



改良剂对酸性植烟土壤固氮菌群落结构和丰度的影响

赵辉¹, 王喜英¹, 谭智勇¹, 刘国权², 刘杰², 艾永峰²

(¹铜仁学院, 贵州铜仁 554300; ²贵州省烟草公司铜仁市公司, 贵州铜仁 554300)

摘要:【目的】研究不同改良剂对酸性植烟土壤固氮菌群落和丰度的影响,从固氮微生物角度为改良剂的筛选及其推广应用提供科学依据。【方法】采用田间单因素随机试验,设4个改良剂处理,分别为硅钙钾镁(T1)、白云石粉(T2)、硅钙钾镁+生物炭(T3)和白云石粉+生物炭(T4),以不施用改良剂为对照(CK)。烟叶旺长期进行烟株农艺性状调查并采集根际土壤样品,以*nifH*基因作为分子标记,应用荧光定量PCR和高通量测序技术,研究不同处理的土壤固氮菌丰度和群落结构变化特征,并分析土壤固氮菌群落结构变化的主要驱动因素。【结果】施用不同改良剂普遍提高了烟株农艺性状及土壤pH、有机碳含量和C/N。施用改良剂可显著提高土壤固氮菌*nifH*基因丰度($P<0.05$,下同),T1、T2、T3和T4处理分别较CK提高2.97、3.32、4.68和3.81倍。施用改良剂也提高了土壤固氮菌群落 α 多样性,且Chao1、ACE、Shannon和Simpson指数均以T3处理最高。相关分析结果表明,固氮菌丰度、Chao1和ACE指数与土壤pH呈显著正相关。在门水平上,共获得5个类群,其中放线菌门(Actinobacteria)、蓝藻门(Cyanobacteria)和厚壁菌门(Firmicutes)为优势类群。改良剂施用对固氮菌群落结构有显著影响,优势门和属发生变化,硅钙钾镁+生物炭处理显著增加放线菌门、蓝藻门、慢生根瘤菌属(*Bradyrhizobium*)和固氮螺菌属(*Azospirillum*)相对丰度。冗余分析结果表明,土壤pH、有机碳、硝态氮和C/N是驱动固氮菌群落结构变化的主要因素。【结论】硅钙钾镁+生物炭混施处理对缓解土壤酸化、改善烟田环境、促进烟株生长及提高固氮菌*nifH*基因丰度、群落 α 多样性和优势类群相对丰度效果显著,适合在酸性植烟土壤中推广应用。

关键词: 改良剂; 植烟土壤; 固氮微生物; *nifH*基因丰度; 群落结构

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Effects of amendment application on structure and abundance of nitrogen-fixing microbial community in acidic tobacco-planting soil

ZHAO Hui¹, WANG Xi-ying¹, TAN Zhi-yong¹, LIU Guo-quan², LIU Jie², AI Yong-feng²

(¹Tongren College, Tongren, Guizhou 554300, China; ²Tongren Branch of Guizhou Tobacco Company, Tongren, Guizhou 554300, China)

Abstract:【Objective】To study the abundance and structure of nitrogen-fixing microbial community in different amendment application in acidic tobacco-planting soils, and to provide a scientific basis for selecting the amendment and applied in flue-cured tobacco production from a microbiological point of view. 【Method】Open-field random test involving a single factor was performed. Four soil conditioner treatments were set, namely, silicon-calcium-potassium-magnesium(T1), dolomite (T2), silicon-calcium-potassium-magnesium and biochar (T3), dolomite and biochar (T4). The treatment without soil conditioner was the control (CK). Agronomic traits were investigated during the exuberant growing period, and rhizosphere soil samples were collected. The fluorogenic quantitative real-time PCR technique was adopted in the experiments using the high-throughput sequencing platform to target the *nifH* gene. The abundance, nitrogen-fixing microbial community composition in acidic tobacco-planting soils werestudied under different treatments. The driving factors for structural changes in the diazotrophic communities of the soil were identified. 【Result】The application of different soil conditioners generally improved the agronomic traits of tobacco plants and soil pH value, organic carbon content and C/N

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第一作者: 赵辉(1983-), <https://orcid.org/0000-0002-9737-9613>, 博士, 教授, 主要从土壤微生物生态研究工作, E-mail: yancao504@163.com

ratio. Soil conditioners significantly improved the *nifH* gene abundance in soil nitrogen-fixing bacteria ($P < 0.05$, the same below). T1, T2, T3 and T4 treatments achieved 2.97, 3.32, 4.68 and 3.81 times increase compared with CK, respectively. Soil conditioners also increased the α -diversity index of the soil nitrogen-fixing bacterial community. Of all treatments, T3 had the highest Chao1, ACE, Shannon and Simpson indexes. The results of correlation analysis showed that the abundance of nitrogen-fixing microbial community, Chao1 and ACE indexes were significant positively correlated with soil pH. On the phylum level, five phyla were identified. Proteobacteria, Cyanobacteria, and Firmicutes were dominant phyla. In contrast, the amendment addition altered the nitrogen-fixing microbial community structure with shifts in the phyla and dominant genus, with significantly higher Proteobacteria, Cyanobacteria, *Bradyrhizobium* and *Azospirillum* in the silicon-calcium-potassium-magnesium and biochar. Soil pH, organic carbon, nitrate nitrogen and C/N were the key factor correlated with change in nitrogen-fixing microbial community structure. The results from redundancy analysis showed that soil pH, organic carbon, nitrate nitrogen and C/N were the key factors correlated with change in nitrogen-fixing microbial community structure. 【Conclusion】Mixed application of silicon-calcium-potassium-magnesium and biochar has multiple effects; alleviating soil acidification, improving tobacco field environment, promoting tobacco plant growth, and increasing *nifH* gene abundance, α -diversity index, and relative abundance of dominant species in soil nitrogen-fixing bacteria. This fertilizer combination is worthy of wider application in acidic tobacco-growing soils.

Key words: amendments; tobacco-planting soil; nitrogen-fixing microbe; *nifH* gene abundance; community structure

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0 引言

【研究意义】烤烟作为我国重要的经济作物之一,种植面积和产量均居世界第一(Niu et al., 2017)。近年来,随着烤烟长期连作和化肥过量施用导致土壤质量退化严重,表现为土壤酸化、污染物积累和生物多样性减少,烟叶产质量下降(李春英等, 2001; Zhang et al., 2016)。目前,土壤酸化已成为烟叶生产中不可忽视的一个问题,严重制约着烟草产业的可持续发展。土壤氮在满足植物生长、降低化肥使用量、减少土壤酸化和改善土壤质量方面发挥着重要作用。大气中的氮在固氮微生物的固氮酶作用下可转化为被植物吸收利用的含氮化合物进入生态系统。由于固氮微生物群落和多样性对环境变化敏感,被广泛用作衡量土壤质量变化的重要指标(Wang et al., 2017a)。研究表明,土壤改良剂通过直接影响土壤理化性质,可间接影响土壤固氮菌群落组成和丰度变化。因此,研究不同改良剂对固氮菌群落结构和丰度的影响,可从固氮微生物角度为酸性植烟土壤调节剂的筛选、评价及其推广应用提供参考。【前人研究进展】施用改良剂是调节土壤酸化常用的农艺措施。目前,普遍采用石灰材料(石灰、方解石和白云石)作为酸化土壤改良剂(Kunhikrishnan et al., 2016)。欧阳延生等(2007)研究表明,与对照相比,施用石灰有利于提高紫花苜蓿根瘤数量。然而,长期或大量施用石灰可引起土壤板结,造成土壤养分失衡,导致烟叶产质量下降(李春英等, 2001)。相关研究认为,硅钙钾镁肥作为碱性肥料,可有效缓解土壤酸化,提高土壤养分,促进烟草生长

