Applied Soil Ecology 98 (2016) 103–111

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Characterization of the bacterial communities of casts from *Eisenia andrei* fed with different substrates

Manuel Aira[a,*], Jessica Olcina[a], Marcos Pérez-Losada[b,c,d], Jorge Domínguez[a]

[a] Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Vigo, E-36310. Spain
[b] CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal
[c] Computational Biology Institute, George Washington University, Ashburn, VA 20147, USA
[d] Department of Invertebrate Zoology, US National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA

ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 1 October 2015
Accepted 5 October 2015
Available online 27 October 2015

Keywords:
Bacterial communities
Bacterial diversity
Bar-coded pyrosequencing
Earthworms
Eisenia andrei
Decomposition

ABSTRACT

Earthworms play a key role during the first stage of decomposition by enhancing the activity of microorganisms. As organic matter passes throughout the earthworm gut, nutrient pools and microbial communities are modified and released in casts. Here we used 16S rRNA pyrosequencing and metagenomic analysis to characterize the bacterial communities of casts from the earthworm *Eisenia andrei* fed with different food sources (cow, horse and pig manure). We found that the bacterial communities of cast strongly depended on the food source ingested by earthworms; although, no differences in α-diversity were detected. Bacterial communities of casts were mainly comprised of a variable amount of OTUs (operational taxonomic unit) belonging to the phyla Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, with minor contributions from the phyla Verrucomicrobia, Chloroflexi, Hydrogenedentes, Latescibacteria, Planctomycetes and Candidatus Saccharibacteria. From these bacterial profiles we found OTUs that worked out as biomarkers for each bacterial community allowing us to discriminate among food sources.

1. Introduction

Earthworms are key components of temperate soil ecosystems, where they constitute the largest biomass and contribute to the key process of decomposition. Although the biochemical decomposition of organic matter is primarily accomplished by microorganisms, earthworms are crucial drivers of the process. Earthworms are involved in the stimulation of microbial populations through ingestion and fragmentation of fresh organic matter, which results in a greater surface area available for microbial colonization, thereby drastically altering biological activity (Edwards, 2004; Domínguez et al., 2010). Earthworm–microbe interaction, and the resulting modified microbial communities (Aira et al., 2008; Gómez-Brandón et al., 2011a) enhances rates of decomposition by, for example, increasing the rates of cellulolytic metabolism (Aira et al., 2006), microbial enzymatic activity (Aira et al., 2007a) or microbial metabolic capabilities (Aira et al., 2007b). Changes in the composition of microbial communities during gut transit play a major role in the decomposition process as the modified microbial communities are released to the environment as part of the earthworm casts. In fact, inoculation of raw residues with earthworm casts modifies the rate of organic matter decomposition in the same way as if earthworms were present (Aira and Domínguez, 2011). During transit through the earthworm gut some bacterial groups may be digested and others may survive and even flourish (Drake and Horn, 2007). Hence, it is important to understand how gut transit modifies the bacterial populations ingested by earthworms. Studies investigating the direct effect of earthworms on microorganisms are in need particularly for epigeic earthworm species because most such studies focus on soil-dwelling endogeic and anecic species. In nature, epigeic earthworms live in fresh organic matter of forest litter, in litter mounds, in herbivore dungs, and in anthropogenic environments such as manure heaps, vegetal debris and vermicomposting beds common in agricultural landscapes. There are several studies characterizing the bacterial communities of casts from epigeic earthworm species. Thus, the composition of bacterial communities of casts from *Lumbricus rubellus* seem to depend on ingested bacterial communities (Furlong et al., 2002; Singleton et al., 2003; Knapp et al., 2009) as is the case with endogeic and anecic species (Egert et al., 2004; Thakuria et al., 2009). However, it is not the case for cast of *Eisenia andrei* fed with different diets

* Corresponding author at: Departamento de Ecología y Biología Animal, Facultad de Ciencias, Campus Universitario As Lagoas, Ourense E-32004, Spain.
E-mail address: aira@uvigo.es (M. Aira).

http://dx.doi.org/10.1016/j.apsoil.2015.10.002
0929-1393/

(Gómez-Brandón et al., 2011bKoubová et al., 2015). Nevertheless, these studies have either used PLFAs, DGGE or cloning and sequencing, which due to their intrinsic or applied technical limitations, underestimate bacterial diversity. Thus, our aims were to characterize the taxonomic and phylogenetic composition of the bacterial communities residing in casts from the earthworm *E. andrei* and to ascertain the contribution of ingested bacteria to its bacterial community composition. To do this we used 16S rRNA pyrosequencing and metagenomic analysis of casts from the earthworm *E. andrei* fed with three substrates that heavily differ in their bacterial composition (cow, horse and pig manure; Ley et al., 2008). We also assess whether bacterial communities of casts from different manures constitute unique bacterial communities (i.e., taxonomic biomarkers) or share a variable proportion of their members.

2. Material and methods

2.1. Animal manures, earthworms and casts sampling

Animal manures (horse, cow and pig manure) were collected from a farm near the University of Vigo (Galicia, NW Spain) and stored under laboratory conditions (20 °C). We sampled five specimens of *E. andrei* (hand-sorted method) from different stock cultures that were fed with the three animal manures from at least 7 years. *E. andrei* was selected as earthworm model species as its importance in vermicomposting and because is one of the most common and abundant epigeic earthworms found in natural (e.g., litter mounds and herbivore dungs) and anthropogenic environments (e.g., manure heaps, vegetal debris and vermicomposting facilities) rich in organic matter (Domínguez et al., 2010). The earthworms were placed in separate sterile plastic Petri dishes (one per dish); each dish was filled (75% of space) with vermicompost from each stock culture) and earthworms were fed ad libitum with one of the three animal manures (breeding dishes). The dishes were stored in random positions in an incubation chamber, at 20 °C and 90% relative humidity. In order to obtain cast samples, earthworms were removed from the dishes, washed three times with sterile distilled water and placed in clean and sterile Petri dishes on moistened sterile filter paper (sampling dishes). This was done under sterile conditions in a laminar flow cabinet. Sampling dishes were placed in the same incubation chamber during 24 h. After that, earthworms were returned to the breeding dishes and casts were recovered from each sampling dish with a sterile spatula, which was sterilized between earthworms from the same diet and between diets. Casts were stored in 1.5 mL Eppendorf tubes at −80 °C. This process was done again under sterile conditions and repeated (a minimum of five times) until attaining 0.25 g of fresh casts per earthworm and manure type.

