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引用本文:

樊代佳, 李成芳, 曹凑贵. 氮肥深施对免耕稻田土壤有机质化学特性和胞外酶活性的影响[J]. 农业环境科学学报, 2021, 40(3): 621-630.

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氮肥深施对免耕稻田土壤有机质化学特性和胞外酶活性的影响

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摘要: 为了探究氮肥深施措施对免耕稻田土壤有机质化学特性及土壤酶活的影响机制, 于2015—2016年在湖北省武穴市花桥镇试验点开展水稻-油菜免耕复种大田试验。本试验通过¹³C核磁共振和热裂解气相色谱/质谱联用法测定土壤有机质的化学特性, 通过冗余分析揭示土壤有机质化学特性及土壤胞外酶活性之间的相互作用关系。结果表明, 与传统表施相比, 氮肥深施提高腐殖质中胡敏酸的有机碳含量(10.5%~30.7%)、轻组有机质的有机碳含量(31.7%~53.5%)、胡敏酸的疏水性(1.3%~11.6%)和轻组有机质中多糖与脂质的含量(9.0%~24.1%和43.4%~68.3%), 降低土壤β-葡萄糖苷酶(BG)(22.2%~48.9%)和β-N-乙酰氨基葡萄糖苷酶(NAG)活性(32.7%~40.4%), 提高L-亮氨酸氨基肽酶(LLA)(37.1%~38.5%)和酚氧化酶(PHO)活性(11.0%~16.0%)。冗余分析显示, 土壤有机质的化学特性与LLA和PHO活性呈正相关, 与BG和NAG活性呈负相关。本研究结果表明, 与传统表施相比, 氮肥深施尤其是深施10 cm主要通过促进作物和微生物群落生长提高土壤胡敏酸和轻组有机质的有机碳含量, 且通过调控土壤BG、NAG和PHO活性改变胡敏酸和轻组有机质的化学组成与性质, 从而改善土壤惰性有机质稳定性且提高活性有机质, 最终提高土壤肥力和质量。因此氮肥深施尤其是深施10 cm值得在免耕稻田生产中得到进一步推广。

关键词: 氮肥深施; 免耕稻田; 腐殖质; 胞外酶; 热裂解气相色谱/质谱

中图分类号: S153.621; S154.2 文献标志码: A 文章编号: 1672-2043(2021)03-0621-10 doi:10.11654/jaes.2020-1125

Effects of nitrogen deep placement on chemical properties of soil organic matter and extracellular enzyme activity in no-tillage paddy fields

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Abstract: This study investigated the impacts of N deep placement on the chemical properties of soil organic matter (SOM) and soil enzyme activity in no-tillage (NT) paddy soil. Multiple cropping of rice and rapeseed was carried out under NT in the experimental site located in Huaqiao County, Wuxue City, Hubei Province from 2015 to 2016. The growing seasons of rice and rapeseed were from June to October and from October to May of the next year, respectively. Five fertilizing treatments, including no N fertilizer (CK), N surface broadcasting (SB), and N deep placement at soil depths of 5 cm, 10 cm (10D), and 20 cm were applied with three replicates per treatment.

收稿日期: 2020-09-24 录用日期: 2020-11-23

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基金项目: 国家重点研究计划项目(2017YFD0301403); 国家自然科学基金项目(31671637); 湖北省自然科学基金项目(2018CFB608)

Project supported: National Key R&D Program of China (2017YFD0301403); The Natural Science Foundation of China (31671637); The Natural Science Foundation of Hubei Province (2018CFB608)

The soil organic carbon (SOC) contents of different SOM fractions, the chemical compositions and properties of humic acid (HA) and light fraction organic matter (LFOM), and the activities of different soil enzymes were determined. Redundancy analysis was carried out to reveal the relationships between the chemical properties of SOM and enzyme activity. The results showed that compared with SB, N deep placement significantly increased the SOC contents in the HA and LFOM by approximately 10.5%~30.7% and 31.7%~53.5%, respectively. The hydrophobicity of HA under N deep placement was 1.3%~11.6% higher than that under SB. Moreover, N deep placement significantly improved the contents of polysaccharides and lipids in the LFOM by 9.0%~24.1% and 43.4%~68.3%, respectively. Compared with those under SB, N deep placement significantly inhibited the activities of β -glucosidase (BG) and β -N-acetylglucosaminidase (NAG) by 22.2%~48.9% and 32.7%~40.4%, respectively, whereas it significantly stimulated the activities of L-leucine aminopeptidase (LLA) and phenol oxidase (PHO) by 37.1%~38.5% and 11.0%~16.0%, respectively. In addition, the redundancy analysis showed that the contents and hydrophobicity of HA and the contents of polysaccharides and lipids in the LFOM were positively correlated with the LLA and PHO activities and negatively correlated with the BG and NAG activities. The results demonstrated that the chemical compositions and properties of humus and LFOM were regulated by the soil enzyme activity. Nitrogen deep placement decreased the humus aromaticity by stimulating the PHO activity and increased the polysaccharide contents in the LFOM by inhibiting the BG and NAG activities. Moreover, compared with those under SB, N deep placement, especially 10D, increased the SOC contents in the HA and LFOM mainly by activating the growth of crops and microbial communities, and altered their chemical properties and compositions mainly through regulating the activities of BG, NAG, and PHO, thereby promoting the soil fertility and quality. Therefore, it is worthwhile to further advocate for and improve the application of 10D placement in NT paddy fields.

