Chapter 8
Vermicomposts Are Biologically Different: Microbial and Functional Diversity of Green Vermicomposts

María Gómez-Brandón, Manuel Aira, and Jorge Domínguez

Abstract Vermicomposting, the process by which organic waste is broken down through the synergistic actions of earthworms and microbial communities, fulfils the dual purpose of environmental protection and fertilizer production. Vermicompost is considered a nutrient-rich and biologically active organic amendment with various beneficial effects when used as a soil conditioner and/or as a plant growth promoter. The underlying biological mechanisms involved in vermicomposting largely determine the dynamics of the process and, consequently, the properties of the end product. However, it is still necessary to further explore the plausible microbial-based mechanisms by which vermicompost may exert a positive influence on plant growth. Therefore, the purpose of this chapter is to compare and provide a detailed characterization of the microbiome of different green vermicomposts by applying 16S rRNA high-throughput sequencing. An in-depth characterization of the taxonomic and functional diversity of bacterial communities and their metabolic functions was assessed in the vermicomposts and the respective parent materials.

Keywords Sustainable agriculture · Plant material · Bacterial community composition · Organic amendment · Soil conditioner · Plant growth promoter

8.1 Introduction

The bio-conversion of biomass waste into organic amendments permits the recycling of large amounts of organic waste that could otherwise cause a serious threat to the environment (Chew et al. 2019). In the last two decades, vermicomposting technology has expanded to seek its potential role in sustainable agriculture (Lazcano and Domínguez 2011) and soil bioremediation (Sánchez-Hernández and Domínguez 2019). Vermicomposting, the process by which organic waste is broken down

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S. A. Bhat et al. (eds.), *Earthworm Assisted Remediation of Effluents and Wastes*,
https://doi.org/10.1007/978-981-15-4522-1_8

through the synergistic actions of earthworms and microbial communities, yields a nutrient-rich and biological active organic amendment known as vermicompost (Domínguez et al. 2010). Vermicomposting therefore fulfils the purpose of disposing of and recycling increasing amounts of organic waste and at the same time offering an alternative to inorganic fertilizers (Lazcano and Domínguez 2011; Gómez-Brandón and Domínguez 2014).

Beneficial effects of vermicompost on plant growth have been documented in a wide variety of agronomic and horticultural crops (Lazcano and Domínguez 2011; Blouin et al. 2019). In addition, extracts and teas made from vermicompost also exhibited positive influence when used as plant growth promoters or soil conditioners (Gómez-Brandón et al. 2015), even though they do not impart the same physical properties as vermicompost amendment. These beneficial effects have been partially attributed to the physico-chemical properties of vermicomposts including their buffering capacity, their high porosity and water-holding capacity and low C to N ratio. The high content of nutrients both in their mineral and organic forms in vermicompost has also been proposed as a plausible, albeit not exclusive, mechanism by which such improvement is achieved (Blouin et al. 2019). Indeed, Lazcano et al. (2013) observed that the beneficial effects of vermicompost amendment on sweet corn yields persisted even when it represented a small portion (25%) of the total amount of nutrients supplied into the soil. This suggests that besides the amount of organic matter and nutrient content that the vermicompost provides, it is necessary to consider other concomitant mechanisms to get the whole picture about the potential role of vermicompost as a plant growth promoter and/or soil conditioner.

Organic amendments contain an endogenous active microbiome that may exert a long-term effect on the productivity and sustainability of agro-ecosystems (Mas-Carrió et al. 2018; Domínguez et al. 2019). Focusing on this aspect is therefore of particular interest in order to broaden our understanding about the role of vermicompost as a plant biostimulant and unravel how its addition into soil may enhance nutrition efficiency and/or crop quality traits, regardless of its nutrient content. In a recent meta-analysis, Medina-Sauza et al. (2019) emphasized the importance of how earthworm-induced changes in the soil microbiota largely impact soil processes, particularly those occurring in the rhizosphere and related to plant growth and health. However, it is still necessary to further evaluate the vermicompost microbiome to shed light onto the plausible microbial-based mechanisms by which the use of vermicompost as an organic amendment may exert a positive influence on plant growth, similar to that when earthworms are present.

The underlying biological mechanisms involved in vermicomposting largely determine the dynamics of the process and, consequently, the properties of the final vermicompost for its further use as a plant growth promoter and in plant disease suppressiveness (Gómez-Brandón and Domínguez 2014). Having this in mind, recent studies from our research group have deeply explored the compositional changes and functional capabilities of the bacterial communities over the course of the vermicomposting process of different plant materials including grape marc (Kolbe et al. 2019; Gómez-Brandón et al. 2019) and the leguminous shrub Scotch broom (Domínguez et al. 2019). Although these substrates have long been

considered to have low value and have usually been discarded, if properly treated, they can be converted into high-nutrient biofertilizers and used as growing media for vegetables and ornamentals (Cai et al. 2018; Gong et al. 2018). Their transformation into green vermicomposts may therefore offer a dual purpose, i.e., environmental protection and fertilizer production.

