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畜禽粪便和桃树枝工业化堆肥过程中微生物群演替及 其与环境因子的关系

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摘要:为了解畜禽粪便和桃树枝工业化堆肥中微生物群落的变化,本研究以猪粪、桃树枝和腐熟有机肥为堆肥原料进行堆肥,通过测定理化指标和利用高通量测序技术,分析了堆肥中理化参数的变化和堆肥微生物群落结构变化. 理化参数结果表明,堆体于第 2 d 快速进入高温期,整个高温期持续 30 d;堆肥过程中有机质含量呈波动性变化,但总体下降;堆肥结束时 TN 含量为 20.58 g·kg $^{-1}$,与堆肥初期相比损失了 5.90%. α 多样性分析表明,不同好氧堆肥时期具有不同的微生物群落多样性. 在细菌门水平上,厚壁菌门(Firmicutes)和放线菌门(Actinobacteria)在整个堆肥过程中占主导地位,其相对丰度所占比例分别为 79.31%~95.09%和 2.98%~19.70%;此外,在堆肥初期,厚壁菌门(Firmicutes)和放线菌门(Actinobacteria)相对丰度分别为87.36%和 9.66%,在堆肥末期,两者的相对丰度分别为 79.38%和 19.70%;在细菌属水平上,随着堆肥的进行,优势类群从 Clostridium_sensu_stricto_1、Terrisporobacter和 Bacillus 演变为 norank_ f_Bacillaceae、Bacillus、Oceanbacillus 和 Pseudogracilibacillus;在真菌门水平上,Ascomycota 始终为优势门类;在真菌属水平上,norank_c_Sordariomycetes 的比例逐渐增加,在堆肥末期成为优势类群、冗余分析结果显示,环境因子对细菌和真菌群落结构影响相关性排序均为 pH > 铵态氮 > 温度 > TOC > TN,其中 pH 对微生物群落组成影响最大. norank_c_Sordariomycetes、norank_o_Sordariales 和 norank_c_Agaricomycetes 可能与铵态氮的挥发有关.

关键词:畜禽粪便;桃树枝;工业化堆肥;群落多样性;冗余分析

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Microbial Community Succession in Industrial Composting with Livestock Manure and Peach Branches and Relations with Environmental Factors

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Abstract: This study sets out to understand the evolution of the microbial community structure in industrial composting with livestock manure and peach branches. Pig manure, peach branches, and decomposed organic fertilizer were used as materials for composting. Changes in physical and chemical indicators and the evolution in the structure of the compost microbial community, determined by highthroughput sequencing, were analyzed. The results of physical and chemical parameters show that the pile reached the high-temperature stage on day 2, and the thermophilic period lasted for 30 days. The changes in total carbon were volatile, and there was an overall decline in the amount of TOC in the whole process of composting; The final content of TN was 20.58 g·kg⁻¹, which was 5.90% lower compared to the initial compost. Alpha analysis indicated that a different microbial community diversity existed at different times during aerobic composting periods. At the bacterial phyla level, Firmicutes and Actinobacteria were the dominant phyla, and the proportion of relative abundance were 79.31%-95.09% and 2.98%-19.70%, respectively, in the entire compost. The relative abundance of Firmicutes and Actinobacteria were 87.36% and 9.66%, respectively, and their respective relative abundances were 79.38% and 19.70% at the end of composting. At the bacterial genus level, the dominant group changed from Clostridium_sensu_stricto_1, Terrisporobacter, and Bacillus to norank_ f_Bacillaceae, Bacillus, Oceanbacillus, and Pseudogracilibacillus; Regarding the fungus phyla, the Ascomycota was the dominant phylum. For the fungus genus, the relative abundance of norank_c_Sordariomycetes gradually increased during composting, and finally was predominant group. The redundancy analysis (RDA) showed that the correlation rank between environmental factors and microbial community structure was: $pH > NH_4^+ - N > T > TOC > TN$, where pH had the greatest impact on the microbial community composition. norank_c_Sordariomycetes, norank_o_Sordariales, and norank_c_Agaricomycetes may be related to the volatilization of ammonium nitrogen.

