Bacterial community dynamics in tropical soil after sewage sludge amendment

Bruna C. Lopes, Raíssa S. Figueiredo, Juliana C. Araújo and Antonio T. Matos

ABSTRACT

Although the widespread use of sewage sludge in developing countries is common, little is known about how sludge disposal can affect the microbial composition and diversity of tropical soils. We evaluated the effects of the sewage sludges of two types of anaerobic digestors differing, by the biological treatment they have undergone (uplow anaerobic sludge blanket and activated sludge digester), and two different disposal methods (surface and incorporated) on tropical soils. Samples were taken from topsoil (0–10 cm) and analyzed by amplifying the 16S rRNA genes to study the microbial community, and physicochemical analysis was performed concomitantly. The results indicated that, in general, sewage sludge amendment (SSA) significantly changed the tropical soil bacterial community by the sludge type and by application method. Moreover, the redundancy analysis diagram indicates that changes in soil chemical parameters over time due to SSA resulted in changes in the bacterial community's composition, increasing the population responsible for recycling nutrients in the soil.

Key words | bacterial community, sewage sludge, sludge disposal, soil amendments, tropical soils

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HIGHLIGHTS

- The impact of different sludges and application methods on tropical soils was evaluated.
- The sewage sludge amendment reduced the bacterial community diversity in general.
- The bacterial community responsible for cycling of nutrients and organic matter decomposition was enhanced.

INTRODUCTION

Sewage sludge is generated during the management of wastewater biological treatment. Due to the expansion of basic sanitation and improved sewage treatment processes, it is expected that sludge production will increase gradually, making it a challenge for sanitation companies to allocate this waste adequately. Land application of sewage sludge can provide organic matter and nutrients that confer agricultural benefits (Suhadolc *et al.* 2010), especially for tropical soils. The biosolid disposal in the ground is a promising alternative in low-income countries, such as Brazil, where it is necessary to replenish the soil organic matter stock because it is a highly weathered soil, provided by the climatic conditions, and with relatively low availability of nutrients (Matos 2014). This practice improves soil structure, drainage, and available water capacity and increases plant-available and soil-extractable nutrients of significant value to agriculture. However, this material constitutes a highly heterogeneous matrix containing many pollutants, such as heavy metals (Börjesson *et al.* 2011), and harmful pathogens (Mattana *et al.* 2014).

For safe use in agriculture as a soil conditioner in Brazil, biosolids must comply with mandatory limits detailed by

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CONAMA 498 (Brazil 2020), such as heavy metal content and stabilization requirements. The upflow anaerobic sludge blanket (UASB) reactor is one of the most widely used processes for sewage treatment in low-income countries and produces a stabilized sludge. On the other hand, activated sludge is commonly used in developing countries and require anaerobic digestion for post-treatment to stabilize the aerobic sludge generated. The application methods of sewage sludge in agricultural land depend mainly on the purpose and nature of the cultivation. Usually, the application methods consist of spreading the sewage sludge on the ground's surface only or, after the surface spreading, using a tractor to disk-incorporate the biosolid in the field. For example, corn crops are harvested by removing all the plant's aerial parts from the soil; then, the biosolid incorporation after soil spreading is desirable. According to a previous study (Matos 2014), the soil/fertilizer incorporation minimizes nitrogen mineral losses due to evaporation and leaching.

It is well established that many soil microorganisms are responsible for fundamental transformations in the biogeochemical cycles of nitrogen, phosphorus, sulfur, and carbon. Different methods are used to verify the sewage sludge amendment (SSA) consequences in soil microbial diversity structure, such as phospholipid fatty acids analysis (Börjesson *et al.* 2011), PCR-DGGE (polymerase chain reaction with denaturing gradient gel electrophoresis) (Mattana *et al.* 2014), and bacterial 16S ribosomal RNA gene amplification (Mendes *et al.* 2015; Lloret *et al.* 2016). The last one completely changed how the scientific community has conceived microbial ecology experiments, being considered the ideal marker for microbial community diversity characterization. Fewer studies have evaluated the impact of biosolids amendments on microbial communities by analyzing the sequencing of the 16S rRNA gene, and showed different results on microbial diversity, impacting negatively (Pedrinho *et al.* 2009) or positively (Mendes *et al.* 2015) depending on the physicochemical properties of the soils (Mhete *et al.* 2020). To our knowledge, only one study (Pedrinho *et al.* 2009) evaluated the effects of biosolid's impact on bacterial communities in tropical soils in Brazil. However, this study focused on describing SSA's diversity with sludge and soil control, without considering the changes in microbial diversity in time, application methods, or sludge type.