Keywords: nitrogen deep placement; no-tillage paddy; humus; extracellular enzyme; pyrolysis-gas chromatography/mass spectrometry

水稻 (*Oryza sativa* L.) 是我国最重要的粮食作物之一, 其种植面积占全国粮食作物总面积的 26.7%^[1]。免耕作为一种新型保护性耕作措施近年来被广泛应用于我国水稻生产^[2-4]。农民常通过表施氮肥提高作物产量。有研究表明, 氮肥表施会造成氮素的大量损失^[5-7]。与传统氮肥表施相比, 氮肥深施可以促进作物生长, 提高作物产量。Ibrahim 等^[8]发现氮肥深施 10 cm 通过增加作物根系体积促进作物对营养元素的吸收, 最终提高作物产量。还有研究表明, 氮肥深施可以提高氮肥利用率, 减少氮素损失。Rychel 等^[9]研究指出, 氮肥深施 20 cm 显著提高作物的含氮量并降低土壤氧化亚氮排放量。Wu 等^[10]研究也发现, 氮肥深施通过促进作物对氮肥的吸收提高氮素利用率。因此, 近年来氮肥深施在我国免耕稻田生产中得到了大面积推广^[11]。

土壤有机质是植物养分的主要来源, 是衡量土壤肥力的重要指标^[12]。研究已表明, 土壤有机质含量与作物产量显著正相关^[12-13]。耕作和施肥等农业措施影响着土壤有机质的含量和质量。有研究发现, 保护性耕作较传统翻耕提高了土壤有机质的含量, 促进了有机碳的固定^[14-16]。同时, 研究表明, 施肥措施显著影响土壤有机质的含量及性质^[17-18]。氮肥深施作为一种高效的氮肥施用方式显著影响着土壤有机质。Zhang 等^[19]研究指出, 氮肥深施可以显著提高土壤有机质的含量; Liu 等^[20]发现氮肥深施可以降低土壤有

机质的分解速率。在众多土壤理化特性中, 有机质的化学组成和性质对土壤肥力和养分循环起决定性作用^[21-22], 因此不同施肥措施对土壤有机质化学特性的影响一度成为研究热点。如史吉平等^[23]发现长期施用有机肥或有机无机肥配施会改变土壤腐植酸中各化学官能团的组成和含量, Francioso 等^[24]的研究结果表明施用粪肥会显著提高土壤有机质中脂肪族和芳香族化合物的含量。然而, 当前有关氮肥深施对土壤有机质化学组成与性质影响的研究寥寥无几。因此, 探讨氮肥深施对免耕稻田土壤有机质化学特性的影响, 对于明确氮肥深施调控土壤肥力和生产力的机制以及对其作出相应的改善对策具有重要意义。

土壤有机质按化学组成可分为腐殖质和非腐殖质两大类。腐殖质由新鲜有机质经微生物分解转化形成, 属于较稳定的惰性有机质, 主要由胡敏素 (Humin, HM)、胡敏酸 (Humic acid, HA) 和富里酸 (Fulvic acid, FA) 组成^[25]。腐殖质含有羟基、羧基、羰基、甲氧基等多种官能团, 这些官能团的含量和占比共同决定腐殖质的化学稳定性和作用^[26], 也是影响土壤肥力的重要因素^[27-28]。HA 是腐殖质中最活跃的成分, 在一定程度上表征腐殖质的芳香性、化学稳定性和有效性, 其含量越高, 表明腐殖质的活性越高, 品质越好^[29]。因此, 研究 HA 含量、化学结构与性质的变化可以揭示腐殖质以及土壤肥力的变化情况。非腐殖质包含小分子有机化合物、可溶性有机碳 (DOC) 和微

生物量碳(MBC)等,属于易分解的活性有机质,对植物养分的供应有最直接的作用^[30-31]。相对密度低于 $2\text{ g}\cdot\text{cm}^{-3}$ 、主要源于未分解及半分解植物残体和部分微生物体的轻组有机质(Light fraction organic matter, LFOM)占活性有机质总含量的76%~96%^[32],因此通过测定LFOM的含量与化学组成可以反映土壤活性有机质及土壤肥力的情况。

本试验旨在通过比较传统表施和氮肥深施之间的差异,明确氮肥深施措施对免耕稻田土壤有机质化学组成和性质的影响机制。本研究假设,与传统表施相比,氮肥深施通过调控作物生长和土壤微生物活性改变土壤惰性和活性有机质的含量与化学特性,改变免耕稻田土壤有机质的质量和有效性,最终影响土壤肥力和生产力。为了验证该假设,本试验采用 ^{13}C 核磁共振(Nuclear magnetic resonance, NMR)和热裂解气相色谱/质谱联用法(Pyrolysis-gas chromatography/mass spectrometry, Py-GC/MS)研究土壤有机质的化学特性,通过 β -葡萄糖苷酶(β -glucosidase, BG)、 β -N-乙酰氨基葡萄糖苷酶(β -N-acetylglucosaminidase, NAG)、L-亮氨酸氨基肽酶(L-leucine aminopeptidase, LLA)、酚氧化酶(Phenol oxidase, PHO)和过氧化物酶(Peroxidase, PEO)5种酶的活性来反映土壤酶活性、能提,通过冗余分析(Redundancy analysis, RDA)研究土壤有机质各组分化学特性与土壤酶活之间的关系。