The purpose of this chapter is therefore to compare and provide a detailed characterization of the microbiome of the green vermicomposts derived from raw and distilled grape marcs of the grape variety *Vitis vinifera* v. Albariño and those obtained from the leguminous shrubs Scotch broom and acacia. We evaluated the taxonomic and functional diversity of bacterial communities and their metabolic functions in the above-mentioned green vermicomposts and the respective raw materials by applying 16S rRNA high-throughput sequencing.

The four types of plant material were separately processed in a pilot-scale vermireactor (6 m^3) housed in a greenhouse with no temperature control located in our research facilities using the epigeic earthworm *Eisenia andrei*. This earthworm species has been widely used in vermicomposting facilities due to its high reproductive rate and its tolerance to a wide range of temperature and moisture conditions (Domínguez and Edwards 2011). The vermireactor set-up and sampling procedure were performed as described in Domínguez et al. (2019).

8.2 How Does Vermicomposting Influence Composition of Bacterial Communities from Dead Plant Material?

For the evaluation of the bacterial community composition of the fresh materials and the resulting vermicomposts, we amplified and sequenced a fragment ~250 bp long of the 16S rRNA gene covering the V4 region using a dual-index sequencing strategy as described by Kozich et al. (2013). DADA2 (version 1.9) was used to infer the amplicon sequence variants (ASVs) present in each sample (Callahan et al. 2016). Default settings were used for ASV inference and chimera detection following the DADA2 pipeline tutorial (https://benjjneb.github.io/dada2/tutorial.html). The taxonomic assignment was performed against the Silva v132 database using the *assignTaxonomy* function in dada2, which implements RDP naive Bayesian classifier (Wang et al. 2007; Quast et al. 2013). The minimum bootstrap confidence for assigning taxonomy was 80. By using an ASV-based approach, it provides a more accurate and reproducible description of amplicon-sequenced communities than an OTU-based approach (Callahan et al. 2017). This relies on the fact that an ASV is defined by a unique sequence that is different from sequences of other ASVs. Moreover, ASVs could differ by as little as one base pair allowing a direct and more straightforward comparison across studies whenever researchers use the same primer set and procedure to obtain the ASVs.

8.2.1 Compositional Changes of Bacterial Communities After Vermicomposting

Vermicomposting led to significant changes in bacterial community composition at phylum level (Fig. 8.1). The fresh plant materials and the respective vermicomposts were found to group separately in two different clades (Fig. 8.1), as shown in the phylogenetic tree inferred from the sequence data using FastTree 2.1 (Price et al. 2010). The raw plant materials were split into two additional clades (Fig. 8.1 top), one comprised by the fresh samples of Scotch broom and acacia substrates and another one by the initial samples of raw and distilled grape marcs. Within each of these clades, there was also a clear differentiation between the two leguminous shrubs and the two winemaking by-products (Fig. 8.1). The dissimilarity of bacterial communities among samples at ASV levels was based on the relative abundance of the dominant bacterial phyla in each sample (Fig. 8.1 barplot). *Proteobacteria* was the most prevalent phylum in the four types of plant material accounting for almost 100% of the sequences in acacia and Scotch broom initial samples and nearly 60–70% of the sequences in the fresh samples of raw and distilled grape marcs (Fig. 8.1). In the case of these two latter substrates, the bacterial community composition was also composed of *Firmicutes*, with minor contributions of *Bacteroidetes* and *Actinobacteria* (Fig. 8.1).

Proteobacteria continued to make up a significant proportion of the bacterial communities within the vermicomposts' microbial composition (Fig. 8.1). However, at ASV level, we observed that 90% of ASVs were exclusively found in the vermicomposts representing in average 90% of the sequences of each vermicompost. Additionally, we tested for differentially abundant bacterial taxa between the fresh plant materials and the respective vermicomposts by using the DESeq2 package (Love et al. 2014). In particular, there was a pronounced reduction in the differential abundance of *Firmicutes* in the vermicomposts derived from raw and distilled grape marcs when compared to the initial samples (*raw*: mean of −9.51 log2 fold change, $p < 0.0001$; *distilled*: mean of −8.56 log2 fold change, $p < 0.0001$, respectively). In contrast, the differential abundance of *Bacteroidetes* (*raw*: mean of 1.50 log2 fold change, $p = 0.029$; *distilled*: mean of 1.79 log2 fold change, $p = 0.007$); *Planctomycetes* (*raw*: mean of 7.81 log2 fold change, $p < 0.0001$; *distilled*: mean of 3.20 log2 fold change, $p < 0.0001$); and *Verrucomicrobia* (*raw*: mean of 4.86 log2 fold change, $p < 0.0001$; *distilled*: mean of 3.92 log2 fold change, $p < 0.0001$) significantly increased after vermicomposting of both raw and distilled grape marcs. Moreover, higher differential abundances of the phyla *Bacteroidetes* (mean: 1.69 log2 fold change, $p = 0.014$), *Verrucomicrobia* (mean: 4.99 log2 fold change, $p < 0.0001$) and *Planctomycetes* (mean: 5.17 log2 fold change, $p < 0.0001$) were also found in the Scotch broom-derived vermicompost when compared to the fresh plant material. Similarly, the acacia-derived vermicompost was characterized by higher abundances of the phyla *Verrucomicrobia* (mean: 3.15 log2 fold change, $p < 0.0001$) and *Bacteroidetes* (mean: 2.99 log2 fold change, $p < 0.0001$) and by

