•研究报告•

古尔班通古特沙漠生物土壤结皮对氨氧化微生物生态位的影响

刘鑫^{ID 1,2}, 荣晓莹^{ID 1}, 张元明^{ID 1*}

1. 中国科学院新疆生态与地理研究所荒漠与绿洲生态国家重点实验室, 乌鲁木齐 830011; 2. 中国科学院大学, 北京 100049

摘要: 生物结皮作为荒漠地表的重要覆被类型, 在荒漠生态系统的氮素循环中扮演重要角色。融雪期为古尔班通古特沙漠生物结皮的复苏和生长提供了充足的水分, 也成为该沙漠氮素固定和转化的重要时期, 但该时期生物结皮如何影响驱动氨氧化转化的微生物群落动态尚未明确。因此, 我们利用荧光定量PCR (fluorescent quantitative PCR, qPCR) 方法分析融雪期生物结皮与去除结皮不同土层(0–2, 2–5, 5–10和10–20 cm)氨氧化菌群丰度特征, 结合潜在硝化速率和土壤理化参数, 探究融雪期生物结皮对荒漠土壤氮素转化作用。结果表明: 氨氧化古菌(ammonia-oxidizing archaea, AOA)是古尔班通古特沙漠土壤优势氨氧化菌, 生物结皮对0–2 cm层土壤中AOA、氨氧化细菌(ammonia-oxidizing bacteria, AOB) *amoA*基因丰度具有显著抑制作用($P < 0.01$), 对10–20 cm层土壤中AOA *amoA*基因丰度具有显著促进作用($P < 0.01$)。冗余分析(redundancy analysis, RDA)表明, AOA、AOB *amoA*基因丰度主要受土壤含水量和铵态氮含量的影响, 占总条件效应的54.90%。氨氧化速率分析发现, 去除生物结皮显著降低古尔班通古特沙漠土壤硝化作用潜力($P < 0.001$), 证实生物结皮对荒漠土壤氮素转化具有重要的调控作用。综上所述, 古尔班通古特沙漠氨氧化微生物的分布规律受环境因子调控, 特别是生物结皮可以通过调节土壤含水量和铵态氮含量影响AOA和AOB的空间生态位分化, 促进沙漠土壤的硝化作用。

关键词: 生物结皮; 融雪期; 氨氧化菌; 荒漠土壤; 氮素转化

刘鑫, 荣晓莹, 张元明 (2021) 古尔班通古特沙漠生物土壤结皮对氨氧化微生物生态位的影响. 生物多样性, 29, 43–52. doi: 10.17520/biods.2019415.
Liu X, Rong XY, Zhang YM (2021) Biocrusts impact niche separation of ammonia oxidizing microorganisms in the Gurbantunggut Desert, northwestern China. Biodiversity Science, 29, 43–52. doi: 10.17520/biods.2019415.

Biocrusts impact niche separation of ammonia oxidizing microorganisms in the Gurbantunggut Desert, northwestern China

Xin Liu^{ID 1,2}, Xiaoying Rong^{ID 1}, Yuanming Zhang^{ID 1*}

1 State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011

2 University of Chinese Academy of Sciences, Beijing 100049

ABSTRACT

Aims: Biological soil crusts (Biocrusts) are complex assemblages of lichens, bryophytes, cyanobacteria, fungi, and heterotrophic microbial organisms in the top few centimetres of desert soils. Biocrusts perform important ecological roles in the nitrogen cycle of desert ecosystems. In desert ecosystems, water sources are vital, and the seasonal melting snow in early spring resurrects biocrusts in the Gurbantunggut desert and begins nitrogen fixation. However, little is known about how biocrusts impact nitrifier distributions across the landscape, specifically ammonia oxidation archaea (AOA) and ammonia oxidation bacteria (AOB).

Methods: We used fluorescent quantitative PCR (qPCR) methods to characterize AOA and AOB *amoA* gene abundances

收稿日期: 2019-12-31; 接受日期: 2020-03-20

基金项目: 国家自然科学基金(31670007)、自治区高层次人才天池百人计划和中国科学院“西部青年学者”项目(2019-XBQNXZ-B-007)

* 通讯作者 Author for correspondence. E-mail: zhangym@ms.xjbg.ac.cn

at different soil depths (0–2, 2–5, 5–10 and 10–20 cm) in biocrust and biocrust-removal soils. Desert nitrification potential and soil physicochemical parameters were researched to understand biocrust impacts on the desert soil nitrogen cycle.

Results: The AOA *amoA* gene abundance was remarkably larger than that of AOB across all soil samples. ANOVA results showed that biocrusts significantly affected AOA and AOB *amoA* gene abundance ($P < 0.01$) while PNR results indicated that biocrust removal significantly reduced soil nitrification potential ($P < 0.001$), which confirmed that biocrusts play an important role in regulating nitrogen transformation in the Gurbantunggut desert. A redundancy analysis confirmed that soil moisture and $\text{NH}_4^+\text{-N}$ were the key environmental factors affecting niche separation of AOA and AOB in desert soil.

Conclusion: Biocrusts coupled with soil moisture and $\text{NH}_4^+\text{-N}$ affected differential distribution of ammonia oxidizing microorganisms in the temperate desert in the early spring.

Key words: biological soil crust; snowmelt period; ammonia-oxidizing microbes; desert soil; nitrogen transformation

生物土壤结皮(biological soil crusts, BSCs, 简称生物结皮)是由蓝细菌、地衣、苔藓和其他微生物与表层土壤颗粒组成的群落复合体,是全球干旱和半干旱地区的重要地表覆盖类型(Belnap, 2003)。生物结皮可增强荒漠土壤肥力(Zhuang et al, 2015),在促进植物种子萌发与生长(Condon & Pyke, 2018)和荒漠碳、氮循环等方面(Weber et al, 2015; Zhang et al, 2018)具有极其重要的作用。已有研究发现,生物土壤结皮以7%的初级生产力固定了全球40%以上的生物氮,而生物结皮固氮酶活性更多地受到破坏程度的影响,因干扰导致的生物结皮固氮酶活性下降了30%–100% (Belnap, 1996; Weber et al, 2015),对荒漠生态系统氮收支产生了重要的影响。同时,干扰也不利于生物结皮集结雨水或凝结水,加速荒漠土壤侵蚀以及土壤碳氮流失(Belnap, 2003; Eldridge et al, 2006; Garcia et al, 2015; Scott et al, 2015)。因此,厘清生物结皮在荒漠生态系统中的氮素转化过程,对深入认识荒漠氮素循环具有重要意义。