发育,也可克服石灰改良土壤存在的不足(马存金, 2020)。白云石粉也具有改善土壤理化性质和提高烟叶产质量的作用,目前,在我国南方烟区普遍采用白云石粉作为改良剂对酸性土壤进行改良(张国等, 2014)。此外,生物炭是一种在缺氧环境通过生物质的热化学转化获得的固体材料,结构稳定,具有较大的比表面积,营养丰富,可减少土壤养分流失,为微生物提供良好的环境条件,提高作物养分利用效率(Gao et al., 2017)。Quilliam等(2013)研究发现,生物炭添加可提高土壤固氮酶活性和固氮微生物数量。生物炭的类型和用量也可对土壤固氮微生物数量和群落结构产生显著影响(孟颖等, 2014; Mia et al., 2014)。在烤烟生产中,生物炭被广泛用作土壤改良剂,在促进烤烟生长、提高土壤肥力、钝化重金属、改善微生物活性和抑制土传病害等方面发挥重要作用(Zhang et al., 2020)。固氮菌在细菌和古细菌分类群中高度保守,包括一些自由生活细菌、硫酸盐还原细菌和共生固氮菌(Xun et al., 2018)。固氮菌中*nifH*基因可对固氮酶的铁蛋白进行编码且高度保守,被广泛作为分子标记基因用于土壤固氮菌群落研究(Hu et al., 2018)。相关研究认为,土壤pH(Wang et al., 2017b)、养分有效性(Li et al., 2022)和C/N(Wang et al., 2018)等理化指标被视为驱动固氮菌群落结构变化的关键因素。综上,不同种类改良剂对土壤酸化改良效果不同,改良剂能通过直接或间接改变土壤理化性质而影响固氮菌数量和群落结构。【本研究切入点】目前,生物炭、硅钙钾镁和白云石粉单独作为改良剂对植烟土壤理化性质(Li et al.,

2022)、酶活性(武晓桐,2020)和烟叶产质量(杜倩等,2021)等方面的研究较多,然而关于生物炭分别与硅钙钾镁和白云石粉混施对植烟土壤固氮菌群落结构和丰度影响的研究尚无报道。【拟解决的关键问题】应用荧光定量PCR和高通量测序技术,研究不同改良剂处理下土壤固氮菌群落结构差异特征及其与环境因子的关系,为缓解烟田土壤酸化、提高土壤质量和筛选出适宜酸性植烟土壤的改良剂提供科学依据。

1 材料与方法

1.1 试验地概况及试验材料

试验于2021年在贵州省江口县狮子口村(108°32'58"E、27°48'32"N,海拔835 m)进行,试验地属典型中亚热带季风气候,年均降水量1340.5 mm,年均湿度80.73%,年均气温16.5℃,有效积温在5000℃以上,年均日照时数1090.7 h,无霜期353 d。供试土壤为黄壤。试验田土壤理化性质:pH 4.8、有机碳27.57 g/kg、全氮1.58 g/kg、全磷1.26 g/kg、全钾12.34 g/kg、速效钾472.8 mg/kg、速效磷112.77 mg/kg。供试改良剂:生物炭(水稻秸秆,比表面积22.5 m²/g、孔径29.4 nm、有机碳75%、氮含量1.4%~1.7%、pH 10.1)、硅钙镁钾(SiO₂≥25%、CaO≥25%、MgO≥12%、pH 8.5)、白云石粉(CaO≥31.86%、MgO≥22.02%、pH 8.8)。供试烤烟品种为云烟87。

1.2 试验方法

采用单因素随机试验设计。设4个改良剂处理,分别为硅钙钾镁(T1)、白云石粉(T2)、硅钙钾镁+生物炭(T3)和白云石粉+生物炭(T4),以不施用改良剂为对照(CK)。每处理重复3次,行株距为1.00 m×0.55 m,每小区面积33 m²,区组设通道,四周设保护行2行。烟田施用专用基肥750 kg/ha、专用追肥225 kg/ha、专用提苗肥47.5 kg/ha,硅钙钾和白云石粉施用量分别为2001 kg/ha,生物炭施用量为4502 kg/ha,均作为基肥条施。烟苗于2021年4月28日采用井窖式技术移栽,其他栽培管理措施按井窖式优质烟叶栽培技术规程进行。

在烟叶旺长期(2021年6月30日)进行根际土壤样品采集和烟株农艺性状调查。每小区采用抖根法采集5株烟株根际新鲜土壤样品,混合形成单一样品。土壤样品过2 mm筛,去除石头和植物残留物。将每个样品分为3份:一份-20℃保存用于土壤DNA提取及高通量测序;一份4℃保存用于铵态氮和硝态氮含量测定;一份风干用于土壤理化性质分析。每处理的每个小区中对采集根际土壤的5株烟株进

行农艺性状调查,取5株平均值为1次重复数据,3次重复。

1.3 测定项目及方法

1.3.1 烟株农艺性状测定 依据烟草行业标准YC/T 142—2010《烟草农艺性状调查测量方法》,采用卷尺和游标卡尺分别测量烟株的株高、茎围、叶长和叶宽等指标。

1.3.2 土壤化学性质测定 土壤化学指标测定参考鲍士旦(2000)的方法。土壤pH采用电位法测定,有机碳含量采用重铬酸钾氧化法测定,全氮含量采用凯氏定氮法测定,铵态氮含量采用靛酚蓝比色法测定,硝态氮含量采用酚二磺酸比色法测定。

1.3.3 土壤固氮菌DNA提取、荧光定量PCR及高通量测序 按照DNA提取试剂盒从0.5 g土壤样品中提取DNA,采用1%琼脂糖凝胶电泳和核酸定量仪对DNA纯度和浓度进行检测。利用荧光定量PCR技术对固氮菌丰度进行分析,扩增引物:*nifH-F*(5'-AAAGGYGGWATCGGYAARTCCACCAC-3')与*nifH-R*(5'-TTGTTSGCSGCRATATSGCCATCAT-3')(Rösch et al.,2002)。每个PCR扩增样品重复3次,通过Minipre Kit获得样品质粒,根据样品质粒的标准曲线,分别计算基因拷贝数。PCR扩增效率为94%,R²为0.99。

采用2%琼脂糖凝胶电泳检测PCR产物,扩增子在Illumina MiSeq平台进行测序。对原始数据进行质量控制,采用Usearch进行序列分析。具有≥97%相似性的序列被分配到相同的操作分类单元(OTU)。同时对OTU代表序列进行分类注释。采用QIIME对固氮菌群落 α 多样性指数进行分析。

