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採货箱泵 (HUANJING KEXUE)

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松嫩平原芦苇湿地退化与修复过程中土壤细菌和甲烷 代谢微生物的群落结构

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摘要:湿地是全球 CH。重要的源与汇. 受人为活动和气候条件影响,我国湿地退化严重,相关部门近年来已逐步开展湿地生态修复的工作. 为研究湿地退化与修复过程中细菌和甲烷代谢微生物群落结构的响应,以松嫩平原芦苇湿地为研究对象,采集原始未退化芦苇湿地土壤、退化的和正在修复的芦苇湿地土壤,采用基于细菌 $168\,$ rRNA 基因、产甲烷菌 mcrA 基因和甲烷氧化菌 pmoA 基因的高通量测序技术研究细菌和甲烷代谢微生物的多样性和群落组成. 结果表明,芦苇湿地退化导致土壤细菌和产甲烷菌的 α 多样性降低,甲烷氧化菌的 α 多样性升高,而细菌和产甲烷菌的 α 多样性与土壤含水率呈显著正相关关系,含水率越高的湿地土壤产甲烷菌的多样性也越高. 原始未退化芦苇湿地土壤中细菌 Rhizobiales 和产甲烷菌 Methanobacteriaceae 的相对丰度较高;湿地退化导致根际促生菌 Rhizobiales 的相对丰度下降,致病菌 Burkholderiaceae、耐污染细菌 Sphingomonas、抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas,抗辐射细菌 Sphingomonas 的相对丰度上升;正在修复的芦苇湿地土壤中细菌 Sphingomonas 的相对丰度上升;正在修复的芦苇湿地土壤中细菌 Sphingomonas 和产甲烷菌 Sphingomonas 和产甲烷菌 Sphingomonas 和 Sphingomonas 的相对丰度上升;正在修复的芦苇湿地土壤中细菌 Sphingomonas 和产甲烷菌 Sphingomonas 和产甲烷 国和产甲烷 国和产甲烷 国和产用 Sphingomonas 和产甲烷 国和产用 Sphingomonas 和产用 Sphingomonas 和产品 Sphingomonas 和产品 Sphingomonas 和 Sphingomonas 和 Sphingomonas 和 Sphingomonas 和 Sphingomonas 和 Sphingomonas

关键词:湿地;退化;修复;甲烷代谢微生物;细菌群落结构

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Microbial Community Structure of Soil Methanogens and Methanotrophs During Degradation and Restoration of Reed Wetlands in the Songnen Plain

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Abstract: Wetlands are an important global source and sink of methane. However, human activities and climatic conditions are causing serious degradation of wetlands in China. In response to this, the relevant departments have progressively carried out wetland restoration projects over the past few years. To investigate the response of microbial communities of bacteria, methanogens, and methanotrophs during degradation and restoration of wetlands, soil samples were collected from undegraded reed wetlands, degraded reed wetlands, and restored reed wetlands in the Songnen Plain. Microbial diversity and community composition were studied by high-throughput sequencing based on the 16S rRNA gene of bacteria, the mcrA gene of methanogens, and the pmoA gene of methanotrophs. The results indicate that the degradation of reed wetlands results in a decrease in bacterial and methanogenic α-diversity and an increase in methanotrophic α-diversity. Bacterial α-diversity and methanogenic α-diversity were both significantly positively correlated with soil water content. At different taxonomic levels, higher relative abundances of Rhizobiales and Methanobacteriaceae were detected in the undegraded wetland soils. Wetland degradation decreased the relative abundance of Rhizobiales but increased that of the pathogenic bacteria Burkholderiaceae and microorganisms resistant to harsh and extreme environments including Sphingomonas, Rubrobacter, Methylobacter, Methylomonas, and Methylococcus. In the restored wetland soils, the relative abundances of Bacillus, Methanosarcinaceae, Methanomicrobiaceae, and the type II methanotroph Methylocystis were higher. Therefore, different wetland conditions can indirectly change soil properties and, consequently, change the community structure of methanogens and methanotrophs.