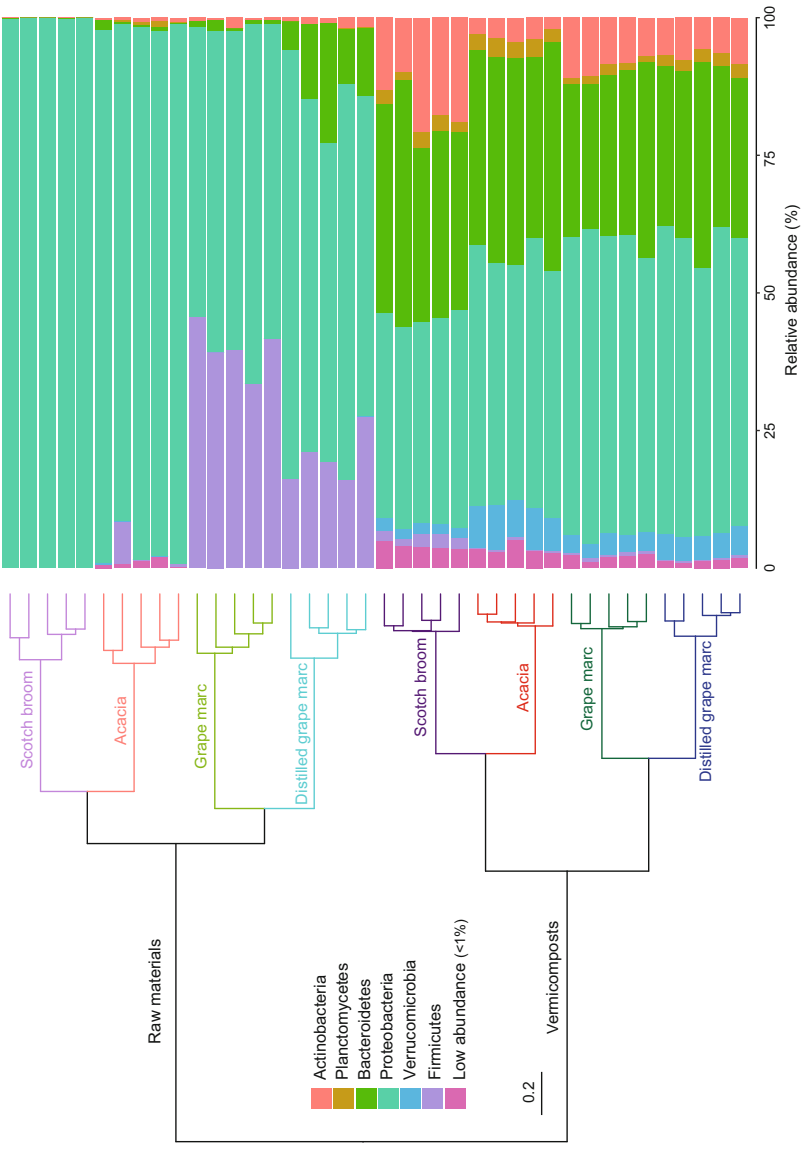

Fig. 8.1 Changes in the bacterial community composition (phylum level) of the fresh plant materials (Scotch broom, acacia, raw and distilled grape marcs) and the respective vermicomposts. The dendrogram represents the dissimilarity of bacterial communities between raw materials and vermicomposts at ASV levels (unweighted UniFrac distances, Ward method). Bars represent the relative abundance of dominant bacterial phyla. Bacterial phyla with relative abundances lower than 1% were grouped together

lower abundances of the phylum *Firmicutes* (mean: −5.14 log2 fold change, $p < 0.0001$).

These differences in microbial community composition between the vermicomposts and the respective raw materials might be a consequence of the successional changes that take place over the course of the vermicomposting process. Such changes will be inextricably linked to the quantity and quality of the available nutrient supplies and constitute an example of heterotrophic ecological succession (Fierer et al. 2010). During vermicomposting, early microbial colonizers (often assimilated to copiotrophs) are characterized by high nutritional requirements and will preferentially consume rich and soluble substrates. As succession progresses, late decomposers (often assimilated to oligotrophs) will take over and will exhibit low growth rates and higher substrate utilization efficiency to metabolize complex carbon compounds (Ho et al. 2017). This could explain why oligotrophic bacterial groups such as *Planctomycetes* and *Verrucomicrobia* had higher differential abundances in the vermicomposts compared to the fresh plant materials while the abundance of *Firmicutes* that is considered as a fast-growing copiotroph group was lower in the vermicomposts. The phylum *Bacteroidetes* is also associated with copiotrophic environments (Yang et al. 2019), but unlike *Firmicutes*, *Bacteroidetes* mostly comprise Gram-negative bacteria. Earlier studies based on phospholipid fatty acid (PLFA) analysis have shown that the passage of organic material through the gut of *E. andrei* reduced the abundance of Gram-positive bacteria to a greater extent than Gram-negative bacteria (Gómez-Brandón et al. 2011, 2012). This feature could have favoured that *Bacteroidetes*, in contrast to *Firmicutes*, appeared in higher abundances in the vermicomposts than in the initial substrates.

