刘玉兵,曹春信,黄洪明,等.降解膜对高粱根际土壤微生物群落结构的影响[J].山西农业大学学报(自然科学版),2024,44(3):66-74.
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doi: 10.13842/j.cnki.issn1671-8151.202311035

降解膜对高粱根际土壤微生物群落结构的影响

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摘　要:[目的]本研究旨在明确降解膜对农田土壤微生物群落结构特征的影响。[方法]设置4个月降解(4J)、6个月降解(6J)、普通黑膜(PT)3种不同降解膜,以不覆膜为对照(CK),利用高通量测序技术分析土壤细菌群落结构变化,探讨降解膜覆盖农田土壤中微生物群落的变化及其对微生物生态环境效应的影响。[结果]与不覆膜处理相比,覆膜后土壤中的全氮(PT处理除外)、速效磷、速效钾以及有机质含量均得到了提升。α-蛋白细菌(*Alphaproteobacteria*)、放线菌(*Actinobacteria*)、酸杆菌Gp1(*Acidobacteria_Gp1*)和伽马杆菌(*Gammmaproteobacteria*)为双季高粱根际土壤中共同优势细菌类群。主季覆膜处理下土壤中放线菌和酸杆菌Gp1和酸杆菌Gp2(*Acidobacteria_Gp2*)的相对丰度均高于不覆膜的土壤;再生季覆膜处理下土壤*Deltaproteobacteria*相对丰度均显著高于不覆膜的土壤,且土壤细菌群落OTU(OperationalTaxonomicUnit)数大于2000,具有更高的微生物多样性。通过KEGG代谢途径分析,再生季覆膜处理下土壤中碳水化合物代谢(Carbohydrate metabolism)、氨基酸代谢(Amino acid metabolism)、辅因子和维生素代谢(Metabolism of cofactors and vitamins)和萜类化合物和聚酮类化合物代谢(Metabolism of terpenoids and polyketides)高于主季覆膜处理,其中以6J处理较佳。[结论]综上,6个月的降解膜(6J处理)可以有效提升土壤理化性质,改善土壤根际细菌群落生物降解,增加微生物多样性和丰富度。

关键词:生物降解膜;根际土壤;微生物群落;高通量测序
中图分类号:S154.37　　**文献标识码**:A　　**文章编号**:1671-8151(2024)03-0066-09

地膜作为农业生产上一种提高作物产量的重要覆盖材料,可以提高土壤表层温度,减少土壤水份蒸发,抑制杂草生长,降低土壤下层温度[1-2],生产中常用的普通农用地膜虽然能够保温保湿,但其不通透性会使雨水难以渗入到土壤中,而且在高温天气土壤表层温度会有所增加[3]。另外,普通农用地膜如果不及时清理出去,之后会形成大量的塑料碎片或微塑料残留在土壤中,影响土壤团粒结构、水分运输和养分循环等,最终阻碍作物生长导致减产[4]。

土壤微生物群落在养分循环、维持土壤结构等方面起着至关重要的作用,其多样性是土壤质量的敏感指标,能反映土壤的细微变化,为评价土壤功能提供信息[5]。不同覆膜方式[6-8]、施肥水平[9-10]、施肥方式[11]及田间栽培、管理措施[12-13]都可能通过影响微生物群落的组成,最终影响作物品质。已有研究主要集中在田间管理措施与地膜覆盖对土壤水分、土壤结构、土壤营养和作物产量的影响,较少报道其对土壤微生态环境和土壤微生物群落演化的影响。本试验使用不同厚度的降解膜覆盖,研究其对土壤微生物的多样性和丰度的影响,为探讨不同降解膜覆膜农业生产模式下土壤群落结构的变化提供依据。

1 材料与方法

1.1 试验材料

降解地膜规格均为宽150 cm、厚0.006 mm,

收稿日期:2023-11-28　　**修回日期**:2024-03-30
基金项目:中国农业科学院金华市院地合作项目(YDHZ2020KY04);浙江省农业重大技术协同推广计划项目(2023ZDXT03-5)
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1.2　试验地概况