生物结皮调控荒漠土壤氮素固定和转化。在已知的氮循环中,氨氧化反应(ammonia oxidation)是土壤氮素固定和转化的限速步骤,平衡和控制土壤中稳定铵态氮(NH_4^+)和不稳定的亚硝酸盐(NO_2^-)以及硝酸盐(NO_3^-) (Kowalchuk & Stephen, 2001; Johnson et al, 2005)。据估计,生物结皮年固氮量介于3.7–13.2 mg/m^2 之间 (Hu et al, 2015),氨氧化反应对荒漠土壤有效氮素的利用、硝酸盐淋失和温室气体如氧化亚氮(N_2O)的排放等环境问题具有重要影响(Matzner & Borken, 2008)。氨氧化菌群包括氨氧化古菌(AOA)和氨氧化细菌(AOB)。近年来,随着对氨氧化过程研究的不断深入,人们发现AOA和AOB广泛分布于多种生态环境(Yao et al, 2013; Liu

et al, 2014; Stempfhuber et al, 2015; Liu et al, 2018)。相对AOB, AOA在寡营养生态系统中具有更强的生态适应性(Leininger et al, 2006)。Magalhaes等(2014)发现在低C/N比的南极土壤和高导电率的McMurdo干谷沙漠土壤中,氨氧化细菌的分布受到限制。Marusenko等(2013)分析生物结皮氨氧化微生物分布发现,美国西部索诺兰、科罗拉多高原、大盆地和奇瓦环沙漠的生物结皮中, AOA与AOB丰度的比值由北部大盆地沙漠至南部索诺兰和奇瓦环沙漠逐渐降低,大尺度上生物结皮中氨氧化菌群演替主要受土壤温度驱动。

土壤有效水分通常是限制生物结皮活性和发挥生态功能的重要因素之一(Belnap et al, 2004; Zhang et al, 2013)。在古尔班通古特沙漠,冬季具有稳定的积雪覆盖期,春季积雪的融化明显增加土壤水分有效性,影响土壤温度、理化性质和微生物生物量(Steinweg et al, 2008; Zhou et al, 2013),促进了生物结皮和荒漠植被群落的发育和维持(Zhao et al, 2018)。近年来,随着放牧、鼠害、旅游、道路施工和石油勘探开采等活动(Eldridge et al, 2013),古尔班通古特沙漠生物结皮遭到了不同程度的破坏,固氮功能受到影响。本研究团队前期工作表明,荒漠区氮输入具有伴随积雪融化和降雨脉冲输入土壤的特征,融雪期不同类型生物结皮的多种氮素形态含量呈现峰值,短时期供给荒漠土壤充足的氮素(Zhou & Zhang, 2014a, b)。值得注意的是,冬季也是生物结皮发育主要的碳截获时段(Kirsten et al, 2012; Wu et al, 2015),因此,融雪期生物结皮氮素转化可能长期影响系统氮素循环,并且更宽泛地关联结皮其他微生物群落及高等生物群落的生态过程。虽然在理解生物结皮对荒漠生态系统氮循环方面取得

了一些进展(Zhou et al, 2016, 2019), 但关于古尔班通古特沙漠生物结皮和氨氧化微生物的相关研究主要集中在沙漠表层土壤坡位变化的微生境差异(Liu et al, 2014)。在融雪期, 生物结皮耦合土壤含水量、pH及土壤碳、氮利用资源等发生变化, 那么干扰引起的固氮作用降低以及荒漠资源分配对氨氧化微生物会产生怎样的影响? 通过分析生物结皮氨氧化微生物菌群丰度的变化, 探究氨氧化微生物群落对生物结皮和土壤环境因子变化的响应, 对进一步了解生物结皮氮输入与流失, 评估荒漠生物结皮长期维持的“肥岛”效应具有重要意义。

目前, 我们对融雪期古尔班通古特沙漠生物结皮氨氧化微生物原生态位相互作用及其氮素转化的环境影响因素了解得仍然有限。本文通过qPCR方法分析古尔班通古特沙漠氮素转化活跃时期AOA、AOB生态位差异及其环境影响因子, 旨在探究温带荒漠生物结皮对氨氧化菌群分布的重要影响, 及融雪期荒漠土壤氮素转化的微生物驱动过程, 进一步认识荒漠土壤氮素转化过程。

1 材料与方法

1.1 研究区概况

研究区位于古尔班通古特沙漠(44°11′–46°20′N, 84°31′–90°00′E)腹地。该沙漠属于温带荒漠生态系统, 是中国第二大沙漠, 也是中国最大的固定和半固定沙漠。年降水量70–150 mm, 年均蒸发量超过2,000 mm。一般情况下, 由11月下旬至第二年3月中旬沙漠被稳定积雪覆盖, 冬春两季降水占全年降水量的30%–45%; 在早春时期, 沙漠冻融循环发生频繁(2019年春季结皮覆盖土壤冻融频次10次, 去除结皮土壤14次)。该沙漠发育有良好的生物结皮, 结皮覆盖度约为30% (Zhang et al, 2007), 并在冻融时期保持较强的生理活性。

1.2 样地设置和土壤样品采集

选取古尔班通古特沙漠腹地地势较为平坦、结皮发育一致且干扰较小的丘间低地设置样方, 每个样方1.5 m × 1.5 m, 样方间距大于10 m。2015年7月, 随机选取4个样方设置为去除结皮处理(Biocrusts-removal), 为避免破坏土壤结构, 使用解剖刀和毛刷轻轻剥离表层生物结皮; 另外选取4个样方作为结皮处理(Biocrusts) (附录1)。根据课题组长期样

地的水分温度监测数据, 于2019年3月13日积雪融化几近完成时(积雪融化 > 90%), 采用5点梅花混合取样法采集不同土层(0–2 cm、2–5 cm、5–10 cm、10–20 cm)土壤样品。清除样品中可见植物根系、凋落物、石子和其他杂物, 低温保存带回实验室。每份土壤样品分为3份, 1份置于–80℃冰箱备用提取DNA, 1份样品置于4℃冰箱, 测定土壤潜在硝化速率(potential nitrification rate, PNR), 剩下的样品风干后用于测定土壤理化性质。