8.2.2 Compositional Changes of Bacterial Communities Among Vermicomposts

The vermicomposts could be differentiated from each other at phylum level in line with the type of plant material (Fig. 8.1 bottom). The percentage of shared ASVs and sequences between each pair of vermicomposts is shown in Fig. 8.2. Testing for differentially abundant bacterial taxa between pairs of vermicomposts was also achieved using the DESeq2 package. When comparing the bacterial community composition of the vermicompost samples derived from the raw grape marc to those obtained from distilled marc, we found that five bacterial phyla significantly differed in abundance. They comprised the phyla *Patescibacteria* (mean: −8.85 log2 fold change, $p = 0.003$), *Acidobacteria* (mean: 2.34 log2 fold change, $p = 0.006$), *Nitrospirae* (mean: 5.95 log2 fold change, $p = 0.011$), *Gemmatimonadetes* (mean: 3.71 log2 fold change, $p = 0.010$) and *Armatimonadetes* (mean: 5.09 log2 fold change, $p = 0.003$). The mean log2 fold change values were either positive or negative depending whether the above-mentioned phyla appeared in higher or lower differential abundances in the raw grape marc vermicompost samples.

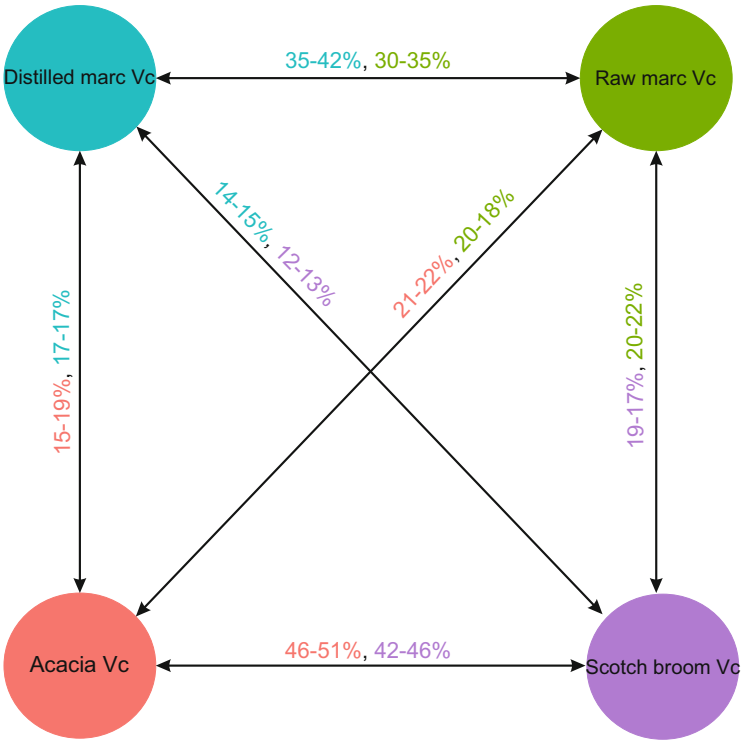

Fig. 8.2 Degree of sharing of native ASVs among vermicomposts (abbreviated as Vc). The shared ASVs and sequences between each pair of vermicomposts are shown as percentage. The colour given to the percentage values is in line with the type of plant material, and it represents the native ASVs (first value before the hyphen) and sequences (second value after the hyphen) shared by this type of vermicompost with respect to the other vermicomposts

Moreover, higher differential abundances of the phyla *Chloroflexi* (mean: 4.10 log2 fold change, $p = 0.0001$), *Proteobacteria* (mean: 1.02 log2 fold change, $p = 0.028$) and *Armatimonadetes* (mean: 4.89 log2 fold change, $p = 0.003$) were detected in the vermicompost samples obtained from the raw grape marc when compared to those derived from the Scotch broom plant material. The opposite trend was found for the phylum *Fibrobacteres* (mean: −7.82 log2 fold change, $p = 0.048$), with lower differential abundances in the raw grape marc-derived vermicompost. If we compared the raw grape marc- and acacia-derived vermicomposts, *Actinobacteria* (mean: 2.01 log2 fold change, $p = 0.004$), *Patescibacteria* (mean: −6.85 log2 fold change, $p = 0.026$), *Spirochaetes* (mean: −6.04 log2 fold change, $p = 0.002$) and *Armatimonadetes* (mean: 3.48 log2 fold change, $p = 0.049$) were found to significantly differ in terms of abundance between this pair of vermicomposts.

The comparison of the bacterial community composition of the distilled grape marc-derived vermicompost and that from Scotch broom- and acacia vermicomposts

resulted in a total of ten and seven phyla that differed in abundance for the pairs distilled marc-Scotch broom and distilled marc-acacia vermicompost samples, respectively. Some of these phyla including *Acidobacteria*, *Spirochaetes*, *Nitrospirae* and *Gemmatimonadetes* had lower differential abundances in the distilled grape marc-derived vermicompost compared to acacia and Scotch broom vermicomposts. Others like *Chloroflexi*, *Verrucomicrobia*, *Patescibacteria* and *Actinobacteria* reached higher abundances in the vermicompost samples from distilled grape marc according to DESeq results.