Key words: livestock manure; peach branch; industrial compost; microbial diversity; redundancy analysis

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我国是畜禽养殖和果树种植大国,近年来随着规模化、集约化的畜禽养殖数量和果树栽培面积的数量不断增加,产生了大量的畜禽粪便和果树修剪枝条等农业有机固体废弃物^[1,2].这些农业有机废弃物如果不能及时有效加以处理,就会成为生态环境污染的重要来源,不仅造成了农业生态环境质量不断恶化,危害人们的身心健康,同时也导致大量附加值高的有用成分和养分资源流失,造成严重的农业面源污染,极大地削弱了我国现代农业可持续发展能力^[3].因此,切实有效地实现不同农业有机固体废弃物的减量化处理与资源化利用已经成为目前研究的重点和热点.

目前实现农业废弃物减量化、无害化和资源化 的方式之一是进行好氧堆肥处理. 好氧堆肥的本质 是微生物分解和转化有机物的生化代谢过程,因此 了解堆肥过程中不同时期微生物丰度、多样性以及 群落演替规律对于揭示堆肥过程中的有机物降解机 制和优化堆肥处理工艺具有重要理论意义和实践价 值. 常用的分析堆肥过程中微生物相对丰度和微生 物群落结构组成的经典技术有克隆测序技术、PCR-DGCE 和 Illumina MiSeq 高通量测序技术[4],其中 Illumina MiSeq 高通量测序技术可以更精确地分析 堆肥过程中微生物相对丰度和微生物群落组成[5]. 本文以典型的畜禽养殖粪便猪粪和果树修剪枝条桃 树枝为主要原料进行堆肥,采用 Illumina MiSeq 高通 量测序技术,研究堆肥化过程中理化参数等环境因 子和微生物相对丰度和微生物群落结构组成的变 化,同时对理化指标进行监测,并分析理化指标与微 生物之间的相关关系,揭示其微生物多样性及其变 化规律,以期为堆肥处理工艺的优化提供理论依据, 整体提升我国农业废弃物资源化利用的理论和技术 水平,并为实现我们农业面源农田综合防治与修复 技术目标提供理论支持.

1 材料与方法

1.1 堆肥实验

样本取自于北京市某有机肥厂,该厂采用槽式好氧堆肥工艺,将猪粪与桃树枝以及腐熟有机肥按体积比(立方):5:2.5:1的比例混合后进行槽式堆肥.其中猪粪来自于北京某农场,桃树枝来源于北京某果园枝条修剪废弃物,腐熟有机肥取自该有机肥厂上一批堆肥结束后的样品,腐熟有机肥取自该有机肥厂上一批堆肥结束后的样品,腐熟有机肥的原料同为猪粪和桃树枝,堆肥原料的理化性质见表1.用铲车将猪粪和辅料均匀搅拌,每隔2d使用翻抛机进行翻堆,进行为期53d的好氧堆肥.调整水分含量为59.07%,有机碳(TOC)含量为390.28g·kg⁻¹,总

氮(TN)含量为 21. 34 g·kg⁻¹. 分别于第 0、2、5、24、30、33、40 和 50 d 从反应堆顶部、中部和底部进行 3 点取样,均匀混合,作为代表性样品. 样品编号分别为 d0、d2、d5、d24、d30、d33、d40 和 d50. 样品分成两份:一份于 4 保存,用于测定理化参数;一份储存于 -20 (,用于微生物多样性分析.