1.3 土壤理化性质的测定

土壤温度(Tem)和土壤含水量(SM)由样地长期水温监测探头EM50获得; 并用烘干法确定土壤含水量。电位法测定土壤pH和电导率(Cond) (土水比1:2.5); 干烧法测定土壤全碳(TC)含量(TOC总有机碳/总氮分析仪, Analytik Jena Multi N/C 3100, 德国); 高氯酸-硫酸消化法测定土壤全氮(TN)含量(全自动定氮仪, Foss 1035, 丹麦)。氯化钙浸提土壤硝态氮(NO₃⁻-N)和铵态氮(NH₄⁺-N), 并用连续流动分析仪测定其含量(BRAN+LUEBBE AA3, 德国)。

1.4 土壤总DNA提取和实时荧光定量PCR

取0.75 g (鲜重)土壤样品, 用土壤专用DNA提取试剂盒(DNeasy® Power Soil® Kit, QIAGEN)提取土壤总DNA, 分光光度计NanoDrop 2000检测核酸质量与浓度。土壤DNA保存于–20℃冰箱用于后续DNA片段扩增和荧光定量PCR (qPCR)。

使用AOA、AOB氨单加氧酶基因特异性引物对Arch-amoA/Arch-amoAR (Francis et al, 2005)和amoA-1F/amoA-2R (Rotthauwe et al, 1997) (附录2), 定量分析荒漠土壤AOA和AOB *amoA*基因丰度。使用琼脂糖凝胶电泳切胶回收的PCR产物, 经克隆、转化后, 筛选出成功插入目的基因的阳性克隆并提取质粒, 制备10倍梯度稀释qPCR标准曲线。qPCR反应体系为: TB Green™ Premix Ex Taq™ II (Takara, 中国) 10 μL, 正、反向引物各0.4 μL (10 μM), 无菌MilliQ水7.2 μL, 质粒或土壤DNA 2 μL。qPCR扩增程序为: 95℃预变性1 min, 95℃变性10 s, 退火30 s, 72℃延伸1 min, 共40个循环; 72℃后延伸1 min。用循环阈值(Ct)确定AOA、AOB *amoA*基因初始模板浓度, 使用公式1转换计算荒漠土壤目的基因的拷贝数:

$$\text{拷贝数} = \frac{\frac{X}{n} \times C \times V}{0.75 \times (1 - SM)} \quad (1)$$

式中, X 为AOA、AOB *amoA*基因初始模板浓度; n 为模板DNA用量(ng); C 和 V 分别是提取0.75 g土壤DNA原始浓度(ng/μL)和体积(μL); SM 为土壤含水量; 本文中qPCR实验扩增效率90%–99%, R^2 值大于0.99。所有土壤样品qPCR独立重复2次。

1.5 土壤潜在硝化速率测定

参照Kurola等(2005)的方法, 采用氯酸盐抑制法测定荒漠土壤潜在硝化速率。取10 g新鲜土壤, 加入装有40 mL磷酸盐的缓冲溶液(NaCl, 8.0 g/L; KCl, 0.2 g/L; Na₂HPO₄, 0.2 g/L; NaH₂PO₄, 0.2 g/L)和10 mL (NH₄)₂SO₄ (6 mM)的150 mL玻璃培养瓶中, 添加10 mL KClO₃ (60 mM), 抑制亚硝酸盐氧化。震荡培养, 并分别于0、3、6 h, 用2 M KCl溶液浸提土壤培养液亚硝酸盐; 经对氨基苯磺酸-α萘胺溶液显色, 读取520 nm吸光度(Zhou et al, 2016); 制定NO₂⁻标准曲线($R^2 = 0.99$), 计算荒漠土壤NO₂⁻产生速率(μg·N day⁻¹·g⁻¹)。土壤样品重复测定3次。

1.6 统计分析方法

使用双因素方差分析(Two-way ANOVA) (SAS 9.2), 检验生物结皮覆盖与土壤深度(0–2 cm、2–5 cm、5–10 cm、10–20 cm)对AOA、AOB *amoA*基因

丰度、土壤理化性质和潜在硝化速率的影响。利用R 3.5.1 vegan包, 将AOA、AOB *amoA*基因丰度经过对数(log)转化后, 对环境参数进行多重共线性和逐步回归分析筛选显著相关环境参数, 并使用ggplot2包绘制氨氧化菌群丰度与土壤理化参数冗余分析(redundancy analysis, RDA)关系图; 利用psych和corrplot包进行Pearson相关性分析。

2 结果

2.1 土壤理化性质

生物结皮对荒漠土壤全碳、氮含量具有显著性影响(表1)。结皮表层TC含量(0–2 cm, 6.72 ± 0.12 mg/g)显著高于去除结皮表层土壤(0–2 cm, 5.86 ± 0.21 mg/g) ($P < 0.05$); 结皮表层TN含量(0–2 cm, 0.38 ± 0.02 mg/g)显著高于去除结皮表层土壤(0–2 cm, 0.23 ± 0.01 mg/g) ($P < 0.01$)及结皮下层土壤(2–5 cm, 0.23 ± 0.02 mg/g; 5–10 cm, 0.25 ± 0.01 mg/g; 10–20 cm, 0.23 ± 0.02 mg/g) ($P < 0.05$)。

结皮与去除结皮表层土壤为富NH₄⁺-N土壤(图1A)。结皮表层NH₄⁺-N含量(0–2 cm, 4.07 ± 0.16 μg/g)显著高于结皮下层土壤(2–5 cm, 2.43 ± 0.12 μg/g; 5–10 cm, 2.13 ± 0.13 μg/g; 10–20 cm, 2.32 ± 0.08 μg/g) ($P < 0.05$); 去除结皮表层土壤NH₄⁺-N含量(0–2

表1 土壤理化性质

Table 1 Soil physical and chemical properties

	温度 Temperature (°C)	含水量 Soil moisture (%)	pH	电导率 Conductivity (μs/cm)	全碳 Total Carbon (mg/g)	全氮 Total Nitrogen (mg/g)
结皮覆盖 Biocrusts						
0–2 cm	0.5 ± 0.0 A**	10.63 ± 0.45 A	8.25 ± 0.05 C	4.55 ± 0.26 A	6.72 ± 0.12 A*	0.38 ± 0.02 A**
2–5 cm	0.2 ± 0.0 B*	6.17 ± 0.23 B**	8.37 ± 0.03 B	3.38 ± 0.17 B	5.76 ± 0.17 A	0.23 ± 0.02 B
5–10 cm	0.2 ± 0.0 ^a B**	3.90 ± 0.12 C	8.55 ± 0.02 A	2.92 ± 0.04 B**	6.74 ± 0.29 A	0.25 ± 0.01 B
10–20 cm	0.1 ± 0.0 B**	2.00 ± 0.21 D	8.57 ± 0.01 A	3.33 ± 0.16 B**	6.47 ± 0.33 A	0.23 ± 0.02 B
去除结皮 Biocrusts-removal						
0–2 cm	0.1 ± 0.0 A	10.75 ± 1.54 A	8.43 ± 0.06 A*	3.96 ± 0.53 A	5.86 ± 0.21 A	0.23 ± 0.01 AB
2–5 cm	0.1 ± 0.0 A	4.90 ± 0.21 B	8.44 ± 0.02 A	3.65 ± 0.24 A	5.83 ± 0.39 A	0.27 ± 0.00 A*
5–10 cm	–0.1 ± 0.0 ^a B	3.65 ± 0.22 B	8.60 ± 0.05 A	2.37 ± 0.13 B	5.82 ± 0.44 A	0.27 ± 0.02 AB
10–20 cm	–0.2 ± 0.0 C	2.48 ± 0.10 B	8.61 ± 0.07 A	2.65 ± 0.06 B	6.04 ± 0.23 A	0.22 ± 0.01 B

