

Characterization of microbial populations associated with different organic fertilizers

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Abstract

Purpose Bacterial and fungal communities represent key bioindicators of soil quality and are essential for the maintenance of nutrient availability. Our purpose was to characterize the bacterial and fungal biodiversity associated with different compost mixtures, comparing microbiological indicators regarding the soil quality.

Methods Compost samples and mixtures were prepared (15:1 final ratio) with chemical fertilizer, animal manures (bovine, swine, and poultry) and green waste (such as tomato waste and leaves). Bacterial and fungal isolation and identification were performed through standard phenotypic methods. Statistical methods to assess differences between treatments included Shannon diversity, Chi-square tests, Bray-Curtis clustering, and Canonical Correspondence Analysis.

Results *Streptomyces* species, Gram-positive bacteria, were commonly found in differing abundances in all samples. Gram-positive species were predominant in soils amended with vegetable green waste while Gram-negative bacteria were more abundant in samples with chemical fertilizer. Fungal abundance increased in poultry manure and chemical fertilizers.

Conclusion Our findings suggested that organic matter recycling and composting resulted in the shifting of biodiversity in bacterial and fungal populations which might be associated with the availability of certain nutrient sources provided by the composting materials.

Keywords Agroecology, Composting, Animal manure, Substrate cycling

Introduction

Agriculture, forestry, and fishing represent important economic activities in Colombia, despite its decline trend to contribute to the Gross Domestic Product from 29.3 % in 1965 to 6.4 % in 2017 (World Bank national accounts data). However, the agricultural expansion still concerns regarding land degradation, slashed and burned areas, soil compaction, and livestock growth especially in the Amazon and Orinoco River Basin areas (Martínez and Zinck 2004; Lavelle et al. 2014).

While crop intensification is growing to supply the global demand for food production, soil degradation represents a future challenge in long-term sustainability. Hence, conventional farming entails the use of large amounts of chemical fertilizers which would have detrimental effects on soil fertility and water quality (Potter et al. 2010; Castanheira et al. 2014).

Alternatively, composting by using animal manures and vegetable residues represents a sustainable strategy for soil recovery and is considered a clean and safe practice for the agriculture and environment (Eghball et al. 2002; Qazi et al. 2009; Hartmann et al. 2015). Altogether, composting might avoid additional problems in agricultural practices such as chemical fertilizer overuse which might alter natural resources leading to environmental disturbance (Lupatini et al. 2016; Shennan et al. 2017).

In Colombia, many farmers recycle their organic green waste derived from intensive farming and animal production and use them as external compost amendments. Benefits of composting practices include the increase of organic matter and nutrients supply for soils as well as the increase of cation exchange

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capacity, enhancement of microbial biomass and reduction of soil pests (Escobar et al. 2012; Chaparro et al. 2012; Zhen et al. 2014). Between animal manures, poultry manure is commonly used in Colombia due to its high content of nutrients including calcium, nitrogen, potassium and phosphorus and a good mineralization rate (>70 %) (Eghball et al. 2002). When composted with *Azotobacter* sp., *Azospirillum* sp. and arbuscular mycorrhizal fungi has a beneficial effect on onion crops (Montenegro-Gomez et al. 2018). Poultry manure has also been recommended as an organic alternative during the fertilization stage of tomato crops (Bojacá et al. 2014).

Consistently, many environmental bacterial and fungal populations (including human and animal pathogens) have been specifically associated with animal manures and green wastes (Agnew and Leonard 2003; Partanen et al. 2010; Awasthi et al. 2018). Moreover, bacterial and fungal biodiversity can shift upon the addition of nutrient sources or compost amendments increasing soil health and plant fertility through a fine-tuning of the soil microbiome (Chaparro et al. 2012; Lupatini et al. 2016). Consequently, this information might be relevant for improving compost quality and consistently contribute to soil health (Cozzolino et al. 2016; Fierer 2017). In a previous study, we identified the microbial biomass associated to several compost sources and mixtures containing the essential or secondary macronutrients (Ca, Mg, Na, Fe, Mn, Cu, Zn, S, B, Si), macronutrients (N, P, K) and organic matter to refine quality parameters. Those studies revealed a high abundance of bacteria

belonging to *Pseudomonas*, *Bacillus*, *Arthrobacter*, and *Streptomyces*; and fungi taxa such as *Aspergillus* and *Penicillium* during organic fertilization of *Zea mays L.* and *Phaseolus vulgaris L.* (Escobar et al. 2012; Escobar et al. 2013a; Escobar and Solarte 2015). These ideas were addressed from the perspective of several homemade organic fertilizers and the possible changes in the soil microbiome (Pineda et al. 2017). Since there is a need to study the succession process of microorganisms in amended soils, our aim was to characterize the microbial population associated with different organic fertilizers.