While lower differential abundances of *Verrucomicrobia* (mean: −2.02 log2 fold change, $p = 0.002$) and *Patescibacteria* (mean: −6.92 log2 fold change, $p = 0.024$) were recorded in Scotch broom vermicompost samples, higher abundances of *Actinobacteria* (mean: 2.08 log2 fold change, $p = 0.003$) and *Firmicutes* (mean: 2.75 log2 fold change, $p = 0.003$) were found in these vermicompost samples compared to those obtained from the acacia plant material.

8.3 How Does Vermicomposting Influence Alpha- and Beta-Diversity of Bacterial Communities from Dead Plant Material?

In this chapter taxonomic α-diversity of bacterial communities was calculated as the number of observed ASVs, while phylogenetic diversity was assessed as Faith's phylogenetic diversity (Faith 1992). An increase in α-diversity was reported for all of the four vermicomposts in comparison with the respective fresh materials at both taxonomic and phylogenetic levels (Fig. 8.3a, b). The Scotch broom-derived vermicompost harboured the highest α-diversity at taxonomic level (689 ± 35), being 17 times greater than in the initial substrate (Fig. 8.3a), while no differences were observed among the other three vermicomposts (Fig. 8.3a). A similar pattern emerged for the Faith phylogenetic diversity (Fig. 8.3b), and in this case the highest values were reported for both the Scotch broom- and acacia-derived vermicomposts (Fig. 8.3b).

The increase in α-diversity was reflected in different patterns of taxonomic and phylogenetic β-diversity (Fig. 8.4a, b). Taxonomic β-diversity at the ASV level was estimated as the difference in the composition of the bacterial taxonomic community between the fresh plant materials and the respective vermicomposts. This was done by coupling principal coordinate analysis (PCoA) with distance matrices that take the abundance of ASVs into account (Bray–Curtis). Phylogenetic β-diversity was estimated by PCoA of weighted (considering abundance of ASVs) UniFrac matrix distances (Lozupone and Knight 2005) by using the phyloseq library (Love et al. 2014). Principal coordinate analysis showed that the fresh plant materials differed from each other in terms of β-diversity at both taxonomic and phylogenetic levels (Fig. 8.4a, b). In addition, they also grouped separately from the respective vermicomposts (Fig. 8.4a, b). This implies that the bacterial community present in