Key words; wetland; degradation; restoration; methanogens and methanotrophs; bacterial community structure

甲烷(CH₄)是大气中第二大重要的温室气体,其全球变暖潜力是二氧化碳(CO₂)的 34 倍,自前工业时代以来,全球变暖大约有 20% 是由 CH₄ 排放造成的^[1].湿地是全球 CH₄ 最大的自然来源^[2],大气中 CH₄ 的浓度取决于土壤中 CH₄ 生成和氧化之间的平衡,产甲烷菌和甲烷氧化菌是驱动 CH₄ 生成和氧化过程的关键微生物,在湿地甲烷循环过程中发挥重要作用^[3-5].产甲烷菌是严格厌氧的古细菌,目前发现的产甲烷菌全部属于广古菌门;甲烷氧化菌包括甲烷好氧氧化菌和甲烷厌氧氧化菌,甲烷好氧氧化菌主

要由变形菌门和疣微菌门组成,甲烷厌氧氧化菌则主要由甲烷厌氧氧化古菌(anaerobic methanotrophic archaea, ANME)、NC10门细菌和未培养细菌(marine benthic group D, MBGD)组成^[6].有研究表明,微生物群落对外界环境因子的变化非常敏感,湿地产甲烷菌

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和甲烷氧化菌群落结构易受温度、pH、有机质、水位和植被等多种环境因子的影响^[5,6].

松嫩平原地处半干旱半湿润气候区^[7],是中国内陆盐碱湿地的集中分布区之一,其盐碱湿地面积约为160×10⁴ hm^{2[8]}. 芦苇湿地是松嫩平原湿地的主要类型,然而,长期气候干旱及人类活动导致芦苇湿地出现了不同程度的退化^[9~12]. 湿地植被退化会影响土壤微生物多样性并使湿地生态系统功能发生改变^[13],同时,湿地的退化将直接导致控制湿地甲烷产生和排放的环境因素和生物因素的改变^[14],将对湿地 CH₄ 排放产生重要影响. 近几年相关部门在松嫩平原扎龙、向海、科尔沁和莫莫格等重要湿地实施了局部生态修复和重建项目^[10,15],目前对于松嫩平原退化湿地及修复湿地的相关研究,主要集中于植被多样性保护、湿地功能提升等方面,而针对土壤微生物群落结构、多样性差异及甲烷代谢微生物组成变化等方面的研究则鲜见报道.

本研究基于细菌 16S rRNA 基因、产甲烷菌 mcrA 基因和甲烷氧化菌 pmoA 基因的高通量测序,解析松嫩平原原始未退化芦苇湿地土壤、退化的和正在修复的芦苇湿地土壤中细菌和甲烷代谢微生物的群落结构特征,并探究细菌和甲烷代谢微生物多样性与环境因子之间的关系,旨在为了解我国东北地区典型湿地退化与修复过程中的土壤质量演变和微生物动态变化提供参考.

1 材料与方法

1.1 土壤采集与处理

本研究所采集土壤位于松嫩平原(43°13′~ 48°40′N, 121°30′~127°00′E), 采样地地上植被均 为芦苇,根据湿地不同状态,选择3种湿地土壤作为 研究对象: ①BL(47°11′55.58″N, 124°14′8.20″E), 原始未退化湿地土壤,位于扎龙自然保护区,芦苇为 优势种,以原生型沼泽土和泥炭土为主;②HL (47°22′33.68″N, 123°21′2.47″E),退化的芦苇湿地 土壤,位于哈拉海湿地保护区外缘,因农田灌溉排水 导致湿地土壤干旱,中生植物蒿子入侵芦苇湿地; ③ML(45°54′0.80″N, 123°36′38.06″E),正在实施 修复的芦苇湿地土壤,位于莫莫格自然保护区,曾因 农耕导致土壤退化和旱生植物入侵. 采样时间为 2018年8月,每种湿地土壤选取3个采样点作为生 物学重复,每个采样点为5 m×5 m的样方. 按照 "L"字形采样路线多点采集各样点表层(0~15 cm) 土壤并混合均匀作为一份样本,共计9份样本.将新 鲜土壤样本在4℃保存下运送至实验室,称取大约 5 g土样置于 - 80℃冰箱中待提取 DNA 及后续实 验,剩余的土壤储存于 - 20℃冰箱中用于土壤理化性质测定.