试验于2022年5月15日-12月14日在浙江省金华市兰溪市梅江镇高粱试验基地(北纬29°5′20″至29°27′30″,东经119°13′30″至119°53′50″)进行双季种植,再生季起始时间为主季收割时间(8月20日),以地上部留1个节位斜砍再生。基地土壤类型为壤质土。试验期间因采用垄作方式,降水可进入膜下被利用,且自然降水及人工分批灌溉水量约800 mm,地块平整,无灌溉条件,排水较好。0～20 cm土壤含有机质11.32 g·kg^{-1}、全氮0.65 g·kg^{-1}、速效磷4.81 mg·kg^{-1}、速效钾114.66 mg·kg^{-1},pH为6.8。

1.3　试验设计

试验共设置4个处理,CK:不覆膜(裸地);4J:4个月降解;6J:6个月降解;PT:普通黑膜。每处理3次重复,共12个小区,各小区随机分布。5月15日地膜覆盖小区先覆膜后人工移栽高粱苗,采用1膜4行种植方式。株距30 cm,行距35 cm,每小区面积15.0 m×1.5 m。田间按大田正常管理。

分别于8月20日、12月14日通过5点取样法采集根际土壤,每组随机采集3份样品,随后立即置于干冰中,带回实验室并置于－80℃冰箱中保存,用于后续测定土壤理化性质和细菌多样性及群落结构变化。

1.4　土样分析

1.4.1　主要试剂和仪器

DNA提取试剂盒(PowerSoil DNA Isolation Kit),核酸纯化试剂盒(Agencourt AMPure XP Kit),PCR纯化试剂盒(MinElute PCR Purification Kit),pH计,EC计,流动分析仪与火焰光度计,紫外可见分光光度计,PCR仪,QuantiFluor-ST荧光定量系统(深圳华大基因科技服务有限公司,武汉);MiSeq PE250高通量测序平台。

1.4.2　土壤养分测定方法

土壤pH测定用土壤pH计,土壤有机质测定用重铬酸钾外加热法,土壤速效氮测定采用碱解扩散法,土壤速效磷测定采用钼锑抗比色法,土壤速效钾测定采用火焰光度法。

1.4.3　土壤DNA提取、扩增和高通量测序

称取1 g土壤样品提取土壤总DNA,使用引物338-F(5′-ACTCCTACGGGAGGCAGCA-3′)和806-R(5′-GGACTACHVGGGTWTCTAAT-3′)对细菌16S rRNA V3－V4区进行扩增。将PCR扩增片段纯化和定量分析后进行高通量测序,测序由北京华大基因生物科技有限公司完成。

1.5　统计分析

KEGG功能预测:使用PICRUSt 2019.10软件通过比对16S测序数据获得的物种组成信息,推测样本中的功能基因组成,并分析不同样本或分组之间在功能上的差异。

所得到数据均利用R软件包"vegan"与SPSS 22.0统计软件进行分析。

2　结果与分析

2.1　不同降解膜覆盖土壤养分含量

由表1可见,与不覆膜处理相比,覆膜后土壤中的全氮(PT处理除外)、速效磷、速效钾以及有机质含量均得到了不同程度的提升。其中全氮含量提升幅度为3.07%～6.15%;速效磷含量增加幅度为0.42%～1.87%;速效钾含量提高2.31%～3.13%;有机质含量提高2.83%～7.51%。