Specific studies in tropical climate regions must better understand particular aspects of the microbial community changes according to the type of sludge used in the SSA. Due to the high diversity of uncharacterized tropical soil, the microbial community's response in the soil after the SSA is still considered a 'dark matter'. To address these questions, we elaborate in this research a comparative study to evaluate two different sludge types (UASB and mesophilic anaerobic digestor) and two different sludge application methods (surface and incorporated). To support the discussion, we included physicochemical soil parameters.

METHODS

Experimental design and soil management

The experiment was carried out under field condition with a soil collected in Federal University of Minas Gerais (UFMG) in Belo Horizonte-MG, Brazil (19° 51′ 46″ S; 43° 57′ 58″ W) and transported to the Centre for Research and Training in Sanitation (CePTS) of the Department of Sanitary and Environmental Engineering, located in Wastewater Treatment Plant of Arrudas River (19° 53′ 43″ S; 43° 52′ 44″ W). The research site is characterized as a subtropical climate (Cwa), according to Köppen and Geiger classification (Júnior *et al.* 2012). The mean annual temperature is 20.5 °C, and the mean annual precipitation is 1,430 mm, with the most precipitation occurring from September to March.

Nearly level soils characterize the landscape with 0–1% slopes. The soil was classified as Oxisol (Red-Yellow Latosol) according to Brazilian Agricultural Research Corporation (EMBRAPA) guidelines methodology (EMBRAPA 2017) and classified as sandy clay according to the United States Department of Agriculture (USDA 1993) (Table S1, Supplementary material). This choice of soil class is associated with the fact that it is typical of sizeable agricultural exploration areas and widely distributed in Brazil. Thirteen plots of 2.0 m^2 each were constructed side by side and delimited by polypropylene. The system consisted of three plots each of incorporated anaerobic digester sludge (ASD) (ASD-I), superficial ASD (ASD-S), incorporated UASB sludge (URS) (URS-I), superficial URS (URS-S), and one plot without sludge (soil control). The incorporation method was done by mixing manually with the soil surface layer (0–10 cm) after the sludge spreading. The amount of sludge corresponded to a nitrogen application rate of 387 kg TN·ha^{-1} for ASD and 250 kg TN·ha^{-1} for URS.

Sewage sludge characterization

The two sludge types were obtained from a full-scale municipal wastewater treatment plant (WWTP) with a capacity of 4.5 $m^3 \cdot s^{-1}$ and which treats the domestic

Table 1 | Physicochemical characteristics of AS and UASB (values on a dry weight basis)

Parameters	AS	UASB	Brazilian limits[b]
pH	6.9 ± 0.2	7.5 ± 0.1	
EC[a] (dS·m^{-1} 25 °C)	20.2 ± 2.2	23.8 ± 1.9	
MC (g·100 g^{-1})	78.2 ± 0.9	74.8 ± 1.1	
TS (g·100 g^{-1})	21.8 ± 0.9	25.2 ± 1.1	
TVS (g·100 g^{-1})	60.2 ± 0.9	61.5 ± 1.4	
OC (g·100 g^{-1})	15.1 ± 2.5	19.3 ± 1.6	
TN (g·100 g^{-1})	1.8 ± 1.3	2.0 ± 0.1	
NH_4^+ (g·kg^{-1})	1.7 ± 0.2	12.1 ± 0.4	
NO_3^- (g·kg^{-1})	14.0 ± 0.6	1.5 ± 0.1	
ON (g·kg^{-1})	2.1 ± 0.2	6.2 ± 0.5	
Total P (%)	3.0 ± 0.2	1.2 ± 0.3	
Total Cd (mg·kg^{-1})	2.2 ± 0.3	2.9 ± 0.2	39–85
Total Pb (mg·kg^{-1})	100.4 ± 1.4	95.4 ± 0.8	300–840
Total Cu (mg·kg^{-1})	334.9 ± 4.7	394.3 ± 6.2	1,500–4,300
Total Zn (mg·kg^{-1})	2,424.6 ± 22.6	2,537.5 ± 31.0	2,800–7,500