1.2 土壤基本理化性质测定

土壤 pH 根据电位法利用 pH 计测定,总碳 (total carbon, TC) 和总氮(total nitrogen, TN)利用元素分析仪进行测定,土壤含水率(soil water content, SWC)通过烘干法进行测定.

1.3 土壤 DNA 提取与高通量测序

土壤总 DNA 的提取使用 FastDNA SPIN 试剂盒 (MP Biomedicals, Santa Ana, California, USA),按照说明书进行操作. DNA 的提取质量通过 1% 的琼脂糖凝胶电泳进行检测, DNA 的浓度及纯度使用NanoDrop 2000 (Thermo scientific, Wilmington, DE, USA)进行测定.

使用通用引物 338F(5'-ACTCCTACGCGAGGC AGCA-3') 和 806R (5'-GGACTACHVGCGTWTCTA AT-3') 扩增原核生物 16S rRNA 基因的 V3-V4 高可 变区[16]; 使用引物 MLfF(5'-GGTGCTGTMGGAT TCACACARTAYGCWACAGC- 3') 和 MLrR (5'-TTCATTGCRTAGTTWGGRTAGTT-3′)扩增产甲烷菌 mcrA 基因[17]; 使用引物 A189F (5'-GGNGACTGG GACTTCTGG- 3') 和 mb661R (5'-CCGGMGCA ACGTCYTTACC-3') 扩增甲烷氧化菌 pmoA 基因[18]. PCR 反应体系为: 5 × TransStart FastPfu 缓冲液 4 μL, 2.5 mmol·L⁻¹ dNTPs 2 μL, 上游引物 (5 μmol·L⁻¹) 0.8 μL,下游引物(5 μmol·L⁻¹) 0.8 μL, TransStart FastPfu DNA 聚合酶 0.4 μL, 模板 DNA 10 ng, ddH₂O 补足至 20 μL. 每个样本 3 个重复. PCR 扩增程序依次为: 95℃预变性 3 min, 95℃变性 30 s, 52℃退火 30 s(mcrA 基因和 pmoA 基因退火温度为 60°C), 72°C延伸30 s,循环30次,之后72°C稳定延伸 10 min. 将同一样本的 PCR 产物混合后使用 2% 琼脂 糖凝胶回收 PCR 产物,利用 AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA)进行回收产物纯化,2%琼脂糖凝胶电泳检测, 并用 Quantus™ Fluorometer (Promega, USA) 对回收产 物进行检测定量. 使用 NEXTFLEX® Rapid DNA-Seq Kit 进行建库,利用 Illumina 公司的 MiSeq PE300 平台 进行测序(上海美吉生物医药科技有限公司).

1.4 测序结果处理

使用 Fastp 软件(https://github.com/OpenGene/fastp,version 0. 20. 0) [19] 对原始测序序列进行质控,使用 FLASH 软件(http://www.cbcb.umd.edu/software/flash, version 1. 2. 7) [20] 进行拼接,使用 UPARSE 软件(http://drive5.com/uparse/,version 7. 1) [21] 根据 97%的相似度对序列进行 OTU

聚类并剔除嵌合体^[21,22]. 利用 RDP classifier (http://rdp. cme. msu. edu/, version 2.2)^[23]对每条序列进行物种分类注释, 16S rRNA 基因序列比对 SILVA 数据库 (https://www. arb-silva. de/, version 132)^[23], mcrA 和 pmoA 基因序列比对 FunGene 数据库 (http://www. fungene-db. fr/, version 9.6)^[24],设置比对阈值为 70%.

