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★江经济带工业污染排放空间分布格局及其影响因素 李芸邑,刘利萍,刘元元



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## 秸秆还田配施化肥对稻-油轮作土壤酶活性及微生物 群落结构的影响

靳玉婷, 李先藩, 蔡影, 胡宏祥\*, 刘运峰, 付思伟, 张博睿

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摘要: 秸秆还田是农业生态系统提高土壤肥力和维持作物生产力的有效管理措施. 为了研究秸秆还田配施化肥对巢湖地区 稻-油轮作农田土壤养分含量、酶活性和微生物群落的影响,开展连续4a(2016~2020年)的田间定位试验,设置无秸秆+ 无施肥( CK)、常规施肥( F)、秸秆还田 + 常规施肥( SF) 和秸秆还田 + 常规施肥减 20% ( SDF) 这 4 个处理,探究不同处理下 影响土壤酶活性与细菌、真菌群落发生变化的关键环境因子. 结果表明, 秸秆还田配施化肥较常规施肥处理能够提高土壤 养分含量, SF 处理的土壤养分含量最高. 与 F 相比, SF 处理的水稻季土壤有机质(OM)和全磷(TP)含量显著提高了 7.94% 和 24.07% (P<0.05),油菜季碱解氮(AN)含量显著提高了 13.62% (P<0.05). SF 较 F 处理的土壤磷酸酶和脲酶 在水稻季显著提高了 28.54% 和 24.13%,在油菜季显著提高了 38.97% 和 30.70%,而 SDF 处理的 4 种土壤酶中仅脲酶活性 较 F 处理显著提高,水稻季和油菜季分别提高了 20.31% 和 24.33% (P < 0.05). 秸秆还田对水稻季土壤细菌的 Chao1 和 Shannon 指数有所增加,油菜季则有所减少,而对真菌群落的 Chaol 和 Shannon 指数均增加. 对于微生物群落结构而言, SF 和 SDF 较 F 处理的变形菌门相对丰度在水稻季分别增加了 8.22% 和 7.88%,油菜季分别增加了 18.53% 和 5.68%. 与 F 相 比, SF和 SDF 处理的绿弯菌门相对丰度在水稻季分别增加了12.00%和11.25%,油菜季分别增加了15.02%和8.43%.水 稻季 SF 和 SDF 处理的担子菌门较 F 相比相对丰度显著提高了 70% 和 43. 42% (P < 0. 05),油菜季 SF 和 SDF 处理的子囊 菌门与 F 相比显著提高了 69.79% 和 43.72% (P < 0.05). 综上, 秸秆还田配施化肥可提高土壤养分含量, 土壤脲酶和磷酸 酶对秸秆还田的响应更为敏感,稻-油轮作农田土壤的细菌群落构成发生改变主要受土壤 TP 和速效磷(AP)影响,而土壤 OM、AN和 pH 则是引起真菌群落构成变化的主要环境因子,因而秸秆还田有利于提高农田土壤肥力和维护生态系统 健康.

关键词:秸秆还田;水稻-油菜轮作;土壤养分;酶活性;微生物群落

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# Effects of Straw Returning with Chemical Fertilizer on Soil Enzyme Activities and Microbial Community Structure in Rice-Rape Rotation

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Abstract: Straw returning is an effective technique for improving soil fertility and maintaining crop productivity in agro-ecosystems. The effects of straw returning, when combined with chemical fertilizer, on soil nutrients, enzyme activity, and microbial community were explored in rice-rape rotation farmland in the Chaohu Area. We carried out a 4-year field experiment (2016-2020) and set up four treatments (no straw + no fertilization, CK; conventional fertilization, F; straw returning + conventional fertilization, SF; and straw returning + conventional fertilization minus 20%, SDF) to explore the key environmental factors affecting soil enzyme activity and microbial and fungal communities. The results showed that straw returning combined with chemical fertilizer could improve soil nutrient content, with the SF treatment resulting in the highest soil nutrient content. Compared with F, the SF treatment significantly increased the organic matter (OM) and total phosphorus (TP) content of the soil, by 7.94% and 24.07%, respectively, in rice seasons (P < (0.05), while the alkaline nitrogen (AN) content was significantly increased by (13.62%) in rape seasons ((P < 0.05)). Compared with F, the SF treatment also significantly increased soil phosphatase and urease, by 28.54% and 24.13% in rice seasons and 38.97% and 30.70% in rape seasons, respectively (P < 0.05). Compared with F, SDF treatments significantly increased urease activity by 20.31% in rice seasons and 24.33% in rape seasons (P<0.05). The results indicated that straw returning increased both the Chaol and Shannon indices of soil bacteria in rice seasons, whereas decreased these indices in rape seasons. However, the Chao1 and Shannon index of the fungal community increased after straw returning. In terms of microbial community structure, the relative abundance of Proteobacteria in SF and SDF treatments increased by 8.22% and 7.88% in rice seasons and 18.53% and 5.68% in rape seasons, respectively, compared with the F treatment. Compared with F, the relative abundance of Chloroflexi in SF and SDF treatments increased by 12.00% and 11.25% in rice seasons and 15.02% and 8.43% in rape seasons, respectively. Compared with

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F, the relative abundance of Basidiomycota in SF and SDF treatments in rice seasons increased by 70% and 43.42% (P < 0.05), respectively, while ascomycetes in rape seasons increased by 69.79% and 43.72% (P < 0.05), respectively. In conclusion, straw returning combined with chemical fertilizer can improve soil nutrient content. Soil urease and phosphatase were more sensitive to straw returning. The compositional changes in the bacterial community of the soil were mainly affected by soil TP and available phosphorus (AP), whereas OM, AN, and pH were the main environmental factors causing changes in the fungal community composition. Consequently, straw returning can improve soil fertility and maintain ecosystem health.

Key words; straw returning; rice-rape rotation; soil nutrients; enzyme activity; microbial community

我国作为一个农业大国,农作物秸秆种类繁多且来源丰富,每年可产生数十亿吨农作物秸秆[1],是世界上秸秆资源最为丰富的国家之一[2].作物秸秆作为物质、能量和养分的载体,含有丰富的碳、氮、磷、钾、中微量元素以及木质素和纤维素等有机物,是一种重要的可再生资源[3],秸秆还田作为改善土壤环境和提高土壤肥力的一种重要方式在全球农业生产活动中广泛应用[4].已有研究表明秸秆还田能够提供作物生长所需的养分元素,还可以调节土壤物理结构,改善土壤生物功能[5].程曼等[6]的研究表明,秸秆还田可以显著提高单季种植作物土壤的全氮、全钾及腐殖质含量,提高土壤肥力[7],Bai等[8]的研究发现稻麦轮作农田实施秸秆还田后提高了土壤氮和有机质含量.

