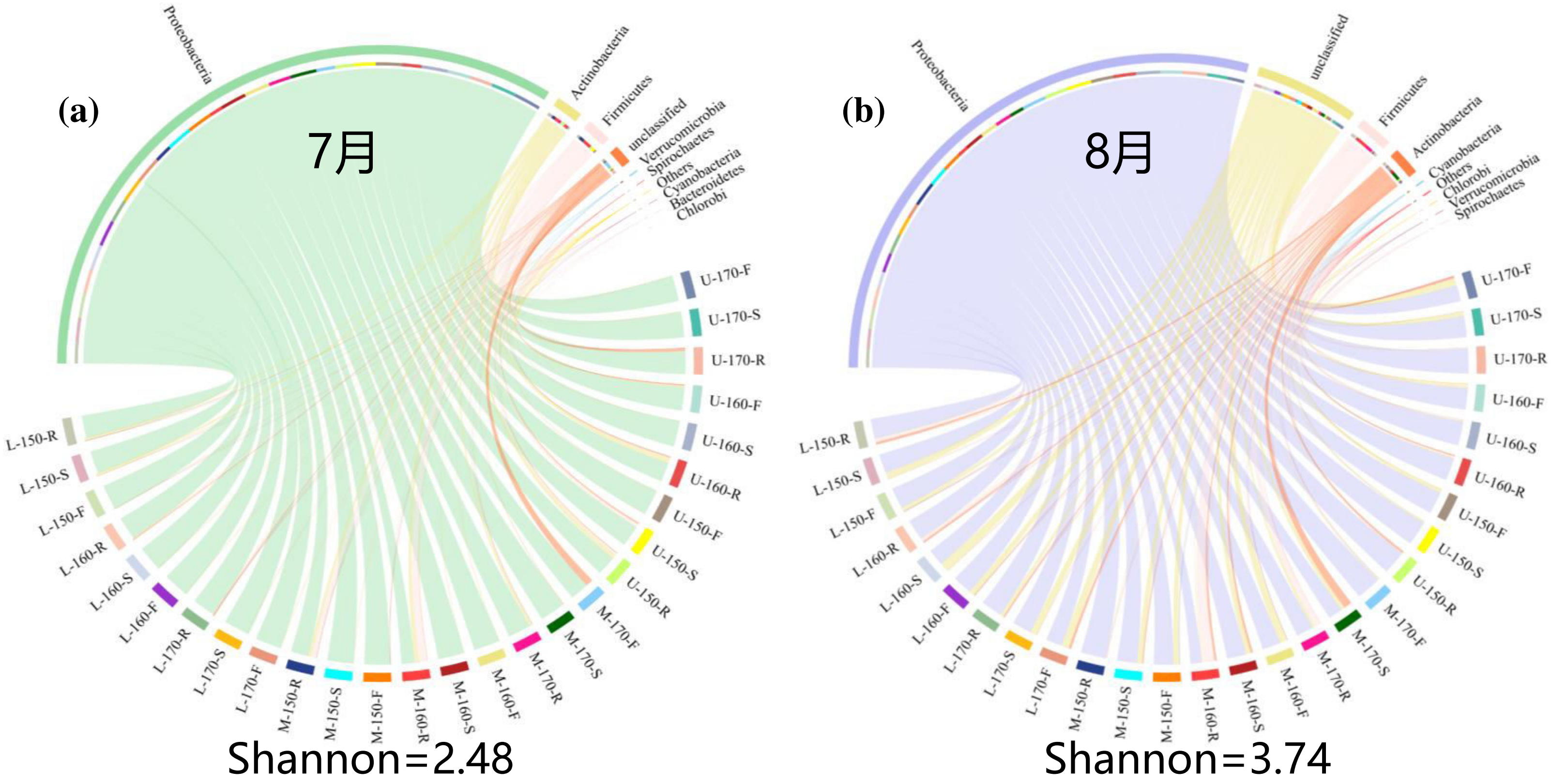


图4.5 内生固氮菌门水平分布

① 门水平上，7月和8月内生固氮菌的优势菌门均为变形菌门（Proteobacteria），在不同样品内的相对丰度范围为 50.86 % ~ 99.61 % 。其次是放线菌门（Actinobacteria）和厚壁菌门（Firmicutes）。