表 1 堆肥各原料的理化性质

Table 1 Physicochemical properties of raw compost materials

	,	pp		
原料	рН	EC /mS·cm ⁻¹	TOC /g•kg ⁻¹	TN /g•kg ⁻¹
猪粪	6. 80	3. 42	363. 2	22. 3
腐熟有机肥	8. 25	2. 65	332. 98	23. 5
桃树枝	_	_	41. 23	9. 02
混合原料	7. 64	4. 39	390. 28	21.87

1.2 理化参数测定

用 DTSW-2 型数字电子温度计(泰安德图自动 化仪器有限公司)测定堆体顶端、中部、底部这 3 个位置的温度. 用水分测定仪 SH1 OA(上海菁海仪器有限公司)检测堆肥样品含水率. 新鲜样品和蒸馏水按比例 1:10(质量比)混合后用 pH 计 PB-10(Sartorius 德国)和 DDS-307A 型电导率仪(上海仪电科学仪器股份有限公司)分别测定堆肥样品的pH 和 EC. 将样品研磨并过筛(40目筛),按照国家标准^[6](HJ 615-2011),采用重铬酸钾-分光光度法测定 TOC;TN 含量参照国家标准^[7](HJ 714-2014)采用凯氏法测定. NH₄+-N含量参考国家标准^[8](HJ 535-2009),使用纳氏试剂分光光度法进行测定. 种子发芽率 根据以下公式计算:

1.3 微生物多样性分析

将堆肥样品送上海美吉生物医药科技有限公司进行 16S rDNA 和 18S rDNA 高通量测序,细菌的通用引物序列为: 338F (5'-ACTCCTACGGGAGGCAG CAG-3')和 806R(5'-GGACTACHVGGGTWTCTAAT-3');真菌的通用引物序列为 SSU0817F (5'-TTAGC ATGGAATAATRAATAGGA-3')和 1196R (5'-TCTG GACCTGGTGAGTTTCC-3'),测序区域分别为 V3-V4区和 V5-V7区,测序平台为 IIIumina MiSeq. 利用 Usearch (vsesion 7.0)软件,对序列进行聚类,并将相似性大于 0.97的序列定义为一个操作分类单元 (OTU),得到 OTU 表,并进行生物信息统计分析. 利用 mothur (version v. 1. 30. 1)进行微生物群落的 α 多样性分析,计算参数为 Chao1、Shannon和 Simpson. Chao1 指数,由 Chao于 1984年提出,用于

计算微生物群落中含 OTU 数量^[10]. Shannon 指数用于提供物种丰富度^[11]. 利用 Qiime 平台进行物种分类,生成不同分类水平上的物种丰度表. 利用 R 语言软件中的 vegan 包进行群落组成分析. 采用 R 语言软件进行主坐标分析(PCoA),研究微生物群落结构相似性分析. 采用 R 语言 vegan 包进行冗余分析(RDA),研究微生物群落和环境因子之间的关系. 多样性指数计算公式如下:

$$S_{\text{Chaol}} = S_{\text{obs}} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)} \tag{1}$$

式中, S_{Chaol} 表示估计的 OTU 数; S_{obs} 表示实际测得 OTU 数; n_1 表示只含一条序列的 OTU 数; n_2 表示只含两条序列的 OTU 数.

$$D_{\text{Simpson}} = \frac{\sum_{i=1}^{S_{\text{obs}}} n_i (n_i - 1)}{N(N - 1)}$$
 (2)

式中, S_{obs} 表示实际测得 OTU 数; n_i 表示第 i 个 OTU 所含序列数;N 表示所有序列数.

$$H_{\text{Shannon}} = -\sum_{i=1}^{S_{\text{obs}}} \frac{n_i}{N} \ln \frac{n_i}{N}$$
 (3)

式中, S_{obs} 表示实际测得 OTU 数; n_i 表示第 i 个 OTU 所含序列数;N 表示所有序列数.

1.4 数据分析

应用统计软件 Excel 和 Origin 进行理化指标统计并绘制图表.