土壤酶和微生物作为评价土壤生物学特性的指 标,在土壤养分循环及有机质分解等方面起重要作 用[9]. 土壤酶来源于根系分泌物、凋落物和微生物 活动[10],是各种生化过程和养分循环的活性成分, 土壤中碳和氮等营养元素的转化主要依赖于土壤酶 系统[11]. 土壤酶是连接土壤微生物和土壤化学过程 的重要媒介,其活性大小可反映出各养分代谢活性 的强度[12],是评价土壤肥力的重要指标之一. 土壤 微生物活动对于营养物质循环和碳固存至关重要, 能够改善土壤结构,分解不易被植物吸收的有机质 和矿物,使土壤中营养元素更好地被植物吸收利 用[13]. 秸秆还田在土壤中的分解转化过程是在土壤 微生物的推动作用下进行的. 秸秆还田为土壤微生 物的繁殖生长提供了充足的碳源,增加了碳源的含 量和种类[14,15]. Li 等[16]的研究表明,秸秆还田改变 了华北平原壤质潮土细菌的群落结构,增加了与复 杂有机物质降解相关细菌的丰度. Zhao 等[17]的研究 发现,秸秆还田使得土壤细菌群落结构发生明显变 化,同时土壤细菌和真菌的丰富度和多样性与土壤 硝态氮呈显著正相关. 6 a 的定位试验结果表明,相

比于单施化肥处理,秸秆还田配施化肥显著提升了土壤肥力,增加了土壤酶活性和细菌丰度,改变了细菌群落结构<sup>[18]</sup>.因此开展土壤微生物相关研究,有利于维护与改良土壤生态系统功能.

目前,有关于秸秆还田的研究主要集中在土壤 微生物总生物量和群落组成变化方面,而不同种植 作物种类和轮作制度下土壤的微生物群落也存在差 异<sup>[19]</sup>,对秸秆还田配施化肥的响应也有所不同. 因 此本研究通过连续 4 a 的田间试验探讨秸秆还田配 施化肥对稻-油轮作模式下土壤酶活性、细菌和真菌 多样性及群落组成结构的影响,以探究土壤养分与 土壤微生物群落变化规律及其与土壤酶活性的关 系,以期为合理利用秸秆资源,调节土壤养分,提高 作物产量,改善土壤生物功能提供科学依据.

#### 1 材料与方法

#### 1.1 试验地概况

试验区位于安徽省巢湖市烔炀镇(东经117°41′37″,北纬31°39′37″),试验点具体位置见图1.属亚热带湿润季风气候,气候特点为常年气候温和,日照充足,雨量充沛.降雨主要集中在夏季,年平均降水量1210 mm,年平均气温18~19℃.土壤类型为潜育型水稻土,初始土壤理化性质见表1.



图 1 试验点区域位置示意

Fig. 1 Location of test site

表 1 土壤理化性质

Table 1 Physical and chemical properties of soil

| 指标 | рН    | TN<br>∕g•kg <sup>-1</sup> | TP<br>∕g•kg <sup>-1</sup> | TK<br>/g•kg <sup>-1</sup> | AN<br>/mg•kg <sup>-1</sup> | AP<br>/mg•kg <sup>-1</sup> | AK<br>/mg·kg <sup>-1</sup> | OM<br>∕g•kg <sup>-1</sup> |
|----|-------|---------------------------|---------------------------|---------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
| 数值 | 6. 03 | 1. 47                     | 0. 45                     | 8. 77                     | 146. 56                    | 12. 87                     | 109. 45                    | 24. 29                    |

#### 1.2 试验设计

定位试验始于 2016 年 6 月,种植制度为水稻-油菜轮作,水稻供试品种为"徽两优 996",于每年 6 月中旬移栽,10 月初收获.油菜供试品种为"秦优十号",于每年 11 月中旬移栽,次年 5 月收获.田间管理按照当地常规生产模式进行.试验共设置 4 个处理:无秸秆+无施肥(CK)、常规施肥(F)、秸秆还田+常规施肥(SF)和秸秆还田+常规施肥减 20%

(SDF),每个处理 3 次重复,共 12 个小区,呈随机区组分布. 试验小区田埂用水泥堆砌而成,面积为 30 m²,长 4 m、宽 7.5 m. 本试验中供试肥料为复合肥(氮-磷-钾:18-10-18)和尿素(氮含量 46.4%),常规施肥参照当地施肥量和方式,具体施肥情况见表 2.每季试验前先将上季作物收获,留茬 10 cm 左右,通过收割机将上一季的秸秆切碎翻耕入土,长度为 5 ~ 10 cm,秸秆还田量为上季作物收获后所有秸秆量.

表 2 不同处理施肥情况

| able 2 Ferti | lization o | f diff | erent | treatments |  |
|--------------|------------|--------|-------|------------|--|
|              |            |        |       |            |  |

| 作物类型 | 处理  | N   | √kg•hm <sup>-2</sup> | $P_2O_5$             | $K_2O$               | 秸秆还田比例 |
|------|-----|-----|----------------------|----------------------|----------------------|--------|
| 作初失型 | 处理  | 基肥  | 追肥                   | /kg·hm <sup>-2</sup> | /kg•hm <sup>-2</sup> | /%     |
|      | CK  | 0   | 0                    | 0                    | 0                    | 0      |
| 油菜   | F   | 196 | 139                  | 109                  | 196                  | 0      |
| 但来   | SF  | 196 | 139                  | 109                  | 196                  | 100    |
|      | SDF | 157 | 110                  | 87                   | 157                  | 100    |
|      | CK  | 0   | 0                    | 0                    | 0                    | 0      |
| 水稻   | F   | 205 | 97                   | 114                  | 205                  | ( 0) 1 |
| AV16 | SF  | 205 | 97                   | 114                  | 205                  | 100    |
|      | SDF | 165 | 78                   | 90                   | 165                  | 100    |

#### 1.3 样品采集与测定方法

在连续秸秆还田的第 4 年,即 2019 年 9 月水稻 收获和 2020 年 5 月油菜收获期,按照五点采样法分 别采集 0 ~ 20 cm 耕层土壤样品,混匀后装于放有冰 袋的保温箱中立即带回实验室.将土壤样品分为 3 份,风干测定土壤养分含量,鲜土测定土壤酶活性,另 一份土壤样品保存于 - 80℃,用于土壤微生物分析.