表1　不同降解膜覆盖土壤养分含量

Table 1　Effects of different degradable films on soil nutrient content

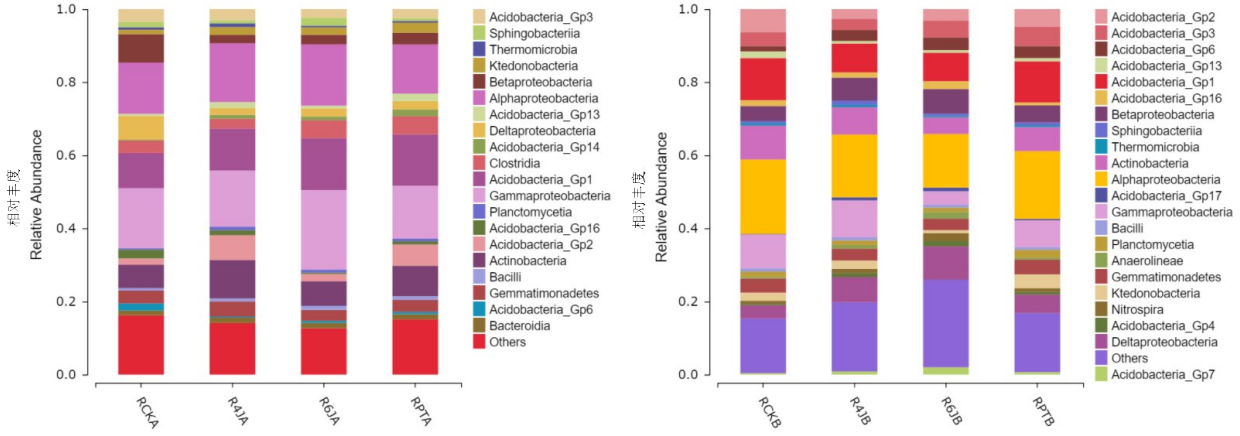
处理 Treatment	全氮/(g·kg^{-1}) Total nitrogen	速效磷/(mg·kg^{-1}) Available phosphorus	速效钾/(mg·kg^{-1}) Available potassium	有机质/(g·kg^{-1}) Organic matter
CK	0.65	4.81	114.66	11.32
4J	0.67	4.85	117.31	11.93
6J	0.69	4.90	117.74	12.17
PT	0.64	4.83	118.25	11.64

注:CK:不覆膜(裸地);4J:4个月降解;6J:6个月降解;PT:普通黑膜

Note:CK: Non－filming (naked ground); 4J: 4－month degradation; 6J: 6－month degradation; PT:common black film

2.2 不同降解膜覆盖双季高粱土壤微生物群落组成

在属分类水平上,α-蛋白细菌(*Alphaproteobacteria*)、放线菌(*Actinobacteria*)、酸杆菌 Gp1(*Acidobacteria_Gp1*)和酸杆菌 Gp2(*Acidobacteria_Gp2*)和伽马杆菌(*Gammmaproteobacteria*)是主季高粱根际土壤优势细菌类群(图1~图2),在CK、4J、6J和PT处理下高粱根际土壤中α-蛋白细菌的相对丰度分别为14.00%、16.11%、16.78%和13.46%;放线菌的相对丰度分别为6.39%、10.53%、6.67%和8.32%;酸杆菌Gp1的相对丰度分别为9.6%、11.53%、14.19%和14.03%;酸杆菌Gp2的相对丰度分别为1.74%、6.77%、2.03%和5.85%;伽马杆菌的相对丰度分别为16.43%、15.37%、21.78%和14.48%。所有处理间差异不显著(图3A)。再生季高粱根际土壤优势细菌类群有:α-蛋白细菌、酸杆菌Gp1、伽马杆菌、放线菌和三角蛋白杆菌(*Deltaproteobacteria*)。在CK、4J、6J和PT处理下高粱根际土壤中α-蛋白细菌的相对丰度分别为20.38%、17.16%、14.68%和18.52%;酸杆菌Gp1的相对丰度分别为11.52%、7.88%、7.80%和11.26%;伽马杆菌的相对丰度分别为9.21%、10.03%、3.65%和7.36%;放线菌的相对丰度分别为9.28%、7.51%、4.57%和6.63%;三角蛋白杆菌的相对丰度分别为3.65%、6.91%、9.16%和5.06%,各处理间均达到显著性差异(图3B)。



RCKA:主季成熟期对照(裸地)处理;R4JA:主季成熟期4个月降解膜处理;R6JA:主季成熟期6个月降解膜处理;RPTA:主季成熟期普通黑膜处理;RCKB:再生季成熟期对照处理;R4JB:再生季成熟期4个月降解膜处理;R6JB:再生季成熟期6个月降解膜处理;RPTB:再生季成熟期普通黑膜处理;图2~7同

RCKA: Control (bare ground) treatment in main season maturity; R4JA: 4—month degradation film treatment in main season maturity; R6JA: 6—month degradation film treatment in main season maturity; RPTA: Ordinary film treatment in main season maturity; RCKB: Control treatment in ratoon season maturity; R4JB: 4—month degradation film treatment in ratoon season maturity; R6JB: 6—month degradation film treatment in ratoon season maturity; RPTB: Ordinary film treatment in ratoon season maturity; It's the same in Fig. 2 ~ 7

图1 不同降解膜覆盖双季高粱土壤细菌在属分类水平的相对丰度

Fig. 1 Relative abundance of bacteria in two-season sorghum soil covered with different degradable films at the genus classification level