2 结果与讨论

2.1 堆体温度、pH和EC的变化

温度是评价好氧堆肥过程的重要指标. 由图 1 可知第 2 d 堆肥迅速进入高温期(>50℃),这是由于好氧微生物快速降解有机物时产生了大量的热^[12]. 第 28 d 达到最高温 60. 4℃,并且高温期持续至第 32 d(高温持续时间>7 d),杀死大量的病原菌,达到堆肥的卫生标准^[13]. 在堆肥高温阶段,随着易分解物质的消耗,微生物代谢强度降低,导致温度下降,但在转化成堆后,高温好氧微生物再次繁殖,开始缓慢消耗可分解的纤维素大分子,从而温度上升^[14]. 从第 33 d 起,堆体进入降温阶段.

pH 反映堆肥过程中微生物所处的酸碱环境,过高或过低的 pH 都会影响微生物生长和有机物的分解,影响堆肥的进程. 由图 2 可知, 堆体初始 pH 为7.69,随着堆肥进行 pH 不断上升, pH 于 30 d 达到最大值 8.87,随后 pH 变化趋于平缓,堆肥结束时 pH 为 8.81. 在堆肥初期,一些易于分解的含氮有机物在微生物作用下发生氨化作用,导致 pH 升高[15]. 随着堆肥过程进行,由于微生物氨化作用减

弱,导致堆肥后期 pH 基本稳定不变[16].

电导率反映了堆肥过程中可溶性盐的变化. 由图 2 可以看出, EC 值总体表现为先下降后升高的趋势, 这可能因为在堆肥初期至高温阶段, 由于有机质的快速分解, 微生物活动消耗堆体的有机质以及利用水溶性盐物质满足自身生长需求, 导致 EC 在高温堆肥过程中先减少^[17]. 随后, 由于堆肥过程中难降解物质的分解, 导致堆体的盐类物质产生使 EC 又逐渐上升^[18], 至堆肥结束, 堆体的 EC 达到最大值 4.5 mS·cm⁻¹, 符合中国固体废弃物处理标准(EC < 9 mS·cm⁻¹), 基本满足农田使用对 EC 要求^[19].

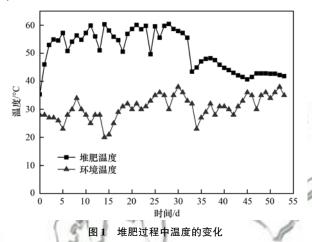


Fig. 1 Temperature changes during composting

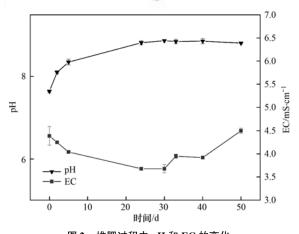


图 2 堆肥过程中 pH 和 EC 的变化

Fig. 2 Changes in pH and EC during composting

2.2 堆肥NH₄ -N、TN 和 TOC 的变化

从图 3 可以看出, NH_4^+ -N含量整体呈现先上升后下降的趋势. 堆肥初期, NH_4^+ -N含量 1.71 $mg \cdot g^{-1}$ 升高到 5.62 $mg \cdot g^{-1}$ (5 d),之后 NH_4^+ -N开始下降至堆肥结束,堆体末期的 NH_4^+ -N含量为 1.92 $mg \cdot g^{-1}$,因为在堆肥初期,微生物分解代谢堆肥中残留的有机氮类物质产生铵态氮,导致 NH_4^+ -N含量升高;而从堆肥的第 10 d 至 30 d,由于高温和 pH 升高导致

 NH_4^+ -N以氨气的形式散失,从而 NH_4^+ -N含量逐渐下降^[20,21]. 与第 30 d 相比,第 33 d 的 NH_4^+ -N含量有所上升,分析可能与氨化细菌的活动有关^[22]. 此后,由于堆肥进入降温阶段, NH_4^+ -N含量下降减缓.