2.2. DNA extraction and bar-coded pyrosequencing

Total DNA from casts (0.25 g) was extracted with the PowerSoil DNA Isolation kit (MoBio Laboratories Inc., Carlsbad, California) according to the manufacturer's protocol. We amplified a fragment of the 16S rRNA gene spanning the V2 (start: 101, end: 361) and V3 (start: 338, end:534) regions by using the primers (forward 5'-AGYGGCGIACGGGTGAGTAA and reverse 5'-ATTACCGCGGCTGCTGG) and touchdown PCR protocol described by Sundquist et al. (2007). Our primers were modified from Sundquist et al. (2007) to include (from 5′–3′) the 21 bp Titanium 454 primer A, the 4 bp key, and the V2 (forward) for our forward primer; while our reverse primer included the Titanium 454 primer B, the 4 bp key, a 10 bp DNA Barcode (MID: Roche Technical Bulletin No. 005-2009) and the V3 (reverse) primer. Using our primers, each sample could proceed directly to pyrosquencing following PCR amplification. We used AccuPrime™ Pfx DNA Polymerase from Invitrogen in a single 14 μl reaction (1.25 μl 10x buffer, 8.5 μl H20, 0.25 μl Taq, 1 μl each of 2.5 μM forward and reverse primer and 2 μl of gDNA). Following successful amplification, samples were submitted to the sequencing center at Brigham Young University. They were cleaned of primer dimer using AMPure beads, pooled in equal amounts according to the total quantity of DNA (as estimated with Quant-iT PicoGreen), and sequenced using a Roche 454 sequencer. We submitted to sequencing 5 samples per treatment (horse, cow and pig) but only 5, 4 and 3 samples were successfully sequenced for cast obtained from earthworms fed with horse, cow and pig manure respectively.

2.3. Processing of pyrosequencing data

Data from raw standard flowgram format (sff) files were processed with mothur (version 1.35.1, Schloss et al., 2009). The default settings were used to minimize the sequencing error described by Schloss et al. (2011). Briefly, the flow grams were separated according to their primer and barcode sequence, and the sequence data were de-noised. The sequence reads were first trimmed to remove barcode and primer sequences. Only sequences ≥200 bp were aligned to the bacterial-subset SILVA alignment available at http://www.mothur.org The sequences were screened to cover the same genetic space and filtered to remove columns without alignment data, upon which the sequences were pre-clustered to remove bad sequences with pyrosequencing errors. Chimeras were checked with the chimera.uchime command in mothur and then removed. Sequences were classified with the naïve Bayesian classifier (Wang et al., 2007) against a RDP reference file version 10 included in mothur, and any contaminants (sequences classified as mitochondria, chloroplasts, archaea, eukaryote or unknown) were removed. To obtain operational taxonomic units (OTUs) at the 0.03 level, we first constructed a distance matrix (cut-off 0.15), clustered the resulting sequences into OTUs and then classified them to obtain their consensus taxonomy. Sequence data (raw sff files) have been uploaded to the GenBank SRA database under accession number SRP059050

2.4. Statistical analysis

In order to remove the effect of sample size on community composition, samples were rarefied to 1178 sequences. We infer an approximately-maximum-likelihood phylogenetic tree with FastTree 2.1 (Price et al., 2010). Taxonomic alpha diversity was calculated as the observed number of OTUs (sobs), estimated diversity (Shannon index) and richness (Chao1 index). Phylogenetic diversity was calculated as Faith's phylogenetic diversity. The effect of manure on both the taxonomic and phylogenetic alpha diversity of bacterial communities from casts was assessed by one-way ANOVA tests over linear models where manure type (pig, horse and cow) was fixed as factor. For each variable we checked normality of residuals and homogeneity of variance across groups. Post-hoc comparisons were performed with Tukey test and the Benjamini–Hochberg FDR multiple test correction method was applied (library multcomp; Hothorn et al., 2008).

Taxonomic beta diversity was estimated as differences in bacterial taxonomic community composition at the OTU level between samples of casts. This was done by principal coordinate analysis (PCoA) with Bray–Curtis (considering abundance of OTUs) and Jaccard (not considering the abundance of OTUs) distance matrixes. Phylogenetic beta-diversity was also calculated by PCoA with weighted (considering abundance of OTUs) and unweighted unifrac distances (Lozupone et al., 2007), which were obtained as averages after sampling the phylogenetic tree 1000 times. All PCoAs were done with function ordinate from library phyloseq

(McMurdie and Holmes, 2013) with distance matrixes obtained with mothur. We used an analysis of similarities (anosim with 10,000 permutations, from the R library vegan; Oksanen et al., 2012) to compare taxonomic and phylogenetic community composition.

In order to check the degree of co-occurrence of OTUs in casts from the three manures we build an OTU network with function make_otu_network.py from QIIME 1.7 using a BIOM table generated with mothur and plotted it with Cytoscape 2.8 (Shannon et al., 2003). The network analysis is an associated *G*-test that checks whether sample-nodes within categories (in our case the three manures) are more connected within each group than expected by chance. In order to know what OTUs define each bacterial community from casts samples we used an indicator test as implemented in mothur. Data are presented as mean ± S.E. All analyses were performed in R 3.1 (R Core Team, 2014), mothur (version 1.35.1) and QIIME 1.7 (Caporaso et al., 2010).