土壤理化性质测定参见文献[20]. 土壤 OM 采用重铬酸钾-外加热法测定;土壤全氮(TN)采用凯氏定氮法测定;土壤 TP 采用高温酸融-钼锑抗比色法测定;土壤 AP 采用 NaHCO<sub>3</sub> 提取-钼锑抗比色法测定;土壤 AN 采用碱解扩散法测定;土壤 pH 采用电极法(水土比 2.5:1).

土壤酶活性测定方法参见文献[21]. 过氧化氢酶活性:高锰酸钾容量法,以1g土壤1h内消耗的0.005  $\text{mol} \cdot \text{L}^{-1} \text{KMnO}_4$  的体积数(mL)表示土壤过氧化氢酶活性;磷酸酶活性:磷酸苯二钠比色法,以24h后1g土壤中释放出的酚的毫克数(mg)表示磷酸酶活性;脲酶活性:苯酚钠-次氯酸钠比色法,以24h后1g土壤中 $\text{NH}_4^+$ -N的毫克数(mg)表示土壤脲酶活性;蔗糖酶活性以24h后每克土壤中葡萄糖的毫克数(mg)表示蔗糖酶活性.

土壤样品微生物的 DNA 提取采用 MOBIO 公司 生产的土壤 DNA 提取试剂盒法 (Power Soil DNA Isolation Kit),采用细菌 16S rRNA 基因的 V3-V4 高 变区序列引物 (5'-ACTCCTAGGGAGGGCAGCAG-3'、5'-GGACTACHVGGGTWTTCTAAT- 3')和真菌 ITS rRNA 区序列引物 (5'- GGAAGTAAAAGTCGT AACAAGG- 3′、5′-GCTGCGTTCTTCATCGATGC- 3′)来进行 PCR 扩增. 用 2% 琼脂糖凝胶电泳检测 PCR 扩增产物,并使用 AxyPrep DNA 凝胶回收试剂盒切割凝胶来回收目标片段,利用 Quantiflur-ST 对 PCR产物进行定量检测. 根据每个样本的测序量需求,将 PCR 扩增产物等摩尔混合,并在 Illumina MiSeq PE300 平台上进行测序分析.

#### 1.4 数据处理

运用 Microsoft Excel 2016 处理数据, SPSS 22.0 软件分析数据, Duncan 进行样本平均数的差异显著性比较, OriginPro 8 作图. 进行冗余分析以评价土壤理化性质及 pH 对土壤酶活性的影响. 使用 SPSS 22.0 对土壤环境因子、酶活性、群落多样性和结构组成进行皮尔逊(Pearson)相关性分析. 图表数据均为 3 次重复的平均值 ± 标准误.

#### 2 结果与分析

#### 2.1 土壤养分含量及 pH 的变化

由表 3 可知,连续进行 4 a 的秸秆还田后,与 F 相比,秸秆还田配施化肥处理(SF 和 SDF)增加了稻-油轮作农田土壤的养分含量.水稻季 SF 较 F 处理的 TN、AP 和 AN 分别增加了 4.83%、6.80% 和 6.22%,其中 OM 和 TP 含量显著提高了 7.94% 和 24.07%(P<0.05), SDF 与 F 处理相比的 TN、AP、AN 和 TP 分别增加了 0.08%、0.61%、1.26% 和 19.33%.油菜季 SF 与 F 处理相比的 TN、TP、AP 和 OM 分别增加了 1.72%、17.69%、37.16% 和 6.15%, SDF 处理的 TN、TP、AP 和 OM 则分别增加

了 0.76%、3.40%、7.40% 和 7.04%,其中 SF 和 SDF 较 F 处理的 AN 含量则显著提高了 13.62% 和 13.10% (P < 0.05).常规与秸秆还田施肥处理均能够使土壤的 pH 值有所下降,CK 处理的 pH 值最

高. 与 CK 相比, SF 和 SDF 处理的 pH 在水稻季显著降低 4.45% 和 7.82% (P < 0.05), F 处理降低不显著,油菜季 F、SF 和 SDF 处理的 pH 值较 CK 显著降低 10.30%、11.67% 和 8.64% (P < 0.05).

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表 3 不同处理对土壤养分及 pH 的影响<sup>1)</sup>
Table 3 Effects of different treatments on soil nutrients and pH

| 作物类型    | 处理  | OM∕g•kg <sup>-1</sup> | $TN/g \cdot kg^{-1}$ | TP/g·kg <sup>-1</sup> | AP/mg•kg <sup>-1</sup> | $AN/mg \cdot kg^{-1}$ | pН                       |
|---------|-----|-----------------------|----------------------|-----------------------|------------------------|-----------------------|--------------------------|
|         | CK  | 26. 68 ± 0. 26c       | $1.63 \pm 0.04$ b    | 0. 42 ± 0. 02 c       | 18. 51 ± 0. 76a        | 154. 22 ± 2. 86b      | $6.52 \pm 0.09a$         |
| 水稻      | F   | 28. 81 $\pm$ 0. 29b   | 1. $82 \pm 0.06a$    | $0.54 \pm 0.02b$      | 23. $85 \pm 1.55a$     | 173. 71 ± 3. 12a      | 6. $48 \pm 0.07$ ab      |
| /J\\/11 | SF  | 31. $10 \pm 0.68a$    | $1.91 \pm 0.03a$     | $0.68 \pm 0.05a$      | $25.47 \pm 3.23a$      | 184. 52 ± 9. 72a      | $6.23\pm0.07\mathrm{bc}$ |
|         | SDF | 28. $56 \pm 0.34$ b   | 1. $82 \pm 0.06a$    | $0.65 \pm 0.03$ b     | 23. 99 $\pm$ 0. 71 a   | $175.90 \pm 2.49a$    | $6.01 \pm 0.01 d$        |
|         | CK  | 27. 10 ± 0. 74b       | 1. 49 ± 0. 10b       | 0.41 ± 0.01b          | 14. 41 ± 1. 13b        | 148. 91 ± 3. 43 c     | $6.60 \pm 0.08a$         |
| 油菜      | F   | 29. $46 \pm 0.68$ ab  | 1. $75 \pm 0.02a$    | $0.49 \pm 0.02 ab$    | 20. 13 $\pm$ 0. 69ab   | $162.48 \pm 3.15$ b   | $5.92 \pm 0.15$ b        |
| 田米      | SF  | $31.28 \pm 0.70a$     | $1.76 \pm 0.03a$     | $0.58 \pm 0.04a$      | 27. 61 ± 3. 71a        | 184. 61 ± 2. 87a      | $5.83 \pm 0.15$ b        |
|         | SDF | $31.54 \pm 1.81a$     | 1. $78 \pm 0.04a$    | $0.51 \pm 0.01a$      | $21.62 \pm 2.35$ ab    | 183. 77 ± 2. 33a      | 6. $03 \pm 0.24$ b       |