2.3 不同降解膜覆盖双季高粱土壤细菌群落α多样性

不同降解膜覆盖下双季高粱土壤细菌群落OTU丰富度稀疏曲线如图4所示,主季成熟时土壤中的物种数小于2000,再生季成熟时土壤中的物种数明显超过2000,说明再生季高粱土壤中具有更高的微生物多样性。可见,随着样本量的增加,稀释曲线趋于平缓,说明测序深度已经达到一定水平,能够比较真实地反映各样品的细菌群落结构组成。另外。双季高粱在不同降解膜覆盖下土壤中细菌群落特有OTU数差异较大。其中RCKA特有的细菌OTU数为138个,RCKB为55;R4JA特有的细菌OTU数为132个,R4JB为104;R6JA特有的细菌OTU数为59,R6JB为80;RPTA特有的细菌OTU数为55个,RPTB为44。但是总的来看,再生季高粱根际土壤具有更丰富的微生物多样性,总数量为1707(图5)。

2.4 不同降解膜覆盖双季高粱土壤细菌KEGG差异分析

图6是基于细菌群落功能丰度结果进行差异

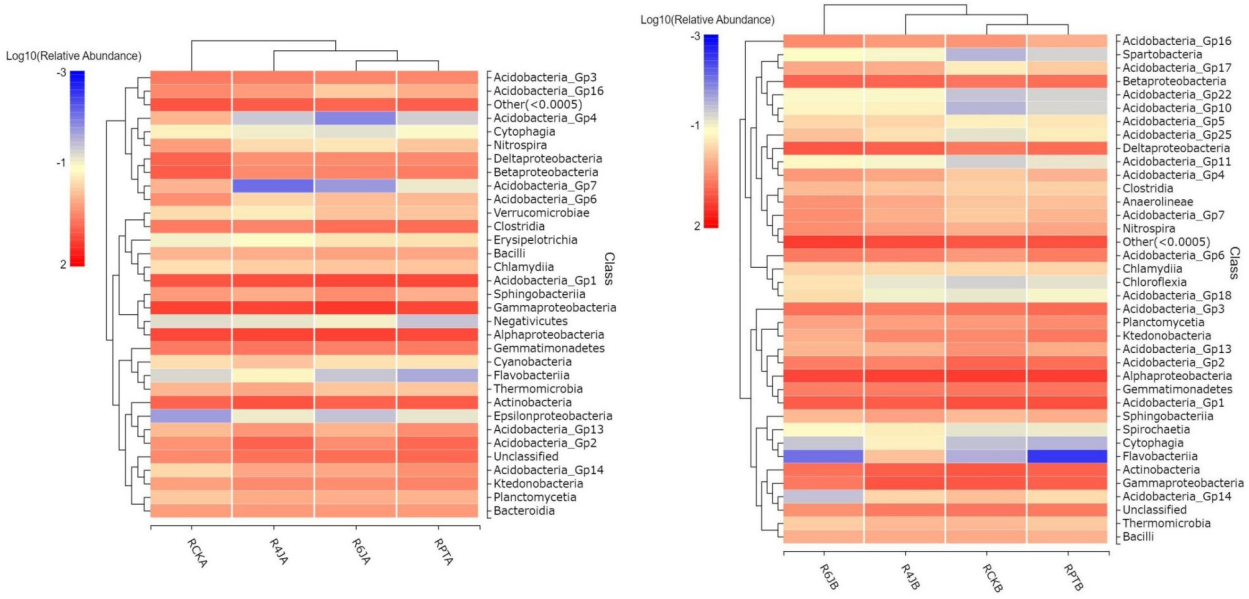
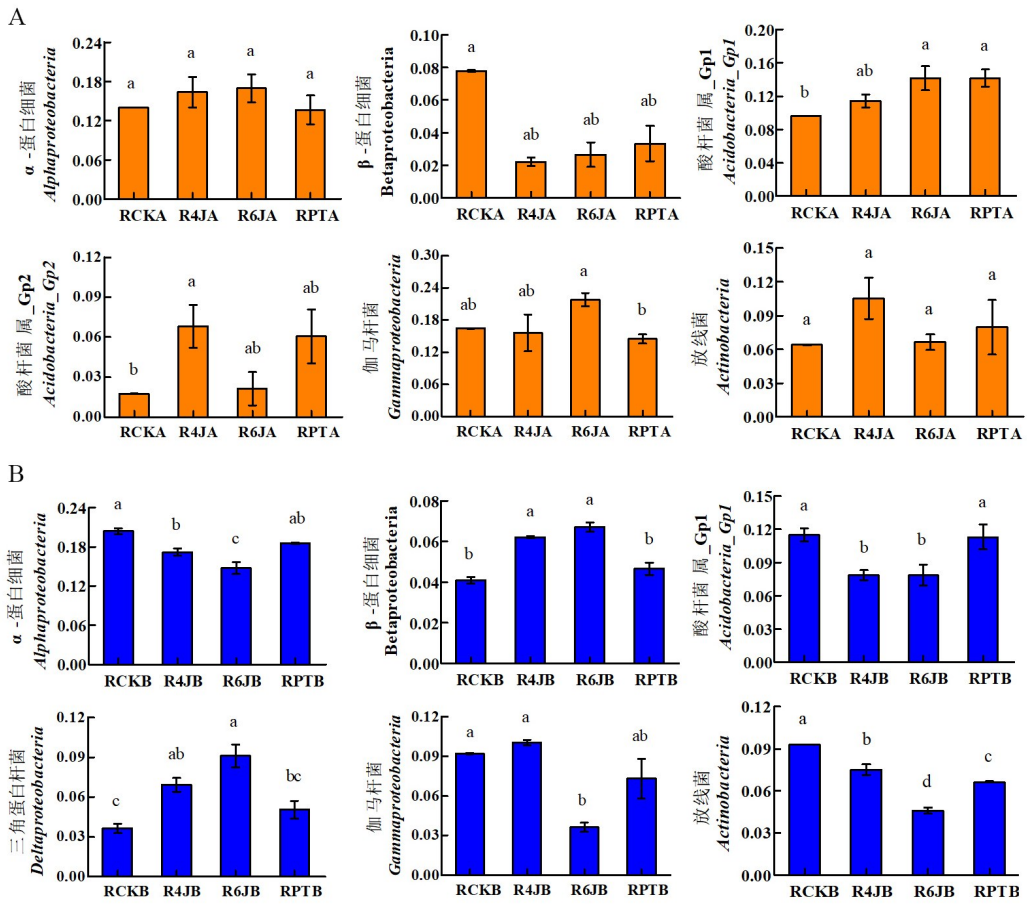