Material and methods

Study area

The study was carried out in “La Esperanza” farm, in the rural area of Fusagasuga municipality (Cundinamarca, Colombia) at 04° 20' 38" N and 074° 22' 04" W, 1,720 m.a.s.l., 25 °C average temperature and 85 % relative humidity.

The study was conducted in six micro-composting units of 2.95 m in length and 0.65 m in width. Controls were organic fertilizer (poultry manure), soil with chemical fertilizer. Treatments were based on mixtures supplemented with three different animal manures (Table 1). The overall design consisted of three repetitions per unit and eighteen experimental units in total. Treatments included materials such as organic fertilizer (soil control), chemical fertilizer and compost mixtures described in a randomized controlled block.

Table 1 Component treatments of three mixtures used as composting substrates

Mixture	Ingredients
Mixture 1	Bovine manure, tomato plants (stems, leaves and remaining tomatoes) and home residues (eggshells, banana leaves and vegetable waste).
Mixture 2	Poultry manure, coffee residues and tomato residues (stems, leaves and remaining tomatoes).
Mixture 3	Swine manure, grass and citric residues (fruits and leaves of orange tree).
Chemical fertilizer	15% nitrogen (Ammoniacal nitrogen), 15% phosphorus (Phosphorus pentoxide) and 15% potassium (Potassium oxide).
Organic fertilizer	Dry poultry manure (Soil control).

Composting mixtures and fertilizers

Three mixtures presented in Table 1 were made by combining vegetable waste (mainly tomato, citrus, and green waste), animal manures (bovine, pig and poultry) and chemical fertilizer. Triple-15®, a chemical (inorganic) fertilizer composed of 15% nitrogen (Ammoniacal nitrogen), 15% phosphorus (Phosphorus

pentoxide) and 15% potassium (Potassium oxide). Mixtures were prepared using suitable solid waste proportions to reach a final ratio of Carbon/Nitrogen 15:1 as previously assessed (Escobar et al. 2013a).

Vegetal residues (leaves, stems) were cut to tiny fragments (< 1 cm) to simplify the decomposition process. The animal manure was collected fresh and spread over on a covered floor for drying at room

temperature for 3 days. Subsequently, the mixtures were made considering a humidity $\leq 40\%$. (Escobar et al. 2013a). All treatments were composted for 70 days indoors at room temperature without microbiological inoculum for promoting the natural decomposition by bacteria and fungi populations. The maturity time of compost was between 60-90 days, monitoring parameters such as temperature, texture, color, C/N ratio and humidity as described previously (Escobar et al. 2013b). The physicochemical properties of compost mixtures are not described in this study. Compost ingredients were selected based on the traditional agroecological practices of rural and farmer communities of promising agricultural productions from the Sumapaz region, Cundinamarca (Escobar and Solarte 2015).

Phenotypic characterization of bacterial and fungal communities

Identification and characterization of bacteria and fungi were carried out in the microbiology laboratory of Tolima University. Samples were taken before and after the addition of fertilizer following the Colombian Technical Standard (NTC 4491-2) regarding microbiological procedures. Bacteria were aseptically isolated from serial dilutions in non-selective media and fungi in PDA (Potato Dextrose Agar).

For each dilution, 100 μL of culture in triplicate were applied in three dilutions (10^{-4} , 10^{-5} and 10^{-6}) to obtain single Count Forming Unit (CFU) (Escobar et al. 2012). CFU were grown and incubated at 30 °C for 24 to 48 hours for bacteria and 25 °C for 48 to 72 hours for fungi.

Bacterial identification included macroscopic characterization of colonies, which followed criteria such as colony size (punctate, small, medium, and large), color, shape (regular, round, oval, irregular, and filamentous rhizoids), elevation (flat, raised, and convex pitching), edge (whole form, wavy, sawing, filamentous and curly), surface, smell (ammonia, fetid, sweet) and growth rate (fast, moderate and slow). Gram staining method was performed to differentiate Gram-negative and positive microorganisms. BBL crystal kit (BD Diagnostic Systems Europe Brand) was used for the identification of aerobic Gram-positive bacteria. This system includes twenty-nine dehydrated chromogenic substrates and fluorescent control. Samples were monitored to identify color changes and fluorescence resulting from microorganisms' metabolic activities.

Fermentation reactions detect the ability of an isolate to metabolize carbohydrates in the absence of atmospheric oxygen and oxidation reactions are based on the ability of an organism to metabolize the substrate with oxygen being the final electron acceptor. Both reactions are frequently detected by using a pH indicator on the substrate of the analysis. Chromogenic substrates undergoing hydrolysis produce color changes, which can be detected visually (Manafi et al. 1991; Kalita and Joshi 2017).

