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有机-无机肥配施对麦玉轮作土壤中细菌氮循环功能基因的影响

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摘　要: 为探讨有机-无机肥配施对小麦-玉米轮作土壤细菌氮循环功能基因的影响,设置单施化肥(NPK)、化肥配施玉米秸秆(NPKS)和化肥配施有机肥(NPKO)3种施肥方式,采用16S rRNA基因的高通量测序技术并结合PICRUSt功能预测分析,探明不同施肥方式下小麦和玉米土壤细菌关键氮循环功能基因的变化特征。结果表明:对于KEGG的二级功能分类,NPKS处理下小麦土壤细菌排泄系统的相对丰度较NPK处理显著提高8.73%,而NPKO处理显著降低了辅酶和维生素代谢的相对丰度,降低幅度达到0.90%;NPKS、NPKO与NPK处理间玉米土壤细菌功能相对丰度差异均不显著。有机-无机肥配施下小麦土壤细菌中具有显著差异的三级功能分类数量明显多于玉米土壤细菌。与NPK处理相比,NPKS处理显著降低了小麦土壤细菌的氨基酸糖与核苷酸糖代谢,丙氨酸、天冬氨酸和谷氨酸代谢,硫胺素代谢,脂多糖生物合成,核黄素代谢和长寿调节途径的相对丰度和玉米土壤细菌Glioma和神经营养素信号通路的相对丰度,但显著提高了玉米土壤细菌突触囊泡循环的相对丰度;NPKO处理显著降低了小麦土壤细菌Cell cycle-Caulobacter、硫胺素代谢和核黄素代谢的相对丰度及玉米季甲烷代谢的相对丰度,但显著提高了小麦土壤细菌碱基切除修复的相对丰度。小麦和玉米土壤细菌均有23个功能基因参与氮循环的KO通路。小麦土壤细菌氮循环功能基因丰度与土壤SOM和TN显著正相关,与土壤NH_4^+-N显著负相关;玉米土壤细菌氮循环功能基因丰度与土壤TN和TP显著正相关。综上所述,小麦和玉米土壤细菌具有功能上的多样性,有机-无机肥配施下小麦土壤细菌发挥的代谢作用更为强烈。小麦和玉米土壤细菌的氮异化还原和氮同化还原潜力最高,反硝化潜力和固氮潜力次之,硝化潜力最弱。土壤细菌氮循环功能基因受轮作体系影响,SOM和TN促进小麦土壤细菌氮循环过程,而NH_4^+-N对氮循环过程产生负面影响;TN和TP在玉米土壤细菌氮循环过程中发挥积极作用。

关键词: 有机-无机肥配施;氮循环;功能基因;小麦-玉米轮作;PICRUSt功能预测
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The effects of combined organic and inorganic fertilizer on the bacterial nitrogen cycling functional genes in wheat and maize soils by PICRUSt functional prediction

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Abstract: A field experiment was conducted in a wheat-maize rotation field to investigate the effects of combined organic and inorganic

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fertilizers on the functional genes involved in soil bacterial nitrogen cycling. Three fertilizer combinations were investigated, including single chemical fertilizer (NPK), chemical fertilizer with maize straw (NPKS), and chemical fertilizer with organic fertilizer (NPKO). The characteristics of the nitrogen cycling functional genes were explored using high-throughput sequencing of the bacterial 16S rRNA gene, followed by PICRUSt functional prediction analysis. Our results showed that, at the level 2 of Kyoto Encyclopedia of Genes and Genomes (KEGG) functional classification, the relative abundance of the wheat soil bacterial excretion system was significantly higher with the NPKS treatment compared to NPK (+8.73%), and the relative abundance of the metabolic cofactors and vitamins significantly decreased with the NPKO treatment (−0.90%). There were no significant differences in the maize soil bacterial functional abundances among treatments. When the organic and inorganic fertilizers were simultaneously applied, the number of level 3 bacterial functions with pronounced differences were significantly higher in wheat soil than in maize soil. Compared to the NPK treatment, the NPKS treatment significantly reduced the relative abundances of the wheat soil bacterial amino sugar and nucleotide sugar metabolism, alanine, aspartate and glutamate metabolism, thiamine metabolism, lipopolysaccharide biosynthesis, riboflavin metabolism and longevity regulating pathway-worm. In maize soil, the relative abundances of the bacterial glioma and neurotrophin signaling pathways were significantly reduced, and the relative abundance of the bacterial synaptic vesicle cycle significantly increased. The NPKO treatment significantly reduced the relative abundances of the wheat soil bacterial cell cycle-Caulobacter, thiamine metabolism and riboflavin metabolism, but significantly improved the relative abundance of the base excision repair. The relative abundance of the maize soil bacteria methane metabolism was also significantly reduced with the NPKO treatment. Twenty-three types of functional genes within the bacterial nitrogen cycling KEGG Orthology (KO) pathway were identified in the wheat and maize soils. The bacterial nitrogen cycling functional gene abundances in wheat soil were significantly positively correlated with the soil organic matter (SOM) and total nitrogen (TN), and significantly negatively correlated with the ammonium-nitrogen (NH_4^+-N) content. The functional gene abundances in maize soil had a significant positive correlation with TN and total phosphorus (TP). The bacteria in wheat and maize soils were functionally diverse, and the wheat soil bacterial metabolism was high when combined application organic and inorganic fertilizers was undertook. For both crops, the soil bacterial nitrogen dissimilation reduction and the nitrogen assimilation reduction potentials were high, the denitrification and nitrogen fixation potentials were less prominent, and the nitrification potential was low. The crop rotation system had a significant influence on the functional genes involved in soil bacterial nitrogen cycling. In wheat soil, SOM and TN promoted bacterial nitrogen cycling, but NH_4^+-N had the opposite effect. In maize soil, TN and TP actively influenced bacterial nitrogen cycling.