1.4 统计分析

采用SPSS 22.0对烟株农艺性状、土壤化学性质、固氮菌数量和优势类群相对丰度进行方差分析和显著性检验($P<0.05$)。使用R软件进行土壤固氮菌群落主坐标分析(Principal coordinates analysis, PCoA)和冗余分析(Redundancy analysis, RDA)。

2 结果与分析

2.1 不同改良剂对烟株农艺性状的影响

由表1可知,施用改良剂对烟株的株高、茎围、叶长、叶宽和叶片数均有明显影响。与CK相比,各改良剂处理的株高显著增加($P<0.05$,下同),表现为T3>T4>T1>T2>CK;T3和T4处理的茎围和叶长也显著增加,茎围分别较CK增加16.17%和12.52%,叶长分别增加14.57%和10.12%。T3处理的各农艺性状指标均为最大值。

表 1 不同改良剂处理的烟株农艺性状

Table 1 Tobacco agronomic traits under different soil amendments

处理 Treatment	株高(cm) Plant height	茎围(cm) Stem girth	叶长(cm) Leaf length	叶宽(cm) Leaf width	叶片数 Leaf number
CK	76.60±11.10b	7.48±1.32b	58.30±2.11b	25.80±0.83b	17.00±0.84b
T1	86.80±3.56a	8.29±1.37ab	62.00±4.42ab	27.20±2.28ab	17.00±0.56b
T2	86.20±3.27a	8.28±0.91ab	62.40±3.78ab	26.20±1.92b	18.00±1.12b
T3	91.60±1.82a	8.69±0.60a	65.60±3.65a	29.20±0.89a	19.00±1.03a
T4	89.00±3.39a	8.57±1.03a	64.20±3.56a	27.80±1.48ab	18.00±0.84b

同列数据后不同小写字母表示差异显著($P<0.05$)。表2~表4同

Different lowercase letters in the same column represented significant difference($P<0.05$). The same was applied in Table 2-Table 4

2.2 不同改良剂对植烟土壤化学性质的影响

由表2可知,施用改良剂对植烟土壤全氮含量无显著影响($P>0.05$,下同),但对其他土壤化学指标产生不同程度的影响。施用改良剂后土壤pH显著增加;有机碳含量以T3处理最高,T4处理其次,二者差异不显著,但均显著高于CK和T1处理;铵态

氮含量以T1处理最高,显著高于其他处理,但T2、T3、T4处理与CK间均无显著差异;施用改良剂导致土壤硝态氮含量显著降低,T1、T2、T3和T4处理分别较CK下降44.17%、54.97%、65.67%和68.69%;改良剂施用后土壤C/N提高,T4处理C/N最高,与CK间差异显著。

表 2 不同改良剂处理的土壤化学性质

Table 2 Chemical properties in flue-cured tobacco under different soil amendments

处理 Treatment	pH	有机碳含量(g/kg) Organic carbon content	全氮含量(g/kg) Total nitrogen content	铵态氮含量(mg/kg) Ammonium nitrogen content	硝态氮含量(mg/kg) Nitrate nitrogen content	C/N
CK	4.65±0.14c	15.99±0.21c	1.58±0.09	0.34±0.05b	31.11±1.98a	10.13±0.49b
T1	5.32±0.27b	16.61±0.15bc	1.57±0.04	0.43±0.04a	17.37±1.94b	10.53±0.35ab
T2	6.11±0.28a	17.21±0.75abc	1.64±0.07	0.34±0.03b	14.01±1.80c	10.49±0.68ab
T3	5.78±0.55ab	18.84±1.93a	1.63±0.06	0.29±0.05b	10.68±1.11d	11.51±1.22ab
T4	6.05±0.40a	18.34±0.44ab	1.57±0.05	0.35±0.06b	9.74±0.65d	11.65±0.65a

2.3 不同改良剂对植烟土壤固氮菌 *nifH* 基因丰度的影响

由图1可知,不同处理的固氮菌 *nifH* 基因丰度范围为 $0.35 \times 10^7 \sim 1.64 \times 10^7$ 拷贝数/g干土。改良剂施用均显著提高了固氮菌丰度,其中T1、T2、T3和T4处理分别较CK显著提高2.97、3.32、4.68和3.81倍。T3处理的固氮菌丰度最高,分别较T1和T2处理显著提高57.69%和40.81%,与T4处理差异不显著。由此可知,改良剂施用对植烟土壤固氮菌数量有促进作用,但不同改良剂之间有明显差异。

2.4 不同改良剂对植烟土壤固氮菌群落 α 多样性指数的影响

由表3可知,施用改良剂均提高了土壤固氮菌群落 α 多样性指数。Chao1和ACE指数在各处理中变化趋势一致,均表现为T3>T4>T2>T1>CK。与CK相比,T1、T2、T3和T4处理的Chao1指数分别显著提高41.31%、57.24%、66.3%和60.4%,ACE指数分别显著提高44.35%、58.82%、68.93%和60.4%,T1、T3和T4处理的Shannon和Simpson指数也均显著升高。由此可知,改良剂施用对土壤固氮菌丰富度和多样性有显著影响,其中以T3(硅钙钾镁+生物炭)处理影响效果最明显。

2.5 不同指标的相关分析结果

为明确土壤化学性质和烟株农艺性状对固氮菌数量的影响,进一步对固氮菌 *nifH* 基因丰度、群落 α 多样性指数与土壤化学性质和农艺性状进行相关分析,结果(图2)表明, *nifH* 基因丰度与土壤pH、有机碳、叶长和叶宽呈极显著正相关($P<0.01$,下同),与硝态氮含量呈极显著负相关,与C/N、株高和叶片数呈显著正相关。土壤固氮菌Chao1和ACE指数分别与土壤pH、有机碳含量呈极显著正相关,与硝态氮含量呈极显著负相关,与C/N、茎围、叶长和叶片数呈显著正相关;Chao1指数与叶宽呈极显著正相

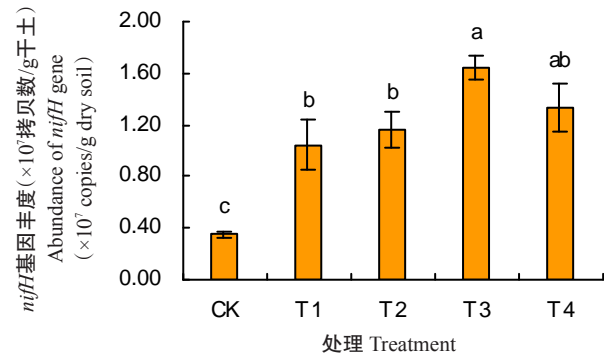


图 1 不同改良剂处理的 *nifH* 基因丰度

Fig.1 Abundance of *nifH* gene under different soil amendments
图柱上不同小写字母表示差异显著($P<0.05$)

Different lowercase letters on the bar represented significant difference ($P<0.05$)

表 3 不同改良剂处理土壤固氮菌群落α多样性指数

Table 3 α diversity index of the nitrogen-fixing microbial community with different soil amendments