1.5 统计分析

本研究使用 Galaxy 数据分析平台 (http://mem. rcees. ac. cn: 8080/) $^{[25]}$ 和 Origin 软件 (version 2018)对测序数据进行分析处理,分析项目包括 α 多样性分析、环境因子与 α 多样性的关联分析和群落结构组间差异分析.

采用 Shannon 指数和 Simpson 指数表征微生物 α 多样性^[26], Shannon 指数与微生物 α 多样性呈正相关, Simpson 指数与微生物 α 多样性呈负相关^[27]. 采用单因素方差分析(ANOVA)检验样品各项理化因子以及 α 多样性指数的组间差异是否显著. 对土壤理化因子和 α 多样性指数进行 Pearson 相关性分析, 以确定土壤理化性质与土壤细菌、产甲烷菌和甲烷氧化菌 α 多样性之间的相关性^[28]. 基于 OTU

水平对微生物群落进行 PCoA 分析,利用 ANOSIM 检验方法判断群落组间差异是否显著^[29].在不同分类学水平上统计各样本的物种丰度,采用 ANOVA 方法检验优势菌群组间差异是否显著,并采用 LEfSe(linear discriminant analysis effect size)分析方法寻找细菌群落组间具有显著性差异的物种^[30].

2 结果与讨论

2.1 土壤理化性质

3组湿地土壤样品的基本理化性质如表1所示.各样本土壤pH均为碱性,其中,修复中的芦苇湿地土壤pH最高,其次是原始湿地土壤,退化湿地的土壤pH最低.原始湿地土壤总碳、总氮指标显著高于退化湿地,这可能与退化湿地地表植物生物量降低导致土壤有机质含量降低有关.原始湿地芦苇生长于淹水沼泽,故含水率最高,平均含水率为67.41%;退化的湿地由于植被退化导致湿地蓄水能力弱,因此含水率下降,平均含水率为44.54%;同样,修复湿地的芦苇土壤样品由于长期植被覆盖退化造成表土含水率显著低于原始芦苇湿地土壤样品,平均值为24.3%.

表 1 土壤样品基本理化性质1)

16	Table 1 Basic	physical and chemical properties	s of the soil samples	1
样品编号	рН	TC/g⋅kg ⁻¹	TN∕g•kg ⁻¹	SWC/%
BL	8.77 ± 0.11a	27.57 ± 2.66a	1.52 ±0.17a	67.41 ±4.89a
一切 担 	$8.38 \pm 0.03 \mathrm{b}$	15.82 ± 2.06 b	$0.81 \pm 0.25 b$	$44.54 \pm 0.95 $ b
ML	$8.85 \pm 0.08a$	$19.7 \pm 0.78b$	$1.2 \pm 0.07 ab$	$24.3 \pm 3.02c$

1)数据为平均值 ±标准偏差, n = 3, 不同小写字母表示差异性显著水平

2.2 土壤细菌和甲烷代谢微生物 α 多样性

各土壤样品细菌、产甲烷菌和甲烷氧化菌的 Shannon 指数和 Simpson 指数如表 2 所示. 原始湿地 土壤的细菌和产甲烷菌群落 Shannon 指数高于退化 湿地和修复湿地土壤样品;对于甲烷氧化菌群落, 退化土壤的 Shannon 指数最高. 原始土壤次之. 修复 土壤最低. 而细菌群落的 Simpson 指数变化趋势与 Shannon 指数相反. 结果表明湿地退化导致土壤细菌和产甲烷菌的多样性降低,甲烷氧化菌的多样性增加. Pearson 相关性分析表明,细菌和产甲烷菌的α多样性与土壤含水率呈显著正相关关系(表3),这可能是由于土壤含水率越高,厌氧程度越大,而产