1)同行数据后不同小写字母表示处理间差异显著(P<0.05),下同

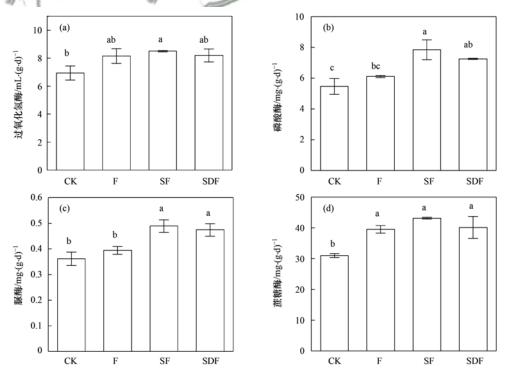
#### 2.2 土壤酶活性

根据图 2 和图 3 可知,秸秆还田配施化肥较常规施肥处理均增加了土壤的酶活性,4 种酶活性均表现为 SF > SDF > F > CK. 与 F 相比, SF 处理的土壤过氧化氢酶和蔗糖酶活性在水稻季增加了4.34%和9.09%,油菜季则增加了4.56%和11.64%.土壤磷酸酶和脲酶活性在水稻季显著提高了28.54%和24.13%,油菜季显著提高了38.97%和30.70%(P<0.05). SDF 处理的土壤过氧化氢酶、磷酸酶和蔗糖酶活性较 F 处理在水稻季分别增

加了 0.65%、18.76% 和 1.38%,油菜季则分别增加了 2.57%、26.19% 和 12.05%.而脲酶活性较 F 处理在水稻和油菜季达显著差异,分别提高了 20.31% 和 24.33% (P < 0.05).可见,稻-油轮作农田土壤的脲酶活性变化对秸秆还田的响应更为敏感,其次是磷酸酶.

#### 2.3 土壤微生物群落多样性

如表 4 所示, 水稻季收获后 SF 和 SDF 处理的 土壤细菌群落的 Chao1 指数较 F 处理增加了 6.55% 和 6.12%, Shannon 指数增加了 2.15% 和



小写字母标注不同处理之间差异显著(P < 0.05),下同

图 2 水稻季不同处理下土壤酶活性变化

Fig. 2 Changes in soil enzyme activities under different treatments in rice season

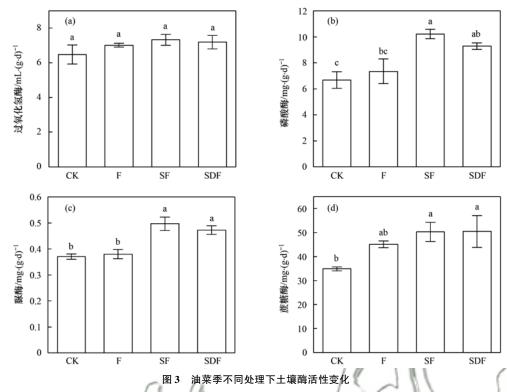


Fig. 3 Changes in soil enzyme activities under different treatments in rape season

2.69%.油菜季 SF 和 SDF 处理土壤的细菌 Chaol 指数较 F 处理减少了 1.69% 和 4.50%, Shannon 指数则减少了 1.22% 和 0.71%,但各处理间差异不显著.对于真菌群落而言,与 F 处理相比,水稻季 SF 和 SDF 的 Chaol 指数增加了 3.65% 和 8.12%, Shannon 指数增加了 1.38% 和 7.83%.油菜季 SF 和

SDF 的 Chaol 指数较 F 处理增加了 10.81% 和 2.87%, SF 处理的 Shannon 指数与 F 相比增加了 7.96%.可见稻-油轮作下,秸秆还田配施化肥可增加水稻季土壤细菌和真菌群落丰富度和多样性,降低油菜季土壤细菌群落丰富度和多样性,增加真菌群落丰富度和多样性.

表 4 稻-油轮作下土壤细菌与真菌 Chaol 指数和 Shannon 指数

| Table 4 | Chao1 | index | and | Shannon | inde | x of | soil | bacteria | and | fungi | under | rice-rape | rotation |
|---------|-------|-------|-----|---------|------|------|------|----------|-----|-------|-------|-----------|----------|
|         |       |       |     |         |      |      |      |          |     |       |       |           |          |

| <i>  </i> | AL TH | 细菌                            |                   | 真菌                          |                  |
|-----------|-------|-------------------------------|-------------------|-----------------------------|------------------|
| 作物类型      | 处理 -  | Chao1 指数                      | Shannon 指数        | Chao1 指数                    | Shannon 指数       |
|           | CK    | 2 778.81 ±51.15a              | 9.22 ± 0.04a      | 1 122.73 ±45.99b            | 6.76 ± 0.32a     |
| 水稻        | F     | 2 641.92 ± 71.22a             | $9.31 \pm 0.14a$  | $1\ 211.76 \pm 44.21 ab$    | $6.51 \pm 0.25a$ |
| 7,141     | SF    | $2814.95 \pm 56.35a$          | $9.51 \pm 0.14a$  | $1155.99\pm37.76\mathrm{b}$ | $6.60 \pm 0.36a$ |
|           | SDF   | $2803.62\pm76.36a$            | $9.56 \pm 0.11a$  | $1310.13\pm48.68a$          | $7.02 \pm 0.04a$ |
|           | CK    | $3\ 140.38 \pm 99.46a$        | $9.80 \pm 0.06a$  | $789.67 \pm 62.25 a$        | $5.19 \pm 0.46a$ |
| 油菜        | F     | $3\ 271.\ 15\ \pm\ 121.\ 97a$ | $9.86 \pm 0.07 a$ | $787.44 \pm 72.49a$         | $5.15 \pm 0.62a$ |
| шж        | SF    | $3\ 215.77 \pm 81.42a$        | $9.74 \pm 0.10a$  | $873.07 \pm 96.90a$         | $5.56 \pm 0.24a$ |
|           | SDF   | $3\ 123.88 \pm 184.37a$       | $9.79 \pm 0.14a$  | $809.59 \pm 44.75a$         | $5.00 \pm 0.94a$ |