Keywords: combined organic and inorganic fertilizers; nitrogen cycling; functional genes; wheat-maize rotation; PICRUSt functional prediction

华北平原作为我国第二大平原，涵盖京津冀和鲁豫皖苏等多个省市[1]，是我国最重要的农作物生产基地之一[2]，其典型的种植制度是“冬小麦-夏玉米”轮作，轮作区耕地面积高达1.22×10^7 hm^2，可为全国供应70%以上的小麦和近30%的玉米[3]，具有“投入高、产量高、环境影响大”的特点[4]。近年来，化肥的大量投入使农田土壤生态系统服务功能和养分循环受到严重影响，生物多样性大幅降低[5]。据报道，单施化肥会降低土壤微生物群落的丰度和活性，并对其组成产生负面影响[6]。在华北平原集约化耕作条件下，有机物质（秸秆和畜禽粪便）的施用是重要的土壤管理措施，其不但可以为植物提供养分，而且可以通过补充有机质（SOM）来维持土壤肥力[7]。小麦、玉米除作为粮食作物外，其副产物——秸秆含有丰富的氮、磷、钾，还田利用后可为后茬作物提供生长所必需的营养元素，提高作物产量[8]。Lori等[9]研究表明，施用有机肥会显著增加土壤细菌丰度和酶活性。此外，与单施化肥相比，有机肥还可以提高土壤微生物群落的多样性[10]。随着化肥的持续施用，其对环境的负面影响逐渐显现，与此同时，为了缓解无机肥料大量施用导致的农田土壤质量下降，有机肥的应用得到不断发展，有机肥与化肥配合施用成为近年来肥料应用研究中较为活跃且发展最快的领域之一[11]。有机-无机肥配施具有速效和长效的优势，可以促进土壤微生物的生长繁殖[12]，并对粮食生产具有显著的积极影响[13]。研究表明，有机-无机肥配施可以影响土壤微生物群落组成，进而影响土壤养分循环[14]和植物生长[15]。土壤微生物群落在有机物质分解中发挥重要作用，影响着营养物质的生物地球化学循环，对维持土壤功能至关重要[16]。此外，季节动态变化也是影响农业生态系统中土壤微生物群落的重要因素之一[17-19]。在小麦-玉米轮作体系中，小麦田土壤理化性质与微生物群落之间的相关性强于玉米田[20]，取样时间对土壤微生物群落的影响远大于施肥制度，施肥效应在小麦田最为显

著[21]。Zhao等[22]的研究同样阐明了轮作体系下施肥时间对土壤微生物的显著影响。因此,在开展轮作体系下土壤微生物群落变化的研究中应该将作物种类和环境条件均视为驱动土壤微生物群落变化的主要因素。

氮循环是土壤生态系统生物地球化学循环中的核心过程之一,在农田生态系统可持续发展方面起着举足轻重的作用[23]。土壤微生物发挥与氮循环有关的生态功能[24]。目前,学者们在有机-无机肥配施对农田土壤微生物影响方面已开展了大量研究工作,初步探明了农田土壤细菌群落的组成、多样性和结构等响应特征及其影响因素。但尚不清楚轮作体系下施肥方式和季节性波动如何影响土壤微生物群落的氮循环功能。因此,本研究选择单施化肥、化肥配施玉米秸秆和化肥配施有机肥3种不同施肥处理,采用16S rRNA基因高通量测序技术,基于生物信息学方法并结合PICRUSt功能预测分析,探究土壤细菌功能分类变化,进而明确氮循环过程功能基因的变化及其与土壤理化性质间的关系,以期为更好地揭示有机-无机肥配施过程中氮循环机制提供理论参考。

1 材料与方法

1.1 研究区概况与试验设计

试验地位于天津市宁河区林场(39°48′N,117°71′E),该地农田耕作方式为华北农耕区常规耕作模式——冬小麦-夏玉米轮作。试验始于2015年,共设3个施肥处理:单施无机肥(NPK)、无机肥配施玉米秸秆(NPKS)和无机肥配施有机肥(NPKO)。各处理小区面积较大(22 m×22 m),故没有设置重复小区,取样时将小区分为3个区域即3次假重复[25-27]。共分小麦季和玉米季两次施肥,每次施肥各处理无机肥施用量相同(140 kg·hm^{-2} N,131.25 kg·hm^{-2} P_2O_5,44.1 kg·hm^{-2} K_2O)。试验中施用的无机肥料包括无机复合肥(N含量28%,P_2O_5含量13%,K_2O含量5%)、磷肥[$Ca(H_2PO_4)_2$,养分含量12%]、钾肥(KCl,养分含量60%)以及尿素(N含量46%)。将168 kg·hm^{-2} N(无机复合肥)作为基肥在冬小麦和夏玉米播种时一次性施入农田,112 kg·hm^{-2} N(尿素)在小麦苗期和玉米拔节期分别追施入农田。NPKO处理施用的有机肥(商品有机肥,养分总含量≥5%,有机质含量≥40%,石家庄市希星肥业科技有限公司生产)量为15 t·hm^{-2},只在小麦季一次性施入。各小区总施肥量见表1。小麦季所有处理产生的小麦秸秆按当地常规耕作方式全部粉碎后覆盖于地表还田,玉米季节NPKS处理产生的玉米秸秆粉碎成小段后随土壤耕翻还田,NPK和NPKO处理的玉米秸秆均不还田。其他田间管理按照当地常规生产模式进行。