Fungi isolation and characterization were performed according to methodologies previously described (Arias and Piñeros 2008; Escobar and Solarte 2015). Taxonomic keys based on macroscopic and microscopic features and microbiological isolation were required to determine to genus level. Sampling was conducted with a method based on adhesive tape strips. A strip (4 cm) was folded and slipped over the surface to remove hyphal segments. Then, the strip containing the fungal segment was covered with lactophenol blue for further microscopic analysis (Winn et al. 2001; Urzi and De Leo 2001).

Statistical Methods

Data analysis was performed for initial soil control and for each treatment based on biodiversity indexes. Shannon diversity tests were assessed with Past program version 3.16 (Hammer et al. 2001). Differences between treatments were assessed with ANOSIM (p-value 0.0009), Chi-square and Bray-Curtis clustering included in R program (Version 9.5). The correlation between all mixtures and microbial biomass was carried out based on Pearson's correlation coefficients ($p < 0.05$). A linear relationship was assessed for the bacterial and fungal communities as variables per each compost mixture to conduct a Canonical Correspondence Analysis (CCA) combined with a Partial Least Squares (PLS) as described previously (Escobar and Solarte 2015).

Results and discussion

We characterized bacterial and fungal populations in compost mixtures and treatment combinations (Tables 5 and 6 in Appendix). To test data variation, the Chi-square test indicated a relationship between variables ($p\text{-value} < 0.05$) and correlation analysis of 72.8 %. Sample combinations with enhanced bacterial diversity were soil plus mixture 2 and 3; chemical fertilizer plus mixtures 1, 2 and 3 and organic fertilizer; organic

fertilizer plus mixture 2 and mixture 1 plus mixture 2 (Table 2). Similarity analyses through a dendrogram clustered in a single branch with all mixtures (treatments) together and chemical fertilizer sharing the highest bacterial diversity followed by the organic fertilizer samples and soil control (Fig. 1).

Remarkably, the compost combinations that were found to be the most diverse were: organic fertilizer with mixture 1; mixture 1 with mixture 3; mixture 2 with mixture 3 and mixture 1 with soil without amendment (Table 3).

Table 2 Compost mixtures containing animal manures, vegetable residues, fertilizers (organic and chemical) and soil samples

Combination		Shannon index (A)	Variance (A)	Shannon index (B)	Variance (B)	t	df*	p-value**
A	B							
Soil	Chemical fertilizer	2.36	0.02	1.97	0.04	1.71	24.21	0.10
Soil	Organic fertilizer	2.36	0.02	2.63	0.01	-1.65	52.27	0.11
Soil	Mixture 1	2.36	0.02	2.51	0.02	-0.83	53.65	0.41
Soil	Mixture 2	2.36	0.02	2.95	0.01	-3.75	51.00	0.00
Soil	Mixture 3	2.36	0.02	2.76	0.01	-2.37	56.03	0.02
Chemical fertilizer	Organic manure	1.97	0.04	2.63	0.01	-3.04	20.20	0.01
Chemical fertilizer	Mixture 1	1.97	0.04	2.51	0.02	-2.36	24.36	0.03
Chemical fertilizer	Mixture 2	1.97	0.04	2.95	0.01	-4.59	19.26	0.00
Chemical fertilizer	Mixture 3	1.97	0.04	2.76	0.01	-3.57	21.82	0.00
Organic fertilizer	Mixture 1	2.63	0.01	2.51	0.02	0.72	59.09	0.47
Organic fertilizer	Mixture 2	2.63	0.01	2.95	0.01	-2.39	85.01	0.02
Organic fertilizer	Mixture 3	2.63	0.01	2.76	0.01	-0.89	77.26	0.38
Mixture 1	Mixture 2	2.51	0.02	2.95	0.01	-2.81	58.34	0.01
Mixture 1	Mixture 3	2.51	0.02	2.76	0.01	-1.48	62.63	0.14
Mixture 2	Mixture 3	2.95	0.01	2.76	0.01	1.34	81.40	0.18

*df = degree freedom. ** Values lower than 0.05 indicate significant differences. Shannon's diversity indexes: <2= Less diverse, 2-3= Normal and >3 highly diverse