大写字母表示相同结皮处理不同土层显著差异性, $\alpha = 0.05$ 。*表示相同土层不同结皮处理之间差异性, * $P < 0.05$, ** $P < 0.01$ 。a表示根据其他土层温度预测值($n = 4$)。

Capital letters indicate significant differences among soil depth in biocrusts or biocrusts-removal treatments, $\alpha = 0.05$. * indicates the difference between biocrusts and biocrusts-removal treatments for the same soil depth, * $P < 0.05$, ** $P < 0.01$. a Predicted value obtained from temperature of other soil depths ($n = 4$).

图1 古尔班通古特沙漠土壤有效态氮含量与氨氧化菌群丰度。ND表示*amoA*基因丰度低于检测标准。大写字母表示相同结皮处理不同土层显著差异性, $\alpha = 0.05$ 。*为相同土层不同结皮处理之间差异性; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ 。

Fig. 1 Available nitrogen content and *amoA* gene abundance in the Gurbantunggut Desert during snow melt period. (A): $\text{NH}_4^+\text{-N}$ ($\mu\text{g/g}$); (B): $\text{NO}_3^-\text{-N}$ ($\mu\text{g/g}$); (C): AOA *amoA* gene abundance (copies/g) and (D): AOB *amoA* gene abundance (copies/g). ND indicates that *amoA* gene abundance is lower than the detection empirical value. Capital letters indicate significant differences among soil depth in biocrusts or biocrusts-removal treatments, $\alpha = 0.05$. * indicates the difference between biocrusts and biocrusts-removal treatments for the same soil depth; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

cm, $2.89 \pm 0.06 \mu\text{g/g}$ 最高, 次表层土壤 $\text{NH}_4^+\text{-N}$ 含量 (2–5 cm, $1.55 \pm 0.01 \mu\text{g/g}$) 低于深层 (5–10 cm, $2.46 \pm 0.01 \mu\text{g/g}$; 10–20 cm, $2.08 \pm 0.18 \mu\text{g/g}$) ($P < 0.05$)。

结皮土壤与去结皮土壤不同土层硝态氮含量均具有显著差异 ($P < 0.05$)。荒漠深层土壤 (10–20 cm: $6.33 \pm 0.10 \mu\text{g/g}$; $3.38 \pm 0.15 \mu\text{g/g}$) 为富硝态氮土壤 (图 1B)。

2.2 荒漠土壤氨氧化古菌与细菌*amoA*基因丰度特征

定量分析结果显示, AOA 是荒漠土壤优势氨氧化菌, AOA *amoA* 基因丰度范围为 1×10^4 – 7×10^4 copies/g, 显著高于 AOB *amoA* 基因丰度 (1×10^3 – 2×10^4 copies/g) ($P < 0.001$, $n = 32$; 图 1C, D; 附录 3)。

结皮土壤不同土层 AOA *amoA* 基因丰度存在显著差异 ($P < 0.05$)。表层 AOA *amoA* 基因丰度 (0–2 cm, 1.1×10^4 copies/g) 最低, 次表层最高 (2–5 cm, 6.6×10^4 copies/g), 显著高于 5–10 cm (4.2×10^4 copies/g)

和 10–20 cm 层 (5.3×10^4 copies/g) 土壤 ($P < 0.05$); 结皮表层土壤 AOB *amoA* 基因丰度为 1.7×10^3 copies/g, 但在 2–20 cm 层土壤中 AOB *amoA* 基因丰度未达到检测限 (低于 1,000 copies/g)。

去除结皮次土层土壤 AOA *amoA* 基因丰度 (2–5 cm, 7.0×10^4 copies/g) 最高, 表层 (0–2 cm, 2.7×10^4 copies/g) 与深层土壤 AOA *amoA* 基因丰度 (5–10 cm, 4.0×10^4 copies/g; 10–20 cm, 3.1×10^4 copies/g) 没有显著差异 ($P > 0.05$); 同样, 在去除结皮 5–20 cm 土壤中, AOB *amoA* 基因丰度也未达到检测标准。去除结皮显著增加表层土壤 AOA (0–2 cm, 2.7×10^4 copies/g)、AOB *amoA* 基因丰度 (0–2 cm, 2.6×10^4 copies/g) ($P < 0.01$), 降低深层土壤 AOA *amoA* 基因丰度 (10–20 cm, 3.1×10^4 copies/g) ($P < 0.001$)。

方差膨胀因子分析结果显示 (图 2), SM、 $\text{NH}_4^+\text{-N}$ 、Cond、pH 和 Tem 5 个环境因子与融雪期古尔班通古特沙漠 AOA 和 AOB *amoA* 基因丰度显著相

图2 AOA和AOB *amoA*基因丰度与环境参数的冗余分析。
Tem表示土壤温度, SM代表土壤含水量, Cond代表土壤电导率, $\text{NH}_4^+\text{-N}$ 代表土壤铵态氮含量。

Fig. 2 Redundancy analysis (RDA) analysis on AOA/AOB *amoA* gene abundance and selected environmental factors. Tem represents soil temperature, SM represents soil moisture, Cond represents soil conductivity, and $\text{NH}_4^+\text{-N}$ represents soil ammonium nitrogen content.