表 2 土壤样品微生物 α 多样性 $^{1)}$

Table 2 Values of α -diversity in microbial communities of the soil samples						
样品编号 -	细菌		产甲烷菌		甲烷氧化菌	
	Shannon	Simpson × 10 ⁻³	Shannon	Simpson × 10 ⁻³	Shannon	Simpson × 10 ⁻³
BL	6.68 ± 0.06a	3.37 ± 0.81a	3.8 ± 0.17a	51.29 ± 13.83a	3.06 ±0.11a	113.54 ± 7.62a
HL	$6.46 \pm 0.04 \mathrm{b}$	$3.64 \pm 0.4a$	$3.48 \pm 0.17 ab$	$73.18 \pm 25.04a$	$3.2 \pm 0.17a$	$90.24 \pm 17.41 a$
ML	5.25 ± 0.39 b	$20.25 \pm 9.52a$	$3.05 \pm 0.22b$	$85.32 \pm 24.32a$	$2.19 \pm 0.84a$	$298.45 \pm 207.35a$

1)数据为平均值 ±标准偏差,n=3,不同小写字母表示差异性显著水平

表 3 土壤理化性质与微生物 α 多样性的 Pearson 相关性 $^{1)}$

Table 3 Pearson correlation coefficients for environmental factors and microbial α -diversity

项目	细菌		产甲烷菌		甲烷氧化菌	
	Shannon	Simpson	Shannon	Simpson	Shannon	Simpson
pH	-0.462	0. 496	- 0. 234	- 0. 00975	-0.457	0. 446
TC	0.31	-0. 193	0. 508	-0.542	0.0232	-0.0164
TN	0. 083 4	0. 001 67	0. 313	-0.477	-0.169	0. 166
SWC	0. 862 **	-0. 727 *	0. 858 **	- 0. 568	0. 601	-0.569

^{1)*}和**表示差异性显著水平,*为P<0.05,**为P<0.01

甲烷菌属于严格厌氧菌,因此含水率越高的土壤产 甲烷菌的多样性也越高^[2,31].

2.3 土壤细菌和甲烷代谢微生物的群落组成 PCoA分析结果(图1)显示3组样本的微生物

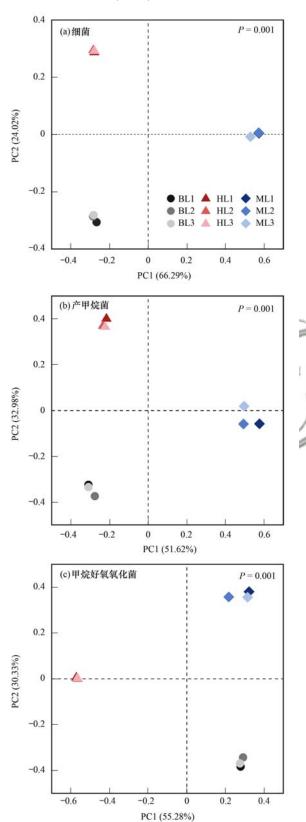


图 1 基于 OTU 水平的微生物群落的 PCoA 分析

Fig. 1 Principal coordinates analysis (PCoA) for microbial communities based at the OTU level

群落结构存在显著差异(P<0.05). 细菌群落的PCoA分析结果如图1(a)所示,PC1和PC2分别解释了66.29%和24.02%的群落结构差异. 产甲烷菌群落的PCoA分析结果如图1(b)所示,PC1和PC2分别解释了51.62%和32.98%的群落结构差异. 甲烷好氧氧化菌群落的PCoA分析结果如图1(c)所示,PC1和PC2分别解释了55.28%和30.33%的群落结构差异.

3组土壤样本门水平细菌群落组成如图 2 所 示. 各样本相对丰度超过 1% 的菌门为 Proteobacteria (变形菌门)、Actinobacteria(放线菌门)、Chloroflexi (绿弯菌门)、Acidobacteria(酸杆菌门)、Firmicutes (厚壁菌门)、Bacteroidetes (拟杆菌门)、 Gemmatimonadetes(芽单胞菌门)、Rokubacteria 和 Nitrospirae(硝化螺菌门),其中相对丰度最高的是 Proteobacteria,其次是 Actinobacteria,这两种菌门总 相对丰度超过50%,与已有的我国东北湿地细菌群 落结构研究结果相符[32,33]. 但退化湿地土壤中 Proteobacteria 和 Actinobacteria 的相对丰度低于原始 土壤和修复土壤, Acidobacteria 的相对丰度高于原 始土壤和修复土壤. Proteobacteria 在营养元素含量 高的土壤中相对丰度更高[34,35],因此由于湿地退化 导致土壤总碳、总氮水平降低, Proteobacteria 的相 对丰度也降低. 而在修复中的芦苇湿地样品中,地上 植物凋落物的水解及转化会使土壤总碳、总氮水平 升高, Proteobacteria 的相对丰度也相应升高. 而 Acidobacteria 相对丰度的变化可能与土壤 pH 有关, 有研究表明土壤中 Acidobacteria 的相对丰度与 pH 呈显著负相关[36],因此在 pH 相对较低的退化土壤