1 材料与方法

1.1 试验地情况

在湖北省武穴市花桥镇现代农业示范中心试验基地($30^{\circ}00'48''\text{N}$, $115^{\circ}47'16''\text{E}$, 海拔19 m)开展大田试验。该地区属于亚热带湿润季风气候,年平均气温 17.8°C ,年平均降雨量1361 mm,年平均日照时长约260 d,土壤为潜育土(FAO土壤分类)。从2011年6月起直至本试验开展期间,该试验田一直进行水稻(黄华占)-油菜(华双3号, *Brassica napus* L.)免耕复种。试验田本底土(0~20 cm)理化性质如下: pH 5.36, 总有机碳含量 $20.92\text{ g}\cdot\text{kg}^{-1}$, 总氮含量 $2.06\text{ g}\cdot\text{kg}^{-1}$, 铵态氮含量 $8.48\text{ mg}\cdot\text{kg}^{-1}$, 硝态氮含量 $0.59\text{ mg}\cdot\text{kg}^{-1}$, 总磷含量 $2.36\text{ g}\cdot\text{kg}^{-1}$, 总钾含量 $3.77\text{ g}\cdot\text{kg}^{-1}$, 土壤容重 $1.08\text{ g}\cdot\text{cm}^{-3}$ 。

1.2 试验设计与大田管理

试验处理包括不施肥(CK)、氮肥传统表施(SB)、以及氮肥深施5 cm(5D)、10 cm(10D)和20 cm(20D), 采取随机区组设计, 3次重复。小区面积 36 m^2 (长

8.0 m, 宽4.5 m), 每小区设两个厢(长7.4 m, 宽1.8 m)。为方便灌溉和排水, 在厢和小区四周挖掘沟渠(宽0.3 m, 深0.25 m)。为防止水肥串流, 小区之间用田埂(宽0.4 m, 高0.4 m, 覆盖塑料薄膜)和保护行(宽1 m)隔开。试验田总面积 1207.5 m^2 (长35 m, 宽34.5 m), 四周设置围沟和保护行, 以方便田间管理。

水稻在每年6月进行人工抛秧($1.8\times10^5\text{ 穴}\cdot\text{hm}^{-2}$), 10月收获。水稻生育期施肥总量为 $180\text{ kg N}\cdot\text{hm}^{-2}$ 、 $90\text{ kg P}_2\text{O}_5\cdot\text{hm}^{-2}$ 和 $180\text{ kg K}_2\text{O}\cdot\text{hm}^{-2}$, 选用复合肥($\text{N}:\text{P}_2\text{O}_5:\text{K}_2\text{O}=15\%:15\%:15\%$)、尿素(含氮量46%)以及氯化钾(K_2O 含量60%)作为肥料。全部磷肥作为基肥一次性施用; 钾肥一半基施, 一半在拔节期追施; 氮肥分别在秧苗期、分蘖期、拔节期和孕穗期施用总量的50%、20%、12%和18%, 其中基肥为复合肥, 追肥为尿素。为了尽量减少肥料施用对土壤的物理扰动, 本试验采用SB-13B3型半自动深施施肥器(台州市农乐塑料有限公司, 中国)进行肥料深施, 施肥点位于水稻之间, 施肥点间距约20 cm。每年6月上旬在稻田淹水前喷洒30%丙草胺乳油除草, 其他时期进行人工除草。水稻生育期内采取间歇灌溉, 维持田间水面在4~8 cm的深度。油菜分别在每年10月和来年5月进行直播和收获, 同样采用免耕栽培。油菜生育期总施肥量为 $180\text{ kg N}\cdot\text{hm}^{-2}$ 、 $90\text{ kg P}_2\text{O}_5\cdot\text{hm}^{-2}$ 和 $150\text{ kg K}_2\text{O}\cdot\text{hm}^{-2}$, 所用肥料类型与水稻一致。在油菜苗期氮肥总量的一半进行深施, 其余氮肥则在抽薹期和开花期进行表施。水稻和油菜季秸秆全部粉碎后覆盖还田。

1.3 土壤采集与测定

在每年的水稻收获期采集土壤样本。每个小区随机选取5个采样点, 用内径7 cm的取土器采集0~20 cm深的土样, 将来自同一小区的土样装入密封袋内形成混合样本并立刻运回实验室进行处理。挑出土样中的石头和动植物残体后将其轻轻捏碎并充分混匀, 分成两个子样本, 一个子样本保存在 -20°C 下用于有机质化学特性的测定, 另一个子样本冷藏于 -80°C 下用于土壤酶活的测定。