关, 其中, 土壤SM和 $\text{NH}_4^+\text{-N}$ 含量与AOA、AOB *amoA*基因丰度相关性最强, 分别占总条件效应的48.96% ($F = 46.49$, $P < 0.001$)和5.94% ($F = 6.62$, $P < 0.05$)。结皮上层土壤AOA *amoA*基因丰度与 $\text{NH}_4^+\text{-N}$ 呈显著正相关关系, 结皮下层土壤AOA *amoA*基因丰度与pH呈显著正相关关系, 与SM、 $\text{NH}_4^+\text{-N}$ 、Cond和Tem呈显著负相关关系; 去结皮表层土壤AOB *amoA*基因丰度和SM、Cond和Tem显著正相关, 与pH显著负相关。

2.3 荒漠土壤潜在硝化作用和环境因子

生物结皮对荒漠土壤潜在硝化作用(PNR)具有显著促进作用($P < 0.001$, 表2)。随土壤深度增加, 结皮和去除结皮土壤PNR均显著降低($P < 0.05$) (图3)。荒漠土壤PNR与环境参数相关性分析显示, 结皮土壤PNR与AOB *amoA*基因丰度、Cond、 $\text{NH}_4^+\text{-N}$ 、SM、Tem和TN显著正相关, 与AOA *amoA*基因丰度、 $\text{NO}_3^-\text{-N}$ 和土壤pH显著负相关; 去结皮土壤PNR与AOB *amoA*基因丰度、Tem和SM显著正相关(表3)。

3 讨论

3.1 生物结皮对荒漠土壤氨氧化速率的影响

氨氧化作用决定荒漠土壤硝化过程的速率与

表2 AOA和AOB *amoA*基因丰度和土壤潜在硝化速率的方差分析

Table 2 Variance analyses of AOA/AOB *amoA* gene abundance and potential nitrification rate (PNR)

	差异来源 Source	自由度 df	f值 f	Pr > F
氨氧化古菌 AOA <i>amoA</i>	处理 Treatment	1	0.13	0.725
	土层 Layer	3	64.00	< 0.001
	处理 × 土层 Treatment × layer	3	9.45	< 0.001
氨氧化细菌 AOB <i>amoA</i>	处理 Treatment	1	118.85	< 0.001
	土层 Layer	3	128.34	< 0.001
	处理 × 土层 Treatment × layer	3	103.57	< 0.001
潜在硝化速率 PNR	处理 Treatment	1	24.03	< 0.001
	土层 Layer	3	50.81	< 0.001
	处理 × 土层 Treatment × layer	3	8.69	< 0.001

图3 结皮覆盖与去除结皮不同荒漠土层潜在硝化速率。大写字母表示相同结皮处理不同土层显著差异性, $\alpha = 0.05$; *表示相同土层不同结皮处理之间差异性; * $P < 0.05$, ** $P < 0.01$ 。

Fig. 3 Potential nitrification rate (PNR) of different soil depths in biocrusts and biocrusts-removal soil. Capital letters indicate significant differences among soil depth in biocrusts or biocrusts-removal treatments, $\alpha = 0.05$; * indicates the difference between biocrusts and biocrusts-removal treatments for the same soil depth; * $P < 0.05$, ** $P < 0.01$.

通量, 生物结皮作为荒漠生态系统重要的地表覆盖类型, 对荒漠土壤氮素固定和转化具有重要影响, 然而, 我们对结皮系统氨氧化微生物驱动的氮素转化的认识仍然有限。本文中, 长期去除生物结皮显著降低了古尔班通古特沙漠土壤的硝化作用潜力 ($P < 0.001$, 表2), 推测干扰首先通过降低固氮活性影响结皮氮素转化(Belnap, 2002; Wu et al, 2009)。Ferrenberg等(2015, 2017)研究发现, 物理踩踏可迅

表3 结皮覆盖土壤和去除结皮土壤的潜在硝化速率与AOA/AOB *amoA*基因丰度及环境参数相关性分析Table 3 Pearson correlation analyses of potential nitrification rate (PNR) and AOA/AOB *amoA* gene abundance and environmental factors in Biocrusts and Biocrusts-removal soil

	潜在硝化速率 Potential nitrification rate	
	结皮覆盖土壤 Biocrusts soil	去除结皮土壤 Biocrusts-removal soil
氨氧化古菌 AOA	-0.73**	-0.06
氨氧化细菌 AOB	0.88**	0.76**
温度 Tem	0.90**	0.66*
含水量 SM	0.91**	0.87**
pH	-0.79**	-0.44
电导率 Cond	0.75**	0.48
全碳 TC	0.05	0.09
全氮 TN	0.80**	-0.26
铵态氮 $\text{NH}_4^+\text{-N}$	0.93**	0.36
硝态氮 $\text{NO}_3^-\text{-N}$	-0.60*	-0.08

* $P < 0.05$, ** $P < 0.01$.

速改变生物结皮的微生物群落组成, 结皮短时期内转变为以蓝细菌为优势细菌的初级藻结皮类型。早期研究表明, 浅色藻结皮固氮速率比深色蓝藻结皮和苔藓结皮的平均固氮速率低3–4倍。荒漠生物结皮氮素固定与转化同时进行, 将生物结皮固定的氮素快速集中释放于结皮地表以下1–10 mm的土壤中, 包括无机硝酸盐(NO_3^-)、铵离子(NH_4^+), 以及其他少量的酰胺、多肽和游离氨基酸等(Garcia-Pichel et al, 2003; Johnson et al, 2007)。融雪期生物结皮显著增加古尔班通古特沙漠表层土壤 $\text{NH}_4^+\text{-N}$ 含量($P < 0.01$), 对比结皮下层土壤(2–20 cm)和去结皮土壤(0–20 cm), 结皮表层潜在硝化速率分别增加96%–204%和72%–99%。这与Johnson等(2005)测定美国大盆地沙漠靠近生物结皮表层土壤的最大氨氧化速率($46 \mu\text{mol N} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$)研究结果一致。以上结果说明, 除氮素固定外生物结皮对荒漠土壤氮素转化具有重要调控作用。

季节性降雪是古尔班通古特沙漠重要水分输入来源, 对维持沙漠短命植被群落至关重要, 结皮组成与分布也明显不同于北美及我国中部地区。本文显示, 土壤含水量是影响古尔班通古特沙漠结皮潜在硝化作用最关键的环境因子(表3, 附录4)。较全年生物结皮处于干旱休眠状态相比, 积雪融水很大程度上解除了荒漠土壤氨氧化作用水分限制, 促使该时期结皮氮循环活跃, 硝化和反硝化作用交替进

行(Zhou & Zhang, 2014a, b)。生物结皮硝化作用潜力同时受到土壤温度、氮素含量(TN、 $\text{NO}_3^-\text{-N}$ 和 $\text{NH}_4^+\text{-N}$)、土壤pH和氨氧化菌群丰度的影响(表3, 图3), 融雪期生物结皮快速推进荒漠土壤氮素转化, 可为春季荒漠植被萌发和生长提供更有利的土壤养分条件, 对维持荒漠生态系统生物结皮的“肥岛”效应具有重要作用。