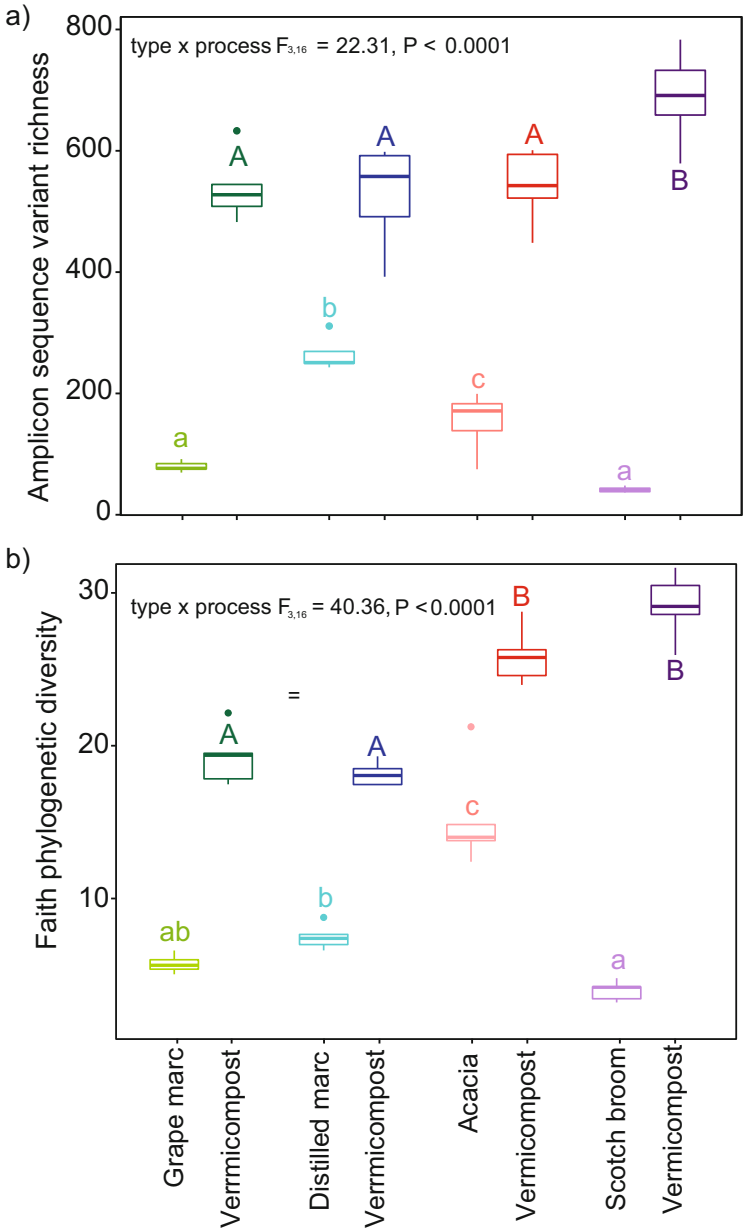

Fig. 8.3 Changes in bacterial α-diversity estimated as (**a**) observed ASV richness and (**b**) Faith phylogenetic diversity of the fresh plant materials (Scotch broom, acacia, raw and distilled grape marcs) and the respective vermicomposts. All of the four plant materials were significantly different from their respective vermicomposts ($P < 0.05$). Different lowercase and capital letters over the boxplots indicate significant differences among fresh plant materials and vermicomposts, respectively (Tukey HSD test, FDR corrected)

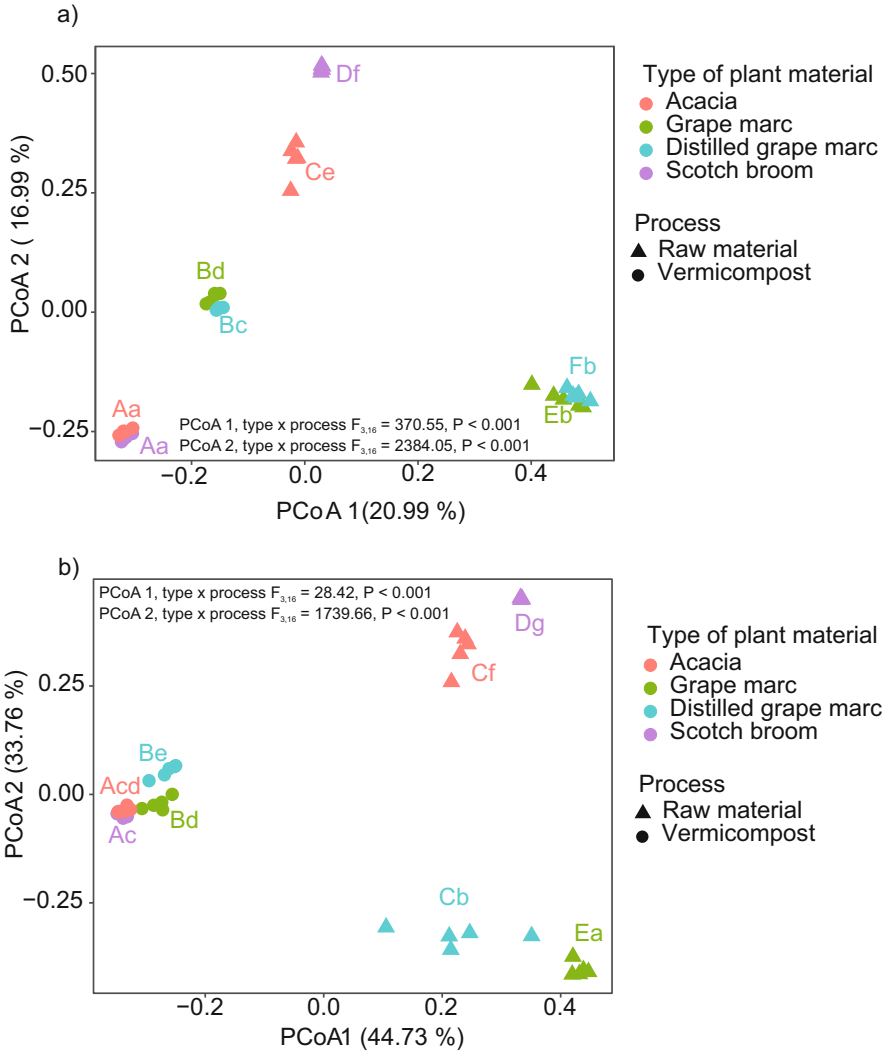

Fig. 8.4 Changes in bacterial β-diversity assessed as principal coordinate analysis of (**a**) Bray–Curtis and (**b**) weighted UniFrac distances of the fresh plant materials (Scotch broom, acacia, raw and distilled grape marcs) and the respective vermicomposts. Capital and lowercase letters indicate significant differences among fresh plant materials and vermicomposts in PCoA1 and PCoA2 scores, respectively (Tukey HSD test, FDR corrected)

the parent materials was strongly affected by the earthworms' activity over the course of the vermicomposting process. Earthworms can directly modify the microbial diversity of the substrate by selectively feeding on and/or stimulating specific taxa during transit through the earthworm gut (Aira et al. 2015). The changes occurred through the gut-associated processes may have further consequences on