[a]EC: electrical conductivity; MC: moisture content; TS: total solids; TVS: total volatile solids; OC: organic carbon; TN: total nitrogen; NH_4^+: ammonium; NO_3^-: nitrate; ON: organic nitrogen; P: phosphorus; Cd: cadmium; Pb: lead; Cu: copper; Zn: zinc.
[b]Limits for soil sludge amendment (Brazil 2020).

wastewater produced by 1.6 million inhabitants using the activated sludge process. Inside this WWTP, the CePTS of the Department of Sanitary and Environmental Engineering, UFMG, is located. CePTS contains several demonstration and pilot-scale wastewater treatment units, including a UASB reactor used to treat raw sewage. The UASB is only used for research purposes and treats sewage equivalent to that produced by 640 inhabitants (80 $m^3 \cdot d^{-1}$). This research used the mesophilic anaerobic digester sludge (ASD), the activated sludge conventional system, and the UASB reactor. ASD was collected after the centrifugation process, and URS was collected after 10 days of dewatering in the air-drying bed. Both sludges were collected and characterized before the sludge soil amendment in triplicate (Table 1).

Soil sampling

Soil samples were collected in May 2018, after the sludge amendment and corresponding to day 0 (T0), and in August 2018 corresponding to day 90 (T90). One composite soil sample was taken from 0–10 cm depth with a trier according to instructions (US EPA 1997), by mixing six random locations in each plot at the experiment's three field replications. Sterilized bags were used to mix 250–300 g of combined soil and sludge. The material was transported at 4 °C for laboratory analysis.

Selected soil and sludge properties

Soil pH and electrical conductivity (EC) were measured in $CaCl_2$ extract (1:5 w/v). Moisture content was measured by drying the sample at 65 °C for 24 hours. Total volatile solids were measured according to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2002). EMBRAPA guidelines were used for soil analysis with the following respective methods: total P, Zn and Cu content was quantified after acid digestion (US EPA 1996); organic carbon was determined by wet oxidation-redox titration (Walkley-Black) method; total nitrogen (TN), nitrate (NO_3^-) and ammonium (NH_4^+) were quantified according to Bremner and Mulvaney method (EMBRAPA 2017). Organic nitrogen was calculated by subtracting the sum of NO_3^- and NH_4^+ from the TN value.

Sludge and soil DNA extraction

Sludge (ASD and URS) and soil samples were stored at −70 °C in falcon tubes until DNA extraction. As described above, the samples collected from the plots submitted to the same treatment were mixed and homogenized to obtain a single sample. The DNA extraction was performed with 0.5 g of the sample as recommended by the FastDNA SPIN Kit for Soil manufacturer (MPBiomedicals, France). Extracted DNA was electrophoresed on 1.5% agarose gels and quantified with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific®, Wilmington, DE, USA).

Sequencing library construction and analysis of sequencing data

To investigate the microbial community changes in SSA, we carried out Illumina sequencing of the V3–V4 region of the 16S ribosomal RNA (rRNA) prepared according to the Illumina MiSeq system instructions. Briefly, the V3–V4 region of the 16S bacterial rRNA gene, which includes Archaea and Bacteria domains, was amplified using PCR with universal primers 515F and 926R. The primer sequence is described in the 16S Metagenomic Sequencing Library Preparation protocol (Illumina 2019). Samples were assessed by high-throughput amplicon sequencing of 2 × 250 bp on the Illumina® MiSeq® platform (Illumina, San Diego, CA, USA). Sequence data were processed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA).

The sequences were briefly filtered for primer removal, sequences less than 150 bp, and ambiguous sequences. Sequences with a similarity of ≥97% were then assigned to the same operational taxonomic unit (OTU). After clustering, the sequences were aligned and taxonomically classified using the BLASTn database against a curated database derived from RDPII and NCBI.