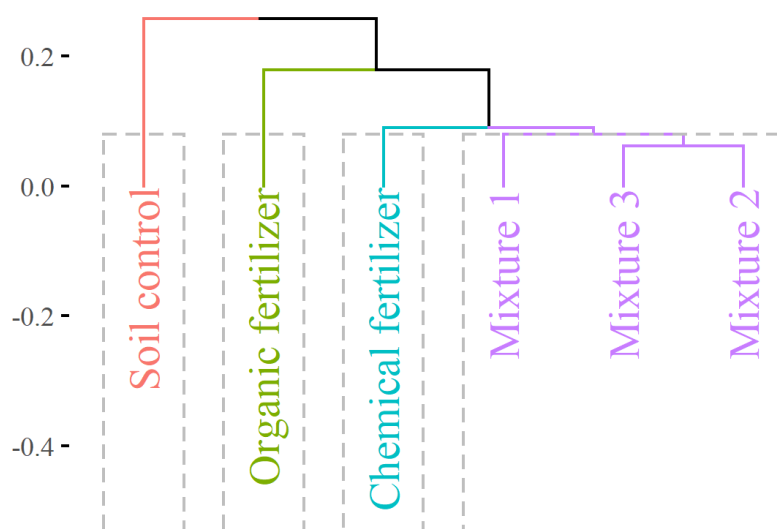


Fig. 1 Similarity dendrogram for compost samples. Similarity values are numbered across the branching clustering (right)

Table 3 Estimates of biodiversity between treatments and soil before amendments

	Chemical fertilizer	Organic fertilizer	Mixture A	Mixture B	Mixture C	Soil control
Chemical fertilizer		0.04	0.10	0.00	0.00	0.30
Organic fertilizer	0.04		0.61	0.02	0.27	0.30
Mixture 1	0.10	0.61		0.01	0.32	0.63
Mixture 2	0.00	0.02	0.01		0.34	0.00
Mixture 3	0.00	0.27	0.32	0.34		0.06
Soil control	0.30	0.30	0.63	0.00	0.06	

Composting of animal manures and vegetable residues have been described to increase yields in plant productivity compared to chemical fertilizers (Lupatini et al. 2016). In Colombia, some rural communities separate organic green waste (e.g. vegetable and fruit green waste) from garbage for composting them with animal manures and proper use as homemade organic fertilizers. In this study, we suggest that composting practices might contribute to improve and restore the soil health regarding biological indicators avoiding the chemical fertilizer overuse.

The role and ecological interactions between certain bacterial and fungal populations are pivotal in the composting process to enhance compost quality and to improve the soil microbiome (Devi et al. 2012; Zhen et al. 2014; Pineda et al. 2017). Species associated with different mixtures provided in this study are identical to microbial biomass identified in other compost recipes (Anastasi et al. 2005; Sánchez et al. 2017; Frac et al. 2018; Sahu et al. 2019). Some studies have shown that microbial communities play an important role as compost inoculants due to their ability to produce hydrolytic enzymes for accelerating the compost maturation and trigger the mineralization process (Chandna et al. 2013; Baćmaga et al. 2015; Awasthi et al. 2018).

Bacterial biodiversity in different compost formulations has been shown to increase beneficial gram-positive bacteria and fungi, and experimental evidence supports that bacterial communities seem to be more important for decomposition process than fungal species (Fries et al. 2005; Storey et al. 2015).

This hypothesis is supported by some studies suggesting that the bacterial density is always higher than the fungal density whatever the age of the compost and soil (Chennaoui et al. 2018). Moreover, microbial consortia formed by species of *Bacillus*, *Pseudomonas* and *Streptomyces* are key producers of antimicrobial secondary metabolites that arrest many co-existing phytopathogenic fungi and promote plant growth (Pathma et al. 2011; Santacoloma-Varón et al. 2017).

Bacterial diversity in compost mixtures was different between treatments supplemented with chemical fertilizer. Gram-positive bacteria were predominant in soils amended with vegetable green waste while Gram-negative species were more abundant in samples containing chemical fertilizer. To test the relationship of some bacterial groups with compost mixtures, we ran a CCA together with PLS. The bacterial communities in control soil samples were less diverse compared to mixtures with chemical fertilizer and composted with animal manure and vegetable residues. The predominant bacterial communities in the soil control sample were *Paucimonas*, *Escherichia* and *Pseudomonas*. On the other hand, dominant bacterial communities identified in the compost mixtures were *Morganella* in mixture 1; *Enterobacter* and *Nitrosomas* in mixture 2 and *Streptomyces* and *Arthrobacter* in mixture 3. Regarding organic and conventional compost, *Proteus* and *Bacillus* were more abundant in chemical fertilizer and *Staphylococcus*, *Nitrobacter* and *Micrococcus* in organic fertilizer (Fig. 2).