3.2 含水量和有效态氮含量是影响荒漠土壤AOA、AOB菌群差异的主要因素

土壤含水量耦合氮素、pH、电导率和温度促使荒漠氨氧化菌群的空间分布呈异质性(图2)。融雪期荒漠土壤AOA丰度与土壤含水量和铵态氮显著负相关, 与pH显著正相关。由结皮表层至深层土壤含水量和铵态氮逐渐降低, pH增加(表1, 图1A), 深层土壤AOA丰度比表层土壤迅速增加6倍, 提示土壤含水量、铵态氮含量和pH是影响结皮氨氧化菌群垂直分布的重要原因。Delgado-Baquerizo等(2016)对澳大利亚东部氨氧化微生物分析发现, 随微生境干旱程度增加, AOA丰度急剧增加, 表明土壤含水量较高对AOA可能具有抑制作用。而且有研究指出, AOA适应的pH范围(2.5–9.0)较宽(Hu et al, 2014), 适宜生长在中性或偏酸性环境中。当pH降低时, 氮的质子化可以降低氮的生物利用度, 从底物利用的角度来看, 这可能更有利于AOA的生长。因此, 土壤含水量与pH的交互作用导致生物结皮表层土壤的AOA *amoA*基因丰度降低, 荒漠土壤次表层(包括结皮和去结皮2–5 cm土层) AOA *amoA*基因丰度最高。

以往的研究发现, 铵态氮的有效性对AOA *amoA*基因丰度有直接的负效应(Delgado-Baquerizo et al, 2016), Ergude等(2010)的研究同样表明高肥力条件有利于AOB的生长, 而低营养环境有利于AOA的生长。Valentine (2007)认为, 氨氧化古菌利用细胞膜低渗透性和特殊分解代谢途径, 形成对寡营养环境较强的生态适应性。Leininger等(2006)对跨越3个气候区的12个未开发土壤与农田土壤氨氧化菌群分析发现, AOA在大多数原位土壤环境氨氧化菌群中占主导地位。综上所述, AOA相对AOB在寡营养生态环境中分布更广泛(Adair & Schwartz, 2008; Tao et al, 2018)。古尔班通古特沙漠生物结皮虽然对维持荒漠“肥岛”效应具有重要作用, 但在大空间尺度上仍然处于氮贫瘠状况, 所以本研究结果

也符合上述规律, 荒漠土壤AOA *amoA*基因丰度相对AOB *amoA*基因占绝对优势($P < 0.001$) (图1C, D; 表2)。因此, 有效态氮含量差异是导致融雪期古尔班通古特沙漠AOA、AOB丰度差异的主要因素之一。

在古尔班通古特沙漠, 由于基质亲和力和硝化途径的差异, AOA和AOB应对环境干扰引起的生物结皮氮素分配可能具有不同的反应。本研究中, 生物结皮显著影响古尔班通古特沙漠AOA和AOB的*amoA*基因丰度, 结皮表层富营养土壤($C/N = 17.68$; $NH_4^+-N = 4.07 \mu g/g$) AOA *amoA*基因丰度低于结皮下层土壤(C/N 比值介于25.04–28.13; $NH_4^+-N = 2.13$ – $2.43 \mu g/g$)和去结皮不同土层土壤(C/N 比值介于21.55–27.45; $NH_4^+-N = 1.55$ – $2.89 \mu g/g$) ($P < 0.05$, $P < 0.01$) (图1 C), 因此对于荒漠土壤, 生物结皮通过介导小尺度范围土壤养分含量, 促使AOA、AOB按照氨底物利用效率差异分布(Verhamme et al, 2011; Prosser & Nicol, 2012)。本研究发现, 古尔班通古特沙漠氨氧化微生物在空间尺度上表现为高肥力表层土壤的AOA *amoA*基因丰度低于下层土壤; 高肥力表层的AOB *amoA*基因丰度高于下层土壤, 这与Di等(2010)对新西兰3个牧场不同土层中氨氧化微生物的研究结果一致。有意思的是, 去除结皮显著增加表层土壤AOB *amoA*基因丰度($P < 0.01$), 我们推测干扰可强制解除氨氧化菌群与结皮微生物群落对氮底物的竞争作用(Xiao et al, 2019), 去除结皮减少了表层土壤 NH_4^+-N 含量, 但相对于去除结皮下层土壤, 仍然为富 NH_4^+-N 土壤, 通过耦合融雪期水分, 迅速增加AOB丰度。因此, 干扰导致的有效态氮素含量的差异也可能是造成古尔班通古特沙漠AOA、AOB空间生态位分化的重要因素之一。

致谢: 感谢课题组尹本丰博士和李永刚对本文工作提出的宝贵意见; 感谢课题组殷进飞、陆永兴和李继文在采集沙漠样地土壤样品过程中提供大力帮助。

ORCID

刘鑫 <https://orcid.org/0000-0001-9612-4830>
荣晓莹 <https://orcid.org/0000-0002-0613-8479>
张元明 <https://orcid.org/0000-0002-7159-6652>

参考文献

- Adair KL, Schwartz E (2008) Evidence that ammonia-oxidizing Archaea are more abundant than ammonia-oxidizing bacteria in semiarid soils of northern Arizona, USA. *Microbial Ecology*, 56, 420–426.
- Belnap J (1996) Soil surface disturbances in cold deserts: Effects on nitrogenase activity in cyanobacterial-lichen soil crusts. *Biology and Fertility of Soils*, 23, 362–367.
- Belnap J (2002) Impacts of off-road vehicles on nitrogen cycles in biological soil crusts: Resistance in different U. S. deserts. *Journal of Arid Environments*, 52, 155–165.
- Belnap J (2003) The world at your feet: Desert biological soil crusts. *Frontiers in Ecology and the Environment*, 1, 181–189.
- Belnap J, Phillips SL, Miller ME (2004) Response of desert biological soil crusts to alterations in precipitation frequency. *Oecologia*, 141, 306–316.
- Condon LA, Pyke DA (2018) Resiliency of biological soil crusts and vascular plants varies among morphogroups with disturbance intensity. *Plant and Soil*, 433, 271–287.
- Delgado-Baquerizo M, Maestre FT, Eldridge DJ, Singh BK (2016) Microsite differentiation drives the abundance of soil ammonia oxidizing bacteria along aridity gradients. *Frontiers in Microbiology*, 7, 505.
- Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology*, 72, 386–394.
- Eldridge DJ, Freudenberger D, Koen TB (2006) Diversity and abundance of biological soil crust taxa in relation to fine and coarse-scale disturbances in a grassy eucalypt woodland in Eastern Australia. *Plant and Soil*, 281, 255–268.
- Eldridge DJ, Soliveres S, Bowker MA, Val J (2013) Grazing dampens the positive effects of shrub encroachment on ecosystem functions in a semi-arid woodland. *Journal of Applied Ecology*, 50, 1028–1038.
- Erguder TH, Boon N, Wittebolle L, Marzorati M, Verstraete W (2009) Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiology Reviews*, 33, 855–869.
- Ferrenberg S, Reed SC, Belnap J (2015) Climate change and physical disturbance cause similar community shifts in biological soil crusts. *Proceedings of the National Academy of Sciences, USA*, 112, 12116–12121.
- Ferrenberg S, Tucker CL, Reed SC (2017) Biological soil crusts: Diminutive communities of potential global importance. *Frontiers in Ecology and the Environment*, 15, 160–167.
- Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences, USA*, 102,