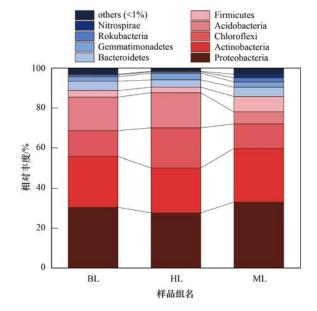


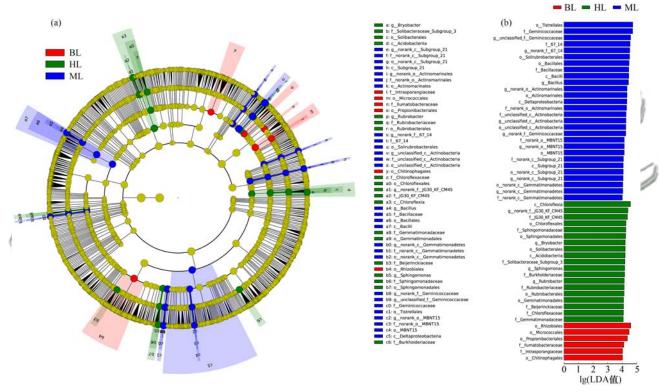
图 2 细菌门水平群落组成

Fig. 2 Community composition of bacteria at the phylum level

中其相对丰度较高.

在纲水平至属水平上,3组样本细菌群落组成差异显著(图3).原始芦苇湿地土壤的差异优势物种包括4目2科,退化土壤的差异优势物种包括2纲5目8科4属,修复土壤的差异优势物种包括3纲8目8科9属.在这些差异优势物种包括3纲8目8科9属.在这些差异优势物种中,Rhizobiales(根瘤菌目)和Bacillus(芽孢杆菌属)属于根际促生菌,是一类能直接或间接促进植物生长及吸收营养物质的根际有益微生物^[37];而Burkholderiaceae(伯克氏菌科)中包含众多动植物病原菌如Burkholderia(伯克氏菌属)和Ralstonia(青枯菌属)^[38].原始芦苇湿地土壤中含有相对丰度较高

的 Rhizobiales,退化湿地土壤中含有相对丰度较高的 Burkholderiaceae,修复湿地土壤中则含有相对丰度较高的 Bacillus,表明湿地退化导致部分根际促生菌相对丰度下降,致病菌相对丰度上升.此外,退化湿地土壤中含有相对丰度较高的 Sphingomonas (鞘氨醇单胞菌属)和 Rubrobacter (红色杆菌属), Sphingomonas 可以利用多环芳烃等有机污染物作为碳源和能源^[39],Rubrobacter(红色杆菌属)具有耐受高强度辐射的特性,可以生长在极端高辐射环境中^[40].由于人类活动造成的松嫩平原湿地退化以及土壤污染、植被覆盖度下降和土壤贫瘠化^[10],而使这些抗逆性强的微生物的相对丰度增加.