土壤有机质各组分的有机碳含量通过重铬酸钾外加热法测定^[33]。用氢氧化钠(NaOH)-焦磷酸钠($\text{Na}_4\text{P}_2\text{O}_7$)提取法分离和提取土壤腐殖质中的HA、FA和HM^[34]。利用固体交叉极化/魔角旋转(CP/MAS) ^{13}C -NMR光谱法对HA的主要化学官能团结构及组成进行测定。通过浮选法从土壤中分离提取LFOM^[35-36]。参考Saiya-Cork等^[37]的方法对土壤中5种胞外酶

(BG、NAG、LLA、PHO和PEO)的活性进行测定。

1.4 数据处理与分析

在对数据进行正态分布和方差齐性检验后,在SPSS 22.0中进行单因素方差分析(ANOVA),对处理影响显著的数据进行Turkey HSD检验以两两比较不同处理间的影响差异。用OriginPro(2017版)进行绘图,用Canoco(5.0版)进行RDA分析。所有统计分析的显著性水平都为 $P<0.05$ 。

2 结果与分析

2.1 腐殖质各组分的有机碳含量

氮肥施用措施显著影响腐殖质中HA和FA的有机碳含量以及二者的比例HA/FA(表1)。与SB相比,氮肥深施下HA的有机碳含量显著高10.4%~30.7% ($P<0.05$);在氮肥深施处理中,与5D和20D相比,10D下HA的有机碳含量显著提高10.7%~18.3% ($P<0.05$)。此外,SB、5D、10D和20D下FA的有机碳含量之间都没有显著差异。与SB相比,10D下的HA/FA显著高22.8%~32.5% ($P<0.05$);氮肥深施处理中,10D下的HA/FA比5D和20D显著高12.7%~21.0% ($P<0.05$)。氮肥施用措施对HM的有机碳含量没有显著影响。

2.2 LFOM的有机碳含量

氮肥施用措施对LFOM的有机碳含量有显著影响(图1)。与SB相比,氮肥深施处理下LFOM的有机碳含量显著提高1.7%~53.5% ($P<0.05$);在氮肥深施处理中,与5D和20D相比,10D下的LFOM有机碳含

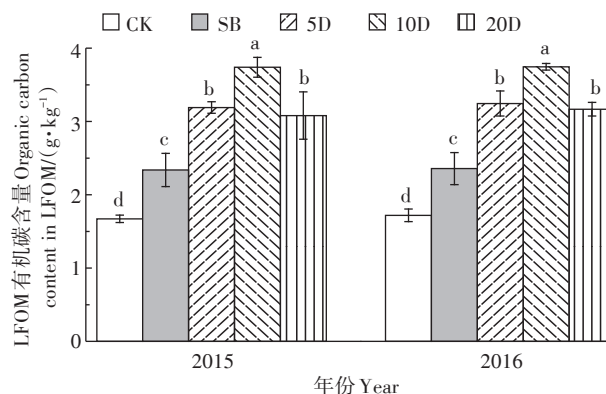


图1 2015和2016年水稻收获期免耕稻田在不同施肥处理下LFOM的有机碳含量

Figure 1 Organic carbon contents in LFOM in no-tillage paddy fields under different treatments at harvest stages in 2015 and 2016

量显著升高2.5%~16.5% ($P<0.05$)。

2.3 HA的化学组成与性质

氮肥施用措施对HA的化学特性有影响(表2)。与SB相比,氮肥深施下烷基碳和羰基碳的含量、二者的比例以及HA的疏水性分别提高3.9%~23.3%、16.7%~225.0%、5.9%~43.7%和1.0%~11.1%,烷氧碳、芳香碳和芳香氧碳的含量以及HA的芳香性则分别降低0.9%~15.2%、1.8%~25.0%、2.3%~27.8%和4.3%~21.7%;此外,与SB相比,5D和10D下羧基碳的含量高2.0%~5.9%,20D下羧基碳的含量则低1.1%~2.0%。氮肥深施处理中,与5D和20D相比,10D下烷基碳、羧基碳、羰基碳、烷基/烷氧以及HA的疏水性分别高12.1%~17.6%、0.2%~7.0%、32.7%~178.6%、

表1 2015和2016年水稻收获期免耕稻田在不同施肥处理下土壤腐殖质中各组分有机碳的含量

Table 1 Organic carbon contents in different humic substances under different treatments in no-tillage paddy fields at harvest stages in 2015 and 2016

年份 Year	处理 Treatments	HA/(g·kg ⁻¹)	FA/(g·kg ⁻¹)	HM/(g·kg ⁻¹)	HA/FA
2015	CK	3.95±0.20d	3.09±0.18b	9.94±0.30a	1.28±0.13b
	SB	4.50±0.25c	3.58±0.11a	10.03±0.30a	1.26±0.11b
	5D	5.31±0.29b	3.70±0.09a	10.10±0.16a	1.44±0.04b
	10D	5.88±0.13a	3.51±0.11a	10.15±0.13a	1.67±0.09a
	20D	4.97±0.28b	3.60±0.14a	10.04±0.20a	1.38±0.12b
2016	CK	3.99±0.14d	3.22±0.20b	9.15±0.29a	1.24±0.12b
	SB	4.48±0.04c	3.64±0.17a	9.09±0.16a	1.23±0.05b
	5D	5.05±0.18b	3.78±0.16a	9.19±0.14a	1.34±0.03b
	10D	5.65±0.13a	3.76±0.25a	9.22±0.41a	1.51±0.07a
	20D	5.05±0.21b	3.78±0.12a	9.18±0.31a	1.34±0.10b

注:同一列中不同字母代表同一年处理间在0.05水平上差异显著。下同。

Note: Different letters in the same column in the same year indicate significant differences among treatments at the level of 0.05. The same below.