1.2 土壤样品采集

分别于2019年5月小麦收获前和9月玉米收获前,使用直径5 cm的土壤采样器采集0~20 cm耕层土壤,将在每个区域随机采集的5个土芯混合成1个土壤样品,剔除石砾和植物残体等杂质后装入灭菌自封袋暂存于保温箱中并迅速带回实验室,土壤样品过2 mm筛后用于提取DNA并测定土壤理化性质。

1.3 土壤理化性质测定

土壤理化指标pH、有机质(SOM)、全氮(TN)、全磷(TP)、速效磷(AP)的测定方法参照文献[28],其中土壤pH在2.5∶1的水土比下采用复合电极测定;SOM采用重铬酸钾容量法测定;TN采用半微量开氏法测定;TP采用$HClO_4$-H_2SO_4消煮-钼锑抗比色法测定;AP采用$NaHCO_3$浸提-钼锑抗比色法测定;硝态氮(NO_3^--N)和铵态氮(NH_4^+-N)经0.01 mol·L^{-1} $CaCl_2$浸提后使用连续流动分析仪(AA3,SEAL Analytical,Germany)测定;将土壤样品于105 ℃下烘干至恒质量,采用重量法测定土壤含水量(WCS)。小麦田和玉米田土壤理化性质见表2。

1.4 高通量测序及生物信息学分析

采用Fast DNA® Spin Kit for Soil(MP Biomedicals,U.S.A)提取土壤DNA,经1%的琼脂糖凝胶电泳检测DNA质量后,使用NanoDrop2000测定DNA浓度和纯度。采用引物338F(5′-ACTCCTACGGGAGGCAGCAG-3′)和806R(5′-GGACTACHVGGGTWTCTA-

表1 各试验小区总施肥量

Table 1 Fertilizer application rate in trail plots

处理 Treatments	N/(kg·hm^{-2})		P_2O_5/(kg·hm^{-2})	K_2O/(kg·hm^{-2})	有机肥 Organic fertilizer/(t·hm^{-2})	玉米秸秆还田比例/% Proportion of maize straw returned to the field
	基肥 Base fertilizer	追肥 Top dressing				
NPK	168	112	262.5	88.2	0	0
NPKS	168	112	262.5	88.2	0	100
NPKO	168	112	262.5	88.2	15	0

表2 不同施肥处理土壤理化性质

Table 2 Physical and chemical properties of soil under different fertilization treatments

作物类型 Crop type	处理 Treatments	pH	SOM/（g·kg^{-1}）	TN/（g·kg^{-1}）	TP/（g·kg^{-1}）	AP/（mg·kg^{-1}）	NH_4^+-N/（mg·kg^{-1}）	NO_3^--N/（mg·kg^{-1}）	WCS/%
小麦田 Wheat field	NPK	8.13±0.02a	29.60±2.76b	0.98±0.02b	0.64±0.03a	14.4±1.72b	2.14±0.16a	16.31±5.62a	13.37±0.66a
	NPKS	8.11±0.05a	34.62±1.79ab	1.03±0.02b	0.64±0.06a	20.69±3.56b	1.82±0.08a	18.75±6.40a	14.98±0.95a
	NPKO	8.08±0.03a	38.31±3.29a	1.16±0.05a	0.70±0.03a	35.1±5.23a	1.75±0.27a	21.17±6.57a	14.20±1.06a
玉米田 Maize field	NPK	8.19±0.01a	20.20±1.78b	0.96±0.05a	0.81±0.02a	36.44±3.72b	1.39±0.17b	15.62±1.72ab	16.05±0.21a
	NPKS	8.20±0.17a	21.10±1.15ab	0.89±0.12a	0.75±0.05a	41.20±2.01b	2.20±0.43ab	12.77±0.82b	16.20±1.22a
	NPKO	8.21±0.02a	24.26±1.83a	0.97±0.08a	0.82±0.04a	48.63±2.34a	3.10±1.08a	19.45±3.81a	15.69±1.41a

注：数据为平均值±SD（n=3）；同列不同小写字母表示不同处理间差异显著（P<0.05）。

Note: The data were mean±SD（n=3）. Different lowercase letters in the same column indicate significant differences between different treatments（P<0.05）.

AT-3′）扩增细菌16S rRNA基因的V3~V4高变区序列[29]，PCR条件如下：95 ℃预变性3 min，27个循环（95 ℃变性30 s，55 ℃退火30 s，72 ℃延伸45 s），然后72 ℃稳定延伸10 min。PCR反应体系包括：5×TransStart FastPfu缓冲液4 μL，2.5 mmol·L^{-1} dNTPs 2 μL，上下游引物（5 μmol·L^{-1}）各0.8 μL，TransStart FastPfu DNA聚合酶1.0 U，模板DNA 10 ng，加ddH_2O补足至20 μL。高通量测序委托上海美吉生物医药科技有限公司在Illumina MiSeq平台上完成。使用Trimmomatic软件对原始测序序列进行质控，并使用FLASH软件进行拼接。拼接时碱基重叠数不得少于20个，设置50 bp的窗口。根据PE序列之间的重叠关系，将成对序列拼接成一条序列，最小重叠长度为10 bp。拼接序列的重叠区允许的最大错配比率为0.2。根据序列首尾两端的条形码和引物区分样品，并调整序列方向，条形码允许的错配数为0，最大引物错配数为2[30]。使用UPARSE软件，根据97%的相似度将高质量的核酸序列聚类到操作分类单元（OTUs）并剔除嵌合体[31]。利用RDP classifier对每条序列进行物种分类注释，设置比对阈值为70%。