(a) 差异物种进化分支,(b) 差异物种 LDA 值

图 3 基于 LEfSe 分析的细菌群落差异物种进化分支和 LDA 值

Fig. 3 Cladogram and LDA scores of different bacterial communities based on LEfSe analysis

各样地产甲烷菌科水平群落组成如图 4 所示.3 组 样 本 共 同 的 产 甲 烷 菌 优 势 菌 科 为 Methanosarcinaceae (甲烷 八 叠 球 菌 科) 和 Methanobacteriaceae (甲烷杆菌科),在其他关于湿地产甲烷菌群落的研究中,这两种产甲烷菌科也占据优势地位^[41].但 3 组样本产甲烷菌优势菌科的相对丰度 存 在 差 异 (图 5).原 始 湿 地 土 壤 中 Methanobacteriaceae 相对丰度最高,约为 63%,其次是 Methanosarcinaceae,约为 30%.退化的和正在修复的芦苇湿地土壤中 Methanosarcinaceae 相对丰度最高,分别为 48%和 46%,而 Methanobacteriaceae 相 对丰度差异较大,分别为 24%和 12%.此外,修复土

壤中还出现了相对丰度较高的 Methanomicrobiaceae (甲烷微菌科),该菌科在修复土壤中相对丰度为 16%,而在原始土壤和退化土壤中的相对丰度很低,均为 1% 左右.在这些优势菌科中, Methanomicrobiaceae 属于氢型产甲烷菌, Methanobacteriaceae 同时具有氢型和甲基型产甲烷的活性, Methanosarcinaceae 则可以利用氢型、乙酸型和甲基型这3种主要途径产甲烷^[42].目前的研究发现只有 Methanosarcinaceae 和 Methanosaetaceae (鬃毛甲烷菌科)能够利用乙酸途径产甲烷^[6],表明湿地退化导致乙酸型产甲烷菌的相对丰度上升.根据上述3类产甲烷菌在3个样地中的相对丰度情

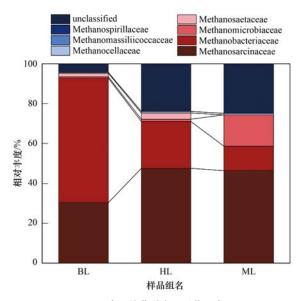
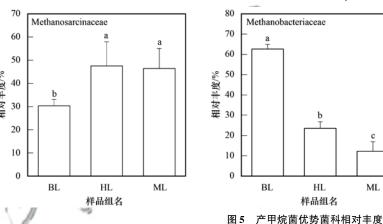
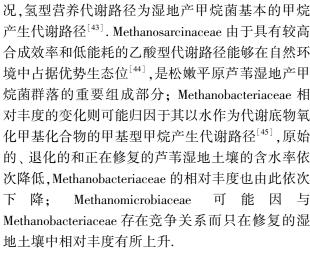


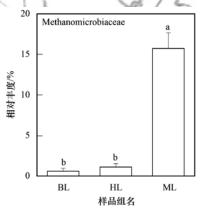
图 4 产甲烷菌科水平群落组成

Community composition of methanogens at the family level



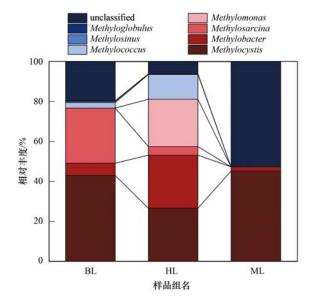


各样地甲烷好氧氧化菌属水平群落组成如图 6 所示,优势菌属相对丰度差异如图 7 所示. 原始湿地 土壤中优势菌属为 Methylocystis (甲基孢囊菌属)和 Methylosarcina(甲基八叠球菌属),相对丰度分别约