- 14683–14688.
- Garcia V, Aranibar J, Pietrasiak N (2015) Multiscale effects on biological soil crusts cover and spatial distribution in the Monte Desert. *Acta Oecologica*, 69, 35–45.
- Garcia-Pichel F, Johnson SL, Youngkin D, Belnap J (2003) Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology*, 46, 312–321.
- Hu BL, Liu S, Wang W, Shen LD, Lou LP, Liu WP, Tian GM, Xu XY, Zheng P (2014) pH-dominated niche segregation of ammonia-oxidising microorganisms in Chinese agricultural soils. *FEMS Microbiology Ecology*, 90, 290–299.
- Hu R, Wang XP, Pan YX, Zhang YF, Zhang H, Chen N (2015) Seasonal variation of net N mineralization under different biological soil crusts in Tengger Desert, North China. *CATENA*, 127, 9–16.
- Johnson SL, Budinoff CR, Belnap J, Garcia-Pichel F (2005) Relevance of ammonium oxidation within biological soil crust communities. *Environmental Microbiology*, 7, 1–12.
- Johnson SL, Neuer S, Garcia-Pichel F (2007) Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. *Environmental Microbiology*, 9, 680–689.
- Coe KK, Belnap J, Sparks JP (2012) Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. *Ecology*, 93, 1626–1636.
- Kowalchuk GA, Stephen JR (2001) Ammonia-oxidizing bacteria: A model for molecular microbial ecology. *Annual Review of Microbiology*, 55, 485–529.
- Kurola J, Salkinoja-Salonen M, Aarnio T, Hultman J, Romantschuk M (2005) Activity, diversity and population size of ammonia-oxidising bacteria in oil-contaminated landfarming soil. *FEMS Microbiology Letters*, 250, 33–38.
- Leininger S, Urich T, Schlöter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, 442, 806–809.
- Liu JJ, Yu ZH, Yao Q, Sui YY, Shi Y, Chu HY, Tang CX, Franks AE, Jin J, Liu XB, Wang GH (2018) Ammonia-oxidizing archaea show more distinct biogeographic distribution patterns than ammonia-oxidizing bacteria across the black soil zone of Northeast China. *Frontiers in Microbiology*, 9, 171.
- Liu RY, Li K, Zhang HX, Zhu JG, Joshi D (2014) Spatial distribution of microbial communities associated with dune landform in the Gurbantunggut Desert, China. *Journal of Microbiology*, 52, 898–907.
- Magalhães CM, Machado A, Frank-Fahle B, Lee CK, Cary SC (2014) The ecological dichotomy of ammonia-oxidizing archaea and bacteria in the hyper-arid soils of the Antarctic Dry Valleys. *Frontiers in Microbiology*, 5, 515.
- Marusenko Y, Bates ST, Anderson I, Johnson SL, Soule TY, Garcia-Pichel F (2013) Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. *Ecological Processes*, 2, 1–10.
- Matzner E, Borken W (2008) Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science*, 59, 274–284.
- Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialisation and differentiation. *Trends in Microbiology*, 20, 523–531.
- Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene *amoA* as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology*, 63, 4704–4712.
- Ferrenberg S, Reed SC, Belnap J (2015) Climate change and physical disturbance cause similar community shifts in biological soil crusts. *Proceedings of the National Academy of Sciences, USA*, 112, 12116–12121.
- Steinweg JM, Fisk MC, McAlexander B, Groffman PM, Hardy JP (2008) Experimental snowpack reduction alters organic matter and net N mineralization potential of soil macroaggregates in a northern hardwood forest. *Biology and Fertility of Soils*, 45, 1–10.
- Stempfhuber B, Engel M, Fischer D, Neskovic-Prit G, Wubet T, Schöning I, Gubry-Rangin C, Kublik S, Schlöter-Hai B, Rattei T, Welzl G, Nicol GW, Schrumpf M, Buscot F, Prosser JI, Schlöter M (2015) pH as a driver for ammonia-oxidizing archaea in forest soils. *Microbial Ecology*, 69, 879–883.
- Tao JJ, Bai TS, Xiao R, Wang P, Wang FW, Duryee AM, Wang Y, Zhang Y, Hu SJ (2018) Vertical distribution of ammonia-oxidizing microorganisms across a soil profile of the Chinese Loess Plateau and their responses to nitrogen inputs. *Science of The Total Environment*, 635, 240–248.
- Valentine DL (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nature Reviews Microbiology*, 5, 316–323.
- Verhamme DT, Prosser JI, Nicol GW (2011) Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *The ISME Journal*, 5, 1067–1071.
- Weber B, Wu DM, Tamm A, Ruckteschler N, Rodríguez-Caballero E, Steinkamp J, Meusel H, Elbert W, Behrendt T, Sörgel M, Cheng YF, Crutzen PJ, Su H, Pöschl U (2015) Biological soil crusts accelerate the nitrogen cycle through large NO and HONO emissions in drylands. *Proceedings of the National Academy of Sciences, USA*, 112, 15384–15389.
- Wu L, Zhang YM, Zhang J, Downing A (2015) Precipitation intensity is the primary driver of moss crust-derived CO₂ exchange: Implications for soil C balance in a temperate desert of northwestern China. *European Journal of Soil Biology*, 67, 27–34.