#### 2.4 土壤微生物群落构成

不同处理下细菌和真菌群落结构在门水平上的物种组成如图 4 所示. 门水平上水稻季各处理土壤细菌按丰富度排列为变形菌门(Proteobacteria)、绿弯菌门(Chloroflexi)、酸杆菌门(Acidobacteria)、硝化螺 旋 菌 门 (Nitrospirae)、芽 单 胞 菌 门 (Gemmatimonadetes)、拟杆菌门(Bacteroidetes)、放线 菌 门 (Actinobacteria)、疣 微 菌 门 (Verrucomicrobia)、黏胶球形菌门(Latescibacteria)和嗜热菌门(Ignavibacteriae)等门类,这 10 类菌占

总细菌的 93. 35%~94. 39%,其中优势细菌门(丰度>5%)变形菌门占比最高,为 36. 10%~39. 06%,其次为绿弯菌门(17. 63%~21. 09%)、酸杆菌门(8. 69%~13. 64%)、硝化螺旋菌门(6. 82%~8. 99%)、芽单胞菌门(5. 06%~5. 66%). 子囊菌门(Ascomycota)和担子菌门(Basidiomycota)是各处理土壤的优势真菌门,相对丰度 45. 08%~54. 01%和3. 23%~9. 98%,其次为接合菌门(Zygomycota)、隐真菌门(Rozellomycota)及球囊菌门(Glomeromyta).在本研究中,与 F 相比,水稻季 SF 和 SDF 处理的细

菌变形菌门相对丰度增加了 8.22% 和 7.88%, 绿弯菌门增加了 12.00% 和 11.25%, 酸杆菌门减少了 11.44% 和 16.83%, 硝化螺旋菌门减少了 3.00% 和 9.45%, 芽单胞菌门则减少了 10.47% 和 3.24%. 对于水稻季真菌群落而言, SF 和 SDF 较 F 处理的担子菌门相对丰度显著提高了 70.00% 和 43.42%.

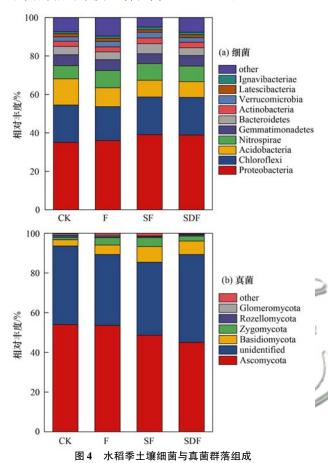
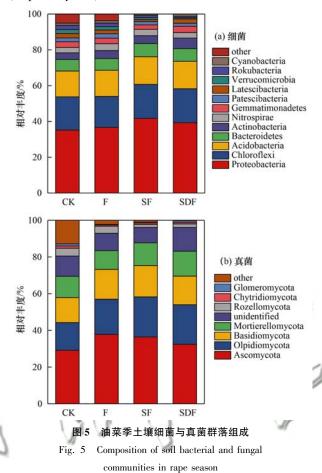


Fig. 4 Composition of soil bacterial and fungal communities in rice season

图 5 中变形菌 (Proteobacteria) (35.59%~ 41.65%)、绿弯菌门(Chloroflexi1)(17.40%~ 19.02%)、酸杆菌门(Acidobacteria)(14.59%~ 15.54%)、拟杆菌门(Bacteroidetes)(6.49%~ 7.33%)和硝化螺旋菌门(Nitrospirae)(3.03%~ 3.82%)为油菜季各处理土壤优势细菌门,共占细 菌总量的 79.29% ~ 91.00%. 子囊菌门 (Ascomycota) (32.42% ~ 37.82%)、油壶菌门 (Olpidiomycota) (15.11% ~ 21.88%)、担子菌门 (Basidiomycota) (13.66% ~ 17.07%)、被孢菌门 (Mortierellomycota) (10.14%~13.55%) 是各处理土 壤的优势真菌门,共占真菌总量的69.48%~ 87.78%.结果表明,与F相比,油菜季秸秆还田处理 (SF和SDF)的变形菌门、绿弯菌门和拟杆菌门分别 增加了 5.68% ~ 18.53%、8.43% ~ 15.02% 和 6.34%~11.07%. 真菌群落中 SF 和 SDF 处理的子 囊菌门(Ascomycota) 较 F 相比,显著提高了69.79% 和 43.72%, SDF 处理的油壶菌门(Olpidiomycota)相对丰度显著降低了29.80%.

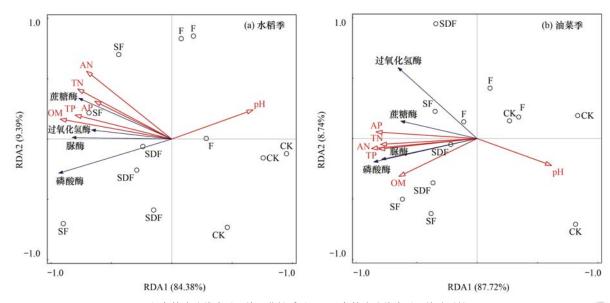


2.5 土壤理化性质的变化与酶活性的 RDA 分析

为解释引起土壤酶活性变化的主要环境因子,对稻-油轮作下土壤酶活性与土壤养分及pH进行了RDA分析(图 6). 结果表明,第一排序轴和第二排序轴分别可以解释水稻季土壤酶活性变化的84.38%和9.39%,油菜季土壤酶活性变化的87.72%和8.74%,前两轴共解释了93.77%的水稻季土壤酶活性总变异和96.46%的油菜季土壤酶活性总变异。由图6(a)可知,秸秆还田后水稻季土壤的OM、TP和TN与土壤酶活性呈正相关,土壤pH与土壤各酶活性呈负相关。根据图6(b)结果显示,油菜季的土壤AN、TP和AP与土壤酶活性呈正相关,土壤pH与土壤各酶活性呈负相关。

# **2.6** 土壤微生物群落与土壤理化性质和酶活性的相关性分析

将各处理间的微生物群落  $\alpha$  多样性和结构组成与土壤理化性质和酶活性进行相关性分析,土壤细菌和真菌  $\alpha$  多样性由 Shannon 指数和 Chao1 指数来反映. 由表 5 可知,细菌与真菌的 Chao 1 指数和 Shannon 指数与土壤理化性质在水稻和油菜季无明