处理 Treatment	Chao1 指数 Chao1 index	ACE 指数 ACE index	Shannon 指数 Shannon index	Simpson 指数 Simpson index
CK	921.25±95.58c	658.37±81.95c	6.07±0.26b	0.94±0.02b
T1	1301.79±174.72b	950.37±141.59b	6.81±0.38a	0.97±0.01a
T2	1448.54±39.82ab	1045.6±27.51ab	6.58±0.54ab	0.96±0.02ab
T3	1532.04±70.36a	1112.16±15.20a	7.04±0.15a	0.98±0.01a
T4	1477.68±102.54ab	1082.57±47.96ab	6.89±0.25a	0.97±0.01a

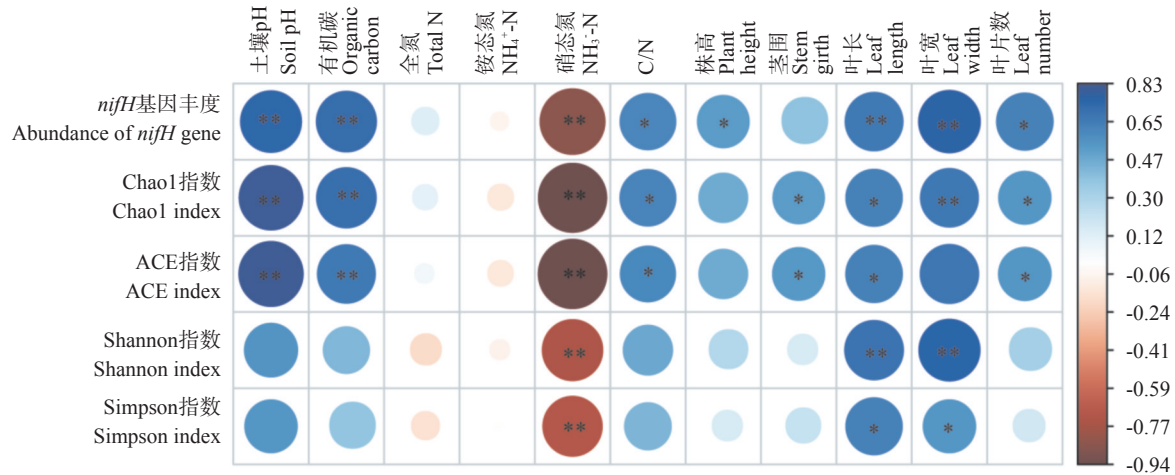


图 2 固氮菌群落α多样性指数、*nifH*基因丰度与土壤化学性质和农艺性状的相关性

Fig.2 Correlation between nitrogen-fixing microbial community α diversity index, *nifH* gene abundance and soil chemical properties, tobacco agronomic traits

*和**分别表示显著相关($P<0.05$)和极显著相关($P<0.01$)

* indicated significant correlation($P<0.05$) and ** indicated extremely significant correlation($P<0.01$)

关;Shannon和Simpson指数与硝态氮含量呈极显著负相关;Shannon指数分别与叶长和叶宽呈极显著正相关;Simpson指数与叶长和叶宽呈显著正相关。

2.6 不同改良剂对植烟土壤固氮菌群落组成的影响

通过对不同处理土壤样品OTUs进行归类,得到5个门、13个纲、24个目、43个科和52个属。在门水平上,分别为变形菌门(Proteobacteria)、蓝藻门(Cyanobacteria)、厚壁菌门(Firmicutes)、拟杆菌门(Bacte-

roidetes)和放线菌门(Actinobacteria)(图3-A)。其中变形菌门、蓝藻门、厚壁菌门和拟杆菌门在不同处理间有极差异显著。放线菌门(61.72%~75.55%)、蓝藻门(8.24%~18.1%)和厚壁菌门(2.42%~14.45%)为优势类群,占固氮菌总类群相对丰度的84.41%~96.51%。在属水平上,得到相对丰度在1%以上的15个类群(图3-B),其中慢生根瘤菌属(*Bradyrhizobium*)、固氮螺菌属(*Azospirillum*)、地杆菌属(*Geobacter*)、念珠蓝

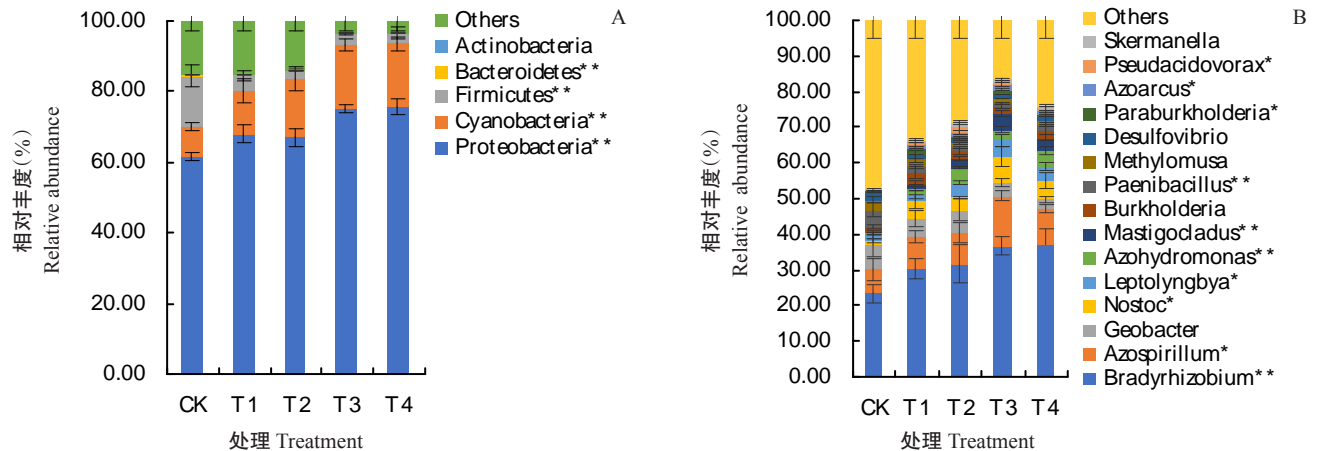


图 3 不同改良剂处理土壤固氮菌门(A)和属(B)水平组成

Fig.3 Nitrogen-fixing microbial community composition at the phylum(A) and genus(B) levels with different soil amendments

*和**分别表示该菌门(属)在不同处理间差异显著($P<0.05$)或极显著($P<0.01$)

* represented significant difference among treatments at phylum(genus) level ($P<0.05$), and ** represented extremely significant difference($P<0.01$)

菌属 (*Nostoc*) 和瘦鞘丝藻属 (*Leptolyngbya*) 为优势类群, 相对丰度分别为23.34%~37.23%、6.73%~14.08%、2.75%~6.76%、1.48%~7.06%和1.45%~4.39%。

由表4可知, T1、T2、T3和T4处理的放线菌门和蓝藻门相对丰度显著提高, 而厚壁菌门相对丰度显著降低, 其中T2和T4处理降幅最大。施用改良剂显

著提高了慢生根瘤菌属相对丰度, 固氮螺菌属、念珠蓝菌属和瘦鞘丝藻属相对丰度也有不同程度的升高, 且均以T3处理为最大值, 与CK差异显著; 而地杆菌属相对丰度在各处理中差异不显著。由此可知, 改良剂对土壤固氮菌门和属优势类群有显著影响, 但不同改良剂处理间差异明显。