堆肥过程中 TN 含量的变化如图 3 所示, 堆肥 初期 TN 快速下降,主要由于初期堆体温度升高较 快,微生物大量消耗有机氮类物质以致产生高浓度 的NH₄ -N加强 NH₃ 的挥发^[23],导致堆肥 TN 下降. 随后由于堆肥有机质大量微生物降解导致堆肥浓缩 效应使得堆肥 TN 逐渐缓慢呈增加趋势^[24]. 第 33 d. TN 含量为 19.82 g·kg⁻¹,相比之前有所下降,可能 是由于高温期结束,堆体的嗜温微生物活性增强,分 解有机物产生了氨气. 第0d时TN含量为21.87 g·kg⁻¹, 堆肥结束 TN 含量为 20.58 g·kg⁻¹, 堆体氮 含量损失了 5.90%. TOC 含量随堆肥的进行呈波动 性变化. TOC 含量下降可能是由于堆肥微生物分解 碳水化合物并以 CO, 的形式挥发[25,26],以及类似总 氮由于堆肥浓缩效应,所以导致堆肥总有机碳初期 先下降,然后逐渐增加,总体下降的变化趋势 (图3).

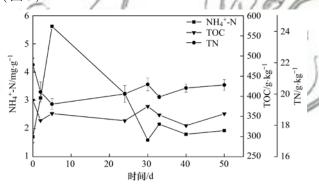


图 3 堆肥过程中铵态氮(NH₄⁺-N)、总有机碳(TOC) 和总氮(TN)的变化

Fig. 3 Changes in NH₄⁺-N, TOC, and TN during composting

2.3 种子发芽指数(GI)变化

GI 是一种反映堆肥植物毒性和腐熟度的重要生物学指标^[27],GI 是植物对于堆肥低毒性(植物根长)和高毒性(发芽率)的综合反映.如图 4 所示,堆肥初期(0~5 d)的 GI 几乎为 0,这与堆肥物料本身具有严重的植物毒性有关^[28].在堆肥中后期(24~40 d),GI 出现了短暂的下降趋势,这与 Liu 等^[14]的研究结果相似,原因可能是堆肥产生了低分子量的酸或挥发性脂肪酸^[29].随着堆肥进行,堆肥中有毒有害物质被逐渐降解,GI 逐渐上升,堆肥结束时,种子发芽指数为 73%.

2.4 堆肥过程中微生物群落相似性和多样性分析

2.4.1 微生物群落相似性分析

微生物群落相似性可以通过主坐标分析

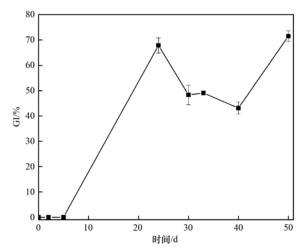


图 4 堆肥过程中种子发芽指数(GI)的变化

Fig. 4 Changes in GI during composting

(principal co-ordinates analysis, PCoA)^[30]. 堆肥过程中不同样品中细菌群落的相似性如图 5(a)所示,结果表明堆肥初期的样品 d0、d2 和 d5 相距较近,说明堆肥前 5 d 细菌群落有高度的相似性;而堆肥高温期的样品(d24、d30、d33)相互间距离远,说明堆肥高温期细菌群落变化明显,可能是堆肥温度

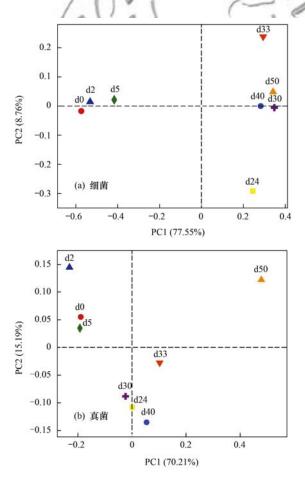


图 5 堆肥过程中各时间段细菌和真菌的 PCoA 分析 Fig. 5 PCoA analysis of bacteria and fungi at various

time points during composting

影响细菌的生长繁殖. 而在堆肥末期的样品(d40、 d50)相聚较近,说明样品中的细菌群落有高度相 似性,降温阶段和腐熟期的细菌群落结构有着高 度稳定性.