23.8%~35.4%和6.4%~10.0%,而烷氧碳、芳香碳和芳香氧碳的含量以及HA的芳香性分别低9.5%~13.4%、4.7%~18.1%、17.0%~24.0%和10.0%~18.2%。

2.4 LFOM的化学组成

LFOM中多糖类化合物的相对含量最高,木质素次之,其次是含氮化合物,脂质的相对含量则最低(表3)。氮肥施用措施对LFOM的化学组成有显著影响。与SB相比,氮肥深施下多糖和脂质的含量分别显著增加9.0%~24.1% ($P<0.05$)和43.2%~68.2% ($P<0.05$),10D下含氮化合物的含量则显著减少31.1%~32.9% ($P<0.05$)。在氮肥深施处理中,与5D和20D相比,10D下多糖的含量显著提高7.9%~12.4% ($P<0.05$),含氮化合物的含量则显著降低31.5%~34.0% ($P<0.05$)。此外,氮肥施用措施对木质素的含量没有显著影响。

2.5 土壤胞外酶活性

氮肥施用措施对土壤中各胞外酶的活性都有显著影响(表4)。与SB相比,氮肥深施下BG和NAG的活性分别显著降低22.2%~48.8% ($P<0.05$)和32.6%~40.4% ($P<0.05$),10D下LLA和PHO的活性则分别显著提高37.1%~38.5% ($P<0.05$)和11.1%~16.0% ($P<0.05$)。氮肥深施处理中,10D下BG的活性在2015年比5D和20D显著降低29.3%~30.3% ($P<0.05$),在2016年只比20D显著降低31.8% ($P<0.05$);10D下LLA的活性则比5D和20D显著提高25.5%~36.2% ($P<0.05$);此外,10D下PHO的活性在2015年比5D和20D显著提高9.5%~13.1% ($P<0.05$),在2016年则只比20D显著提高11.4% ($P<0.05$)。氮肥施用措施对PEO的活性没有显著影响。

表2 2015和2016年水稻收获期免耕稻田在不同氮肥施用措施下HA中各化学官能团的相对含量(%)

Table 2 Relative abundance of different chemical functional groups in HA in no-tillage paddy fields under different treatments at harvest stages in 2015 and 2016(%)

年份 Year	处理 Treatments	烷基碳 C-H	烷氧碳 C-O	芳香碳 Ar	芳香氧碳 Ar-O	羧基碳 -COOH	羰基碳 C=O	烷基/烷氧 C-H/C-O	疏水性 HYD	芳香性 ARM
2015	CK	25.04	38.63	12.05	9.45	13.43	1.40	0.65	0.87	0.25
	SB	30.98	35.75	10.27	8.46	12.28	2.26	0.87	0.99	0.22
	5D	33.27	33.82	9.13	7.63	12.97	3.18	0.98	1.00	0.20
	10D	37.64	30.33	8.70	6.11	13.00	4.22	1.24	1.10	0.18
	20D	32.19	34.65	10.09	7.78	12.15	3.14	0.93	1.00	0.21
2016	CK	25.75	37.25	11.81	10.00	13.20	1.99	0.69	0.91	0.26
	SB	31.49	34.87	10.88	9.42	12.26	1.08	0.90	1.07	0.23
	5D	34.65	33.05	9.38	8.42	12.51	1.99	1.05	1.10	0.21
	10D	38.83	29.92	8.16	6.99	12.59	3.51	1.30	1.17	0.18
	20D	33.03	34.54	9.96	9.20	12.01	1.26	0.96	1.09	0.22

表3 2015和2016年水稻收获期免耕稻田在不同氮肥施用措施下LFOM中各主要化合物的相对含量(%)

Table 3 Relative abundance of dominant compounds in LFOM in no-tillage paddy fields under different treatments at harvest stages in 2015 and 2016(%)