表 4 不同改良剂处理土壤固氮菌门和属水平优势类群的相对丰度

Table 4 Relative abundance of dominant nitrogen-fixing microbial at the phylum and genus levels with different soil amendments

处理 Treatment	菌门 Phylum			菌属 Genus				
	Proteobacteria	Cyanobacteria	Firmicutes	<i>Bradyrhizobium</i>	<i>Azospirillum</i>	<i>Geobacter</i>	<i>Nostoc</i>	<i>Leptolyngbya</i>
CK	61.72±1.28c	8.24±1.08c	14.45±3.22a	23.34±2.37b	6.73±3.25b	6.76±2.93	1.47±0.64b	1.45±0.74b
T1	68.07±2.74b	12.35±3.33b	4.08±1.56b	30.41±2.80a	8.92±1.81b	5.06±2.01	4.81±1.81ab	1.83±0.96b
T2	67.16±2.58b	16.24±3.43ab	2.42±0.20b	31.71±5.23a	8.89±2.92b	6.21±3.21	3.63±0.31ab	3.97±0.52a
T3	75.26±1.08a	18.1±1.72a	3.12±0.38b	36.62±2.49a	14.08±1.52a	3.97±1.02	7.06±2.78a	4.39±1.63a
T4	75.55±2.31a	17.98±1.74a	2.98±1.60b	37.23±4.16a	9.89±1.31b	2.75±0.94	5.04±2.34ab	4.12±1.16a

2.7 固氮菌群落结构及其影响因子分析

由图4固氮菌群落结构的主坐标分析(PCoA)可知, 前2个主坐标累积解释群落结构总变异量的36.8%。其中, CK的固氮菌群落与其他处理明显分离; T1、T2、T3和T4处理聚集在一起。表明施用改良剂显著改变了烟田土壤固氮菌群落结构。固氮菌群落结构与土壤化学性质和烟株农艺性状的冗余分析(RDA)结果(图5)表明, RDA1和RDA2分别解释固氮菌群落变异的64.65%和6.77%, 共解释总变异的71.42%。土壤pH、有机碳、硝态氮和叶片数对土壤固氮菌群落结构有极显著影响, C/N、株高、叶长和叶宽对土壤固氮菌群落结构有显著影响。

3 讨论

3.1 不同改良剂处理对土壤化学性质的影响

土壤酸化对土壤微生物活性及有机质分解起重要作用, 酸性过低导致土壤养分流失加快和肥力

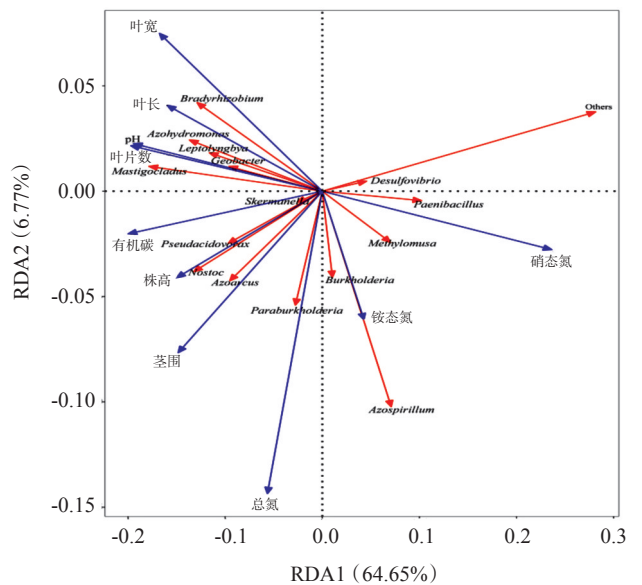


图 5 土壤固氮菌群落RDA分析结果
Fig.5 RDA analysis of the soil nitrogen-fixing microbial community

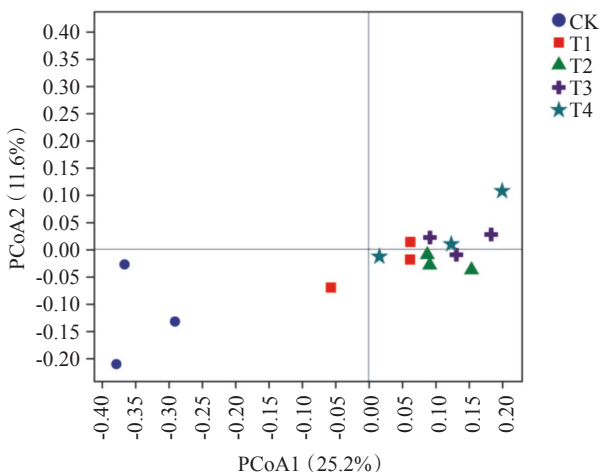


图 4 土壤固氮菌群落PCoA分析结果
Fig.4 PCoA analysis of the soil nitrogen-fixing microbial community

下降, 影响烟株生长发育(杜倩等, 2021)。在酸性植烟土壤中, 施用白云石粉、硅钙钾镁和生物炭均可提高土壤pH(张国等, 2014; 杜倩等, 2021)。本研究结果也表明, 施用不同改良剂均可显著提高植烟土壤pH, 其中, 白云石粉、白云石粉+生物炭、硅钙钾镁+生物炭使土壤pH提高到适合优质烟叶生长的5.5~6.5范围(邓小华等, 2021)。白云石粉和白云石粉+生物炭对土壤pH提高效果明显, 可能是因为白云石粉本身pH较高, 对土壤pH提高有较大影响, 与李昱等(2006)研究得出施用白云石粉可显著提高土壤pH和改善烟叶品质的结果相似, 但与姜超强等(2015)研究认为白云石粉对提高土壤pH作用较弱、效果不显著的研究结果不同, 产生差异的原因可能与土壤质地、改良剂类型及添加量、成土因素和气候

条件等有关。改良剂施用均提高了土壤有机碳含量,且T3和T4处理对有机碳提高作用高于其他处理,可能与含有生物炭有关,有利于提高土壤碳含量(Zhang et al., 2016)。改良剂施用后,土壤硝态氮含量均有所下降,T3和T4处理下降最明显,可能与土壤pH急剧上升有关,导致土壤氨氧化微生物数量减少,对硝化过程有抑制作用(Wang et al., 2017c);同时,生物炭混施也有利于促进烟株生长,增强烟株对硝态氮的吸收。