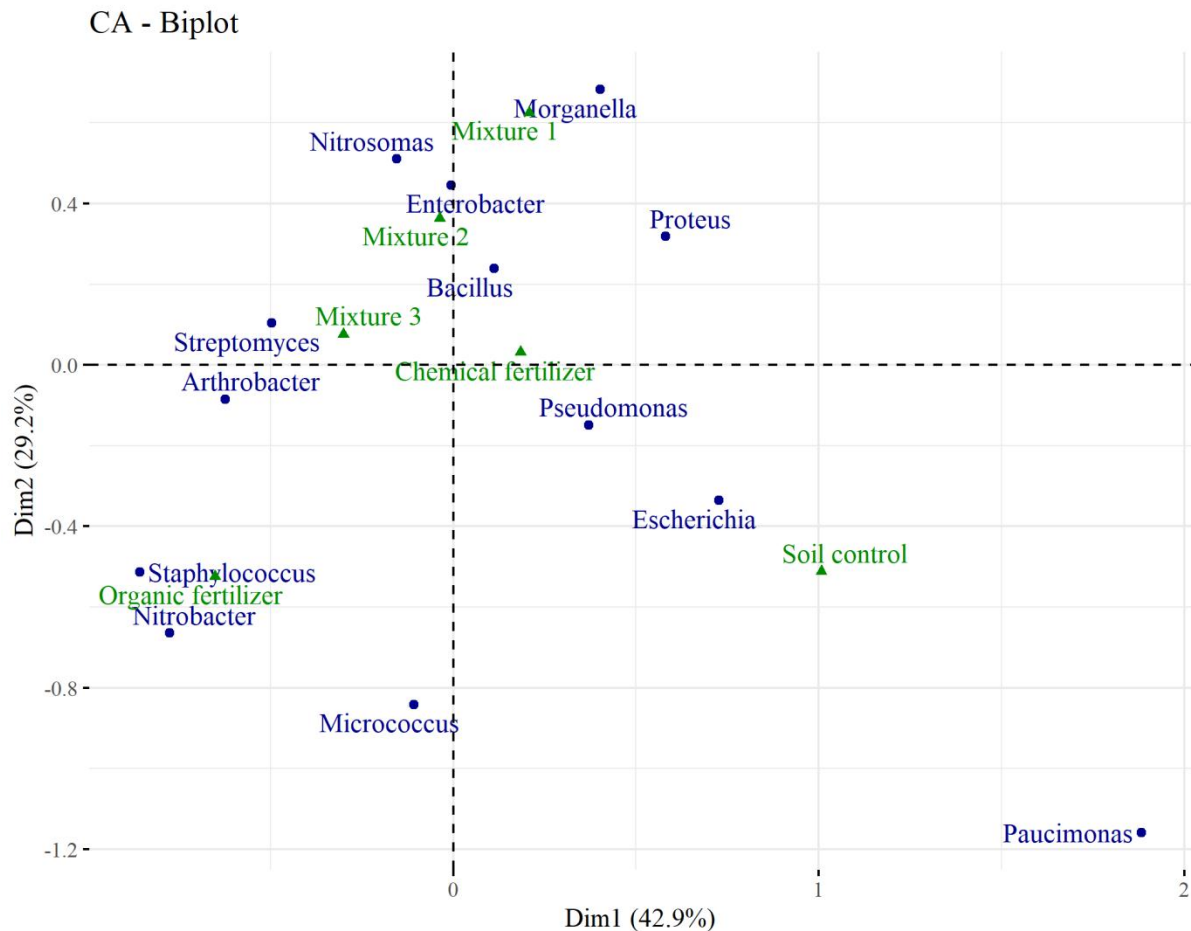


Fig. 2 Correspondence analysis of bacterial communities. Bacterial groups were colored in blue and substrates in green. Proximity in the map represents association to each substrate or mixture

Cluster analysis of fungal diversity showed some communities that were restricted to some compost samples as found in bacteria. Fungal diversity identified in mixture 1 was *Rhizopus*, *Sordaria*, and *Trichoderma*. For soil and mixture, 2 contained mainly *Aspergillus*. Mixture 3 showed *Nigrospora*, *Alternaria*, *Sistotrema*, and *Cladosporium* as dominant communities. The fertilizer sample presented *Moniliella* and *Thielavia*. Pearson's Chi-squared test showed that poultry manure had the lowest fungal diversity and the bacterial and fungal groups changed significantly upon composting amendment (Fig. 3). Soil control contained *Cephalophora*, *Macrosporium*, *Thysanophora* as fungal communities and *Penicillium*, *Humicola*, *Staphylotrichum* and *Zygorhynchus* in organic fertilizer.

Some bacterial species were more abundant upon certain manure and vegetable sources. First, compost mixtures containing bovine manure harbored *Proteus*

and *Morganella* and fungal genera such as *Rhizopus*, *Sordaria*, *Trichoderma*, and *Aspergillus*.