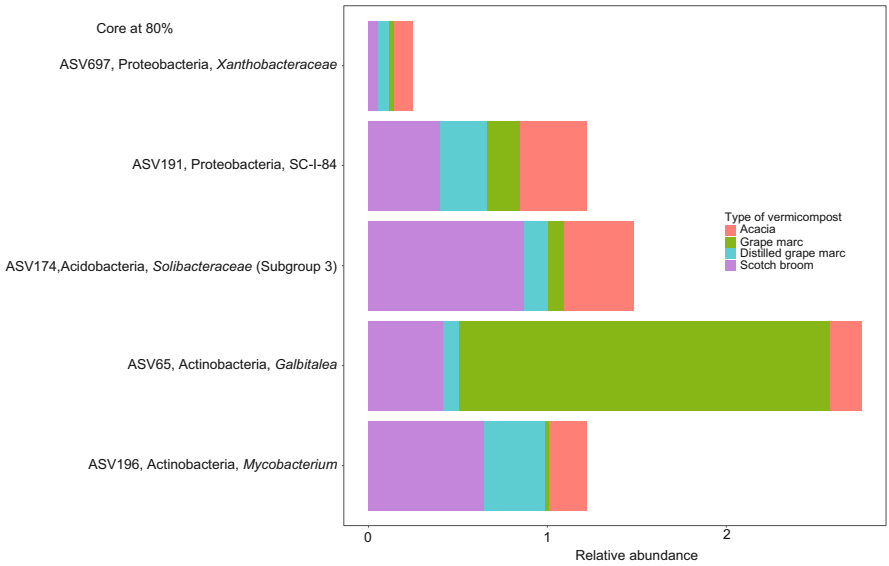

Fig. 8.5 Relative abundance (%) of ASVs (phylum and genus or most inclusive taxonomy found) from the core microbiome of vermicomposts obtained from Scotch broom and acacia plant materials and those derived from raw and distilled marcs of the grape variety *Vitis vinifera* v. Albariño

the decomposition process because microorganisms are released again into the vermicompost matrix as part of the earthworm casts (Aira and Domínguez 2011). Later on, natural ageing processes known as cast-associated processes take place during vermicomposting with implications on nutrient availability and, consequently, on the structure of the microbial community (Aira et al. 2010).

Furthermore, we found that the initial differences among bacterial communities in terms of β-diversity were markedly reduced, mainly at phylogenetic level, once transformed into vermicomposts (Fig. 8.4b). All of the four vermicomposts grouped closely together in the negative side of the first axis (Fig. 8.4b). These similarities at phylogenetic level also held true for the microbiome of fresh casts of *E. andrei* feeding on different types of animal manure as reported by Aira et al. (2015). The presence of a core of common bacteria was evidenced in the four vermicomposts (Fig. 8.5), even though this core only represented a small percentage (1.74%) of all sequences. Core microbiome can be defined arbitrarily at any threshold for presence and/or abundance (Shade and Handelsman 2012), but in our case, we chose the most restrictive option, that is, one ASV should be present in all of the four vermicomposts to be considered within the core microbiome regardless of its abundance. The fresh plant materials were not considered within the core microbiome because these samples were not subjected to the action of earthworms. After removing them, the core microbiome of the studied vermicomposts comprised only five ASVs that were affiliated to the phyla *Proteobacteria*, *Actinobacteria* and *Acidobacteria* (Fig. 8.5).

8.4 How Does Vermicomposting Influence Functional Diversity of Bacterial Communities from Dead Plant Material?

Functional diversity includes the wide range of metabolic activities carried out by the microorganisms in an ecosystem and can describe the way in which diverse microorganisms interact as a meta-organism to perform specific functions (Goswami et al. 2017). Assessing functional diversity is of high ecological importance because it can influence both the ecosystem dynamics and functioning (Tilman 1999). In the present chapter, the functional prediction of the fresh materials and the respective vermicomposts was performed by using a gene-based computational tool known as PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) that allows to computationally infer metagenome functional contents from 16S rRNA gene sequences (Langille et al. 2013). Predicted metagenomes were collapsed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway metadata (Kanehisa et al. 2019). Putative functional genes involved in cellulose metabolism, hormone synthesis and antibiotic production, which can be considered as a proxy for plant growth and development, were also predicted using PICRUSt tool.

Overall, we detected significant predicted increases in genes classified only as "metabolism" in KEGG functional hierarchies in all four vermicomposts in comparison with the respective fresh plant materials (Fig. 8.6a). The same pattern emerged for specific genes related to cellulose metabolism (Fig. 8.6b). The degree of such effect varied with the type of plant material, and lower gene abundances were found in the distilled grape marc-derived vermicompost compared to the other three vermicomposts (Fig. 8.6a, b). This indicates that the bacterial communities initially contained in the parent materials had an influence on the metabolic functions in the resulting vermicomposts. Indeed, previous works have underlined the importance of the starting material for driving bacterial succession during vermicomposting (Fernández-Gómez et al. 2010; Yakushev et al. 2011). Other factors shaping the functional diversity of vermicompost microbiomes may include the earthworm species and/or the vermicomposting procedure (Domínguez et al. 2019).