3.2 植烟土壤固氮菌丰度和群落结构及其与环境因子的关系

土壤pH、有机碳、植物及其根系分泌物等均会影响固氮菌数量(杨亚东等, 2017; 王磊等, 2020)。Wang等(2017b)研究认为,土壤酸化对微生物生长和代谢有显著影响,可抑制微生物活性,降低微生物固氮能力。本研究发现,施用不同改良剂均显著提高土壤固氮菌*nifH*基因丰度,通过提高土壤pH和改善土壤养分,促进固氮微生物生长和繁殖。土壤pH与*nifH*基因丰度呈正相关,与Pereira e Silva等(2011)的研究结果一致。生物固氮除受土壤pH和有效氮等指标影响外,能量来源也是其中一个重要限制因素(DeLuca et al., 2002)。Liu等(2019)研究表明,施用生物炭可提高土壤固氮菌数量。T3和T4处理土壤固氮菌数量较高,可能与生物炭混施土壤有机碳含量增加有关,可为固氮微生物生长提供充足碳源。固氮菌数量与有机碳呈正相关,与Wang等(2018)研究结果一致。碳利用率对氮固定有显著影响,而碳利用率增加通常会提高生物氮固定率(Chen et al., 2019)。生物炭混施后,生物炭中不稳定有机碳可被土壤固氮微生物直接利用;同时,还可促进植物生长,通过根系分泌物进一步降低有机碳稳定性(Liu et al., 2019)。高雪峰和韩国栋(2021)研究认为,植物长势好,根系分泌物多,有利于土壤微生物数量增加。硅钙钾镁+生物炭混施处理的烟株农艺性状(株高、茎围、叶长、叶宽和叶片数)显著高于对照,可能是烟株通过根系分泌物为固氮菌生长提供较多养分供应。

本研究中,土壤固氮菌群落在门水平上以变形菌门、蓝藻门、厚壁菌门为主,与李旭等(2021)的研究结果相似。变形菌门的相对丰度在一定程度上可反映土壤有机质等养分含量,且在pH较高土壤中生长较好(Zhang et al., 2014)。变形菌门相对丰度以T3和T4处理最高,可能与土壤有机碳含量及pH较高有关。蓝藻门为水生环境中优势类群,且偏好于湿润环境(Zehr et al., 2003)。蓝藻门相对丰度以T3和T4处理较高,可能与生物炭混施和取样时期有关。生物炭施用可显著提高土壤保水能力(吴愉萍等,

2019),且本研究取样期降雨较多,有利于蓝藻门固氮菌生长繁殖。厚壁菌门可生活在极端环境,通过分泌有害物质,干扰植物氮代谢,抑制植物正常生长(Karelová et al., 2011)。本研究中,对照处理厚壁菌门相对丰度显著高于其他处理,可能与土壤酸化和烟株生长缓慢有关。在属水平上,慢生根瘤菌属相对丰度高于其他类群,可能是由于慢生根瘤菌可排泄碱性化合物,在酸性环境中比其他类群具有竞争优势(Fujihara, 2008)。施用改良剂均显著提高了慢生根瘤菌相对丰度,生物炭混施处理慢生根瘤菌相对丰度最高,与Liu等(2019)生物炭单施的研究结果不一致,说明生物炭混施和单施对其有差异影响。本研究结果表明,随硝态氮含量增加,慢生根瘤菌属相对丰度逐渐降低,与Favero等(2022)的研究结果一致。固氮螺菌属具有促进植物生长的作用(Steenhoudt and Vanderleyden, 2000; Veresoglou and Menexes, 2010)。T3处理的固氮螺菌属相对丰度显著高于其他处理,推测T3处理烟株生长较快可能与该菌群有关。地杆菌属在土壤厌氧环境分布广泛,可驱动铁锰等金属矿物生物还原,对部分芳香烃化学物有降解作用,具有较强的生态适应性(田丽丽和王鑫, 2020)。T3和T4处理地杆菌属相对丰度低于其他处理,由于生物炭具有改善土壤通透性的作用(王宇龙, 2021),在一定程度上抑制地杆菌生长,进一步验证了地杆菌生长倾向于厌氧环境。

4 结论

改良剂可通过调节土壤pH,驱动土壤性质变化从而引起固氮菌群落结构发生变化。其中,硅钙钾镁+生物炭混施处理对缓解土壤酸化、改善烟田环境、促进烟株生长及提高固氮菌*nifH*基因丰度、群落α多样性和优势类群相对丰度效果显著,适合在酸性植烟土壤中推广应用。

参考文献:

- 鲍士旦. 2000. 土壤农化分析[M]. 第3版. 北京:中国农业出版社. [Bao S D. 2000. Analysis of soil agrochemicals[M]. The 3rd Edition. Beijing: China Agriculture Press.]
- 邓小华,何铭钰,陈金,彭曙光,刘勇军,王振华,彭德元,符昌武,邓永晟,粟戈璇,夏振. 2021. 山地酸性土壤耕层重构的理化性状及酶活性动态变化[J]. 中国烟草科学, 42(4): 17-23. [Deng X H, He M Y, Chen J, Peng S G, Liu Y J, Wang Z H, Peng D Y, Fu C W, Deng Y S, Su G X, Xia Z. 2021. Dynamic of soil physico-chemical properties and enzymatic activities after restructured arable layer of mountainous acidic soil[J]. Chinese Tobacco Science, 42(4): 17-23.] doi: 10.13496/j.issn.1007-5119.2021.04.003.
- 杜倩,黄容,李冰,王昌全,文登鸿,谢云波,陈玉蓝,冯浪. 2021. 生物炭还田对植烟土壤活性有机碳及酶活性的影