图2　不同降解膜覆盖双季高粱土壤细菌群落物种组成

Fig. 2　Species composition of bacteria community in double-season sorghum soil covered with different degradable films



A为主季,B为再生季;不同字母表示差异达5%显著水平

A: main season maturity; B: ratoon season maturity; The different letters indicated significant differences of at $P<0.05$ level

图3　双季高粱土壤中优势细菌类群的相对丰度

Fig. 3　Relative abundance of dominant bacterial groups in double season sorghum soil

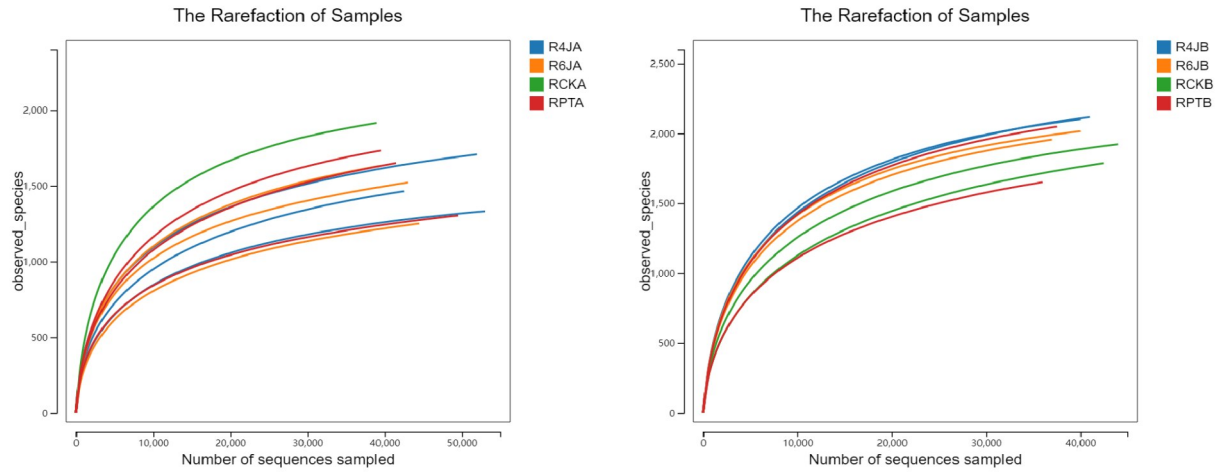


图4 不同降解膜覆盖双季土壤细菌群落OTU丰富度稀疏曲线

Fig. 4 OTU richness and sparsity curve of soil bacteria community covered with different degradable film in two seasons

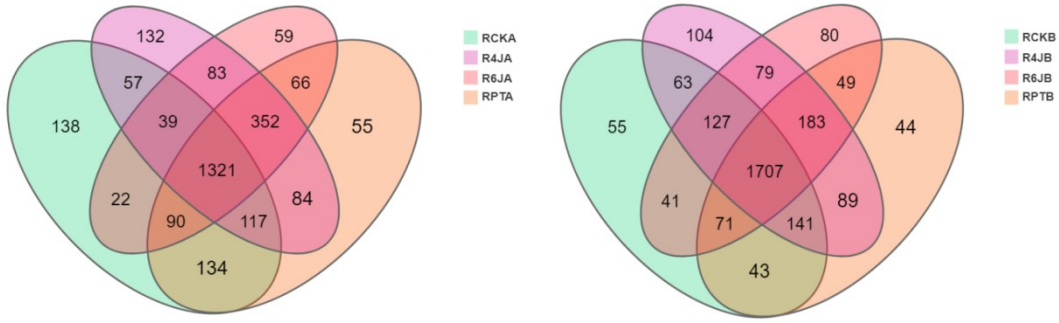


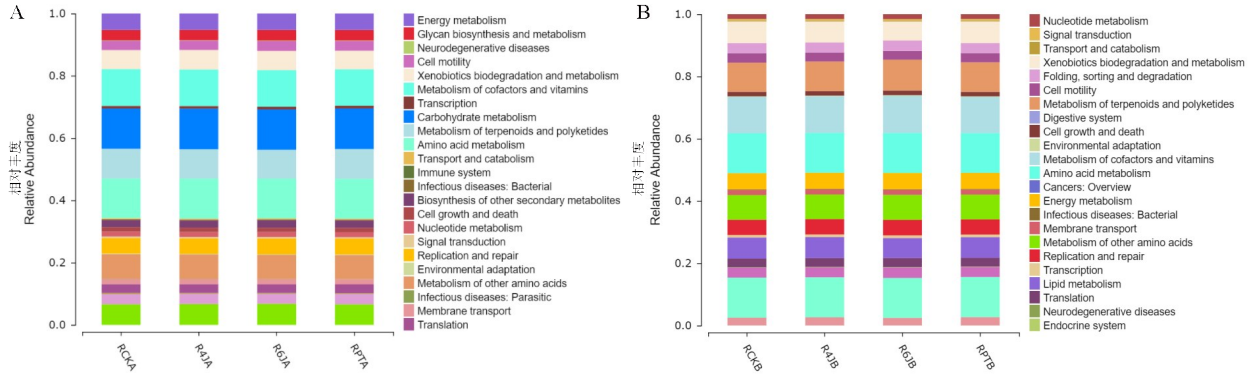
图5 不同降解膜覆盖双季土壤细菌群落特有OTU数量

Fig. 5 Number of unique OTU of soil bacteria communities covered with different degradable films in two seasons