年份 Year	处理 Treatments	多糖 Polysaccharide	含氮物 N-bearing	木质素 Lignin	脂质 Lipid	其他 Others
2015	CK	27.83±0.82d	11.44±0.75a	17.45±1.08a	0.83±0.12b	42.45±0.94a
	SB	31.00±0.71c	8.92±0.51b	17.11±0.16a	0.85±0.05b	42.12±0.24a
	5D	35.02±0.56b	9.32±1.10b	17.51±1.05a	1.37±0.10a	36.77±1.90b
	10D	38.48±0.56a	6.15±0.47c	17.60±0.60a	1.43±0.14a	36.35±0.38b
	20D	34.25±0.73b	9.01±1.27b	17.18±1.01a	1.32±0.07a	38.24±2.64b
2016	CK	31.04±0.57d	11.25±1.01a	16.90±0.28a	0.89±0.15b	39.92±1.81a
	SB	34.38±0.72c	8.37±0.46b	16.86±0.34a	0.95±0.07b	39.44±0.13a
	5D	38.19±0.43b	8.40±0.46b	16.87±0.82a	1.41±0.03a	35.13±0.96a
	10D	41.22±1.52a	5.62±0.90c	16.76±1.12a	1.46±0.16a	34.95±3.05a
	20D	37.48±0.90b	8.20±0.78b	16.70±0.65a	1.36±0.19a	36.25±1.44a

2.6 土壤有机质化学特性与土壤胞外酶活性之间的关系

RDA结果表明,氮肥深施尤其是氮肥深施10 cm对土壤酶活有显著影响(图2)。各胞外酶与HA、HA/FA、C-H/C-O、HYD和ARM密切相关。HA、HA/FA、C-H/C-O、HYD、ARM与LLA、PHO、PEO呈正相关,与BG、NAG呈负相关。另外,LFOM、Ps、Lp与LLA、PHO、PEO呈正相关,而与BG、NAG呈负相关;与之相反的是,Nb与BG、NAG呈正相关,而与LLA、PHO、

PEO呈负相关;此外,Lg与NAG呈正相关,但与其他胞外酶相关性较低。

3 讨论

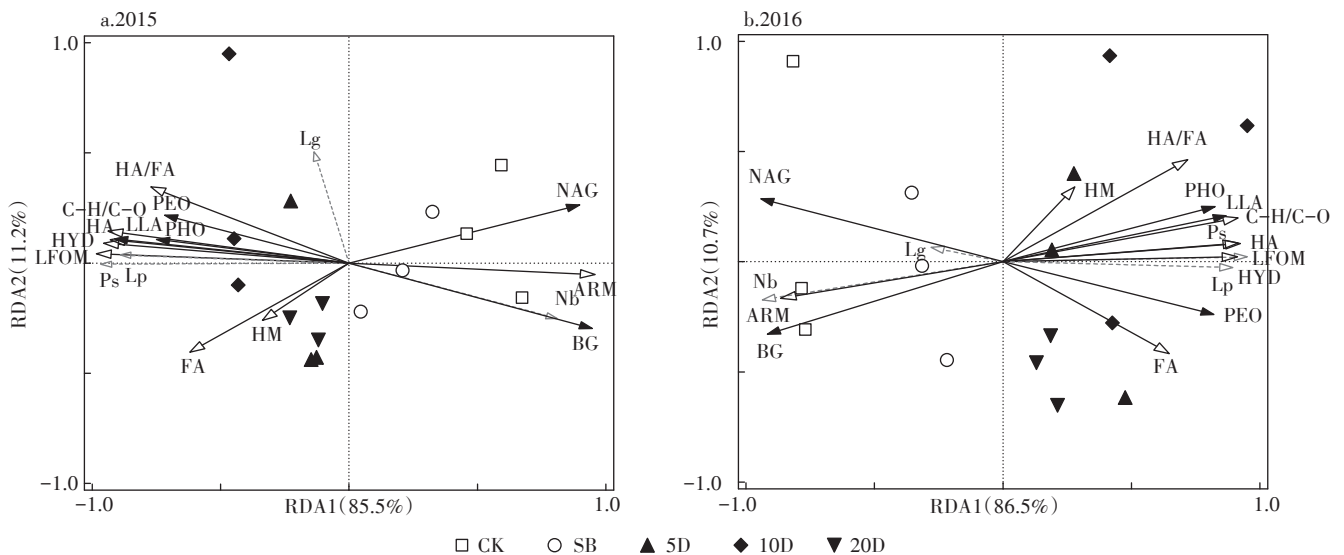
3.1 氮肥深施对土壤腐殖质有机碳含量及其化学性质的影响

有研究表明,HA是腐殖质中活性最高的成分,可以和无机微量元素螯合形成有机盐而免于淋失,是植

表4 2015和2016年水稻收获期免耕稻田在不同氮肥施用措施下土壤的酶活性($\text{nmol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$)

Table 4 Enzyme activity in soil in NT paddy fields under different treatments at harvest stages in 2015 and 2016($\text{nmol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$)

年份 Year	处理 Treatments	BG	NAG	LLA	PHO	PEO
2015	CK	152.7±21.6a	98.5±4.1a	1.62±0.21c	228.4±11.1c	498.3±22.3b
	SB	120.2±11.7b	69.3±12.5b	2.37±0.03b	243.6±8.0bc	535.3±13.3a
	5D	92.2±11.9c	46.7±6.4c	2.59±0.14b	258.0±15.8b	548.6±28.8a
	10D	65.2±13.4d	43.0±14.3c	3.25±0.05a	282.6±13.0a	545.8±14.8a
	20D	93.5±7.9c	46.0±6.9c	2.46±0.27b	249.8±12.0bc	539.0±16.1a
2016	CK	147.2±14.0a	99.9±18.4a	1.75±0.13c	225.7±12.6c	472.2±5.5b
	SB	121.4±15.5b	66.1±10.8b	2.39±0.17b	255.9±12.6b	507.9±14.9a
	5D	81.4±10.2cd	41.1±10.5c	2.53±0.13b	268.3±15.0ab	512.8±12.9a
	10D	62.1±18.5d	39.4±9.5c	3.31±0.09a	284.2±9.1a	516.3±8.3a
	20D	91.0±6.9c	44.1±1.9c	2.43±0.16b	255.2±11.5b	509.2±13.2a