红色箭头连线表示土壤理化性质及 pH,蓝色箭头连线表示土壤酶活性

#### 图 6 土壤酶活性与理化性质的 RDA 分析

Fig. 6 RDA analysis of soil enzyme activity and physical and chemical properties

显相关性,水稻季细菌的 Shannon 指数与土壤脲酶显著正相关(P < 0.05). 对于稻-油轮作下门水平上的优势细菌与真菌群落构成而言,水稻季的变形菌门与土壤 TP 和磷酸酶明显正相关(P < 0.05),与脲酶极显著正相关(P < 0.01),酸杆菌门与土壤 AP 和过氧化氢酶显著负相关(P < 0.05). 子囊菌门与土壤 TP 和脲酶明显正相关(P < 0.05), 与磷酸酶极显著正相关(P < 0.05), 与磷酸的极显著

油菜季的皮尔逊相关性分析结果显示(表6),

变形菌门与土壤 TP、AP 和磷酸酶显著正相关(P < 0.05),与脲酶明显负相关(P < 0.05).酸杆菌门和拟杆菌门与土壤磷酸酶极显著正相关(P < 0.01),与脲酶显著正相关(P < 0.05).油壶菌门与土壤OM、过氧化氢酶和蔗糖酶极显著正相关(P < 0.05),而担子菌门与土壤 pH 极显著负相关(P < 0.05),而担子菌门与土壤 pH 极显著负相关(P < 0.01).综上可知土壤优势细菌、真菌群落构成与土壤理化性质和酶活性密切相关,土壤环境因子的变化能引起土壤优势细菌和真菌群落构成发生改变.

#### 表 5 细菌与真菌 $\alpha$ 多样性与门水平上优势菌群与土壤理化性质和酶活性之间的相关性(水稻季) $^{1)}$

Table 5 Correlation between α diversity of bacteria and fungi and dominant flora at the phylum level,

with physical and chemical properties of soil and enzyme activities (rice season)

| 类别 | α 多样性与门<br>水平上优势菌群 | OM     | TN     | TP       | AP      | AN     | pН        | 过氧化<br>氢酶 | 磷酸酶       | 脲酶        | 蔗糖酶    |
|----|--------------------|--------|--------|----------|---------|--------|-----------|-----------|-----------|-----------|--------|
|    | Chao 1 指数          | 0. 053 | -0.248 | 0. 167   | -0.134  | 0.091  | -0.332    | -0.007    | 0. 518    | 0. 547    | 0.047  |
|    | Shannon 指数         | -0.401 | -0.540 | -0.500   | -0.459  | -0.272 | 0. 228    | 0.793     | 0. 937    | 0. 974 *  | 0.801  |
|    | Proteobacteria     | 0.765  | 0.816  | 0. 973 * | 0.811   | 0.854  | -0.907    | 0.815     | 0. 979 *  | 0. 997 ** | 0.834  |
| 细菌 | Chloroflexi        | 0.058  | -0.077 | 0. 242   | -0.314  | -0.021 | -0.508    | -0.133    | 0.406     | 0.437     | -0.079 |
|    | Acidobacteria      | -0.765 | -0.916 | -0.949   | -0.951* | -0.934 | 0.788     | -0.957*   | -0.859    | -0.877    | -0.944 |
|    | Nitrospirae        | 0.788  | 0.895  | 0.689    | 0. 914  | 0.868  | -0.261    | 0.912     | 0. 572    | 0. 528    | 0.894  |
|    | Gemmatimonadetes   | -0.884 | -0.750 | -0.798   | -0.672  | -0.766 | 0. 522    | -0.663    | -0.899    | -0.845    | -0.720 |
|    | Chao 1 指数          | 0.096  | 0. 288 | 0.403    | 0. 028  | 0. 482 | -0.308    | 0. 494    | 0. 370    | 0.470     | 0. 440 |
| 真菌 | Shannon 指数         | -0.486 | -0.477 | -0.120   | -0.453  | 0.013  | -0.120    | -0.157    | 0. 142    | 0. 263    | -0.171 |
| 四六 | Ascomycota         | -0.447 | -0.564 | -0.830   | -0.593  | -0.620 | 0. 999 ** | -0.604    | -0.817    | -0.886    | -0.606 |
|    | Basidiomycota      | 0.891  | 0.898  | 0. 982 * | 0.874   | 0. 923 | -0.786    | 0.873     | 0. 999 ** | 0. 987 *  | 0.900  |

<sup>1) \*</sup> 和 \*\* 分别表示在 0.05 和 0.01 水平显著相关,下同

#### 3 讨论

3.1 秸秆还田配施化肥对土壤理化性质的影响 本研究结果显示秸秆还田可增加稻-油轮作农 田氮磷及有机质养分含量,均以 SF 处理最高. SF 处 理的有机质含量在水稻季达到显著提高(P < 0.05),这与 Zhu 等[19]的研究结果—致. 秸秆中养分是提高土壤有机碳的重要来源,外源碳的持续加入激发了土壤微生物的活性,加强了微生物矿化和固定过程,促进秸秆中碳在土壤中的积累[22]. 旱地土

#### 表 6 细菌与真菌 α 多样性与门水平上优势菌群与土壤理化性质和酶活性之间的相关性(油菜季)

Table 6 Correlation between  $\alpha$  diversity of bacteria and fungi and dominant flora at phylum

level and soil physical and chemical properties and enzyme activities (rape season)