- 响[J]. 核农学报, 35(6): 1440-1450. [Du Q, Huang R, Li B, Wang C Q, Wen D H, Xie Y B, Chen Y L, Feng L. 2021. Effect of biochar returning on labile organic carbon and enzyme activity in tobacco-growing soil[J]. Journal of Nuclear Agricultural Sciences, 35(6): 1440-1450.] doi: 10.11869/j.issn.100-8551.2021.06.1440.
- 高雪峰, 韩国栋. 2021. 短花针茅根系分泌物对荒漠草原土壤细菌群落及土壤养分的影响[J]. 中国草地学报, 43(6): 76-84. [Gao X F, Han G D. 2021. Effects of *Stipa breviflora* root exudates on soil bacterial community and soil nutrients in desert steppe[J]. Chinese Journal of Grassland, 43(6): 76-84.] doi: 10.16742/j.zgdx.20190310.
- 姜超强, 董建江, 徐经年, 沈嘉, 薛宝燕, 祖朝龙. 2015. 改良剂对土壤酸碱度和烤烟生长及烟叶中重金属含量的影响[J]. 土壤, 47(1): 171-176. [Jiang C Q, Dong J J, Xu J N, Shen J, Xue B Y, Zu C L. 2015. Effects of soil amendment on soil pH, plant growth and heavy metal accumulation of flue-cured tobacco in acid soil[J]. Soils, 47(1): 171-176.] doi: 10.13758/j.cnki.tr.2015.01.027.
- 李春英, 刘添毅, 刘奕平, 熊德中, 邢世和, 唐莉娜. 2001. 酸性植烟土壤施用石灰的后效探讨[J]. 烟草科技, (7): 38-41. [Li C Y, Liu T Y, Liu Y P, Xiong D Z, Xing S H, Tang L N. 2001. Discussion on after effects of applying lime in acidic tobacco planting soil[J]. Tobacco Science & Technology, (7): 38-41.] doi: 10.3969/j.issn.1002-0861.2001.07.018.
- 李旭, 董炜灵, 宋阿琳, 李艳玲, 卢玉秋, 王恩召, 刘雄舵, 王萌, 范分良. 2021. 秸秆添加量对土壤生物固氮速率和固氮菌群落特征的影响[J]. 中国农业科学, 54(5): 980-991. [Li X, Dong W L, Song A L, Li Y L, Lu Y Q, Wang E Z, Liu X T, Wang M, Fan F L. 2021. Effects of straw addition on soil biological N₂-fixation rate and diazotroph community properties[J]. Scientia Agricultura Sinica, 54(5): 980-991.] doi: 10.3864/j.issn.0578-1752.2021.05.010.
- 李昱, 何春梅, 林新坚. 2006. 施用沸石、白云石对植烟土壤及烟叶品质的影响[J]. 烟草科技, (4): 50-54. [Li Y, He C M, Lin X J. 2006. Effects of zeolite and solomite application on tobacco growing soil and tobacco leaf quality[J]. Tobacco Science & Technology, (4): 50-54.] doi: 10.3969/j.issn.1002-0861.2006.04.014.
- 马存金. 2020. 硅钙钾镁肥不同用量对酸性土壤pH值及烟草根系发育的影响[J]. 江苏农业科学, 48(19): 83-86. [Ma C J. 2020. Effects of different dosages of silicon-calcium-potassium-magnesium fertilizers on acid soil pH and tobacco root development[J]. Jiangsu Agricultural Sciences, 48(19): 83-86.] doi: 10.15889/j.issn.1002-1302.2020.19.018.
- 孟颖, 王宏燕, 于崧, 刘志华, 赵成森, 陶月. 2014. 生物黑炭对玉米苗期根际土壤氮素形态及相关微生物的影响[J]. 中国生态农业学报, 22(3): 270-276. [Meng Y, Wang H Y, Yu S, Liu Z H, Zhao C S, Tao Y. 2014. Effect of biochar on nitrogen forms and related microorganisms of rhizosphere soil of seedling maize[J]. Chinese Journal of Eco-Agriculture, 22(3): 270-276.] doi: 10.3724/SP.J.1011.2014.30750.
- 欧阳延生, 徐桂花, 于徐根, 李翔宏, 叶德胜, 刘水华. 2007. 施用根瘤菌及石灰对紫花苜蓿生长的影响[J]. 江西农业学报, 19(3): 29-30. [Ouyang Y S, Xu G H, Yu X G, Li X H, Ye D S, Liu S H. 2007. Effects of nodule bacteria and lime on growth of alfalfa[J]. Acta Agriculturae Jiangxi, 19(3): 29-30.] doi: 10.19386/j.cnki.jxnyxb.2007.03.010.
- 田丽丽, 王鑫. 2020. 地杆菌属微生物在厌氧污水处理中的应用潜力[J]. 给水排水, 46(S): 201-206. [Tian L L, Wang X. 2020. Potential applications of *Geobacter* in anaerobic wastewater treatment[J]. Water & Wastewater Engineering, 46(S): 201-206.] doi: 10.13789/j.cnki.wwe1964.2020.S2.030.
- 王磊, 王静, 张爱君, 张辉, 张永春. 2020. 小麦—甘薯轮作长期增施有机肥对碱性土壤固氮菌群落结构及多样性的影响[J]. 生态学报, 40(16): 5771-5782. [Wang L, Wang J, Zhang A J, Zhang H, Zhang Y C. 2020. Effects of long-term organic fertilization on soil diazotrophic community structure and diversity under wheat-sweet potato rotation system[J]. Acta Ecologica Sinica, 40(16): 5771-5782.] doi: 10.5846/stxb201907231551.
- 王宇龙. 2021. 减氮配施腐植酸-生物炭对土壤理化性质及玉米养分吸收的影响[D]. 哈尔滨: 东北农业大学. [Wang Y L. 2021. Effects of nitrogen reduction combined with humic acid and biochar on soil physical and chemical properties and nutrient absorption of maize[D]. Harbin: Northeast Agricultural University.] doi: 10.27010/d.cnki.gdbnu.2021.000544.
- 吴愉萍, 王明湖, 席杰君, 潘洁, 陈玲瑜, 连瑛, 孙辉, 周萍. 2019. 不同农业废弃物生物炭及施用量对土壤pH值和保水保氮能力的影响[J]. 中国土壤与肥料, (1): 87-92. [Wu Y P, Wang M H, Xi J J, Pan J, Chen L Y, Lian Y, Sun H, Zhou P. 2019. The effects of different agricultural waste biochars and application rates on soil pH, water holding capacity and N adsorption[J]. Soil and Fertilizer Sciences in China, (1): 87-92.] doi: 10.11838/sfsc.1673-6257.18094.
- 武晓桐. 2020. 生物炭对牛粪堆肥中固氮微生物多样性及功能的影响[D]. 哈尔滨: 东北农业大学. [Wu X T. 2020. Effect of biochar on the diversity and function of diazotrophs in cattle manure composting[D]. Harbin: Northeast Agricultural University.] doi: 10.27010/d.cnki.gdbnu.2020.000782.
- 杨亚东, 冯晓敏, 胡跃高, 任长忠, 曾昭海. 2017. 豆科作物间作燕麦对土壤固氮微生物丰度和群落结构的影响[J]. 应用生态学报, 28(3): 957-965. [Yang Y D, Feng X M, Hu Y G, Ren C Z, Zeng Z H. 2017. Effects of legume-oat intercropping on abundance and community structure of soil N₂-fixing bacteria[J]. Chinese Journal of Applied Ecology, 28(3): 957-965.] doi: 10.13287/j.1001-9332.201703.007.
- 张国, 朱启法, 郭熙盛, 相智华, 王世济, 沈思灯, 季学军. 2014. 皖南烟区白云石粉对酸性植烟土壤的改良研究[J]. 土壤, 46(3): 534-538. [Zhang G, Zhu Q F, Guo X S, Xiang Z H, Wang S J, Shen S D, Ji X J. 2014. Amelioration of dolomite on acidity of flue-cured tobacco soil in south Anhui[J]. Soils, 46(3): 534-538.] doi: 10.13758/j.cnki.tr.2014.03.023.
- Chen J, Wang P F, Wang C, Wang X, Miao L Z, Liu S, Yuan Q S. 2019. Dam construction alters function and community composition of diazotrophs in riparian soils across an environmental gradient[J]. Soil Biology and Biochemistry, 132: 14-23. doi: 10.1016/j.soilbio.2019.01.020.
- DeLuca T H, Zackrisson O, Nilsson M C, Sellstedt A. 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests[J]. Nature, 419(6910): 917-920. doi: 10.1038/nature01051.
- Favero V O, de Carvalho R H, Leite A B C, dos Santos D M T, de Freitas K M, Boddey R M, Urquiaga S. 2022. *Bradyrhizobium* strains from Brazilian tropical soils pro-