堆肥过程中不同样品中真菌群落的相似性如图 5(b)所示, 堆肥初期的样品(d0、d2、d5)、高温期 的样品(d24、d30、d33)和腐熟期的样品(d50)同细 菌群落的相似性类似,第0、2、5 d 的样品相距较 近,说明堆肥前5d真菌群落有高度的相似性,而与 后期高温阶段的样品(d24、d30 和 d33)相距明显 远,说明堆肥初期和高温期的真菌微生物发生了明 显的群落演替现象. 堆肥末期(50 d)的真菌组成与 前两个时期发生了明显变化.

2.4.2 微生物群落多样性分析

Chao1 指数反映样品中群落的丰富度, Shannon 指数反映的是群落的多样性,受样品群落中物种丰 富度和物种均匀度的影响. 如表 2 所示,随着堆肥的 进行,0~30 d 堆体的细菌丰富度降低;堆肥30 d 后,随着堆温下降,细菌丰富度增加,在第40 d 丰富 度最大. 相比建堆初期, d5、d30、d33、d40 和 d50 样品的细菌多样性升高. 真菌群落的丰富度变化总 体呈先上升后下降的趋势;与0 d 相比,高温阶段的 真菌群落多样性上升,第50 d 多样性下降. 这表明: ①温度的变化会影响微生物群落多样性:②不同好 氧堆肥时期的微生物群落多样性不同,这与王秀红 等[31]的研究结果一致.

表 2 堆肥过程中不同时间点细菌群落和真菌群落的相对丰度和多样性指标

Table 2	Indices of relat	tive bacterial	and fungal	abundances	and diversity	vat different	times during	composting
							17267	540

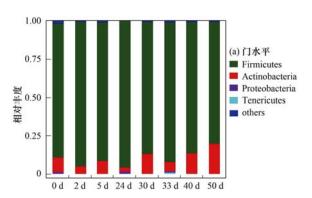
项目	d0	d2	d5	d24	d30	d33	d40	d50 F
细菌 Chaol 指数	358. 5	319. 307 7	352. 5	284. 04	281. 035 7	310. 121 2	374. 823 5	359
真菌 Chaol 指数	29. 5	35. 5	32	38	28	29.5	35. 5	28
细菌 Shannon 指数	3. 219 788	2. 851 634	3. 330 901	2. 997 379	3. 231 737	3. 650 301	3. 511 426	3. 458 467
真菌 Shannon 指数	1. 388 013	1. 334 36	1. 482 551	1. 925 251	1. 776 095	1. 696 824	1. 891 112	1.386 371

2.5 堆肥过程中细菌群落结构的变化

通过高通量测序技术可获得环境中微生物群落 在数量和结构方面的信息,分析细菌群落结构的动 态变化,结果如图 6(a) 所示,在细菌门分类水平上, 厚壁菌门(Firmicutes)和放线菌门(Actinobacteria) 在整个堆肥过程中占主导地位,为整个堆肥过程中 的优势门类,其相对丰度所占比例分别为79.31%~ 95.09%和2.98%~19.70%,在一些报道中,厚壁菌 门(Firmicutes)、放线菌门(Actinobacteria)均为堆肥 过程中的细菌优势门类的一部分[12,18,32]:其中. others 代表相对丰度小于 0.01 的物种集合. 在不同 的堆肥时期,细菌群落在各细菌门类上有不同的相 对丰度:第0d时,厚壁菌门(Firmicutes)、放线菌门 (Actinobacteria)和变形菌门(Proteobacteria)所占比 例分别为 87. 28%、9. 64% 和 1. 38%. 随着堆肥的进

行,厚壁菌门(Firmicutes)和变形菌门 (Proteobacteria)的相对丰度在第24 d 均达到最大值 分别为 95.09% 和 19.77%. 厚壁菌门(Firmicutes) 可以形成耐热孢子以抵抗高温,同时厚壁菌门 (Firmicutes)在高温期是最优势菌门,其可以在高温 下(>55℃)存活,并能参与各种代谢活动^[32]. 另外 放线菌门(Actinobacteria)的相对丰度呈上升趋势, 其可以通过分泌各种抗生素抑制病原微生物[33]. 软 壁菌门(Tenericutes)在堆肥第33d时出现,相对丰 度为1.11%.