3. Results

After processing raw files we obtained 61,998 sequences which resulted in 1180 OTUs defined at 97% similarity (558 after rarefaction at 1178 sequences per sample). Rarefaction curves indicated that sampling depth was suboptimal, although curves showed a sign of reaching an asymptote (Fig. S1). The composition of bacterial communities of casts strongly depended on the type of manure ingested by the earthworms, being the effect evident at the phylum (Fig. 1) and class levels (Fig. 2, Fig. S2). Bacterial communities of cast from earthworms fed with cow and horse manure showed more OTUs from the phylum Proteobacteria (ANOVA, $F_{2,9} = 26.25$, $P < 0.001$) than the ones fed with pig manure. Furthermore, bacterial communities of casts from earthworms fed with cow manure showed a higher abundance of OTUs from the phylum *Chloroflexi* (ANOVA, $F_{2,9} = 6.24$, $P = 0.019$) than cast from earthworms fed with the other two animal manures. On the other hand, bacterial communities of casts from earthworms fed with pig manure were dominated by OTUs belonging to the phylum *Firmicutes* (ANOVA, $F_{2,9} = 343.07$, $P < 0.001$) (Fig. 1). At class level, bacterial of communities of casts clustered based on the manure they came from, with high abundance of OTUs from classes Clostridia, Actinobacteria, Gammaproteobacteria and Alphaproteobacteria (Fig. S2). In fact, there were significant differences in the abundance of OTUs in the casts of earthworms fed on different manures in the classes Flavobacteriia (phylum Bacteroidetes, ANOVA, $F_{2,9}$=4.36, P=0.047); Thermomicrobia (phylum Chloroflexi, ANOVA, $F_{2,9} = 9$, $P = 0.007$), Clostridia (phylum Firmicutes; ANOVA, $F_{2,9} = 397.92$, $P < 0.001$), Erysipelotrichia (phylum Firmicutes; ANOVA, $F_{2,9} = 6.29$, $P = 0.019$), Betaproteobacteria (ANOVA, $F_{2,9} = 4.32$, $P = 0.048$) and Gammaproteobacteria (ANOVA, $F_{2,9} = 6.21$, $P = 0.020$, both from phyla Proteobacteria) (Fig. 2). The most abundant OTUs in cast of *E. andrei* were those classified as *Acinetobacter*,*Nocardioides*, *Anaerobacter*, *Chryseobacterium*, *Amaricoccus*, *Luteolibacter*, *Clostridium sensu stricto* and *Pseudomonas*.

Interestingly, all bacterial communities of cast from *E. andrei* fed with different manures showed similar levels of α-diversity for number of OTUs (overall mean and S.E. 87 ± 8 OTUs; sobs $P = 0.59$), estimated richness (205 ± 19 OTUs; Chao1 $P = 0.54$), diversity (2.28 ± 0.18; Shannon $P = 0.41$) and phylogenetic diversity (16.11 ± 1.16; Faith $P = 0.13$). However, it is important to point out that this lack of differences in α-diversity does not imply that bacterial communities are similar in their composition. In fact, we found that bacterial communities of casts from earthworms fed with the three manures had different taxonomic (Jaccard, ANOSIM, $R = 0.79$, $P < 0.0001$; Fig. 3a) and phylogenetic composition (unweigthed UnifracANOSIM, $R = 0.78$, $P < 0.001$; Fig. 3b) at the OTU level, as indicated by presence-absence (qualitative) indexes. The same was true for taxonomic and phylogenetic bacterial community composition when abundance of OTUs was considered (quantitative, Fig. S3).

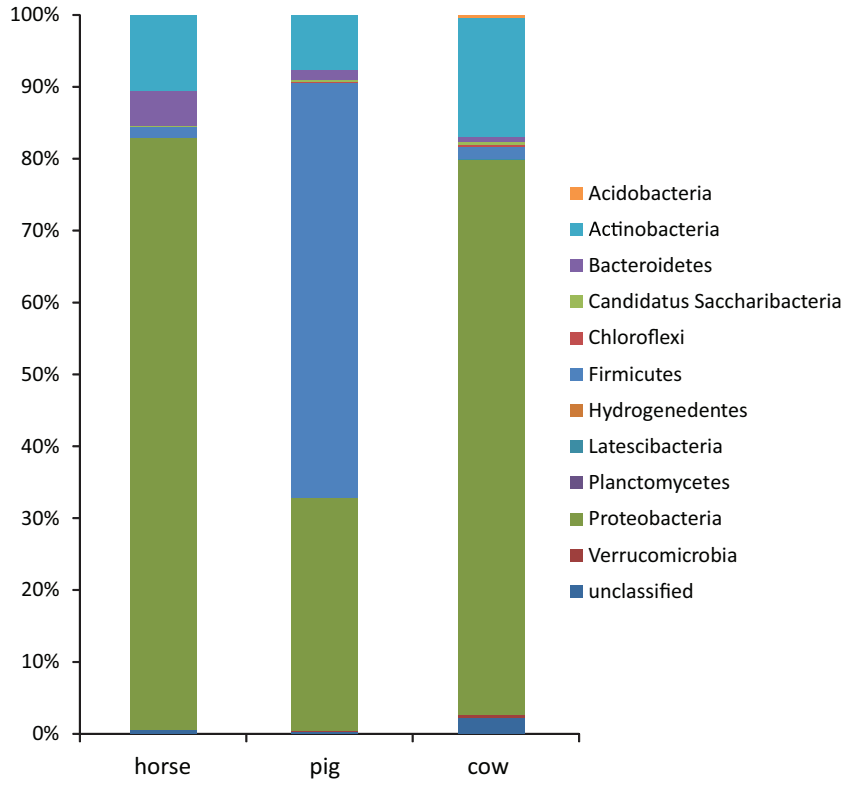

Fig. 1. Bacterial community composition (phylum level) of bacterial communities from casts of the earthworm *Eisenia andrei* fed with pig, horse and cow manure.