With regard to the genes involved in the synthesis of plant hormones, the vermicomposts obtained from the raw grape marc and Scotch broom plant material had higher abundances than the respective initial substrates (Fig. 8.6c). An increase in the abundance of genes related to antibiotic production was also observed in the vermicompost samples, except for the distilled grape marc (Fig. 8.6d). Generally speaking, processing of dead plant material through vermicomposting resulted in increases in specific metabolic processes potentially beneficial for plant growth and development. Indeed, antibiotic production by beneficial bacteria has been proposed as a plausible mechanism by which vermicompost addition may confer disease

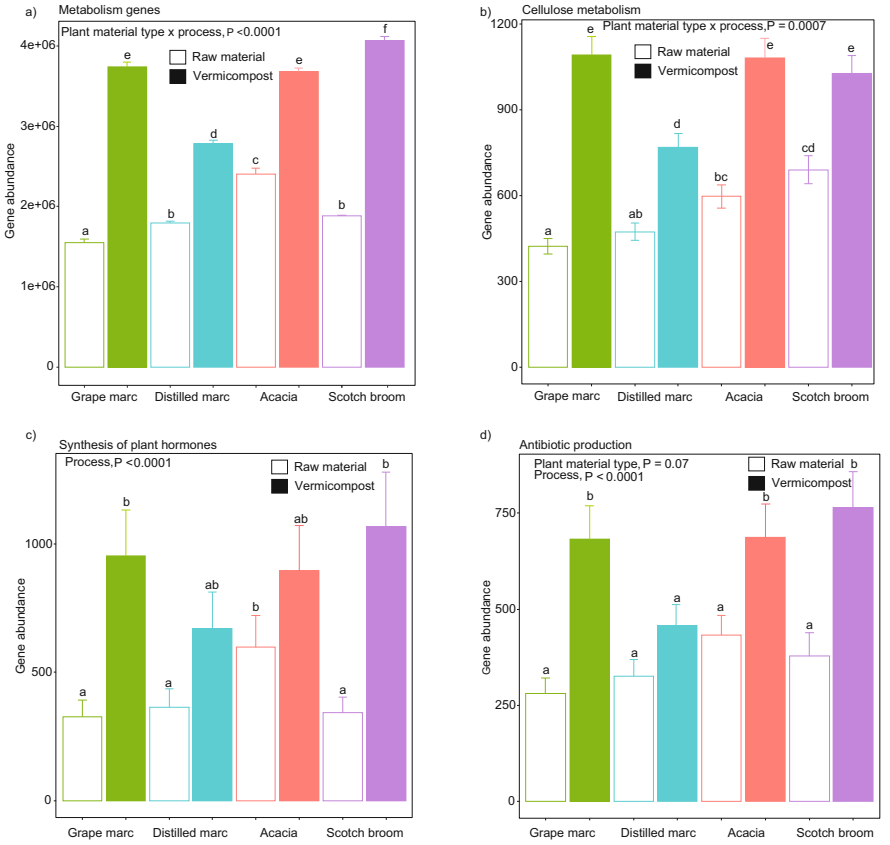

Fig. 8.6 Changes in gene abundance of PICRUSt-predicted KEGG orthologies classified as (**a**) "metabolism," (**b**) cellulose metabolism, (**c**) plant hormone synthesis, and (**d**) antibiotic production of the fresh plant materials (Scotch broom, acacia, raw and distilled grape marcs) and the respective vermicomposts (abbreviated as "Vc" in the x-axis). For each starting material, different letters indicate significant differences between the fresh plant material and the resulting vermicompost (Tukey HSD test, FDR corrected)

resistance in some plants (Lazcano and Domínguez 2011). In this sense, Gopalakrishnan et al. (2011) identified five actinomycete isolates from the genus *Streptomyces* in herbal vermicomposts that contained antagonistic potential against *Fusarium* wilt of chickpea under both greenhouse and field conditions. The selected *Streptomyces* strains were also shown to act as plant growth-promoting agents (Gopalakrishnan et al. 2013). Song et al. (2015) also found that adding vermicompost enhanced the beneficial effects of plant growth-promoting rhizobacteria on both soil and crop, but the extent of this promotion varied with the dose of vermicompost and the crop type.

8.5 Conclusions

The present chapter provides an in-depth characterization of bacterial communities inhabiting green vermicomposts from a taxonomic and functional perspective. Our findings showed that the studied vermicomposts were biologically different not only from the parent plant materials but also among them with regard to the abundance of certain bacterial phyla. Such differences in bacterial community composition may help to define the potential usefulness of each vermicompost as a soil conditioner and/or as a plant growth promoter. Moreover, the vermicomposts exhibited higher functional diversity and higher abundances of putative genes related to specific metabolic processes potentially beneficial for plant growth and development. This reinforces the role of vermicomposts as bioactive organic materials and provides evidence of microbial functions that may explain the positive influence of vermicompost on soil and plants.

Acknowledgements The authors would like to thank Hugo Martínez, Alberto Da Silva and Natalia Ribao for their help with the vermicomposting process, sample collection and DNA extraction. The authors also acknowledge Paul Fraiz for his valuable help regarding the English wording and style. This study was supported by the Spanish Ministerio de Economía y Competitividad (grant numbers CTM2013-42540-R and AGL2017-86813-R) and the Xunta de Galicia (grant numbers ED431F 2018/05 and ED431B 2019/038). MGB acknowledges support by the Programa Ramón y Cajal (RYC-2016-21231; Ministerio de Economía y Competitividad).

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