- Wu N, Zhang YM, Downing A (2009) Comparative study of nitrogenase activity in different types of biological soil crusts in the Gurbantunggut Desert, Northwestern China. *Journal of Arid Environments*, 73, 828–833.
- Xiao R, Qiu YP, Tao JJ, Zhang XL, Chen HH, Reberg-Horton SC, Shi W, Shew HD, Zhang Y, Hu SJ (2019) Biological controls over the abundances of terrestrial ammonia oxidizers. *Global Ecology and Biogeography*, 29, 384–399.
- Yao HY, Campbell CD, Chapman SJ, Freitag TE, Nicol GW, Singh BK (2013) Multi-factorial drivers of ammonia oxidizer communities: Evidence from a national soil survey. *Environmental Microbiology*, 15, 2545–2556.
- Zhang BC, Zhang YM, Su YG, Wang JZ, Zhang J (2013) Responses of microalgal-microbial biomass and enzyme activities of biological soil crusts to moisture and inoculated *microcoleus vaginatus* gradients. *Arid Land Research and Management*, 27, 216–230.
- Zhang CP, Niu DC, Song ML, Elser JJ, Okie JG, Fu H (2018) Effects of rainfall manipulations on carbon exchange of cyanobacteria and moss-dominated biological soil crusts. *Soil Biology and Biochemistry*, 124, 24–31.
- Zhang YM, Chen J, Wang L, Wang XQ, Gu ZH (2007) The spatial distribution patterns of biological soil crusts in the Gurbantunggut Desert, Northern Xinjiang, China. *Journal of Arid Environments*, 68, 599–610.
- Zhao RM, Hui R, Liu LC, Xie M, An LZ (2018) Effects of snowfall depth on soil physical-chemical properties and soil microbial biomass in moss-dominated crusts in the Gurbantunggut Desert, Northern China. *CATENA*, 169, 175–182.
- Zhou XB, Smith H, Giraldo SA, Belnap J, Garcia-Pichel F (2016) Differential responses of dinitrogen fixation, diazotrophic cyanobacteria and ammonia oxidation reveal a potential warming-induced imbalance of the N-cycle in biological soil crusts. *PLoS ONE*, 11, e0164932.
- Zhou XB, Tao Y, Yin BF, Tucker C, Zhang YM (2020) Nitrogen pools in soil covered by biological soil crusts of different successional stages in a temperate desert in Central Asia. *Geoderma*, 366, 114166.
- Zhou XB, Zhang YM (2014a) Season and nitrogen effects on activities of three hydrolytic enzymes in soils of the gurbantunggut desert, northwest China. *Communications in Soil Science and Plant Analysis*, 45, 1699–1713.
- Zhou XB, Zhang YM (2014b) Seasonal pattern of soil respiration and gradual changing effects of nitrogen addition in a soil of the Gurbantunggut Desert, northwestern China. *Atmospheric Environment*, 85, 187–194.
- Zhou XQ, Chen CR, Wang YF, Xu ZH, Duan JC, Hao YB, Smaill S (2013) Soil extractable carbon and nitrogen, microbial biomass and microbial metabolic activity in response to warming and increased precipitation in a semiarid Inner Mongolian grassland. *Geoderma*, 206, 24–31.
- Zhuang WW, Downing A, Zhang YM (2015) The influence of biological soil crusts on ^{15}N translocation in soil and vascular plant in a temperate desert of northwestern China. *Journal of Plant Ecology*, 8, 420–428.

(责任编辑: 张丽梅 责任编辑: 时意专)

附录 Supplementary Material

附录1 本研究的样方示意图

Appendix 1 Plot quadrat diagram of this study
<http://www.biodiversity-science.net/fileup/PDF/2019415-1.pdf>

附录2 本研究所用引物序列

Appendix 2 Primer sequences used in this study
<http://www.biodiversity-science.net/fileup/PDF/2019415-2.pdf>

附录3 AOA和AOB *amoA* 基因丰度配对T检验

Appendix 3 Paired T test for AOA and AOB *amoA* gene abundance
<http://www.biodiversity-science.net/fileup/PDF/2019415-3.pdf>

附录4 AOA、AOB *amoA* 基因丰度与潜在硝化速率及环境因子的冗余分析

Appendix 4 Redundancy analysis (RDA) analysis on AOA, AOB *amoA* gene abundance potential nitrification rate (PNR) and environmental factors
<http://www.biodiversity-science.net/fileup/PDF/2019415-4.pdf>

刘鑫, 荣晓莹, 张元明 (2021) 古尔班通古特沙漠生物土壤结皮对氨氧化微生物生态位的影响. 生物多样性, 29(1): 43–52. <http://www.biodiversity-science.net/CN/10.17520/biods.2019415>

附录1 本研究的样方示意图
Appendix 1 Plot quadrat diagram of this study

刘鑫, 荣晓莹, 张元明 (2021) 古尔班通古特沙漠生物土壤结皮对氨氧化微生物生态位的影响. 生物多样性, 29(1): 43–52. <http://www.biodiversity-science.net/CN/10.17520/biods.2019415>

附录2 本研究所用引物序列

Appendix 2 Primer sequences used in this study

基因	引物	序列(5' – 3')	退火温度	参考文献
Gene	Primers	Sequence (5' – 3')	Annealing temperature (°C)	Reference
氨氧化古菌	Arch-amoAF	STAATGGTCTGGCTTAGACG	60	Francis et al, 2005
AOA <i>amoA</i>	Arch-amoAR	GCGGCCATCCATCTGTATGT		
氨氧化细菌	amoA-1F	GGGGTTTCTACTGGTGGT	57	Rotthauwe et al, 1997
AOB <i>amoA</i>	amoA-2R	CCCCTCKGSAAAGCCTTCTTC		

刘鑫, 荣晓莹, 张元明 (2021) 古尔班通古特沙漠生物土壤结皮对氨氧化微生物生态位的影响. 生物多样性, 29(1): 43–52. <http://www.biodiversity-science.net/CN/10.17520/biods.2019415>

附录3 AOA和AOB *amoA*基因丰度配对T检验
Appendix 3 Paired T test for AOA and AOB *amoA* gene abundance

分组	差的平均值	标准误	自由度	<i>t</i> 值	Pr> t
Group	Mean's differences	Standard error	df	<i>t</i> value	
AOAv.s. AOB	38674.7	4253.3	31	9.09	< 0.001

附录4 AOA、AOB *amoA*基因丰度与潜在硝化速率及环境因子的冗余分析
Appendix 4 Redundancy analysis (RDA) analysis on AOA, AOB *amoA* gene abundance potential nitrification rate (PNR) and environmental factors