腐殖质各组分的有机碳含量与化学性质用黑色实线空心箭头表示,轻组有机质的有机碳含量与化学组成用灰色虚线空心箭头表示,土壤各胞外酶的活性用黑色实心箭头表示;不同处理下的单个样本用不同形状的数据点表示。HA:胡敏酸,FA:富里酸,HM:胡敏素,HA/FA:胡敏酸和富里酸的比例,C-H/C-O:烷基碳和烷氧基碳的比例,HYD:疏水性,ARM:芳香性,LFOM:轻组有机质,Ps:多糖,Nb:含氮化合物,Lg:木质素,Lp:脂质;BG:β-葡萄糖苷酶,NAG:β-N-乙酰氨基葡萄糖苷酶,LLA:L-亮氨酸氨基肽酶,PHO:酚氧化酶,PEO:过氧化物酶

Organic carbon contents and chemical properties of humic substances are denoted by black hollow arrows, organic carbon contents and chemical compounds in LFOM are denoted by gray dashed hollow arrows, and extracellular enzyme activity is denoted by black solid arrow. Individual samples under different treatments are denoted by data points in different shapes. HA, humic acid; FA, fulvic acid; HM, humin; HA/FA, ratio of HA to FA; C-H/C-O, ratio of alkyl carbon to alkoxy carbon; HYD, hydrophobicity; ARM, aromaticity; LFOM, light fraction organic matter; Ps, polysaccharide; Nb, N-bearing compound; Lg, lignin; Lp, lipid. BG, β-glucosidase; NAG, β-N-acetylglucosaminidase; LLA, L-leucine aminopeptidase; PHO, phenol oxidase; PEO, peroxidase

图2 2015年和2016年水稻收获期免耕稻田在不同氮肥施用措施下土壤中有有机质的化学组成和性质与土壤酶活之间的关系

Figure 2 Relationships among the chemical composition and properties of soil organic matter fractions and enzyme activity in soil in no-tillage paddy fields under different treatments at harvest stages in 2015 and 2016

物运输营养物质和水分的重要介质;此外,它也可以和土壤黏粒结合形成稳定的聚集体,提高土壤物理结构的稳定性,因此HA对促进作物生长以及提高土壤肥力和质量都有很大的作用^[38-39]。HA的积累也意味着土壤腐殖化程度的提高^[26]。本研究结果显示,与传统表施相比,氮肥深施显著提高免耕稻田土壤腐殖质中HA的有机碳含量以及HA和FA的比例(表1),说明氮肥深施特别是深施10 cm可以显著提升腐殖质的品质和土壤的肥力。Zhang等^[40]研究也发现,施用氮肥能提高土壤HA的有机碳含量。HA主要是植物的凋落物、根系等经微生物降解转化而形成^[41-42],因此氮肥深施能激发作物生长,从而促使更多植物残体、凋落物或作物残茬和秸秆输入土壤^[43],导致腐殖质中HA有机碳含量及其在腐植酸中比例的上升。同理,本研究中氮肥深施10 cm下HA的有机碳含量比深施5 cm和20 cm时更高(差异显著,表1),其原因可能是深施10 cm比深施5 cm和20 cm对作物生长的促进作用更强^[44]。

本研究表明,氮肥深施显著改变免耕稻田土壤腐殖质的化学性质(表2)。与传统表施相比,氮肥深施尤其是深施10 cm提高了HA中烷基碳的含量和HA的疏水性(表2)。烷烃属于土壤碳库中一种较难分解的脂肪族化合物,其疏水性对土壤有机质的分解和稳定性起着至关重要的作用^[45],因此氮肥深施下烷基碳含量以及HA疏水性的升高说明氮肥深施相对于传统表施更有利于土壤腐殖质化学稳定性的提高。然而,本研究发现氮肥深施下芳香族碳含量以及HA的芳香性都显著降低(表2),意味着HA中复杂有机化合物的减少,化学结构变简单^[29]。与之类似的是,Řezáčová等^[46]也发现施肥会降低土壤腐殖质的芳香性和分子量。这可能是氮肥深施下更多的外源碳氮输入激发了特定土壤微生物群落的活性^[44],从而促进了其对芳香族化合物的氧化分解。此外,本研究结果表明,氮肥深施10 cm土壤PHO的活性比深施5 cm和20 cm更高(表4),而PHO酶催化反应的底物酚类属于芳香族化合物,导致本研究中深施10 cm HA的芳香性比深施5 cm和20 cm更低(表2),RDA结果中PHO活性与HA芳香性呈显著负相关,进一步支撑该推论(图2)。