② 8月内生固氮菌的微生物多样性大于7月。

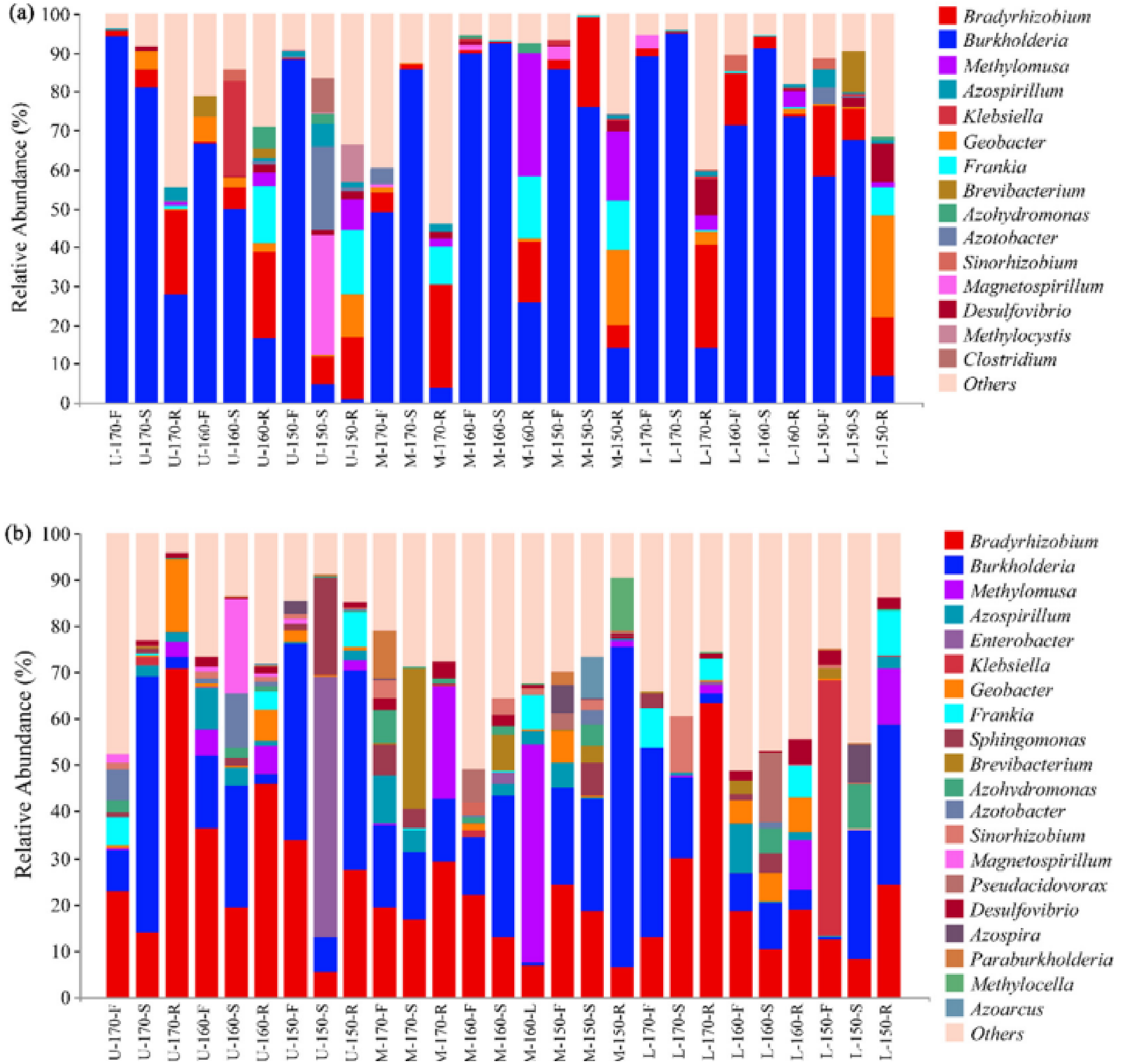
研究结果

Results

内生固氮菌群落组成Community composition

7月

8月



内生固氮菌属水平分布

Fig. 3. Relative abundance of endophytic nitrogen-fixing bacteria of *C. dactylon*. The relative abundances are given at the genus level in (a) July, and (b) August. The abscissa labels are composed of the sampling point (U, M, L)-sampling altitude (170, 160, and 150 m)-plant tissue (F-foliage, S-stem, R-root).