Diversity, richness and statistical analysis

Alpha diversity indices (Chao1, Shannon, and Simpson) and the Bray–Curtis beta diversity index were calculated in MicrobiomeAnalyst (Chong *et al.* 2020). Redundancy component analysis (RDA) was performed to compare the microbial community with the soil's physical and chemical variables after the SSA using Microsoft Excel™ and XLSTAT package. We tested the impact of time and application method by the Mann–Whitney test, with 95% confidence.

RESULTS AND DISCUSSION

Overview of the Illumina dataset and bacterial community structures

Sequencing of the 16S rRNA gene was used to evaluate the microbial composition and diversity in sludge, soil, and sludge (ASD and URS) amended soils from two sampling events. A total of 914,657 high-quality reads and 11,777 OTUs were obtained from 11 samples (Table 2). Compared to the control soil, the SSA decreased OTUs by 18.8 and 16.8% in ASD and URS, respectively. The community richness index (Chao1) and diversity indices (Shannon and Simpson) indices also reflected the negative impact of SSA procedures.

In this study, comparing the control soil sample and SSA samples, the sludge amendment showed a trend to reduce microorganisms' diversity. These results agree with previous studies (Pedrinho *et al.* 2009; Hu *et al.* 2019). However, the impact on the diversity of SSA is still imprecise. Different studies (Chen *et al.* 2016; Liu *et al.* 2017; Bai *et al.* 2019) reported the increase of diversity of microbial communities in the soil after the sludge amendment; meanwhile, Urra *et al.* (2019) verified that soil microbial diversity was not significantly affected by the anaerobic sludge application. According to van Elsas (2019), soils are highly variable and dynamic in time and space, and this characteristic could justify the different soil responses after the sludge amendment.

The bacterial community changed over 90 days of treatment according to the sludge type used in the soil amendment. Comparing samples collected in day 0 and day 90 from both applications evaluated, the URS superficial method decreased the OTUs values by 4%, and the incorporation method increased the OTUs values by 8%. Meanwhile, the ASD applications increased the OTUs values by 18 and 15% for superficial and incorporation methods, respectively. Despite this increase, the 90 days was not sufficient for SSA to reach the soil richness and diversity index of the control.

To better understand how the OTU clusters overlap within the treatment evaluated, Venn diagrams were applied

Table 2 | Estimation of the richness and diversity of 16S rRNA sequencing libraries from Illumina sequencing analysis

Samples	No. of sequences	No. of normalized sequences	OTUs	Chao1	Shannon	Simpson
AS	70,988	55,673	2,904	2,825.03	5.20	0.977
URS	77,652	55,673	2,957	2,779.11	5.36	0.986
Control soil	91,447	55,673	5,450	4,748.57	6.87	0.996
ASD I-D0	98,959	55,673	3,857	3,460.74	5.11	0.967
ASD S-D0	55,673	55,673	3,519	3,333.89	5.57	0.983
ASD I-D90	79,152	55,673	4,539	4,267,73	5.69	0.979
ASD S-D90	73,101	55,673	4,312	4,061.59	5.57	0.978
URS I-D0	69,836	55,673	3,908	3,723.09	5.65	0.987
URS S-D0	121,731	55,673	4,997	4,343.78	5.49	0.977
URS I-D90	94,714	55,673	4,270	3,912.11	5.59	0.987
URS S-D90	81,404	55,673	4,802	4,374.40	6.06	0.988

ASD: mesophilic anaerobic digester sludge; URS: sludge from UASB reactor; S: superficial; I: incorporated; D: day.

to exhibit the microbial diversity difference for each treatment group (Figure 1).

In SSA with URS sludge, 18.3% of OTUs were common to all samples, and 20.1–20.5% of OTUs were exclusive to control soil. Comparing the SSA after 90 days, 64% of OTUs in the superficial sample and 66% in the incorporated sample were in common with control soil. The SSA with ASD sludge shared 17.0–17.2% of OTUs in common, and 14.4–16.7% of OTUs were exclusive to control soil. Also, comparing the SSA after 90 days, 55% of OTUs in the superficial sample and 59% of OTUs in the incorporated sample were in common with control soil. This result suggests that 90 days was not enough for the bacterial community in SSA samples to achieve the diversity and richness of soil before the sludge amendment.