在细菌属水平上,如图 6(b) 所示,堆肥初 期,主要优势菌属为 Clostridium sensu stricto 1, Terrisporobacter 和 Bacillus. 从第 24 d 一直到堆肥 结束, 优势菌群为 norank _ f _ Bacillaceae, Bacillus, Oceanbacillus 和 Pseudogracilibacillus, 这



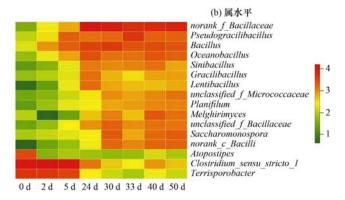


图 6 堆肥过程中细菌群落组成变化

Fig. 6 Changes in bacterial community composition during composting

说明堆肥过程中细菌群落组发生了明显变化. Clostridium_sensu_stricto_1 在堆肥过程中呈逐渐减少,而 norank_ f_Bacillaceae 逐渐成为堆肥过程中的主导菌属,并且从堆肥初期至堆肥结束,Bacillus 呈 现增加,然后维持相对稳定. Oceanbacillus 菌属的变化情况类似 Bacillus. 并且其他菌属在堆肥过程中总体逐渐减少. 而自动物肠道的微生物(Clostridium_sensu_stricto_1 和Terrisporobacter 菌属)经过堆肥后相对丰度下降,这与黄雅楠等[34]的研究结果一致.

2.6 堆肥过程中真菌群落结构的变化

如图 7(a),在真菌门水平上,堆肥初期和高温期后主要真菌门类有 Ascomycota 和 Basidiomycota,并且在整个堆肥过程中 Ascomycota 始终为优势门类,主要是因为 Ascomycota 能够分泌多种纤维素、半纤维素降解酶,能高效利用堆肥中的营养元素^[35].在整个堆肥过程中,Basidiomycota 相对丰度

呈现先上升后下降的趋势, 在第 40 d 时其相对丰度 达到最大值 13.71%.

从真菌属水平上看,堆肥过程中各时间点的真菌群落变化如图 7(b) 所示,堆肥过程中真菌优势类群分别为 $unclassified_f_Trichocomaceae \ norank_c_Sordariomycetes \ Scopulariopsis 和 <math>norank_o$ 0 Sordariales,其中 $unclassified_f_Trichocomaceae$ 比例在堆肥过程中基本呈现总体减少的趋势,Scopulariopsis 没有明显的变化,但是在堆肥过程中 $norank_o$ 2 Sordariomycetes 的比例逐渐增加,在堆肥末期成为优势类群,据相关研究报道, $norank_o$ 2 Sordariomycetes 是分解堆肥中有机质的主要真菌类群,可能由于Sordariomycetes 具有耐高温和抗旱的微生物结构特征和孢子[36,37]. 另外, $norank_o$ 2 Sordariales 的比例在堆肥过程中逐渐增加,高温中期(24 d)达到最大随后略减少比例维持稳定,研究表明在堆肥的嗜热阶段 Sordariales 有较高的相对丰度[38].