Second, bacterial specimens in poultry manure from *Enterobacter*, *Nitrosomonas*, *Bacillus* genera and fungal communities including *Cephalophora*, *Macrosporium*, *Thysanophora*, *Penicillium*, *Humicola* and *Zygorhynchus* were identified. Finally, bacterial diversity in swine manure was represented by *Streptomyces*, *Arthrobacter* and fungal specimens such as *Sistotrema* and *Alternaria* genera.

To describe the potential contribution from each bacterial and fungal population in compost mixtures, we compared their expected roles in the soil (Table 4). Positive effects of both compost mixtures and fertilization (organic and chemical) have been reported for both bacterial and fungal species. In consensus, it can be assumed that the use of animal manure and green waste treated-soils harbored certain bacterial species that promote biocontrol, nitrogen fixation and control of soil pests.

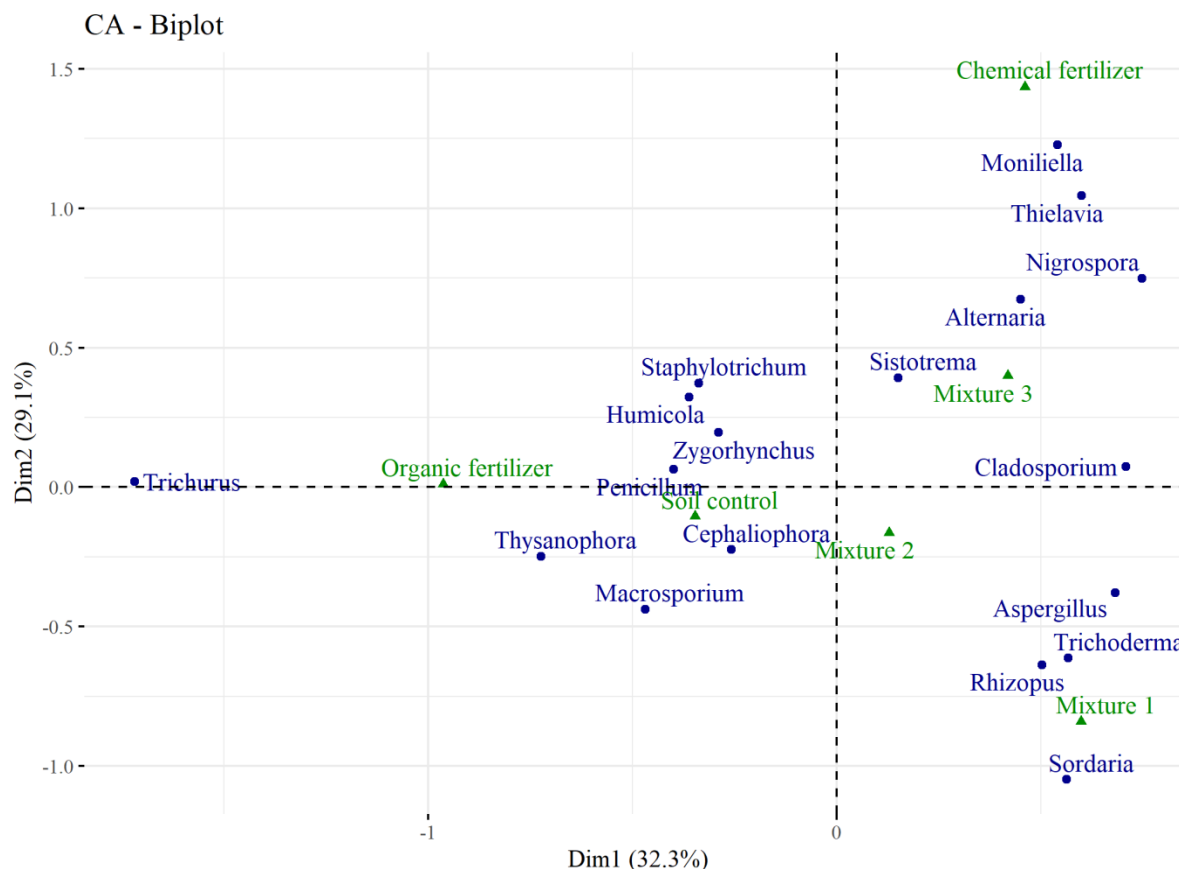


Fig. 3 Correspondence analysis for fungal groups identified in soil and compost samples. Proximity between fungal groups and samples represent their association. Fungal groups were colored in blue and substrates in green