Principal coordinate analysis, based on the dissimilarity index of Bray–Curtis (Figure S1, Supplementary material), emphasized the changes in bacterial community composition and diversity over 90 days of SSA. In general, there was a trend of grouping the samples according to the type of sludge applied, but the application method presented some differences. The soil collected shortly after SSA (day 0) did not show similarity to those samples collected after 90 days, indicating that changes occurred in the microbial community composition and diversity over time. In contrast, the incorporated URS-soil collected at T0 was close to T90. Furthermore, concerning the application mode, the incorporated ASD-soil did not show similarity to the surface ASD-soil, indicating that the application mode affected the soil microbial community composition differently.

Taxonomic complexity of the bacterial community in sludge and control soil before SSA

Sludges and soil bacteria communities are now routinely characterized by analyzing 16S rRNA genes to avoid the required laboratory cultivation. Overall, 34 phyla were identified in sanitary sludges (URS and ASD) and 36 phyla in control soil. Phyla with relative abundance higher than 1% amounted to 9 in URS, 10 in ASD, and 11 in soil control (Figure 2). In sanitary sludges, *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Bacteroidetes*, *Synergistedes*, and *Actinobacteria* were among the significant phyla (relative abundance >1%) identified in URS and ASD samples (Figure 2(a). However, sanitary sludges shared similar phyla but different proportions due to treatment technology (Goberna *et al.* 2018).