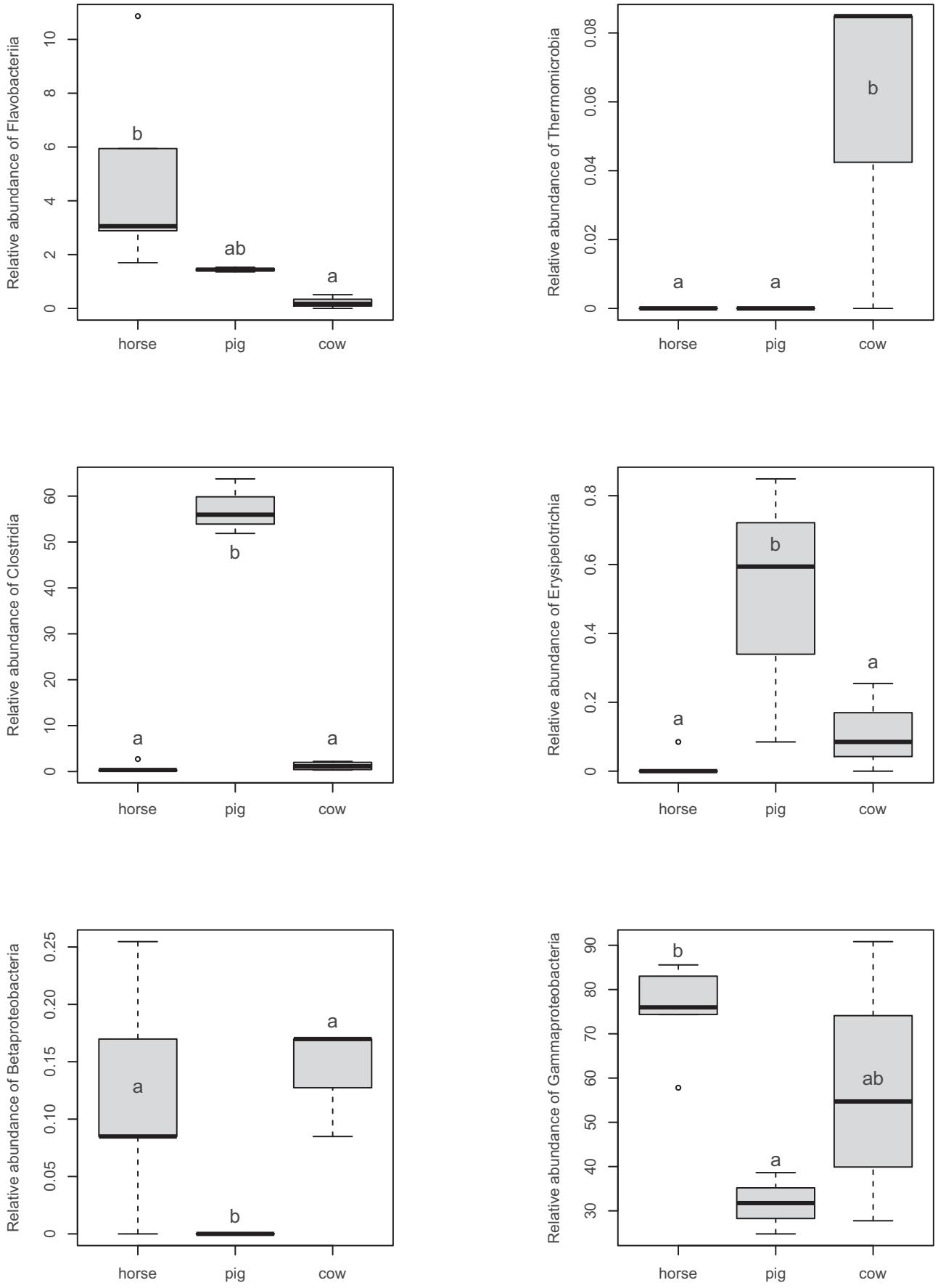

Fig. 2. Differences in relative abundance of bacterial classes from bacterial communities of casts of the earthworm *Eisenia andrei* fed with pig, horse and cow manure. Differences between manures (ANOVA-test, Benjamin–Hochberg FDR corrected) are marked with different letters.

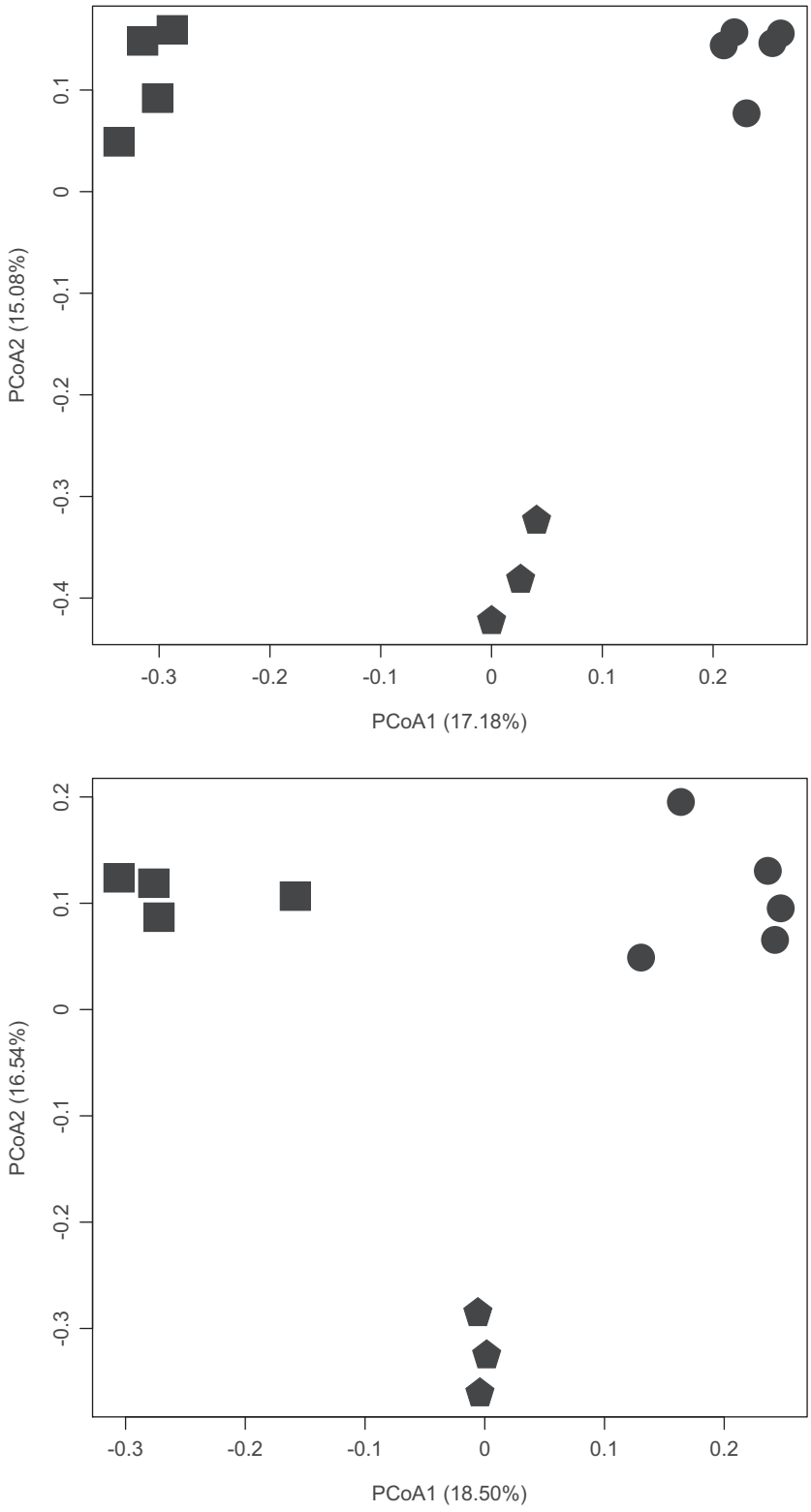

Fig. 3. Principal coordinate analysis of (a) taxonomic (Jaccard), and (b) phylogenetic (unweighted UniFrac) β-diversity of bacterial communities from casts of the earthworm *Eisenia andrei* fed with pig (pentagons), horse (circles) and cow (squares) manure.