① 属水平上，7月的优势菌属为伯克氏菌属（*Burkholderia*），样品间的平均丰度可达56.20 %。 At the genus level, the dominant bacterium in July was *Burkholderia*, with an average abundance of 56.20% among samples.

② 8 月 的 优 势 菌 属 为 伯 克 氏 菌 属（ *Burkholderia* ）， 和 慢 生 根 瘤 菌 属（*Bradyrhizobium*），样品间的平均丰度分别可达 23.25 % 和 20.54 % 。In August, the dominant bacteria genera were *Burkholderia* and *Bradyrhizobium*, and the average abundance between samples was 23.25% and 20.54%, respectively.

研究结果
Results

内生固氮菌空间分布

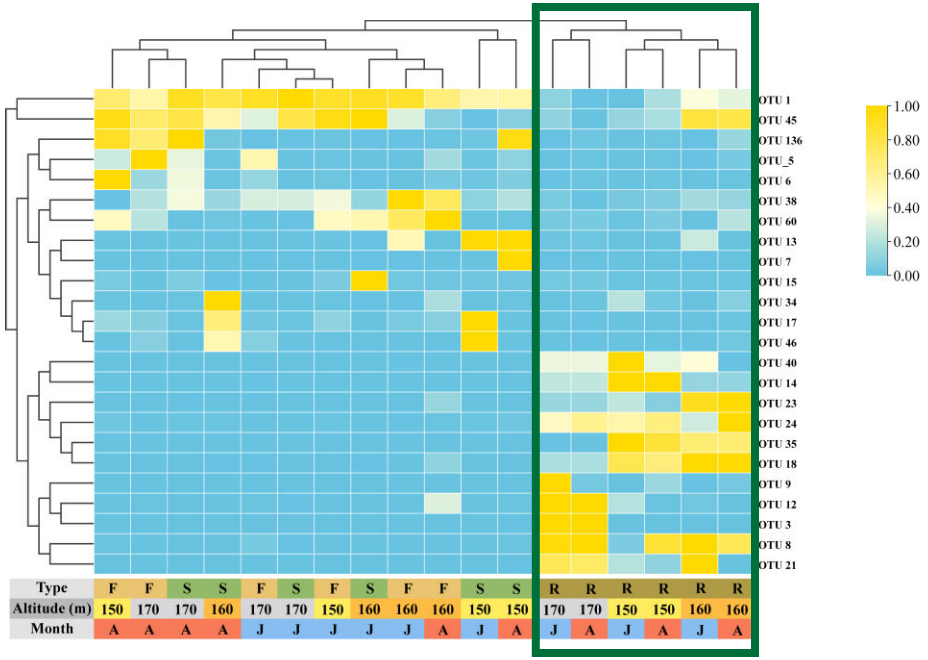


图4.7 内生固氮菌相对丰度热图

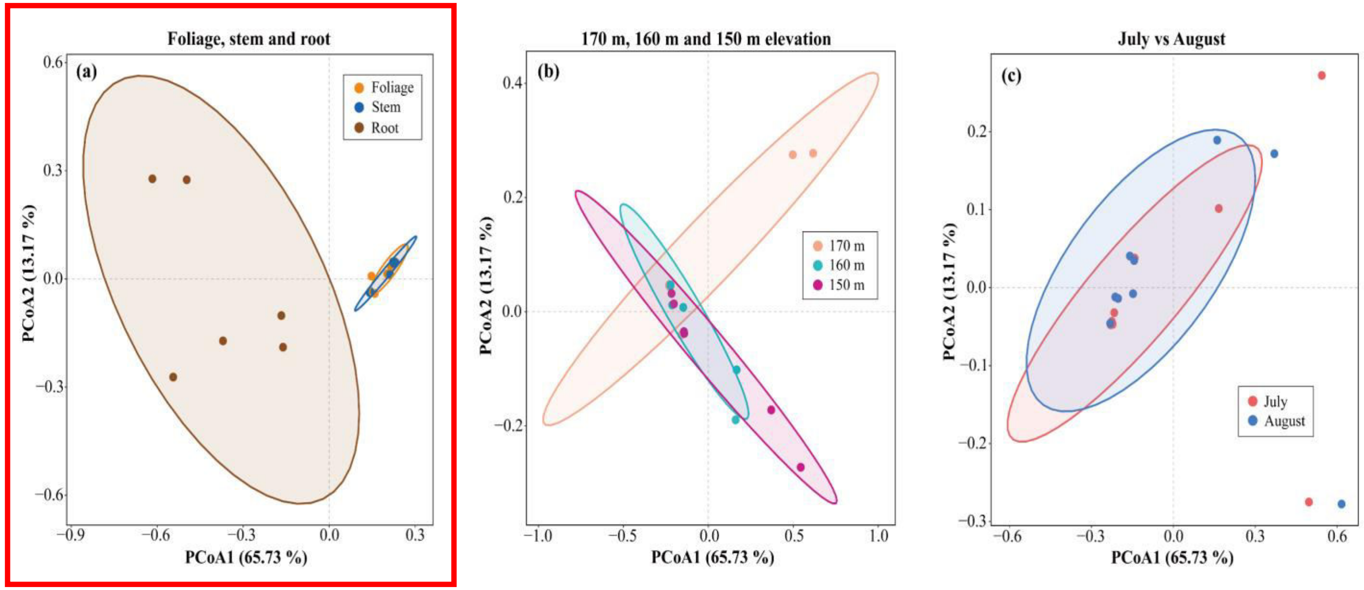


图5 内生固氮菌PCoA分析

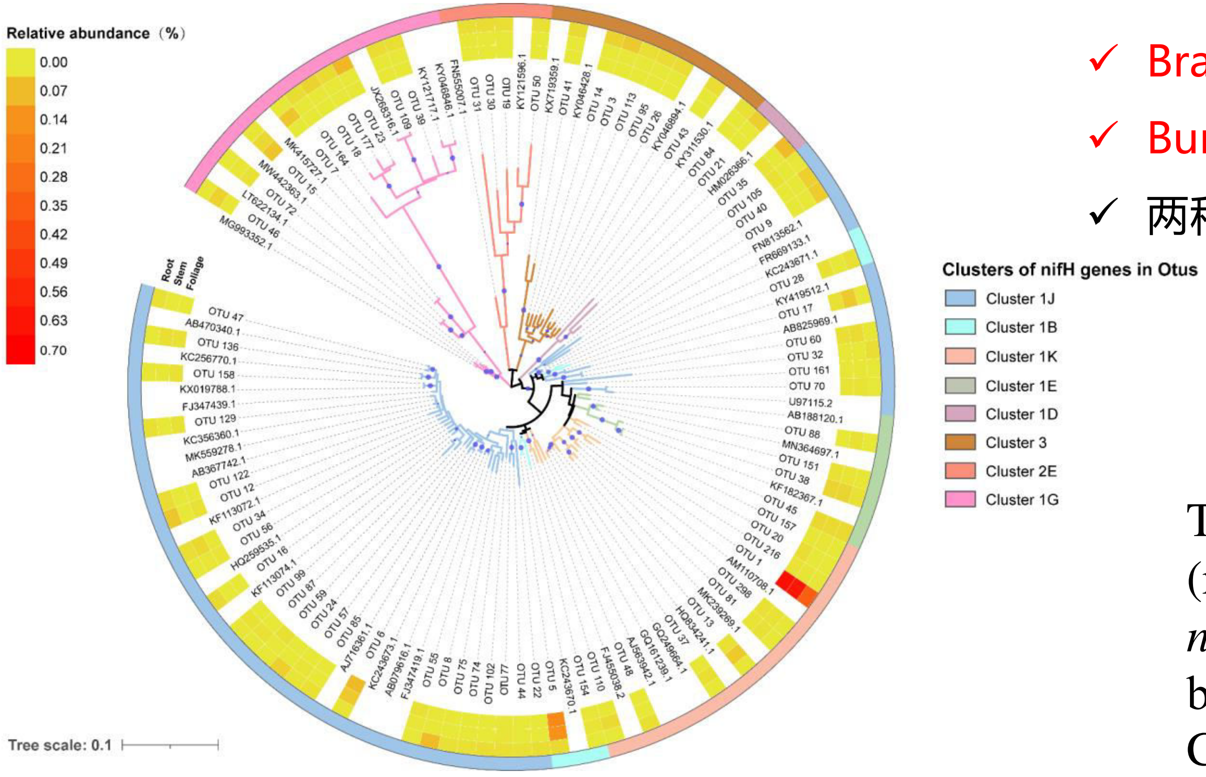
① 内生固氮菌群落组成仅在狗牙根植物根组织与叶、茎组织间存在显著差异。
② 狗牙根植物根和茎、叶组织间的优势菌属不同。