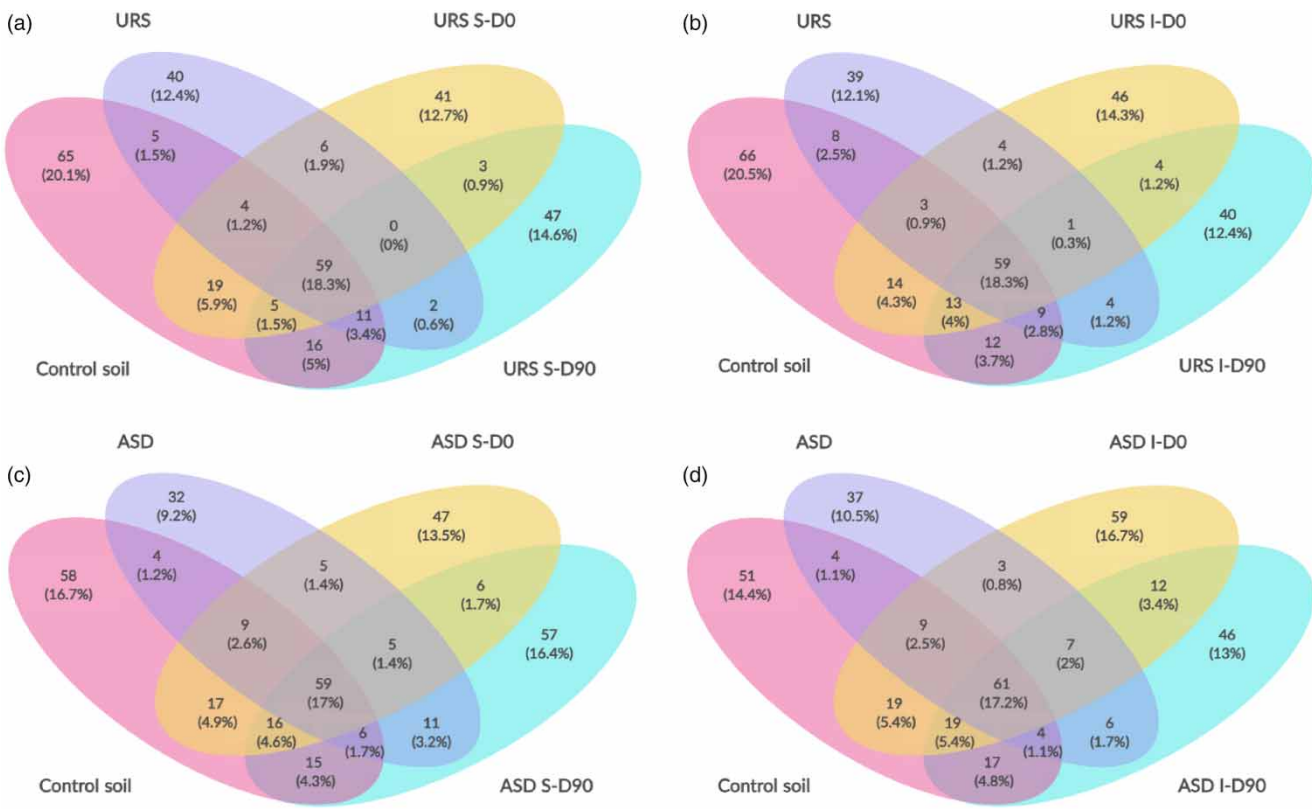

Figure 1 | Venn diagrams showing the common and exclusive bacterial OTUs of sludges analyzed (URS and ADS) and studied soil before the SSA.

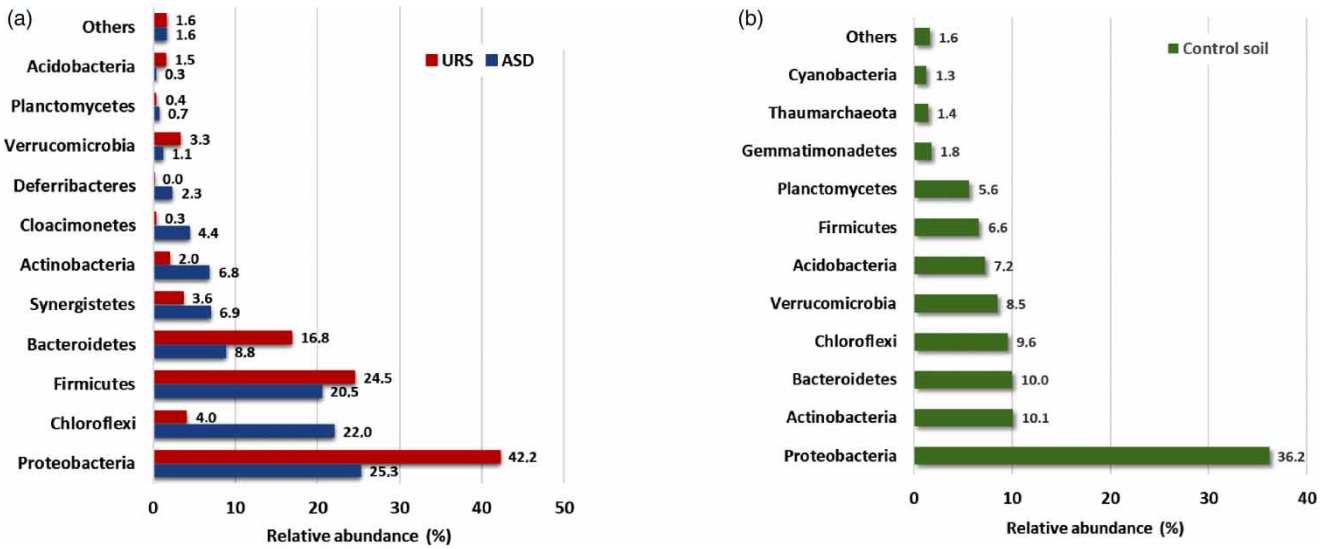

Figure 2 | The microbial composition at the phylum level of relative abundance higher than 1% for sludge (a) and control soil (b) before SSA.

Among the bacteria present in soil (Figure 2(b)), *Proteobacteria* were the most abundant, followed by *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia*, *Acidobacteria*, *Firmicutes*, and *Planctomycetes*. These observations are in line with studies worldwide analyzing anaerobic digestion technologies (Paul 2015; Lloret *et al.* 2016; Goberna *et al.* 2018).