功能分析。从图中可以看出,辅因子和维生素代谢(Metabolism of cofactors and vitamins)、碳水化合物代谢(Carbohydrate metabolism)、氨基酸代谢(Amino acid metabolism)和萜类化合物和聚酮类化合物代谢(Metabolism of terpenoids and polyketides)是双季高粱根际土壤中预测菌群功能共有的优势菌群代谢功能(图6)。在主季CK、4J、6J和PT处理下高粱根际土壤中辅因子和维生素代谢相对丰度分别为11.84%、11.74%、11.72%和11.72%;碳水化合物代谢相对丰度分别为12.92%、13.03%、12.95%和13.01%;氨基酸代谢相对丰度分别为12.75%、12.85%、12.77%和12.78%;萜类化合物和聚酮类化合物代谢相对丰度分别为9.52%、9.42%、9.27%和9.57%。再生季CK、4J、6J和PT处理下高粱根际土壤中辅因子和维生素代谢相对丰度分别为11.68%、11.78%、11.96%和11.61%;碳水化合物代谢相对丰度分别为12.87%、12.84%、12.81%和12.88%;氨基酸代谢相对丰度分别为12.84%、12.79%、12.85%和12.80%;萜类化合物和聚酮类化合物代谢相对丰度分别为9.35%、9.51%、9.89%和9.48%(图7)。总之,与主季相比,再生季土壤中细菌群落功能丰度更高。

3 讨论

可降解地膜作为一种绿色、环保的新型地膜,可以有效改善作物生长的水肥环境,加速作物吸收氮素,提高作物产量,对改善土壤养分可起到良好的促进作用[14-15]。本研究结果表明,与塑料地膜相比可降解地膜提高了土壤有机质含量,有利于降低土壤硝态氮累积量[16]。由于降解膜在使用一段时间后,受光和热等影响会自降解成CO_2和



A为主季,B为再生季

A:main season maturity; B: ratoon season maturity

图6　不同降解膜覆盖双季土壤细菌KEGG差异分析

Fig. 6　Analysis on the difference of soil bacteria KEGG under different degradable film mulching in two seasons

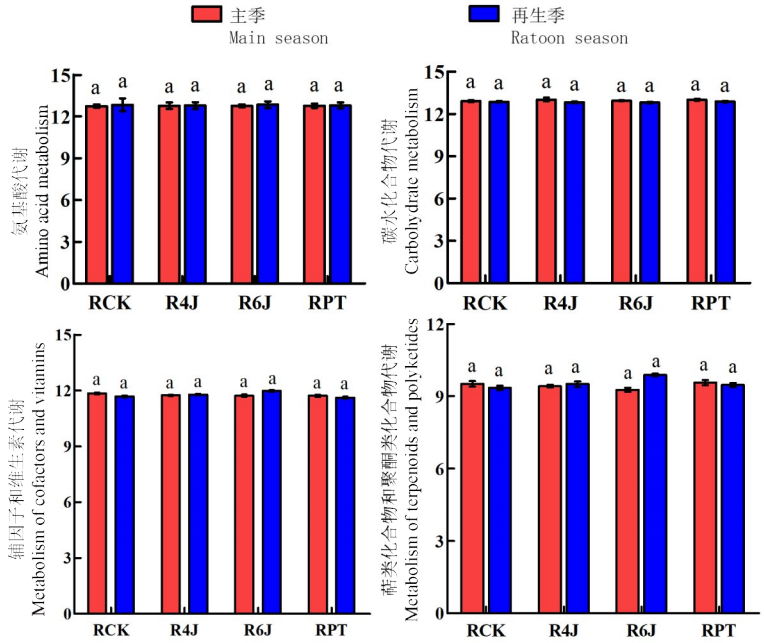


图7　不同降解膜覆盖双季土壤细菌KEGG差异分析的相对丰度

Fig. 7　Relative abundance of KEGG difference analysis of soil bacteria covered with different degradable films in two seasons

H_2O,最终以还原形式重新进入土壤环境中,增加土壤微生物的多样性和丰度,不易影响下一步的种植和污染土地[17-18]。目前在农业生产中,大力发展降解膜已成为趋势[19-20]。本研究中,与不覆膜处理相比,覆膜后土壤中的全氮(PT处理除外)、速效磷、速效钾以及有机质含量均得到了不同程度的提升,可能是降解膜在自然条件下经长时间的风吹和暴晒降解成CO_2和H_2O,然后活化了土壤中被固定的氮磷钾养分,最终提高了土壤的养分含量[21-22]。