1.5 数据分析

使用SPSS 23.0对细菌氮循环功能基因与土壤理化性质进行皮尔逊（Pearson）相关性分析，并利用单因素方差分析（One-way ANOVA）中的Tukey′s方法对不同处理的土壤理化性质进行方差分析和显著性检验。使用Origin 2018绘制细菌氮循环功能基因丰度差异图，使用STAMP软件并采用Welch′s-test方法比较不同处理间细菌二级功能分类和三级功能分类中存在显著差异的功能丰度。

2 结果与讨论

2.1 土壤细菌功能分类

基于KEGG数据对比样品中的细菌二级功能分类，NPKS、NPKO与NPK处理间存在显著差异的小麦土壤细菌功能丰度见图1。与NPK处理相比，NPKS处理中排泄系统的相对丰度显著升高，升高幅度达到8.73%，而NPKO处理中辅酶和维生素代谢的相对丰度显著降低，降低幅度达到0.90%。NPKS、NPKO与NPK处理间玉米土壤细菌的功能丰度差异均不显著。

根据细菌三级功能分类，小麦土壤和玉米土壤NPKS、NPKO与NPK处理间相对丰度存在显著差异的细菌代谢功能见图2。从整体来看，NPKS和NPK处理中，小麦土壤细菌氨基酸糖与核苷酸糖代谢（Amino sugar and nucleotide sugar metabolism）的相对

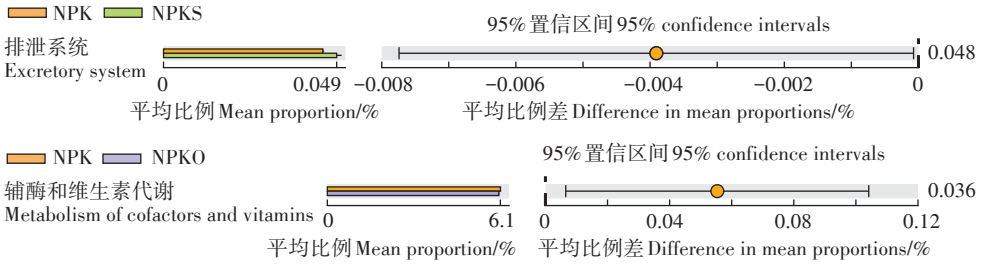


图1 小麦土壤KEGG功能差异（二级功能分类）

Figure 1 Differences of KEGG function in wheat soil（secondary functional classification）

丰度比其他代谢功能更大，其次是丙氨酸、天冬氨酸和谷氨酸代谢（Alanine，aspartate and glutamate metabolism）、硫胺素代谢（Thiamine metabolism）、脂多糖生物合成（Lipopolysaccharide biosynthesis）、核黄素代谢（Riboflavin metabolism）和长寿调节途径（Longevity regulating pathway-worm），这些功能的相对丰度均表现出NPKS处理显著低于NPK处理。与NPK处理相比，NPKO处理显著降低了Cell cycle-Caulobacter所占的相对丰度，但显著提高了碱基切除修复（Base excision repair）所占的相对丰度。NPKO处理中的硫胺素代谢和核黄素代谢所占的相对丰度均显著低于NPK处理。对于玉米土壤细菌，Glioma和神经营养素信号通路（Neurotrophin signaling pathway）在NPK处理中的相对丰度显著高于NPKS处理，而突触囊泡循环（Synaptic vesicle cycle）表现相反；甲烷代谢（Methane metabolism）在NPK和NPKO处理中存在显著差异。小麦季有机添加处理与无机处理下相对丰度具有显著差异的土壤细菌三级功能分类中的代谢功能个数（NPK与NPKS处理对比为12个，NPK与NPKO处理对比为10个）多于玉米季（NPK与NPKS处理对比为3个，NPK与NPKO处理对比为1个），表明有机添加对细菌代谢功能的影响在小麦土壤中发挥的作用更加复杂，这可能受两个取样时间的土壤理化性质不同所影响。这与先前的报道中小麦田土壤理化性质与土壤微生物群落之间的相关性强于玉米田相一致[20]。此外，有机肥于冬小麦种植时施入农田，且整