To understand how OTUs were distributed among samples, we performed a network analysis to represent clustering of samples in terms of their shared OTUs (Fig. 4). We found that samples from casts of *E. andrei* fed with the three manures only shared a small fraction of OTUs (*G*-test, $G = 0$, $P = 1$), which indicates that there was not co-occurrence. Moreover, the indicator analysis found 28 OTUs (i.e., biomarkers) that distinguished bacterial communities of casts from earthworms fed with horse (13 OTUs), pig (7 OTUs) and cow manure (8 OTUs) (Fig. 5). Biomarkers of casts from horse manure belonged to the phyla Proteobacteria (Moraxellaceae,

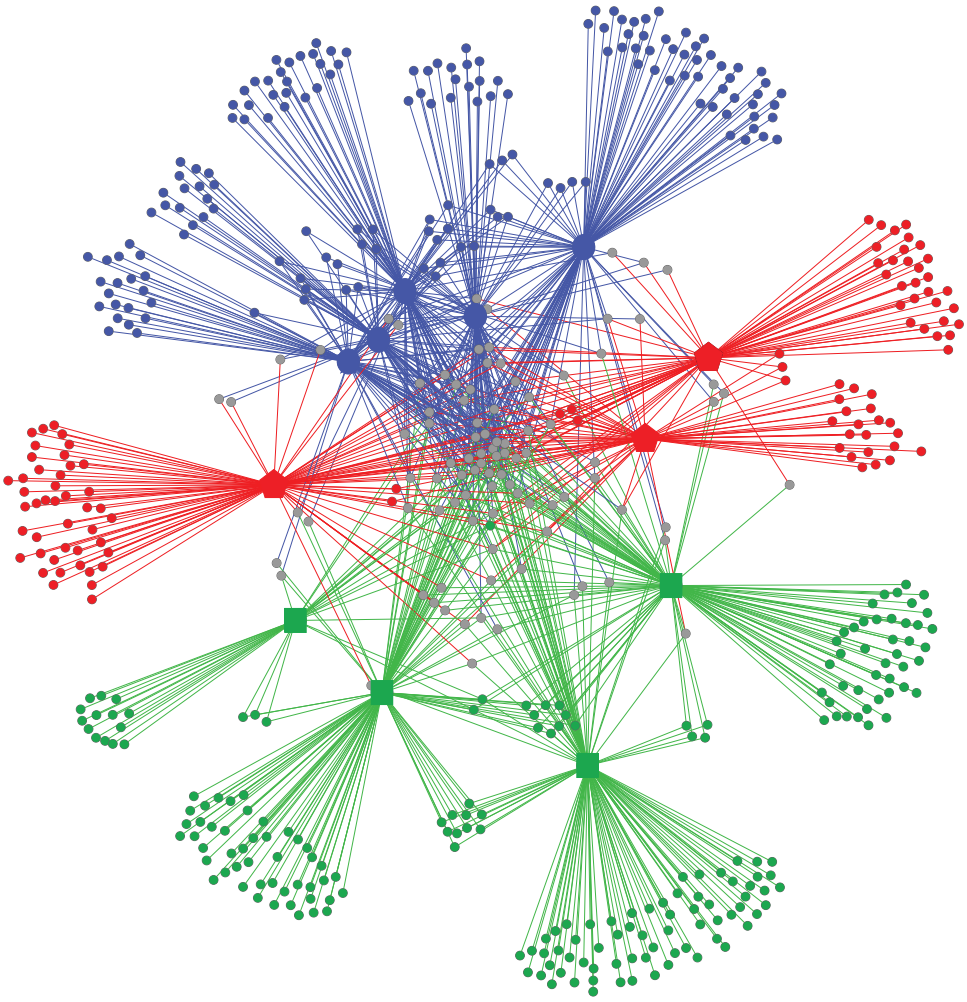

Fig. 4. Network of OTUs from bacterial communities of casts of the earthworm *Eisenia andrei* fed with pig (red pentagons), horse (blue circles) and cow manure (green squares). Small circles represent bacterial OTUs, which are colored attending to they are shared between samples of different manures (grey) or not (same color as samples they belong to). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Acinetobacter, *Pseudomonas*, *Brevundimonas* and *Kaistia*), Bacteroidetes (Flavobacteriaceae and *Chryseobacterium*), Actinobacteria (Actinomycetales and *Demequina*) and Firmicutes (*Caryophanon*). Biomarkers of casts from earthworms fed on cow manure belonged to the phyla Proteobacteria (*Pseudomonadaceae*, Rhodobacteraceae, *Rhodobacter* and *Amaricoccus*), Actinobacteria (*Nocardioides*) and Chloroflexi (Thermomicrobia); whereas biomarkers of cast from earthworms fed on pig manure belonged to the phyla *Firmicutes* (*Clostridiaceae_1*, *Clostridium_XI*, *Turicibacter* and *Clostridium sensu stricto*) and Actinobacteria (Actinomycetales and *Mycobacterium*) (Fig. 5).

4. Discussion

We have descriptively analyzed the bacterial communities in cast from the earthworm *E. andrei* fed on different types of manure. Our results indicate that the composition of bacterial communities in the casts differs widely depending on the food ingested by the earthworms (horse, cow and pig manures). This contradicts previous results by Gómez-Brandón et al. (2011b), where there were not differences in microbial community structure in casts of *E. andrei* fed with the same type of manures. In that study, transit through the gut of earthworms reduced the abundance of gram-positive bacteria more than it did gram-negative bacteria. We could not find differences due to the low resolution of PLFAs. Opposed to this, Koubová et al. (2015) detected differences in bacterial communities in casts of *E. andrei* obtained from composting and vermicomposting piles using PLFAs, but not when using DGGE. Gram-positive bacteria comprises bacterial phyla Firmicutes and Actinobacteria, whereas gram-negative bacteria includes most of the remaining phyla like Proteobacteria, Bacteroidetes, Planctomycetes, Chloroflexi and Acidobacteria. Previous studies involving the effects of epigeic earthworms on microorganisms have also shown that gram-negative bacteria can survive the transit through the earthworm gut (Hendriksen, 1995; Daane et al., 1997; Williams et al., 2006). The high resolution of 454 pyrosequencing technique could track differences in survival and/or viability of bacteria ingested, thus discriminating bacterial communities of casts. Our findings for the epigeic *E. andrei* are also consistent with those reported for other epigeic, endogeic and anecic earthworm species in soil ecosystems. Those studies have shown that bacterial communities of casts from the endogeic (*Aporrectodea caliginosa* and *Aporrectodea* longa) and anecic species (*Lumbricus terrestris* and *Lumbricus* friendi) are a subset of those found in soils (Egert et al., 2004; Thakuria et al., 2009). Moreover, as we have found here, changes in the composition of bacterial communities of soils and/or organic matter added to soils were mirrored by the bacterial communities found in the cast (Egert et al., 2004; Thakuria et al., 2009).