微生物群落特征是影响土壤质量和植物病害的关键因素[23-24]。前人的研究结果显示,在长期覆膜的土壤中变形菌的相对丰度低于不覆膜的土壤,放线菌的相对丰度高于不覆膜的土壤[25-27]。与不覆膜相比,麻地膜显著降低了番茄根际土壤微生物酸杆菌的相对丰度[28]。本研究中,α-蛋白细菌、放线菌、酸杆菌Gp1和伽马杆菌为双季高粱根际土壤中共同优势细菌类群。主季覆膜处理下土壤中放线菌和酸杆菌的相对丰度均高于不覆膜的土壤。相比主季土壤,α-蛋白细菌的相对丰度在

再生季土壤得到了不同程度的提升,但R6JB处理除外,可能是6个月的降解膜在再生季时的土壤温度较低,再加上雨水的侵入,稀释了α-蛋白细菌中的蛋白质含量,从而导致α-蛋白细菌的相对丰度略低于主季。再生季覆膜处理下土壤中三角蛋白杆菌的相对丰度均显著高于不覆膜的土壤(RPTB处理除外),可能是普通黑膜在降解后有部分未能被土壤中的微生物分解。

在物种数量方面,再生季土壤中的物种要多于主季土壤,具有更高的微生物多样性,但是各处理下特有的细菌OTU数目却低于主季(R6JB除外)。说明再生季处理生物降解膜的性能最好。主季成熟时土壤中的物种低于2000,再生季成熟时土壤中的物种明显超过2000,且与其它处理比较,6J处理下明显增多。表明随着时间的延长,降解膜的降解性能更强,高粱土壤中具有更丰富的微生物多样性。

通过KEGG代谢途径分析,观测不同处理土壤细菌群落的功能基因在代谢途径上的差异和变化,发现再生季覆膜处理下土壤中碳水化合物代谢、氨基酸代谢、辅因子和维生素代谢和萜类化合物和聚酮类化合物代谢高于主季覆膜处理,其中以6J处理较佳。

4 结论

本试验条件下,使用降解膜覆膜对土壤的代谢途径有影响,可增强一部分有益代谢功能。比较各处理,发现在双季种植中,6J处理下可以更有效的提高土壤氮、磷、钾和有机质含量,从而改善根际细菌群落生物降解、新陈代谢功能,增加了土壤中微生物的多样性和丰富度等功能,更好的为高粱生长提供物质和能量基础。

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Effects of biodegradable films on rhizosphere soil microbial community structure in sorghum

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Abstract: [Objective] The film is an important covering material for increasing crop yield and income in agricultural production. Biodegradable ground film, as a new type of soil covering material, can decompose into small molecules, eventually converting into carbon dioxide, water, and organic matter, playing an irreplaceable role in improving soil. This study aimed to clarify the influence of the biodegradable ground film on the characteristics of microbial community structure in farmland soil. [Methods] Three different degradable films were set: 4-month degradation (4J), 6-month degradation (6J), and common black film (PT), with non-filming field as the control (CK). The variation of soil bacterial community structure were analyzed using high-throughput sequencing technology, and the changes in microbial community and its effects on microbial ecological environment under the coverage of biodegradable films in farmland soil were explored. [Results] Compared with the non-filming treatment, the contents of total nitrogen (except for the PT treatment), available phosphorus, available potassium, and organic matter in the soil were all increased after filming. *Alphaproteobacteria*, *Actinobacteria*, *Acidobacteria_Gp1* and *Gammmaproteobacteria* were the common dominant bacterial groups in the rhizosphere soil of dual-season sorghum. Under the filming treatments of the first season, the relative abundance of *Actinobacteria*, *Acidobacteria_Gp1* and *Acidobacteria_Gp2* in the soil was higher than that in the non-filming soil; Under the filming treatment of the second season, the relative abundance of *Deltaproteobacteria* in the soil was significantly higher than that in the non-filming soil, and the number of Operational Taxonomic Unit (OUT) of soil bacteria was more than 2000, showing higher microbial diversity. Through KEGG metabolic pathway analysis, soil carbohydrate metabolism, amino acid metabolism, metabolism of cofactors and vitamins, and metabolism of terpenoids and polyketides were higher under filming treatment of the second season than that of the first season, among which the 6J treatment was optimal. [Conclusion] In summary, a 6-month biodegradable film (6J treatment) can effectively improve soil physicochemical properties, enhance biodegradation of soil rhizosphere bacterial communities, and increase microbial diversity and richness.

Keywords: Biodegradable film, Rhizosphere soil, Microbial community, High-throughput sequencing

(编辑:郭玥微)