Both soil and sludge samples contain different phyla that play essential roles in the nutrient cycle in sludge or soil, including photoautotrophs, chemolithoautotrophs, and generalist heterotrophs – a highly metabolically diverse group. For example, *Proteobacteria* are predominant in sludge and soil samples and play an essential and active role in the nitrogen and carbon cycle, encompassing organisms with different lifestyles (Garrity 2005). Also, members of the *Bacteroidetes* phylum exhibit chemoautotrophic characteristics capable of degrading various polymers, such as cellulose, chitin, and pectin (Madigan *et al.* 2019). Understanding these microorganisms' behavior when facing the challenge of sewage sludge application in the soil is vital to elucidate the soil response.

The response of soil bacterial community activity after 90 days of SSA

Figure 3 shows the URS and ASD response after 90 days of soil amendment by two application methods (superficial and incorporated). In general, the SSA introduced exogenous bacterial phyla, increased others, and tended to reduce the diversity of soil samples (Figure 3 and Table 2).

SSA practice introduced some microorganisms from *Synergistetes* and *Cloacimonetes* phyla. *Synergistetes* are related to fermentative metabolisms in gastrointestinal animals, and the human tract (Madigan *et al.* 2019), and *Cloacimonetes* are related to methane production (Aida *et al.* 2014; Goberna *et al.* 2018; Nazina *et al.* 2018; van Elsas 2019). Due to lower abundance in URS sludge (Figure 2(a)) when the sludge was applied to the soil, *Synergistetes* phylum also remained in low abundance after 90 days of SSA. The high abundance of *Synergistetes* phylum in ASD sludge (Figure 2(a)) enhanced its soil presence. However, after the 90 days of SSA by surface application method, this phylum decreased the relative abundance by 27.6%. The relative abundance of *Cloacimonetes* phylum after 90 days of SSA with ASD remained at 10.2% for the surface disposal method, and 4.2% for the incorporation disposal method. These results suggest that new microorganisms introduced by the SSA could not persist in the tropical soil environment.

According to Garrity (2005), *Proteobacteria* is one of the major phyla of Gram-negative bacteria and is divided into five subclasses (*α*-, *β*-, *γ*-, *δ*- and *ϵ*-*proteobacteria*). Although the *Proteobacteria* phylum was predominant in all SSA samples, the relative abundance decreased by 19% in ASD-I and 3% in URS-S. Also, *Proteobacteria* subclasses changed over time (Figure 4), and for SSA with URS sludge, *δ*-*proteobacteria* was predominant. This subclass includes bacteria that are essential contributors to the sulfur cycle's anaerobic stages (Garrity 2005), and it was predominant in URS sludge in agreement with a previous study (Nascimento *et al.* 2018).