As shown in the left figure, PCoA indicated that the azotobacter community composition in the root differed from that in the foliage and stems (Fig. 5a), the azotobacter community composition in the 170 m differed from that in the 160 m and 150 m (Fig. 5b), whereas no significant differences existed between sampling times.
The dominant bacteria genera were different among the root, stem and leaf tissues of the plant

研究结果

Results

内生固氮菌系统发育



- ✓ Bradyrhizobium（慢生根瘤菌属）是Cluster 1J中的优势菌属。
- ✓ Burkholderia（伯克氏菌属）是Cluster 1K中的优势菌属。
- ✓ 两种固氮菌均被广泛发现于各种生境，且被验证具有固氮能力。

The phylogenetic tree can be divided into eight clusters (represented by colored branches and outer bands). Overall, the *nifH* sequence analysis revealed that most of the OTUs belonged to the α, β, and γ variants of Proteobacteria, thus Cluster 1 was the most abundant *nifH* cluster.

图4.9 内生固氮菌系统发育树

- ☐ Cluster 1J（α-变形菌纲）；Cluster 1K（β-变形菌纲）；Cluster 1G（γ-变形菌纲）的分布最广.

研究结果

Results

内生固氮菌共生模式

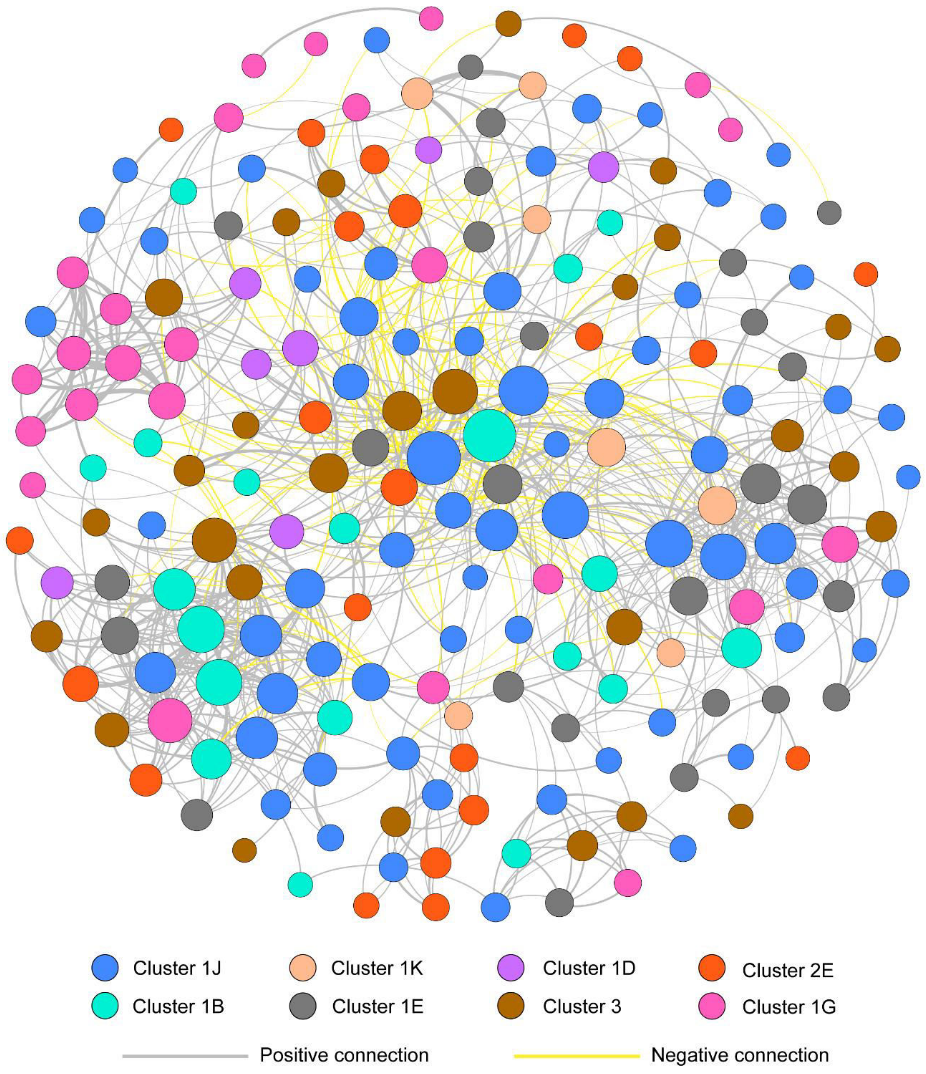


图4.9 内生固氮菌共现网络图

- 内生固氮菌之间合作大于竞争。
- 不同固氮菌分支间的相互协作大于同一固氮菌分支内的菌属。
- Cluster 1J是共生网络中的核心内生固氮菌。

The results indicated that the clusters of these three groups of nitrogen-fixing bacteria were more active in the overall plant environment and had stronger co-occurrences with other bacterial genera.

主要结论

Main Conclusions

- 研究发现，三峡库区消落带狗牙根具有通过增加内生固氮细菌丰度来克服长期淹水的生存策略。
- 狗牙根植物内生固氮菌的丰度在叶片中含量最高，并且8月份的丰度高于7月份。
- 内生固氮菌更适宜低NH_4^+-N、低NO_3^--N、高总磷、高含水率的土壤环境。
- 狗牙根内生固氮菌通过不同菌属之间的协同作用，构建复杂的共生网络系统，来抵抗环境扰动的影响。
- 综上，本研究揭示了长江三峡库区优势植物狗牙根在频繁的水位波动中生存的抗逆机制，对三峡库区脆弱生态系统的恢复具有重要意义。
- This study found that the *C. dactylon* in the riparian zone of the TGR had strategies to overcome the long-term flooding by increasing the abundance of endophytic nitrogen-fixing bacteria.