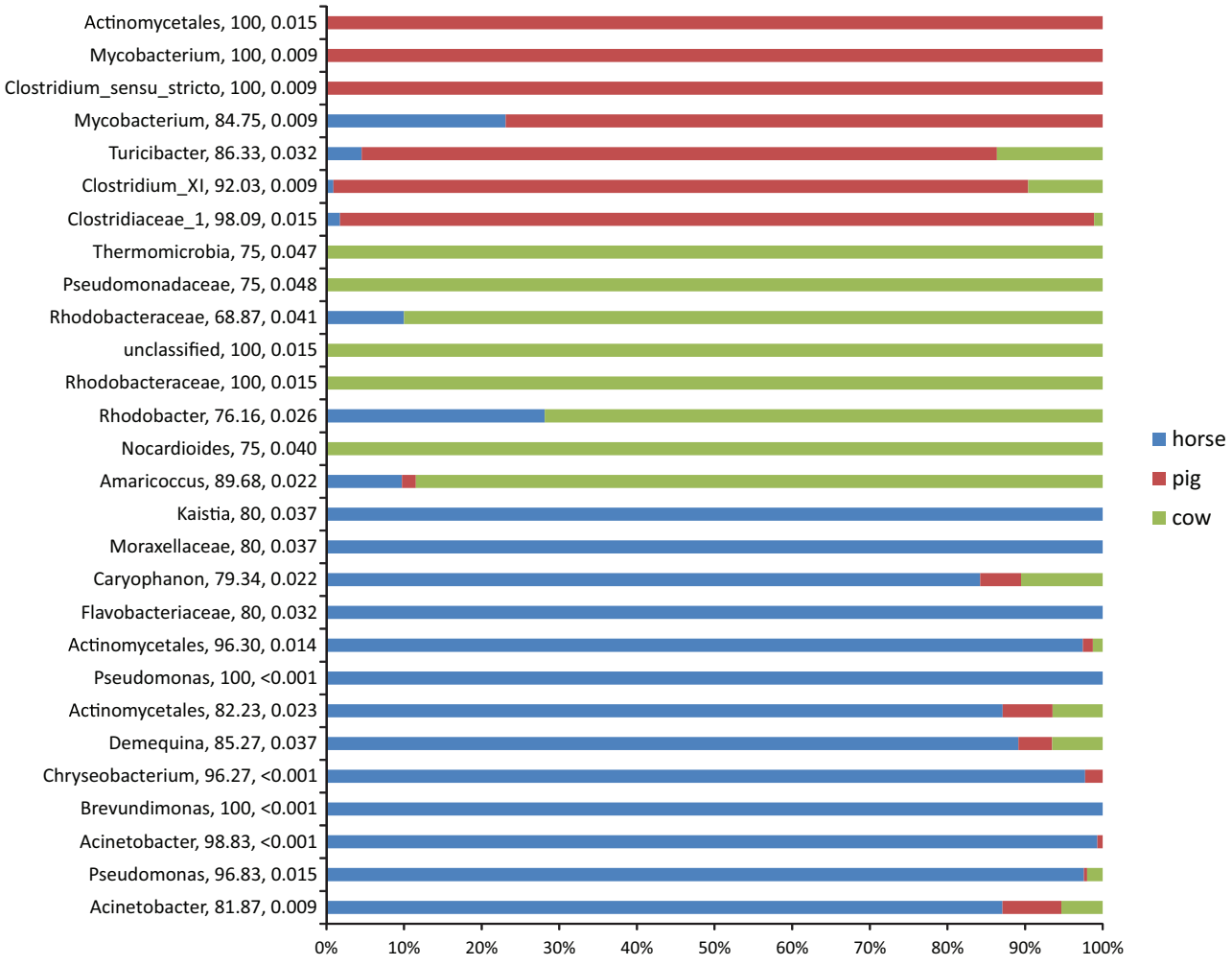

Fig. 5. Relative abundance of OTU biomarkers of bacterial communities from casts of the earthworm *Eisenia andrei* fed with pig, horse and cow manure. For each OTU we give the most accurate taxonomy found, and the indicator and associated *P*-values.

Previous studies have been shown that the microbial flora in the gut of the earthworm *E. fetida*, a species closely related (Domínguez et al., 2005Pérez-Losada et al., 2005), fed with cattle manure are dominated by *Entomoplasma somnilux*, *Bacillus licheniformis* with minor contributions of *Aeromonas*, *Bacillus*, *Clostridium*, *Ferrimonas*, *Photobacterium* and *Shewanella* (Hong et al., 2011). This bacterial taxa did not match with those we have found in *E. andrei* casts; this could be due not only to differences in earthworm diet but also on differences due to coevolution between earthworms and microbes, as it occurs for example in primates (Ochman et al., 2010). Other studies have revealed that the gut of *E. fetida* is composed by bacteria from phyla *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia*, *Firmicutes*, *Planctomycetes* and *Proteobacteria* in different proportions due to different ingested substrates (Vivas et al., 2009; Yasir et al., 2009; Rattray et al., 2010; Huang et al., 2014). The composition of the bacterial communities of casts from *E. andrei* is relatively similar to that of both the epigeic earthworm species such as *L. rubellus* (Knapp et al., 2009) and the anecic earthworm *L. terrestris* (Wüst et al., 2011). Thus, and according to our results, these studies have found that bacterial communities of casts from these two earthworm species have different proportions of OTUs belonging to phyla Proteobacteria, Firmicutes, Actinobacteria, Planctomycetes, Chloroflexi, Gemmatimonadetes, Bacteroidetes and Cytophagales among others. Although these differences in bacterial abundance and composition are related to earthworm diet, bacterial communities of casts from *L. rubellus* and *L. terretris* showed higher proportions of Proteobacteria (>30%), Actinobacteria, Acidobacteria, Bacteroidetes and Firmicutes (17–30%) than of any other bacterial phyla, which agrees with what we have found in *E. andrei* casts.