- mote increases in nodulation, growth and nitrogen fixation in mung bean[J]. *Applied Soil Ecology*, 175: 104461. doi: 10.1016/j.apsoil.2022.104461.
- Fujihara S. 2008. Biogenic amines in rhizobia and legume root nodules[J]. *Microbes and Environments*, 24(1): 1-13. doi: 10.1264/jsme2.ME08557.
- Gao L, Wang R, Shen G, Zhang J, Meng G, Zhang J. 2017. Effects of biochar on nutrients and the microbial community structure of tobacco-planting soils[J]. *Journal of Soil Science and Plant Nutrition*, 17(4): 884-896. doi: 10.4067/S0718-95162017000400004.
- Hu X J, Liu J J, Zhu P, Wei D, Jin J, Liu X B, Wang G H. 2018. Long-term manure addition reduces diversity and changes community structure of diazotrophs in a neutral black soil of northeast China[J]. *Journal of Soils and Sediments*, 18(5): 2053-2062. doi: 10.1007/s11368-018-1975-6.
- Karelová E, Harichová J, Stojnev T, Pangallo D, Ferianc P. 2011. The isolation of heavy-metal resistant culturable bacteria and resistance determinants from a heavy-metal-contaminated site[J]. *Biologia*, 66(1): 18-26. doi: 10.2478/s11756-010-0145-0.
- Kunhikrishnan A, Thangarajan R, Bolan N S, Xu Y, Mandal S, Gleeson D B, Naidu R. 2016. Functional relationships of soil acidification, liming, and greenhouse gas flux[J]. *Advances in Agronomy*, 139: 1-71. doi: 10.1016/bs.agron.2016.05.001.
- Li C J, Ahmed W, Li D F, Yu L, Xu L J, Xu L, Xu T Y, Zhao Z X. 2022. Biochar suppresses bacterial wilt disease of flue-cured tobacco by improving soil health and functional diversity of rhizosphere microorganisms[J]. *Applied Soil Ecology*, 171: 104314. doi: 10.1016/j.apsoil.2021.104314.
- Liu X Y, Liu C, Gao W H, Xue C, Guo Z H, Jiang L, Liu Y. 2019. Impact of biochar amendment on the abundance and structure of diazotrophic community in an alkaline soil[J]. *Science of the Total Environment*, 688: 944-951. doi: 10.1016/j.scitotenv.2019.06.293.
- Mia S, Van Groenigen J W, Van de Voorde T F J, Oram N J, Bezemer T M, Mommer L, Jeffery S. 2014. Biochar application rate affects biological nitrogen fixation in red clover conditional on potassium availability[J]. *Agriculture, Ecosystems & Environment*, 191: 83-91. doi: 10.1016/j.agee.2014.03.011.
- Niu J J, Chao J, Xiao Y H, Chen W, Zhang C, Liu X D, Rang Z W, Yin H Q, Dai L J. 2017. Insight into the effects of different cropping systems on soil bacterial community and tobacco bacterial wilt rate[J]. *Journal of Basic Microbiology*, 57(1): 3-11. doi: 10.1002/jobm.201600222.
- Pereira e Silva M C, Semenov A V, van Elsland J D, Salles J F. 2011. Seasonal variations in the diversity and abundance of diazotrophic communities across soils[J]. *FEMS Microbiology Ecology*, 77(1): 57-68. doi: 10.1111/j.1574-6941.2011.01081.x.
- Quilliam R S, DeLuca T H, Jones D L. 2013. Biochar application reduces nodulation but increases nitrogenase activity in clover[J]. *Plant and Soil*, 366(1): 83-92. doi: 10.1007/s11104-012-1411-4.
- Rösch C, Mergel A, Bothe H. 2002. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil[J]. *Applied and Environmental Microbiology*, 68(8): 3818-3829. doi: 10.1128/AEM.68.8.3818-3829.2002.
- Steenhoudt O, Vanderleyden J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: Genetic, biochemical and ecological aspects[J]. *FEMS Microbiology Reviews*, 24(4): 487-506. doi: 10.1016/S0168-6445(00)00036-X.
- Veresoglou S D, Meneses G. 2010. Impact of inoculation with *Azospirillum* spp. on growth properties and seed yield of wheat: A meta-analysis of studies in the ISI Web of Science from 1981 to 2008[J]. *Plant and Soil*, 337(1): 469-480. doi: 10.1007/s11104-010-0543-7.
- Wang C, Zheng M M, Hu A Y, Zhu C Q, Shen R F. 2018. Diazotroph abundance and community composition in an acidic soil in response to aluminum-tolerant and aluminum-sensitive maize (*Zea mays* L.) cultivars under two nitrogen fertilizer forms[J]. *Plant and Soil*, 424(1): 463-478. doi: 10.1007/s11104-017-3550-0.
- Wang C, Zheng M M, Song W F, Wen S L, Wang B R, Zhu C Q, Shen R F. 2017a. Impact of 25 years of inorganic fertilization on diazotrophic abundance and community structure in an acidic soil in southern China[J]. *Soil Biology & Biochemistry*, 113: 240-249. doi: 10.1016/j.soilbio.2017.06.019.
- Wang Y S, Li C N, Kou Y P, Wang J J, Tu B, Li H, Li X Z, Wang C T, Yao M J. 2017b. Soil pH is a major driver of soil diazotrophic community assembly in Qinghai-Tibet alpine meadows[J]. *Soil Biology & Biochemistry*, 115: 547-555. doi: 10.1016/j.soilbio.2017.09.024.
- Wang Z Y, Chen L, Sun F L, Luo X X, Wang H F, Liu G C, Hu Z H, Jiang Z X, Pan B, Zheng H. 2017c. Effects of adding biochar on the properties and nitrogen bioavailability of an acidic soil[J]. *European Journal of Soil Science*, 68(4): 559-572. doi: 10.1111/ejss.12436.
- Xun W B, Li W, Huang T, Ren Y, Xiong W, Miao Y Z, Ran W, Li D C, Shen Q R, Zhang R F. 2018. Long-term agronomic practices alter the composition of symbiotic diazotrophic bacterial community and their nitrogen fixation genes in an acidic red soil[J]. *Biology and Fertility of Soils*, 54(3): 329-339. doi: 10.1007/s00374-018-1264-y.
- Zehr J P, Jenkins B D, Short S M, Steward G F. 2003. Nitrogenase gene diversity and microbial community structure: A cross-system comparison[J]. *Environmental Microbiology*, 5(7): 539-554. doi: 10.1046/j.1462-2920.2003.00451.x.
- Zhang J, Huang Y, Lin J, Chen X, Li C, Zhang J. 2020. Biochar applied to consolidated land increased the quality of an acid surface soil and tobacco crop in Southern China[J]. *Journal of Soils and Sediments*, 20(8): 3091-3102. doi: 10.1007/s11368-019-02531-z.
- Zhang J X, Zhang Z F, Shen G M, Wang R, Gao L, Kong F Y, Zhang J G. 2016. Growth performance, nutrient absorption of tobacco and soil fertility after straw biochar application[J]. *International Journal of Agriculture & Biology*, 18(5): 983-989. doi: 10.17957/IJAB/15.0197.
- Zhang Y G, Cong J, Lu H, Yang C Y, Yang Y F, Zhou J Z, Li D Q. 2014. An integrated study to analyze soil microbial community structure and metabolic potential in two forest types[J]. *PLoS One*, 9(4): e93773. doi: 10.1371/journal.pone.0093773.
- Zhang Y T, He X H, Liang H, Zhao J, Zhang Y Q, Xu C, Shi X J. 2016. Long-term tobacco plantation induces soil acidification and soil base cation loss[J]. *Environmental Science and Pollution Research*, 23(6): 5442-5450. doi: 10.1007/s11356-015-5673-2.