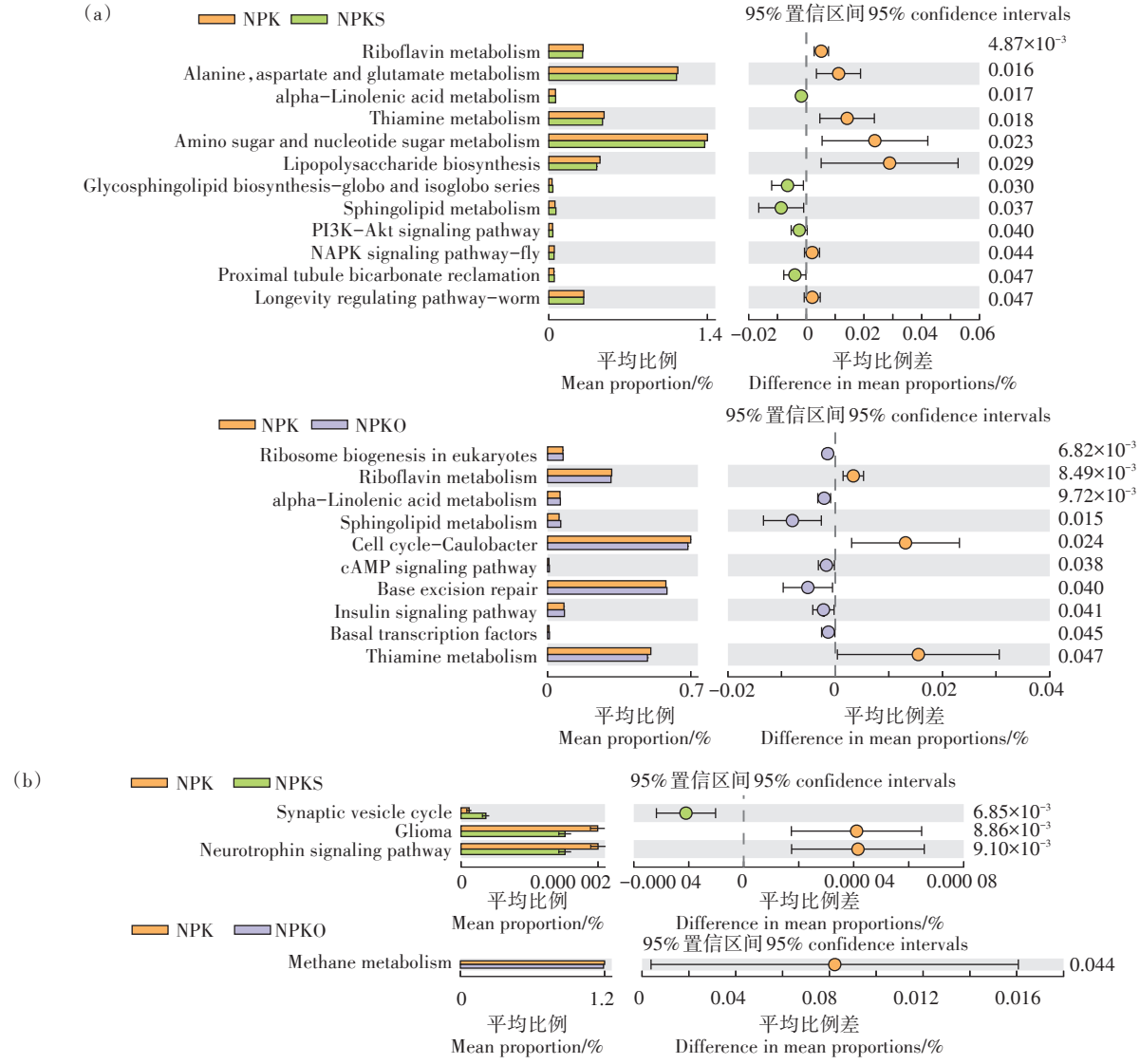


图2 小麦土壤（a）和玉米土壤（b）KEGG功能差异（三级功能分类）

Figure 2 Differences of KEGG function in wheat (a) and maize (b) soils (three-level functional classification)

个轮作体系下仅施入一次，冬小麦的生长周期较长且需越冬，气候变化强烈；而夏玉米的生长周期较短且气候炎热，降水量高。推测认为有机肥和气候条件差异同样可能造成细菌代谢功能的改变。

2.2 土壤细菌氮循环

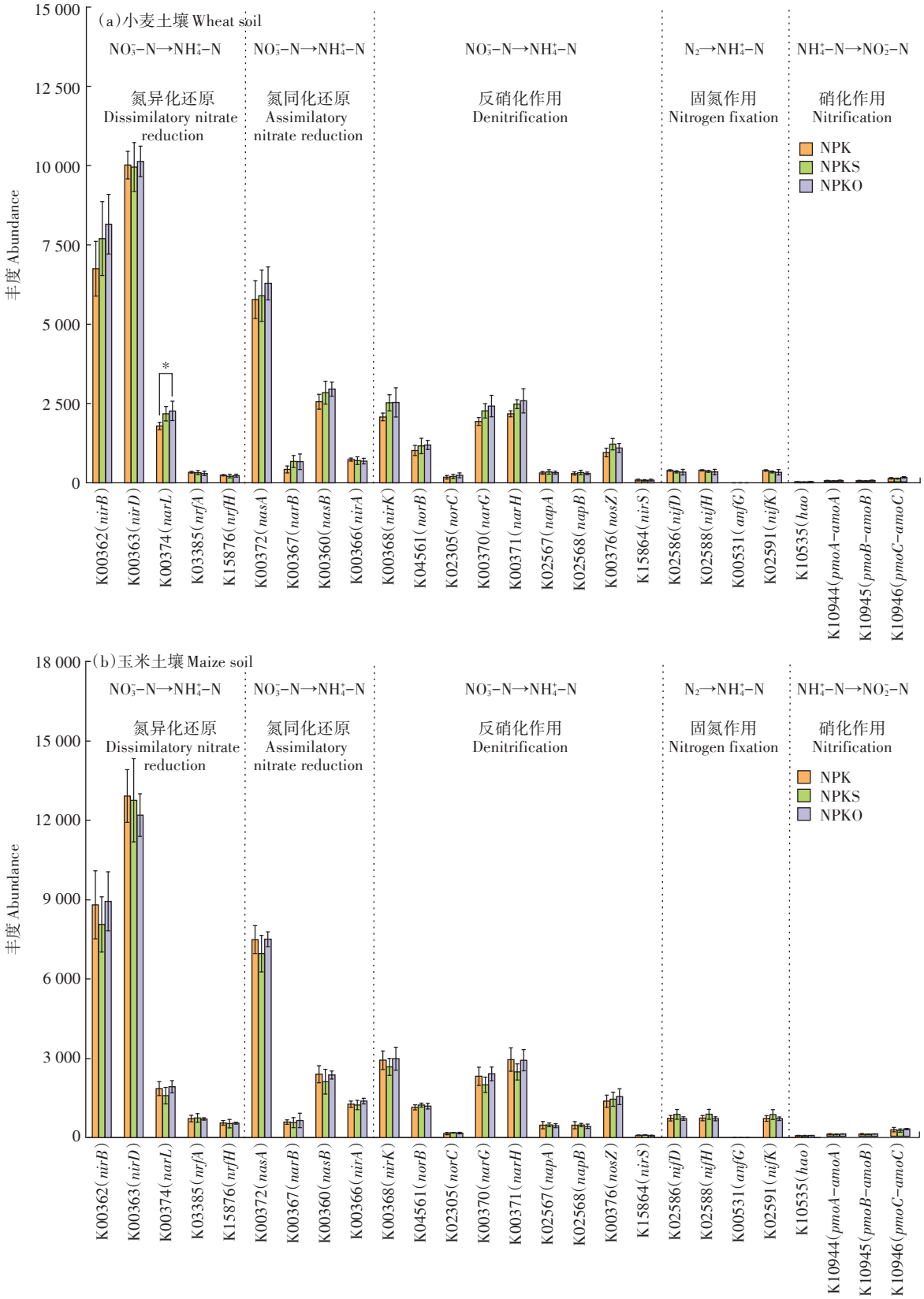
进一步研究有机-无机肥配施下麦玉轮作田细菌参与氮循环途径（K00910）的基因差异。根据氮循环中氮价位的变化，将氮循环过程分为5个阶段，分别是氮异化还原、氮同化还原、反硝化作用、固氮作用和硝化作用。氮异化还原过程由*nirB*、*nirD*、*narL*、*nrfA*和*nrfH*基因参与完成，氮同化还原过程由*nasA*、*narB*、*nasB*和*nirA*基因参与完成，反硝化作用由*nirK*、*norB*、*norC*、*narG*、*narH*、*napA*、*napB*、*nosZ*和*nirS*基因参与完成，固氮作用由*nifD*、*nifH*、*anfG*和*nifK*基因参与完成，硝化作用由*hao*、*pmoA-amoA*、*pmoB-amoB*和*pmoC-amoC*基因参与完成。