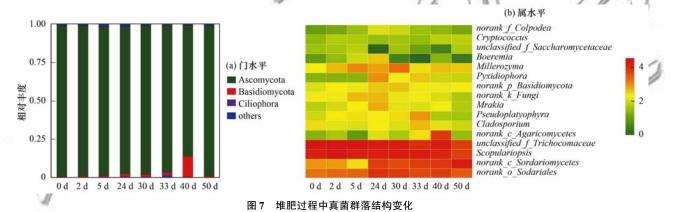


Fig. 7 Changes in fungal community composition during composting

2.7 环境因子与微生物群落结构的相关关系

采用冗余分析(RDA)分析堆肥体理化参数(温 度、铵态氮、pH、TOC和TN)与主要菌属之间的关 系. 两个排序轴分别解释种群与环境积累变化率为 85.10%、4.73%和69.14%、11.61%,理化参数分别 共同解释89.83%和80.75%的样本信息,说明堆肥 过程中理化参数与细菌和真菌群落结构变化有关. 如图 8 所示,环境因子对细菌和真菌群落结构影响 相关性排序均为 pH > 铵态氮 > 温度 > TOC > TN,该 结果表明一方面堆肥过程中的微生物会造成有机酸 的积累[39],另一方面 pH 的变化会导致堆肥中钙和 镁的变化,从而间接影响微生物群落[40]. 含碳物质 与含氮物质与细菌群落结构的相关性表明, 堆肥中 的C、N含量会随着微生物代谢产物的不断转化而 发生变化[32];另外氮源的质量和数量也可以控制细 菌的群落结构变化[41]. 如图 8 所示,虽然温度不是 与微生物群落结构变化相关性最大的环境因子,但

其仍可通过影响微生物的生物活性从而导致微生物 群落结构的改变[42]. 在堆肥初期(第2d和第5d), 细菌群落结构较为相似:高温阶段和降温阶段,细菌 群落结构变化剧烈. 此外,在整个堆肥过程中,真菌 群落结构变化明显,其中这可能与温度影响了真菌 生长繁殖有关[43]; Duan 等[44]的研究结果表明, 温度 在堆肥的高温期和腐熟阶段均会影响真菌的群落结 构. 从图 8(a) 可以看出, 在细菌群落结构中, TOC、 TN、铵态氮与 Clostridium _ sensu _ stricto _ 1、 Terrisporobacter 呈正相关,与 Bacillus 和 norank_ f_ Bacillaceae 呈负相关, 而 Bacillus 和 norank _ f _ Bacillaceae 与 pH 呈正相关. 如图 8 (b) 中显示: norank_c_Sordariomycetes、norank_o_Sordariales 和 norank_c_Agaricomycetes 与 TN、pH 呈正相关,与 TOC、 铵 态 氮 呈 负 相 关, 说 明 norank _ c _ Sordariomycetes、norank_o_Sordariales 和 norank_c_ Agaricomycetes 可能与铵态氮的挥发有关.

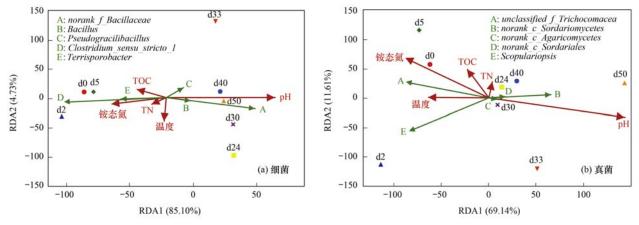


图 8 堆肥过程中细菌和真群落结构与理化参数之间的冗余分析(属水平)

Fig. 8 Redundancy analysis (RDA) between bacterial and fungal community structure and physical and chemical parameters during composting (genus level)

3 结论

- (1)堆肥高温期持续了30 d(>7 d),达到粪便处理卫生标准;堆肥结束时EC为4.5 mS·cm⁻¹,符合中国固体废弃物处理标准(EC<9 mS·cm⁻¹).利用好氧堆肥工艺,以腐熟有机肥为外源添加物,实现了对畜禽粪便和桃树枝堆肥的无害化和资源化.
- (2)利用高通量测序技术对堆肥过程中微生物的变化进行监测,结果显示,厚壁菌门和放线菌门是堆肥中的细菌优势门类;子囊菌门为真菌的优势门类;芽孢杆菌属是堆肥中后期的优势菌属.
- (3)对堆肥的理化指标进行冗余分析,环境因子对细菌和真菌群落结构影响相关性排序均为 pH > 铵态氮 > 温度 > TOC > TN,其中 pH 对微生物群落组成影响最大. norank_c_Sordariomycetes、norank_o_Sordariales 和 norank_c_Agaricomycetes 可能与铵态氮的挥发有关,这为堆肥工艺的调节提供了理论参考.

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