Bacterial communities in *E. andrei* cast showed the same level of α-diversity independently of the manure type. Since production of casts is the first step in the earthworm-microorganisms interactions and its microbial α-diversity is related to ecosystem function (Naeem et al., 2012), this has important consequences for organic matter decomposition. For example, decomposition rates are increased due to increases in bacterial diversity (Gómez-Brandón et al., 2011a). Once casts are egested, the decomposition is entirely controlled by microorganisms; given our results, we predict that this process should not be limited by the bacterial diversity of the starting material, at least for the three animal manures studied here. Interestingly, bacterial communities in casts strongly differ at taxonomic and phylogenetic levels, with both quantitative and qualitative indexes. Quantitative changes imply differences in the abundance of OTUs in the casts of *E. andrei* fed on the three animal manures. These changes may be related to temporal factors (Aira et al., 2005; Lozupone et al., 2007) such as variability of nutrients within casts among the three manures

(Gómez-Brandón et al., 2011b). On the other hand, qualitative changes imply that specific OTUs thrive exclusively in each of the casts analyzed, and that may be due to uneven microbial growth or different founder bacterial populations (Lozupone et al., 2007). Moreover, these differences still appear when phylogenetic information is incorporated, indicating that the bacterial communities derived from casts of E. andrei fed with the three manures are phylogenetically distant. This indicates that bacteria found in each manure have developed specific adaptations to proliferate/grow in the horse, cow and pig digestive systems, and that earthworm gut passage did not alter them. Moreover, we found several OTUs that function as indicators for each of these communities. Hence, the presence and abundance of these indicators would allow us to track casts from animal manures in a similar way as that proposed by Lores et al. (2006), but with greater taxonomic detail.

Casts are the first step in vermicomposting (Domínguez et al., 2010), so bacterial communities in the vermicompost should be a subset of the bacterial communities in the casts. According to this, previous studies have shown that vermicomposts are also characterized by different abundances and/or presence/absence of OTUs from phyla Firmicutes, Bacteroidetes, Acidobacteria, Actinobacteria, Planctomycetes, Gemmatimonadetes, Verrucomicrobia, Actinobacteria, Chloroflexi and Proteobacteria depending whether the raw materials are vegetable wastes (Huang et al., 2014), olive-mill wastes (Vivas et al., 2009), mixtures of paper sludge and dairy sludge (Yasir et al., 2009) or mixtures of straw and goat manure (Pathma and Sakthivel, 2013). Up to date, there are only two studies taking advantage of next-generation sequencing techniques to study bacterial communities of vermicompost (Neher et al., 2013; Romero-Tepal et al., 2014). In both cases, the studies were able to detect more than 10 bacterial phyla in the vermicomposts, revealing the high diversity of bacteria that controls the vermicomposting process. As we found in bacterial communities of casts, these studies have shown that Proteobacteria (>50%, both studies), Actinobacteria (15%; Romero-Tepal et al., 2014) and Firmicutes (23%; Neher et al., 2013) were the dominant bacterial phyla. Remarkably, both studies revealed that these two phyla reduced their dominance through time, both in the short time (28 days, Romero-Tepal et al., 2014) and in the long time (203 days, Neher et al., 2013), whereas the dominance of other phyla like Bacteroidetes, Actinobacteria or Verrucomicrobia was increased. This is reflected in changes in bacterial diversity, thus it has been reported that vermicomposts can have higher (Sen and Chandra, 2009; Huang et al., 2014; Neher et al., 2013; Romero-Tepal et al., 2014) or lower bacterial diversity (Vivas et al., 2009) than the raw initial materials. So, during the vermicomposting process we could expect that maturation of casts will lead to changes in the composition of bacterial communities according to the changes in quantity and quality of substrate nutrient pools.

5. Conclusion

We have descriptively analysed the bacterial communities of casts from the earthworm *E. andrei* and found that the bacterial community found in the cast depended on the animal manure ingested by the earthworm. Moreover, we found several OTUs that do function as indicators for each of these communities. Bacterial communities of cast showed the same level of α-diversity independently of the manure type, which is related with ecosystem function, in this case the rate of decomposition during the organic matter decomposition. Our results are important because bacterial communities of casts are able to alter decomposition rates once they are inoculated into raw substrates (Aira and Domínguez, 2011).

Acknowledgements

This study was supported by grants from the Ministerio de Economía y Competitividad (CTM2013-42540-R) and the Xunta de Galicia (CN2012/305)

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2015.10.002.

References

Aira, M., Domínguez, J., 2011. Earthworm effects without earthworms: inoculation of raw organic matter with worm-worked substrates alters microbial community functioning. PloS One 6, e16354.
Aira, M., Monroy, F., Domínguez, J., 2005. Ageing effects on nitrogen dynamics and enzyme activities in casts of *Aporrectodea caliginosa* (Lumbricidae). Pedobiologia 49, 467–473.
Aira, M., Monroy, F., Domínguez, J., 2006. *Eisenia fetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microb. Ecol. 52, 738–747.
Aira, M., Monroy, F., Domínguez, J., 2007a. Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application rates of pig slurry. Sci. Total Environ. 385, 252–261.
Aira, M., Monroy, F., Dominguez, J., 2007b. *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microb. Ecol. 54, 662–671.
Aira, M., Sampedro, L., Monroy, F., Domínguez, J., 2008. Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. Soil Biol. Biochem. 40, 2511–2516.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Gonzalez Pena, A., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Daane, L.L., Molina, J.A.E., Sadowsky, M.J., 1997. Plasmid transfer between spatially separated donor and recipient bacteria in earthworm-containing soil microcosms. Appl. Environ. Microbiol. 63, 679–686.
Domínguez, J., Ferreiro, A., Velando, A., 2005. Are *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Oligochaeta, Lumbricidae) different biological species? Pedobiologia 49, 81–87.
Domínguez, J., Aira, M., Gómez-Brandón, M., 2010. Vermicomposting: earthworms enhance the work of microbes. In: Insam, H., Franke-Whittle, I., Goberna, M. (Eds.), Microbes at Work: from Wastes to Resources. Springer, Berlin, Heidelberg, pp. 93–114.
Drake, H.L., Horn, M.A., 2007. As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. Annu. Rev. Microbiol. 61, 169–189.
Edwards, C.A., 2004. Earthworm Ecology, second ed. CRC Press, London.
Egert, M., Marhan, S., Wagner, B., Scheu, S., Friedrich, M.W., 2004. Molecular profiling of 16S rRNA genes reveals diet-related differences of microbial communities in soil, gut, and casts of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae). FEMS Microbiol. Ecol. 48, 187–197.
Furlong, M.A., Singleton, D.R., Coleman, D.C., Whitman, W.B., 2002. Molecular and culture-based analyses of prokaryotic communities from an agricultural soil and the burrows and casts of the earthworm *Lumbricus rubellus*. Appl. Environ. Microbiol. 68, 1265–1279.
Gómez-Brandón, M., Aira, M., Lores, M., Domínguez, J., 2011a. Changes in microbial community structure and function during vermicomposting of pig slurry. Bioresour. Technol. 102, 4171–4178.
Gómez-Brandón, M., Aira, M., Lores, M., Domínguez, J., 2011b. Epigeic earthworms exert a bottleneck effect on microbial communities through gut associated processes. PloS One 6, e24786.
Hendriksen, N.B., 1995. Effects of detritivore earthworms on dispersal and survival of the bacterium *Aeromonas hydrophilo*. Acta Zool. Fenn. 196, 115–119.
Hong, S.W., Kim, I.S., Lee, J.S., Chung, K.S., 2011. Culture-based and denaturing gradient gel electrophoresis analysis of the bacterial community structure from the intestinal tracts ofearthworms (*Eisenia fetida*). J. Microbiol. Biotechnol. 21, 885–892.
Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363.
Huang, K., Li, F., Wei, Y., Fu, X., Chen, X., 2014. Effects of earthworms on physicochemical properties and microbial profiles during vermicomposting of fresh fruit and vegetable wastes. Bioresour. Technol. 170, 45–52.
Knapp, B.A., Podmirseg, S.M., Seeber, J., Meyer, E., Insam, H., 2009. Diet-related composition of the gut microbiota of *Lumbricus rubellus* as revealed by a molecular fingerprinting technique and cloning. Soil Biol. Biochem. 41, 2299–2307.