Table 4 The potential biological contribution to the soil of bacterial and fungal species found in this study according to earlier reports (Anastasi et al. 2005; Sánchez et al. 2017; Fraç et al. 2018; Sahu et al. 2019)

Sample	Bacteria	Expected role	Fungi	Expected role
Control	<i>Escherichia</i> , <i>Paucimonas</i>	Plant growth and nutrient uptake, bioremediation	<i>Penicillium</i> , <i>Zygorhynchus</i> , <i>Cephalophora</i> , <i>Thysanophora</i> , <i>Macrosporium</i>	Antibiotic production. Bioremediation. Phosphorus mobilization.
Mixture 1	<i>Morganella</i> , <i>Nitrosomonas</i>	Indicator of Nitrogen rich soils. Insecticide degradation. Nitrifying bacteria.	<i>Sordaria</i> , <i>Rhizopus</i> , <i>Trichoderma</i>	Reduction in soils affected by drought. Bio-stimulants and biocontrol agents
Mixture 2	<i>Bacillus</i> , <i>Enterobacter</i>	Biocontrol of <i>Colletotrichum fulcatum</i> and <i>Macrophomina</i> sp. Nitrate reduction.	<i>Aspergillus</i>	Recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers.
Mixture 3	<i>Streptomyces</i> , <i>Arthrobacter</i>	Disease suppressive soils	<i>Sistotrema</i> , <i>Alternaria</i> , <i>Nigrospora</i> , <i>Cladosporium</i>	Mycorrhizal fungi associated to nutrient uptake for plants. Phytopathogens.
Organic fertilizer	<i>Staphylococcus</i> , <i>Nitrobacter</i> , <i>Micrococcus</i>	Oxidation of organic nitrogen	<i>Trichurus</i>	Bioremediation
Chemical fertilizer	<i>Pseudomonas</i>	Promote fermentation and mineralization of nutrients and biocontrol	<i>Moniliella</i> , <i>Thielavia</i>	Lead tolerant species. Biodegradation

High frequency of some Gram-negative bacteria such as *Escherichia* and *Pseudomonas* in the soil before adding compost sources was identified. Nevertheless, another report has explained the abundance of Gram-positive in sample soils due to their ability to persist via endospore formation which survive by degrading specific sources either vegetable green waste, or animal manures (Fries et al. 2005; Pan et al. 2012). Those findings are consistent with our clustering patterns across the bacterial communities which might indicate a diversification related to the carbon and nitrogen availability (Fierer 2017; Meng et al. 2019).

The most diverse compost samples were those mixtures containing several carbon and nitrogen sources such as poultry manure. Thus, our results are consistent with mineral and nutrient contribution associated with specific abundance of certain bacterial and fungal species (Eghball et al. 2002; Yanagi and Shindo 2016). Some mixtures containing certain green waste products promoted specific bacterial communities as estimated with our biodiversity indexes. The ecological succession occurs during compost maturation due to factors such as: mineral and nutrient availability, environmental conditions, physicochemical properties, and bacterial and fungal competition (Neher et al. 2013; Tiquia and Tam 2000). Besides, animal manures contain not only beneficial microorganisms, but also essential minerals and organic material resulting from the animal metabolism (Fischer and Glaser 2012; Montenegro-Gomez et al. 2018). Thus, mineral content in animal manures from Colombia revealed that bovine manure was rich in C source because of its fibrous forage content; poultry and swine manure had a higher content of macronutrients, secondary nutrients such as Ca and Mg, and micronutrients including B, Zn, Cu, Mn and Fe which might explain their higher bacterial diversity (Escobar and Solarte 2015).

The addition of specific C and N sources together with chemical fertilizers has been reported to selectively favor particular bacterial groups for the uptake of available nutrients (Souza et al. 2014; Nigussie et al. 2015). However, depending on compost mixtures detrimental effects for plant productivity might result from pathogen proliferation which affects the efficiency of symbiotic relations between soil microorganisms and plants (Cozzolino et al. 2016).

Hence, bioremediation was another role for microbial biomass that might alleviate pollutants in

the soil. Some of the fungal communities found (e.g. *Rhizopus* sp., *Penicillium* sp., *Aspergillus* sp.) have been proven to perform a key role in the removal of pollutants from the soil and contribution in alleviating phosphorus fraction in soils evidencing adaptability to harsh conditions (Srivastava et al. 2011; Gaiad 2014). The results showed the bacterial and fungal enrichment through different compost mixtures might improve the soil microbiome as reported in organic farming systems compared to conventional farming systems (Hepperly et al. 2009; Hartmann et al. 2015; Lupatini et al. 2016; Finkel et al. 2017). In a similar study, Chinese mollisols only reached a minor recovery attributable to soil fungal diversity impairment upon the addition of chemical fertilizers combined with animal manures (Ding et al. 2017).