小麦和玉米土壤细菌氮循环相关基因的丰度变化如图3所示。从整体来看，小麦和玉米土壤细菌的氮异化还原和氮同化还原潜力最高，反硝化潜力和固氮潜力次之，硝化潜力最弱。在小麦土壤细菌氮异化还原过程中，亚硝酸盐还原酶*nirD*基因最为丰富，NPK和NPKO处理中*nirD*基因丰度高于NPKS处理；其次是亚硝酸盐还原酶基因*nirB*和硝酸盐还原酶基因*narL*，NPKS和NPKO处理中*nirB*和*narL*基因丰度高于NPK处理；异化还原酶基因*nrfA*和*nrfH*所含基因丰度较低。氮同化还原过程中，硝酸盐还原酶基因*nasA*和*nasB*丰度高于*narB*和*nirA*，*nasA*和*nasB*基因丰度大小均表现为NPKO>NPKS>NPK。反硝化过程中，亚硝酸还原酶基因*nirK*、*narH*和硝酸还原酶基因*narG*丰度较高，大小同样表现为NPKO>NPKS>NPK；其次是一氧化氮还原酶基因*norB*和氧化亚氮还原酶的编码基因*nosZ*；丰度较低的基因是亚硝酸盐还原酶基因*norC*、异化还原酶基因*napA*和*napB*。固氮酶基因*nifD*、*nifH*和*nifK*在固氮作用中丰度较低。硝化作用基因*hao*、*pmoA-amoA*、*pmoB-amoB*和*pmoC-amoC*明显低于其他过程氮循环相关基因。玉米土壤细菌氮循环相关基因丰度高低整体上与小麦土壤细菌一致，但在各处理中的差异有所变化。氮异化还原过程中，NPK处理中*nirD*基因丰度高于NPKS和NPKO处理，NPK和NPKO处理中*nirB*和*narL*基因丰度高于NPKS处理。氮同化还原过程中，NPK和NPKO处理中*nasA*和*nasB*基因丰度高于NPKS处理。反硝化过程中，NPK和NPKO处理中亚硝酸还原酶基因*nirK*、*narH*和硝酸还原酶基因*narG*丰度高于NPKS处理。固氮酶基因*nifD*、*nifH*和*nifK*在NPKS处理中的丰度高于NPK和NPKO处理。

在小麦土壤细菌中，与单施化肥相比，配施秸秆和有机肥提高了反硝化基因丰度，这与前人的研究结果相一致[23,32]。反硝化微生物大多是异养微生物，有机物料可以为土壤提供更均衡和稳定的营养，促进反硝化微生物的生长繁殖[23]。尽管氮循环主要由土壤微生物驱动，但是植物可以通过释放根系分泌物来控制根际及其附近的微生物群体催化氮转化[33]。根系分泌物主要对氮循环过程中的硝化作用和固氮作用起控制作用[34]。不同取样时间，不同农田种植作物，小麦和玉米根际分泌物可能成为影响氮循环功能基因丰度变化的制约因素之一。例如，小麦根系分泌物可以释放生物硝化抑制剂来控制土壤硝化作用[35]，但在玉米根系中并没有发现生物硝化抑制作用[36]。此外，有报道表明高温条件下，一些生态系统的根系分泌物增强，这可能导致与土壤有机质分解和根际氮周转相关的微生物活动加速[37]。因此，小麦和玉米生长季所面临的气候差异也可能导致根系分泌物对氮循环的影响发生变化。在未来的研究中，可以建立“植物-土壤-微生物”有机串联与协调统一机制，构建土壤氮循环网络体系。