- The abundance of endophytic nitrogen-fixing bacteria was highest in foliage of *C. dactylon*, and was greater in August than in July.
- Endophytic nitrogen-fixing bacteria is more suitable for soil environments with low NH_4^+-N, low NO_3^--N, high total phosphorus and high water content.
- The complex interaction network system constructing between different genera of the endophytic nitrogen-fixing bacterial communities could be the reason that *C. dactylon* is able to resist the influence of environmental disturbance.
- Our study revealed a possible mechanism that the dominant plant species of *C. dactylon* can survive the frequent fluctuating water level in the riparian zone of the TGR, and will provide significant implications for the restoration of the fragile ecosystem.

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Located at the boundary between land and water, the terrestrial-aquatic interface is home to large and complex exchanges of water, materials, and energy. This transitional zone is the site of dynamic interactions between the geosphere, atmosphere, hydrosphere, and biosphere through the processing and cycling of materials, nutrients and pollutants. The terrestrial-aquatic margin includes wetlands – forests and marshes, riparian areas, mudflats and sandbars, and subaqueous shallow water habitats including submerged aquatic vegetation. In this special issue, we aim to bring together papers that describe the physical, chemical, and biological attributes of these critically important ecosystems and the diverse array of aerobic and anaerobic processes that characterize them.

Your contribution is highly welcomed!

Guest Editors

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