Relative abundances of dominant methanogens at the family level

ML

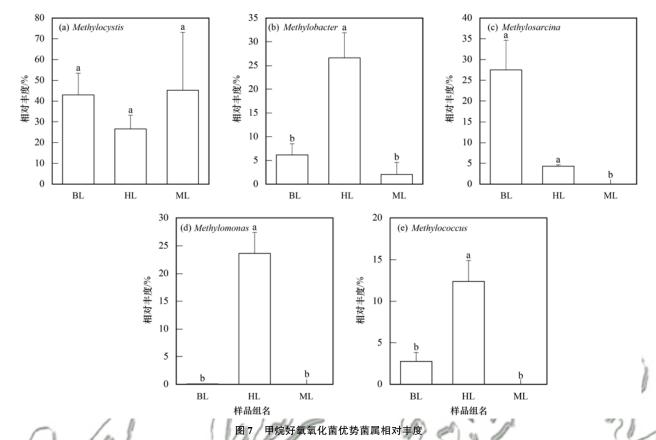


甲烷好氧氧化菌属水平群落组成

Fig. 6 Community composition of methanotrophs at the genus level

为43%和28%.退化湿地土壤中优势菌属为 Methylocystis、 Methylobacter (甲基杆菌属)、

Methylomonas(甲基单胞菌属)和 Methylococcus(甲基 球菌属),相对丰度分别约为 27%、27%、24% 和 12%. 修复湿地土壤中优势菌属为 Methylocystis (甲 基孢囊菌属),相对丰度约为45%. Methylocystis 属 于 Type Ⅱ型甲烷好氧氧化菌,普遍存在于湿地等多 种生态系统中[22,46,47],该菌属是3组湿地土壤样本 的共同优势菌属. Methylocystis 能够在非常低的甲烷 浓度下生长,是贫营养型甲烷氧化菌,某些 Methylocystis 菌株甚至可以在大气中氧化甲烷长达3 个月[48],并且 Methylocystis 在周期性排水的生态系 统中普遍存在. 因此, Type Ⅱ型甲烷好氧氧化菌在 松嫩平原芦苇湿地中发挥着重要作用. Methylosarcina 属于 Type I 型甲烷好氧氧化菌,最初 分离自垃圾填埋场土壤[49],在已有的关于松嫩平原 湿地甲烷氧化菌的研究中,该菌属不占据主要地 位[50],然而在本研究中, Methylosarcina 在原始芦苇 湿地土壤中占优势地位,在退化湿地土壤和修复湿 地土壤中的相对丰度较低. 原始湿地土壤中出现高



g. 7 Relative abundances of dominant methanotrophs at the genus level

丰度的 Methylosarcina 可能与样地土壤理化性质及土壤性状有关,这种甲烷好氧氧化指示菌可能是该湿地区域潜在的发挥碳汇作用的微生物资源.

此外,在退化湿地样品中占优势地位的Methylobacter、Methylomonas 和 Methylococcus 其相对丰度均显著高于原始湿地和修复湿地.这3种菌属均属于 Type I型甲烷好氧氧化菌.其中,Methylobacter和 Methylomonas可以适应极端低温环境[51],研究者在西伯利亚永久冻土和高寒湿地中检测到了大量的 Methylobacter [52,53],Methylomonas 分类下也已经分离到了能在深层地下水中生存的嗜冷菌株[54]。Methylococcus则可以耐受极端高温环境,有研究者从热泉中发现了该属下的嗜热菌株[55]。可见,芦苇湿地退化会导致耐受极端环境的甲烷氧化菌属的相对丰度增加.

3 结论

- (1)与原始芦苇湿地相比,松嫩平原退化的芦苇湿地土壤理化性质发生了变化.具体表现为:退化湿地土壤总碳、总氮和土壤含水率均显著降低.
- (2)芦苇湿地退化导致土壤细菌和产甲烷菌的 α 多样性降低,甲烷氧化菌的 α 多样性升高,而细菌和产甲烷菌的 α 多样性与土壤含水率呈显著正相关关系,含水率越高的湿地土壤产甲烷菌的多样性

也越高.

(3)湿地退化导致根际促生菌 Rhizobiales 的相对丰度下降,致病菌 Burkholderiaceae、耐污染细菌 Sphingomonas、抗辐射细菌 Rubrobacter 以及 Type I型耐受极端环境的甲烷好氧氧化菌 Methylobacter、Methylomonas 和 Methylococcus 的相对丰度上升. 而在原始及修复的芦苇湿地中优势菌属为 Type II型甲烷好氧氧化菌 Methylocystis. 因此,不同的湿地状态可以间接改变土壤性状进而改变湿地甲烷代谢菌群落结构.

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