3.2 氮肥深施对LFOM的有机碳含量及化学组成的影响

本研究发现,与传统表施相比,氮肥深施显著提高了免耕稻田LFOM的有机碳含量(图1),而LFOM

与微生物生长和营养供给紧密相关^[47-48],因此该结果表明氮肥深施可以通过改变LFOM的含量来提高土壤肥力。同样,有很多研究发现长期施肥会提高土壤中活性有机质的有机碳含量^[49-51]。这是因为LFOM等活性有机质更易被微生物分解利用且周转速率更高,因此更易受到施肥等农业管理措施的影响^[52];此外,LFOM主要来源于植物和微生物残体^[53-54],因此,氮肥深施相对于传统表施能促进作物生长,从而提高作物残茬、凋零物、秸秆的还田,并激发土壤微生物活性,提高LFOM有机碳含量^[43]。同理,氮肥深施10 cm比深施5 cm和20 cm显著提高作物生长与微生物群落丰度^[44]也可能是本研究中深施10 cm时LFOM有机碳含量比深施5 cm和20 cm更高(差异显著,图1)的原因。

本研究发现,氮肥深施显著影响免耕稻田LFOM的化学组成。与传统表施相比,氮肥深施提高LFOM中多糖和脂质的含量,而降低含氮化合物的含量(表3)。氮肥深施下多糖的增多一方面可能是因为氮肥深施促进作物的生长,促使更多的植物残茬、秸秆和凋落物等外源碳的输入;另一方面氮肥深施对微生物活性的激发也会导致更多糖的产生^[43,55];此外还可能是因为氮肥深施下土壤DOC和MBC等小分子活性有机碳的增加使得微生物对多糖等大分子有机碳源的需求降低,即减少微生物对多糖的分解利用,导致LFOM中多糖含量增加^[43,56]。氮肥深施下BG和NAG的显著降低以及BG和NAG活性与多糖含量的负相关都进一步论证了这一点(表4和图2)。同理,与深施5 cm和20 cm相比,氮肥深施10 cm提高作物生长、微生物群落丰度及小分子活性有机碳含量^[44],这也可能是导致氮肥深施10 cm下LFOM中多糖含量比深施5 cm和20 cm更高(表3)的原因。本研究表明,与传统表施相比,氮肥深施提高了脂质含量。这可能与氮肥深施促进作物根系的生长有关。作物根系经微生物降解后会产生稳定性较强的脂质化合物^[44,57],因此提高了LFOM中脂质的累积量。本研究表明,与传统表施及氮肥深施5 cm和20 cm相比,氮肥深施10 cm降低了含氮化合物的含量(表3)。其原因可能是氮肥深施10 cm对微生物活性的激发促使其对氮素的需求增加^[43-44],从而提高LLA等分解转化含氮化合物的酶类的含量,最终导致LFOM中含氮化合物的消耗。本研究结果中氮肥深施10 cm处理下更高的LLA活性以及LLA活性与含氮物之间的显著负相关也印证了这一点(表4和图2)。

3.3 氮肥深施对土壤胞外酶活性的影响

本研究发现,与传统表施相比,氮肥深施显著降低了免耕稻田土壤中BG和NAG的活性,而氮肥深施10 cm显著提高了LLA和PHO的活性(表4),说明氮肥深施尤其是深施10 cm显著改变土壤的酶活性。相对于动植物等同样能产生酶的有机体,土壤胞外酶主要由微生物群落产生;与动植物相比,土壤微生物群落的代谢活性更高且生命周期更短^[58]。据此可以推断,氮肥深施主要通过影响土壤微生物群落来改变土壤胞外酶的活性。有研究表明,施肥提高土壤微生物的活性和酶活性^[59],但本试验结果显示氮肥深施对各胞外酶活性的影响并不一致(表4)。如前所述,与传统表施相比,氮肥深施下更高的土壤微生物群落丰度可能是本研究中更高的LLA、PHO和PEO活性的原因,而BG和NAG活性的下降则可能与氮肥深施下含量更高的DOC和MBC会在一定程度上替代多糖成为微生物群落的有机碳源有关^[44]。同理,不同氮肥深施措施下(深施5、10 cm与20 cm)微生物群落丰度和DOC、MBC含量的差异^[44]可能是造成氮肥深施10 cm具有更低BG活性和更高LLA、PHO活性的主要原因(表4)。

4 结论

(1)与传统表施相比,氮肥深施通过促进作物和微生物群落生长提高土壤有机质各组分的有机碳含量。

(2)与传统表施相比,氮肥深施通过调控 β -葡萄糖苷酶和 β -N-乙酰氨基葡萄糖苷酶的活性改变土壤有机质的化学特性。

(3)与传统表施相比,氮肥深施提高土壤惰性有机质稳定性,促进活性有机质的有效性,最终改善土壤肥力和质量。

(4)3种氮肥深施措施中深施10 cm改善土壤肥力和质量的效果最佳,因此氮肥深施尤其是深施10 cm值得在免耕稻田生产中得到进一步推广。

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