2.3 土壤细菌氮循环功能基因与土壤理化性质的相关性

由土壤细菌氮循环功能基因丰度与土壤理化性质的相关性分析可知，与小麦土壤细菌氮循环功能基因关系最为密切的土壤理化性质为SOM、TN和NH_4^+-N（表3），玉米土壤细菌氮循环功能基因与土壤TN和TP相关性最为显著（表4）。在小麦土壤细菌氮异化还原相关基因中，*nirB*和*narL*基因与土壤SOM和TN呈显著正相关，*narL*基因与土壤NH_4^+-N呈显著负相关；氮同化还原相关基因中，*nasA*和*narB*基因与土壤SOM呈显著正相关，*nasB*基因与土壤TN呈显著正相关；反硝化作用相关基因中，*nirK*、*norB*、*norC*、*narG*和*narH*基因均与土壤SOM呈显著正相关，*nirK*基因还与土壤NH_4^+-N呈显著负相关，*narG*和*narH*基因还与土壤TN呈显著正相关，与土壤NH_4^+-N呈显著负相关；硝化作用中*pmoC-amoC*基因与土壤TN呈显著正相关，与NH_4^+-N呈显著负相关。在玉米土壤细菌氮异化还原相关基因中，*nirB*基因与土壤TP呈显著正相关，*narL*基因与土壤TN和TP呈显著正相关，*nrfH*基因与土壤pH呈显著负相关；氮同化还原相关基因中，



*表示在0.05水平上显著相关

* indicates a significant correlation at the level of 0.05

图3 小麦土壤和玉米土壤细菌氮循环途径

Figure 3 Bacteria nitrogen cycling pathway in wheat and maize soil

nasA、*narB* 和 *nasB* 基因与土壤 TN 和 TP 呈显著正相关；反硝化作用相关基因中，*nirK* 基因与土壤 TP 呈显著正相关，*narG* 和 *narH* 基因与土壤 TN 和 TP 呈显著正相关；硝化作用相关基因均与土壤 TP 呈显著正相关。土壤细菌氮循环功能基因丰度与土壤理化性质的相关性表明氮循环过程受多种氮循环途径调控，土壤 SOM、TN 和 TP 促进氮循环过程，而 NH_4^+-N 对氮循环过程产生负面影响。

土壤有机质和氮含量对调节土壤氮循环发挥着重要作用[38]。研究表明，土壤有机碳是影响反硝化作用的重要因素[39]，土壤有机质提升显著提高了反硝化过程还原酶功能基因 *nar*、*nir* 和 *nor* 的丰度[40]。郭安宁等[41]的研究发现土壤硝化作用与 NH_4^+-N 含量呈显著负相关，廖李容等[42]的研究发现，*narG* 基因丰度与 NH_4^+-N 含量呈显著负相关，这与本研究小麦季的结果相一致。土壤 C、N、P 等养分供应及其理化指标可以通过改变氮循环功能基因的丰度来影响土壤氮循环过程[23]。小麦和玉米土壤细菌所表现的相关性差异表明影响氮循环功能基因丰度的理化性质并非唯一且不固定，季节动态性变化和施肥方式不同可能会造成时间变异性和环境差异性。轮作体系下不同作物类型会导致土壤微环境的改变，进而影响土壤氮循环功能微生物群落[38,43]。小麦的须根在其生长阶段可以释放丰富的分泌物，为功能微生物提供内源性碳源[44]，推测玉米的须根同样可以通过释放分泌物来为氮循环微生物提供内源性碳源。

3 结论

（1）小麦和玉米土壤细菌具有功能上的多样性，有机-无机肥配施在小麦土壤细菌发挥的代谢作用

表3 细菌氮循环功能基因与土壤理化性质的相关系数（小麦土壤）

Table 3 Correlation coefficients between bacterial nitrogen cycling function genes and soil physical and chemical properties (Wheat soil)

氮循环过程 Nitrogen cycle	功能基因 Functional genes	pH	SOM	TN	TP	AP	NH_4^+-N	NO_3^--N	WCS
氮异化还原 Dissimilatory nitrate reduction	K00362(*nirB*)	−0.389	0.841**	0.687*	−0.164	0.465	−0.526	0.131	0.121
	K00363(*nirD*)	−0.539	0.115	0.096	−0.172	0.352	0.195	−0.112	−0.664
	K00374(*narL*)	−0.533	0.742*	0.783*	−0.045	0.484	−0.852**	0.194	0.609
	K03385(*nrfA*)	0.665	0.015	−0.053	−0.59	−0.338	−0.26	0.152	0.026
	K15876(*nrfH*)	0.491	0.137	0.139	−0.424	−0.071	−0.195	0.207	−0.285
氮同化还原 Assimilatory nitrate reduction	K00372(*nasA*)	−0.256	0.677*	0.565	−0.234	0.344	−0.325	0.127	−0.157
	K00367(*narB*)	−0.528	0.720*	0.549	−0.231	0.265	−0.503	−0.251	0.468
	K00360(*nasB*)	−0.393	0.646	0.667*	−0.036	0.567	−0.648	0.531	0.081
	K00366(*nirA*)	0.473	0.217	−0.08	−0.399	−0.245	0.183	−0.053	−0.304
反硝化作用 Denitrification	K00368(*nirK*)	−0.422	0.757*	0.654	−0.189	0.289	−0.694*	−0.03	0.600
	K04561(*norB*)	−0.436	0.704*	0.516	−0.326	0.349	−0.356	−0.066	−0.017
	K02305(*norC*)	−0.364	0.719*	0.630	−0.281	0.286	−0.447	−0.024	0.100
	K00370(*narG*)	−0.506	0.738*	0.813**	−0.021	0.490	−0.850**	0.192	0.592
	K00371(*narH*)	−0.349	0.799**	0.784*	−0.058	0.396	−0.811**	0.223	0.592
	K02567(*napA*)	−0.077	0.446	0.224	−0.605	−0.022	−0.267	−0.033	−0.039
	K02568(*napB*)	−0.123	0.413	0.138	−0.635	−0.064	−0.175	−0.14	−0.063
	K00376(*nosZ*)	−0.492	0.540	0.284	−0.339	0.073	−0.353	−0.378	0.476
	K15864(*nirS*)	−0.051	0.361	0.307	−0.452	−0.076	−0.125	−0.085	−0.185
固氮作用 Nitrogen fixation	K02586(*nifD*)	0.609	−0.029	−0.122	−0.63	−0.552	−0.012	−0.221	0.012
	K02588(*nifH*)	0.639	−0.040	−0.138	−0.633	−0.574	−0.045	−0.167	0.064
	K00531(*anfG*)	0.215	−0.165	−0.128	−0.655	−0.071	−0.205	−0.210	−0.193
	K02591(*nifK*)	0.600	−0.020	−0.11	−0.627	−0.541	−0.016	−0.210	0.003
硝化作用 Nitrification	K10535(*hao*)	−0.009	0.085	0.521	0.411	0.494	−0.454	0.601	−0.016
	K10944(*pmoA-amoA*)	−0.113	0.074	0.531	0.148	0.425	−0.529	0.514	−0.039
	K10945(*pmoB-amoB*)	−0.099	0.047	0.51	0.147	0.414	−0.502	0.499	−0.068
	K10946(*pmoC-amoC*)	−0.257	0.28	0.707*	0.175	0.587	−0.688*	0.436	0.093