Koubová, A., Chroňáková, A., Pižl, V., Sánchez-Monedero, M.A., Elhottová, D., 2015. The effects of earthworms *Eisenia* spp. on microbial community are habitat dependent. Eur. J. Soil Biol. 68, 42–55.
Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D., Knight, R., Gordon, J.I., 2008. Evolution of mammals and their gut microbes. Science 320, 1647–1651.
Lores, M., Gómez-Brandón, M., Pérez, D., Domínguez, J., 2006. Using FAME profiles for the characterization of animal wastes and vermicomposts. Soil Biol. Biochem. 38, 2993–2996.
Lozupone, C.A., Hamady, M., Kelley, S.T., Knight, R., 2007. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl. Environ. Microbiol. 73, 1576–1585.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8 (4), e61217.
Naeem, S., Emmett Duffy, J., Zavaleta, E., 2012. The functions of biological diversity in an age of extinction. Science 336, 1401–1406.
Neher, D.A., Weicht, T.R., Bates, S.T., Leff, J.W., Fierer, N., 2013. Changes in bacterial and fungal communities across compost recipes, preparation methods, and composting times. PLOS One 8, e79512.
Ochman, P., Worobey, M., Kuo, C., Ndjango, J., Peeters, M., Hahn, B., Hugenholtz, P., 2010. Evolutionary relationships of wild hominids recapitulated by gut microbial communities. PLoS Biol. 8, e1000546.
Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, H., Wagner, H., 2012. Vegan: community ecology package. R Package Version 2, 0–5.
Pérez-Losada, M., Eiroa, J., Mato, S., Domínguez, J., 2005. Phylogenetic species delimitation of the earthworms *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Oligochaeta, Lumbricidae) based on mitochondrial and nuclear DNA genes. Pedobiologia 49, 317–324.
Pathma, J., Sakthivel, N., 2013. Molecular and functional characterization of bacteria isolated from straw and goat manure based vermicompost. Appl. Soil Ecol. 70, 33–47.
Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS One 5, e9490.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria ISBN 3-900051-07-0, http://www.R-project.org/.
Rattray, R.M., Perumbakkam, S., Smith, F., Morrie Craig, A., 2010. Microbiomic comparison of the intestine of the earthworm *Eisenia fetida* fed ergovaline. Curr. Microbiol. 60, 229–235.
Romero-Tepal, E.L., Contreras-Blancas, E., Navarro-Noya, Y.E., Ruíz-Valdiviezo, V.M., Luna-Guido, M., Gutiérrez-Miceli, F.A., Dendooven, L., 2014. Changes in the bacterial community structure in stored wormbed leachate. J. Mol. Microb. Biotechnol. 24, 105–113.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microb. 75, 7537–7541.
Schloss, P.D., Gevers, D., Westcott, S.L., 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16s rRNA-based studies. PLoS One 6, e27310.
Sen, B., Chandra, T.S., 2009. Do earthworms affect dynamics of functional response and genetics structure of microbial community in a lab-scale composting system. Bioresour. Technol. 100, 804–811.
Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504.
Singleton, D.R., Hendrix, P.F., Coleman, D.C., Whitman, W.B., 2003. Identification of uncultured bacteria tightly associated with the intestine of the earthworm *Lumbricus rubellus* (Lumbricidae, Oligochaeta). Soil Biol. Biochem. 35, 1547–1555.
Sundquist, A., Bigdeli, S., Jalili, R., Druzin, M.L., Waller, S., Pullen, K.M., El-Sayed, Y.Y., Taslimi, M.M., Batzogloul, S., Ronaghi, M., 2007. Bacterial flora-typing with targeted, chip-based pyrosequencing. BMC Microbiol. 7, 108.
Thakuria, D., Schmidt, O., Finan, D., Egan, D., Doohan, F.M., 2009. Gut wall bacteria of earthworms: a natural selection process. ISME J. 4, 357–366.
Vivas, A., Moreno, B., García-Rodríguez, S., Benítez, E., 2009. Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. Bioresour. Technol. 100, 1319–1326.
Wüst, P.K., Horn, M.A., Drake, H.L., 2011. Clostridiaceae and Enterobacteriaceae as active fermenters in earthworm gut content. ISME J. 5, 92–106.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.
Williams, A.P., Roberts, P., Avery, L.M., Killham, K., Jones, D.L., 2006. Earthworms as vectors of *E. coli* O157:H7 in soil and vermicomposts. FEMS Microbiol. Ecol. 58, 54–64.
Yasir, M., Aslam, Z., Kim, S., Lee, S.-W., Jeon, C., Chung, R., 2009. Bacterial community composition and chitinase gene diversity of vermicompost with antifungal activity. Bioresour. Technol. 100, 4396–4403.