Our results also suggest that poultry manure showed a positive effect in the enrichment of certain bacterial communities such as Gram-negative bacteria upon different nutrient composition which was statistically supported by similarity analysis (Escobar et al. 2012). Interestingly, *Streptomyces* and *Arthrobacter* species have been determined as being beneficial in suppressing tobacco bacterial wilt (Wu et al. 2014). Particularly, *Arthrobacter* species are well-known for producing substances that positively promote plant growth and development (Pathma et al. 2011). Altogether, microbial abundance might serve as indicators of soil status regarding physicochemical properties and health (Trivedi et al. 2016). Regarding swine manure, Firmicutes and Proteobacteria have been reported as the most abundant phyla with significant shifts to anaerobic populations during maturation (Ma et al. 2018). Furthermore, bovine manure has been shown to be useful through the increase of aerobic microbiome which promotes beneficial plant-interactions in avocado (Ramírez-Gil et al. 2013).

A final consideration, it is important to consider the collateral risk of composting manure from animals exposed to intensive antibiotic pressure due to the enrichment of antibiotic resistance genes (ARGs) in the soil and environment (Gillings et al. 2015; Chen et al. 2016; Xie et al. 2018). Hence, composting with excessive amounts of those manures might increase ARGs, decrease soil microbial activity, and impair biogeochemical cycles in soil microenvironments (Lin et al. 2016; Kuppasamy et al. 2018). This situation also entails actions to promote organic production and training in antibiotic

stewardship during animal husbandry before using manure as a compost source.

Conclusion

Our result showed that diversification of microbial populations occurs between compost mixtures and upon soil amendments (organic residues and chemical fertilizers) in comparison with soil control. Mixtures which seem to be more efficient are those including soil bacteria and fungi species from nitrogen-fixing genera and decomposer communities. Additionally, compost combinations were more diverse and abundant when combined with different sources (including chemical fertilizer) as seen in the clustering pattern and CCA. Thus, our results showed that Gram-positive species were commonly found in mixtures supplemented with vegetable residues while Gram-negative bacteria and fungal populations were more abundant in samples with chemical fertilizer. Further studies are needed to evaluate the best formula to

promote beneficial plant-microbe interactions and to a better understanding of the behavior of beneficial microbial populations.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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Appendix

Table 5 Count of bacterial taxa in each compost mixtures and treatment combinations. Shannon index was calculated based in the relative abundance of individuals according to colony count obtained in the single CFU (dilutions 10^{-4} , 10^{-5} and 10^{-6}) and each taxa identified per treatment. Taxa identification was performed at genus level

Treatments	No. taxa	Fungi taxa
Mixture 1	7	<i>Alternaria, Aspergillus, Cladosporium, Rhizopus, Staphylotrichum, Sordaria, Trichoderma</i>
Mixture 2	11	<i>Alternaria, Aspergillus, Cephalophora, Cladosporium, Humicola, Macrosporium, Penicillium, Rhizopus, Sordaria, Thysanophora, Zygorhynchus</i>
Mixture 3	7	<i>Aspergillus, Alternaria, Cladosporium, Penicillium, Nigrospora, Rhizopus, Sistotrema</i>
Organic fertilizer	7	<i>Cephalophora, Humicola, Macrosporium, Penicillium, Staphylotrichum, Thysanophora, Zygorhynchus</i>
Chemical fertilizer	4	<i>Alternaria, Moniliella, Thielavia, Trichurus</i>

Table 6 Count of fungi taxa in each compost mixtures and treatment combinations. Shannon indexes were determined as described with bacterial taxa

Treatments	No. taxa	Bacterial taxa
Mixture 1	10	<i>Actinobacteria, Arthrobacter, Bacillus, Enterobacter, Morganella, Nitrobacter, Proteus, Pseudomonas, Staphylococcus, Streptomyces</i>
Mixture 2	10	<i>Actinobacteria, Arthrobacter, Bacillus, Enterobacter, Micrococcus, Nitrosomas, Nitrobacter, Pseudomonas, Staphylococcus, Streptomyces</i>
Mixture 3	9	<i>Actinobacteria, Arthrobacter, Bacillus, Enterobacter, Micrococcus, Nitrobacter, Pseudomonas, Staphylococcus, Streptomyces</i>
Organic fertilizer	7	<i>Actinobacteria, Bacillus, Enterobacter, Escherichia, Paucimonas, Pseudomonas, Streptomyces</i>
Chemical fertilizer	5	<i>Actinobacteria, Bacillus, Escherichia, Nitrosomonas, Streptomyces</i>

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