注：**表示在0.01水平上显著相关，*表示在0.05水平上显著相关。下同。

Note: ** indicates a significant correlation at the level of 0.01, and * indicates a significant correlation at the level of 0.05. The same below.

表4 细菌氮循环功能基因与土壤理化性质的相关系数(玉米土壤)

Table 4 Correlation coefficients between bacterial nitrogen cycling function genes and soil physical and chemical properties(Maize soil)

氮循环过程 Nitrogen cycle	功能基因 Functional genes	pH	SOM	TN	TP	AP	NH_4^+-N	NO_3^--N	WCS
氮异化还原 Dissimilatory nitrate reduction	K00362(*nirB*)	0.19	−0.037	0.597	0.763*	0.403	0.279	0.443	−0.194
	K00363(*nirD*)	0.496	−0.161	0.409	0.107	−0.487	−0.069	−0.296	−0.112
	K00374(*narL*)	0.505	0.208	0.895**	0.883**	0.296	0.296	0.4	−0.155
	K03385(*nrfA*)	−0.659	0.059	−0.541	−0.38	0.15	−0.233	−0.266	0.081
	K15876(*nrfH*)	−0.675*	0.03	−0.425	−0.177	0.179	−0.24	−0.041	0.091
氮同化还原 Assimilatory nitrate reduction	K00372(*nasA*)	0.474	0.089	0.780*	0.856**	0.321	0.136	0.382	0.059
	K00367(*narB*)	0.37	−0.153	0.762*	0.757*	0.302	0.454	0.352	−0.312
	K00360(*nasB*)	0.661	0.123	0.922**	0.721*	0.097	0.139	0.241	0.105
反硝化作用 Denitrification	K00366(*nirA*)	−0.183	0.407	−0.002	0.199	0.647	0.135	0.413	0.262
	K00368(*nirK*)	0.167	−0.091	0.624	0.818**	0.345	0.293	0.43	−0.295
	K04561(*norB*)	0.395	0.006	0.433	0.337	0.311	0.409	−0.151	−0.258
	K02305(*norC*)	0.139	0.256	−0.049	−0.023	0.587	0.34	0.056	0.047
	K00370(*narG*)	0.417	0.224	0.825**	0.903**	0.39	0.339	0.477	−0.235
	K00371(*narH*)	0.274	0.04	0.744*	0.878**	0.259	0.221	0.409	−0.285
	K02567(*napA*)	0.081	−0.22	0.107	0.257	0.262	0.053	0.088	0.023
	K02568(*napB*)	0.026	−0.324	0.096	0.226	0.166	0.028	0.027	−0.048
	K00376(*nosZ*)	0.105	0.005	0.465	0.553	0.503	0.4	0.218	−0.193
	K15864(*nirS*)	0.076	−0.257	−0.293	−0.364	−0.065	−0.326	−0.349	0.487
固氮作用 Nitrogen fixation	K02586(*nifD*)	−0.655	−0.329	−0.624	−0.496	0.024	−0.049	−0.341	−0.161
	K02588(*nifH*)	−0.648	−0.362	−0.604	−0.496	0.002	−0.054	−0.339	−0.155
	K00531(*anfG*)	−0.404	−0.382	−0.233	0.128	−0.129	0.118	0.375	−0.602
	K02591(*nifK*)	−0.648	−0.331	−0.614	−0.486	0.031	−0.046	−0.334	−0.157
硝化作用 Nitrification	K10535(*hao*)	0.611	0.297	0.595	0.713*	0.526	0.202	0.33	0.192
	K10944(*pmoA-amoA*)	0.456	0.343	0.504	0.692*	0.594	0.228	0.334	0.109
	K10945(*pmoB-amoB*)	0.445	0.333	0.513	0.709*	0.607	0.267	0.352	0.056
	K10946(*pmoC-amoC*)	0.407	0.201	0.566	0.785*	0.508	0.238	0.38	−0.038

中更为强烈。

(2)小麦和玉米土壤细菌的氮异化还原和氮同化还原潜力最高,反硝化潜力和固氮潜力次之,硝化潜力最弱。其中,氮异化还原过程中*nirD*和*nirB*基因,氮同化还原过程中*nasA*基因,反硝化过程中*nirK*、*narH*和*narG*基因相对丰富。

(3)土壤细菌氮循环功能基因受轮作体系影响,SOM和TN促进小麦土壤氮循环过程,而NH_4^+-N抑制氮循环过程;TN和TP在玉米土壤细菌氮循环过程中发挥积极作用。

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