| 种类 | α 多样性与门<br>水平上优势菌群 | OM        | TN     | TP       | AP      | AN       | рН       | 过氧化 氢酶    | 磷酸酶       | 脲酶       | 蔗糖酶       |
|----|--------------------|-----------|--------|----------|---------|----------|----------|-----------|-----------|----------|-----------|
|    | Chao 1 指数          | -0.112    | -0.147 | 0.040    | 0.032   | -0.010   | -0.375   | 0. 297    | -0.033    | -0.168   | 0.188     |
|    | Shannon 指数         | -0.413    | -0.345 | -0.024   | 0.039   | -0.165   | -0.295   | -0.382    | -0.745    | -0.787   | -0.340    |
|    | Proteobacteria     | 0. 849    | 0.682  | 0. 956 * | 0.965 * | 0.910    | -0.739   | 0.883     | 0. 980 *  | -0.951*  | 0.834     |
| 细菌 | Chloroflexi        | 0. 213    | -0.180 | 0. 189   | 0.218   | 0.376    | 0. 230   | 0.09      | 0.516     | 0.609    | 0. 104    |
|    | Acidobacteria      | 0.858     | 0.651  | 0.918    | 0.926   | 0. 929   | -0.674   | 0.858     | 0. 993 ** | 0. 983 * | 0.827     |
|    | Bacteroidetes      | 0.865     | 0.664  | 0.925    | 0.932   | 0. 932   | -0.688   | 0.867     | 0. 994 ** | 0. 982 * | 0.835     |
|    | Actinobacteria     | 0. 775    | 0.698  | 0.400    | 0.359   | 0.728    | -0.454   | 0.614     | 0.547     | 0.588    | 0.736     |
|    | Nitrospirae        | 0. 200    | 0.511  | 0.428    | 0.417   | 0.089    | -0.687   | 0.409     | 0.052     | -0.083   | 0.316     |
|    | Chao 1 指数          | 0. 251    | 0.000  | -0.027   | -0.008  | 0. 193   | -0.065   | 0. 694    | 0.877     | 0.850    | 0.607     |
|    | Shannon 指数         | 0.405     | 0.007  | -0.070   | -0.055  | 0. 126   | -0.005   | 0.321     | 0.469     | 0.408    | 0.171     |
| 真菌 | Ascomycota         | 0. 520    | 0.757  | 0.694    | 0.679   | 0.423    | -0.893   | 0.696     | 0.374     | 0. 249   | 0.618     |
| 共四 | Olpidiomycota      | 0. 993 ** | 0. 944 | 0.927    | 0.907   | 0. 971 * | -0.901   | 0. 991 ** | 0.907     | 0.870    | 0. 998 ** |
|    | Basidiomycota      | 0.806     | 0.884  | 0. 941   | 0.931   | 0.764    | -0.981 * | 0. 927    | 0.748     | 0.654    | 0.862     |
|    | Mortierellomycota  | 0. 589    | 0. 254 | 0.364    | 0.364   | 0.684    | -0.081   | 0.408     | 0.689     | 0.780    | 0.486     |

壤通气性优于淹水土壤,秸秆还田加速了微生物呼 吸作用,使其微生物生物量和活性提高[23],不利于 根际土壤有机质累积,水稻土壤由于淹水厌氧环境 限制了有机质分解消耗,使土壤养分得以积累[24] 本研究表明,与 F 处理相比,秸秆还田显著提高水 稻季土壤全磷含量,其原因是稻-油轮作种植过程 中,秸秆中养分的投入活化了土壤磷库[25],水稻季 淹水环境中土壤 pH 趋向中性,中性范围内磷的有 效性最高[26].淹水状态下土壤处于还原条件,土壤 中铁、锰等氧化物在还原条件下会释放出与其结合 的闭蓄态磷,提升土壤磷的扩散能力和有效性[27]. 另一方面, 秸秆腐解过程中也为土壤输入了磷 素[28],导致土壤中磷素含量增加.本试验显示秸秆 还田处理可提高土壤固氮能力, SF 和 SDF 处理的 油菜季碱解氮含量与 F 相比显著提高. 秸秆还田通 过提高微生物活性,调节矿质氮的固定与转化,将易 流失的无机氮转化为相对稳定的有机氮,使土壤中 的氮素积累[29]. 另一方面作物生长期落叶和残根增 加了土壤中的有机物料,其氧气充沛的土壤条件加 速分解,有利于氮素固存,这与 Yang 等[30]的研究结 果一致. 本研究中常规施肥与秸秆还田配施化肥处 理均可降低土壤 pH 值,但与背景值并无较大差异, 秸秆还田短期内对土壤的 pH 值无显著影响,这与 Zhu 等[19]的研究结果一致. 由于秸秆在分解过程中 产生了大量的有机酸,如乙酸和腐植酸-黄腐酸等, 但很快会因土壤自身的酸碱缓冲能力而有所恢复, 短期内不会影响土壤酸碱度[19].

#### 3.2 秸秆还田配施化肥对土壤酶活性的影响

本研究表明,与不还田相比, SF 和 SDF 处理的 土壤过氧化氢酶、脲酶、磷酸酶和蔗糖酶活性在水稻

和油菜季均有所提高,其中SF处理的土壤脲酶和磷 酸酶较 F 处理达到显著水平. 土壤酶是土壤中物质 的转化、养分释放和固定等过程的重要参与者,对土 壤肥力的形成也具有重要作用[31]. 脲酶是促进土壤 氮素转化的专一酶,磷酸酶对促进土壤碳、氮、磷元 素转化有显著作用,蔗糖酶和过氧化氢酶主要驱动 土壤有机质的分解[32]. 秸秆还田腐解过程中,有机 物料的投入改变了土壤的团聚体和孔隙结构,提高 缓冲作用和持水力,为各种酶提供载体[33].另一方 面秸秆的输入为土壤中相关微生物提供了丰富的能 量原料[5],优化了土壤微生物生存环境[34],促进微 生物新陈代谢活动,能够刺激微生物分泌参与碳氮 磷循环相关的土壤酶,使其随着旺盛的根系分泌和 土壤微生物活动进入土壤,导致土壤中酶活性增 加[35],加快土壤中碳氮周转.张涵等[12]和冯慧琳 等[36]的研究表明土壤碳氮比是影响土壤酶活性的 关键影响因素, Wang 等[37] 的试验得出秸秆还田与 化肥配施,加速了土壤有机碳的矿化分解进程,对土 壤酶活性均有一定程度的提高. 这与本研究结果一 致. 本试验结果显示相比于水稻季, 秸秆还田在油菜 季对土壤酶活性的影响更为显著. 由于秸秆中含有 较多纤维素及木质素降解菌[18],油菜季良好的土壤 通气环境能有效促进秸秆碳氮养分的分解释放,为 土壤酶提供更多和更丰富的酶促反应基质.

# 3.3 秸秆还田配施化肥对土壤微生物多样性及群落结构的影响

本试验结果表明秸秆还田配施化肥对水稻、油菜土壤的细菌和真菌多样性和群落结构具有一定的影响,能够提升水稻季土壤细菌的 Chaol 指数和Shannon 多样性指数,对油菜季土壤细菌的 Chaol

指数和 Shannon 多样性指数有所降低. Liu 等[38] 的 研究表明长期单施化肥显著降低细菌的丰富度和多 样性. 秸秆的加入为以异养方式生存的细菌提供了 外源有机物料等营养物质,有利于其繁殖生长,减少 各细菌群落之间的竞争[39],增强稻田土壤细菌群落 的多样性,这与 Wang [40] 和 Zhang 等[41] 的研究结果 一致. 刘文静等[42]的研究表明细菌在淹水条件的细 菌 α 多样性显著高于旱作生态系统. 由于水稻季厌 氧环境下电子受体的多样化增强了各细菌群落之间 的稳定性[43].油菜季频繁的翻耕活动以及特定施肥 方式导致了特定微生物才能在此环境过滤作用下存 活,抵抗外界干扰能力相对较小,秸秆的加入增加了 微生物分解负担,从而降低了微生物多样性.而对于 真菌而言,秸秆还田配施化肥较单施化肥处理增加 稻-油轮作农田土壤真菌的 Chao1 指数和 Shannon 多样性指数. 真菌适宜在 pH < 5 的酸性土壤中生 存,土壤 pH 下降会刺激嗜酸性真菌的生长繁 殖[44],弱碱性环境并不利于真菌的生长[45]. 研究结 果显示秸秆还田配施化肥短期内会降低土壤的 pH 值,但无显著差异性,故短期内会增加稻-油轮作农 田土壤的真菌 α 多样性.

从微生物群落结构来看,稻-油轮作下农田土壤 微生物优势细菌门为变形菌门、绿弯菌门和酸杆菌 门等,秸秆还田配施化肥增加了土壤变形菌门和绿 弯菌门群落的相对丰度,这与前人的研究结果-致[46]. 秸秆还田过程中养分的供应决定了土壤微生 物种群生长的关键因素,其中有机质作为微生物繁 殖的重要碳源[14],是引起微生物群落发生改变的影 响因素. 秸秆还田提高了土壤 C/N, 也为微生物生长 提供了基质. 变形菌门在秸秆还田处理土壤中占用 较高的比例,其包含多种代谢固氮细菌种类[47],能 够广泛参与土壤矿物碳氮等多种营养元素的生物化 学循环过程. 秸秆还田带来的有机物料通过矿化和 降解释放氮,从而补充了土壤氮库,促进了固氮细菌 的生长繁殖,从而刺激了变形菌门中固氮微生物种 群相对丰度的增加[48],有利于土壤肥力的保持和植 物生长. 绿弯菌门中的微生物主要为厌氧类细菌,可 将糖和多糖发酵成有机酸和氢[49],从而加快土壤中 有机物料的分解与吸收. Wegner 等[50]的研究表明, 绿弯菌门对秸秆中半纤维素的降解具有促进作用. Yu 等<sup>[51]</sup>的研究表明经过连续 6 a 的秸秆还田后,土 壤中绿弯菌门相对丰度显著高于无秸秆还田处理.

本研究中真菌群落丰度最高的优势菌门为子囊菌门,秸秆还田配施化肥能够提高水稻-油菜轮作土壤中子囊菌门和担子菌门真菌群落的相对丰度,这与文献[41,52]的研究结果一致.秸秆施用后土壤

真菌群落丰度都有一定程度地增加,这很可能是由 于秸秆还田过程中有机质的矿化,消耗土壤中的氧 气,使土壤中微生物兼性厌氧微生物或厌氧微生物 的相对丰度增加[13]. 因为真菌群落中的子囊菌门是 一类腐生菌,其产生的纤维素酶能够分解土壤中的 有机物,如纤维素和木质素等,秸秆还田后的植物残 茬在子囊菌门的降解作用下充分释放养分[53]. 结果 显示油菜季经秸秆还田处理的子囊菌门较常规施肥 处理显著提高,这是由于子囊菌门适宜在干旱环境 下生存[54],油菜季土壤通气性较淹水状态的水稻季 更利于其生长. 水稻季经秸秆还田处理的担子菌门 相对丰度较常规施肥处理显著提高,可能由于担子 菌门中的白腐真菌能够有效分解秸秆中碳氮比高的 木质素[17],秸秆还田为担子菌门提供了良好的生长 环境,能够充分利用降解作物残留物,促进其快速增 长,从而改变真菌群落的物种组成.

#### 4 结论

水稻-油菜轮作模式下,秸秆还田配施化肥能够 有效提高土壤养分含量,水稻季土壤 OM 和 TP 显著 提高,油菜季表现为 AN 含量显著提高. 秸秆还田可 显著提高稻-油轮作农田土壤脲酶和磷酸酶活性. 在 秸秆还田的基础上,相比于水稻季,油菜季土壤酶活 性对秸秆还田的响应更为显著. 其中,水稻季土壤 OM、TP及TN是影响土壤酶活性的主要环境因子, 油菜季土壤 AN、TP及 AP为主控因子. 土壤 pH与 土壤各酶活性呈负相关. 采用高通量测序技术表明, 秸秆还田配施化肥能够提升水稻季土壤细菌与真菌 的 Chao1 和 Shannon 多样性指数,降低油菜季土壤 细菌的 Chao1 和 Shannon 多样性指数,增加真菌的 Chao1 和 Shannon 多样性指数. 变形菌门、绿弯菌 门、酸杆菌门和硝化螺旋菌等是主要细菌类群,子囊 菌门和担子菌门是主要的真菌类群,其中土壤 TP 和 AP 是影响稻-油轮作下农田土壤细菌群落构成的 主要因子,土壤 OM、AN 和 pH 是影响土壤真菌群 落发生改变的主要因素,土壤磷酸酶与脲酶和土壤 微生物之间关系密切. 因此,持续性秸秆还田配施化 肥能够改善稻-油轮作农田土壤的生态环境和维持 生态系统功能的稳定,进而保障农业的可持续发展.

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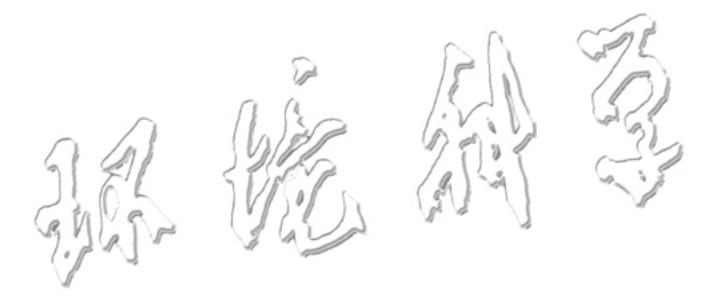
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