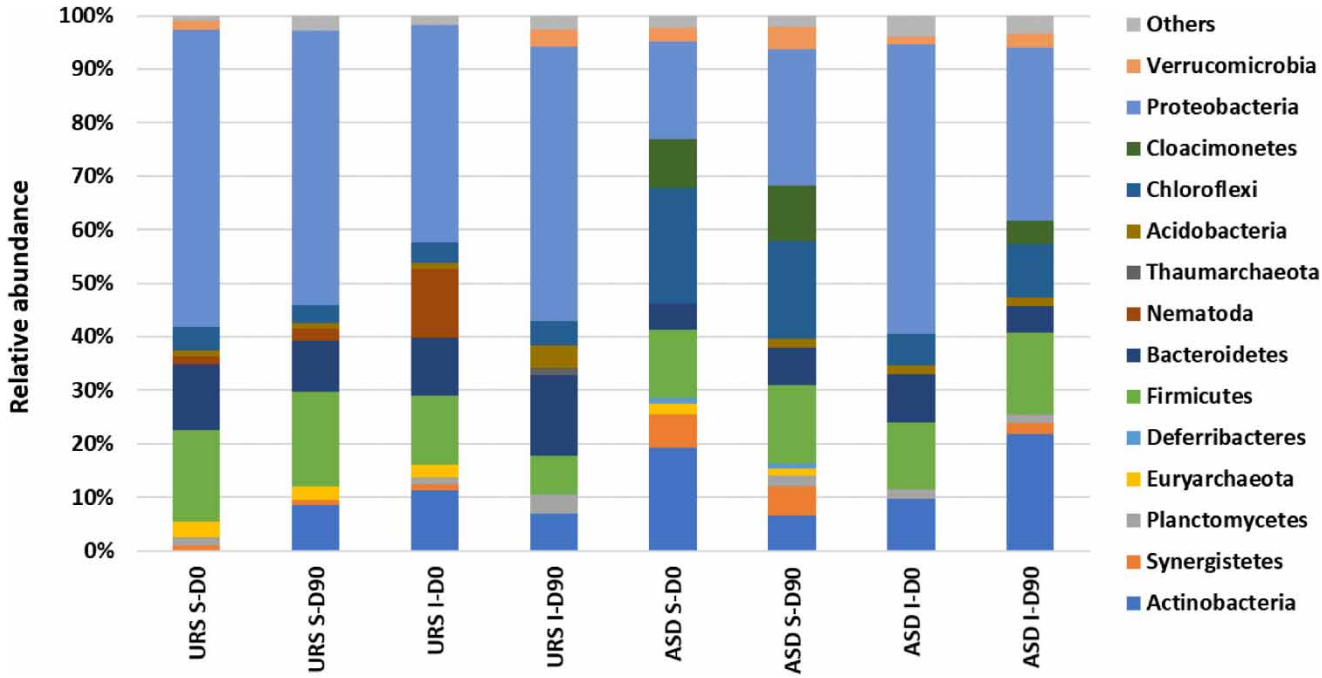

Figure 3 | Relative abundance of different bacterial community structures at phylum level in the samples after 90 days of SSA (relative abundance >1%).

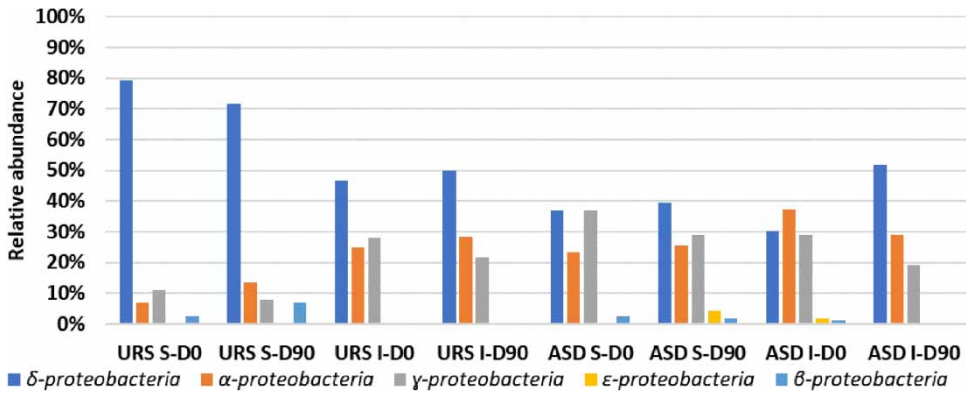

Figure 4 | Relative abundance of *Proteobacteria* subclasses in SSA after URS and ASD disposal by surface and incorporated method.

The number of *δ-proteobacteria* was affected by sludge type and application method. In general, SSA-URS showed higher relative abundance, and incorporation methods increased this population.

The *α-proteobacteria* and *γ-proteobacteria* were also relevant in SSA samples, especially in ASD samples, in agreement with a previous study (Curci *et al.* 2020). According to Garrity (2005), this subclass includes essential bacteria capable of inducing nitrogen fixation in symbioses with plants agriculturally. These results showed that SSA by incorporation method could enhance the microbial proteobacteria community responsible for the nitrogen and sulfur environment cycle.

Another frequently found phylum found in this study was the *Firmicutes*. Figure 5 shows the relative abundance of *Firmicutes* families in SSA samples. *Clostridiaceae* and *Syntrophomonadaceae* were predominant families in SSA samples, showing different relative abundance among the sewage sludge type evaluated, but not by the application method. Concerning *Clostridiaceae*, SSA-URS showed relative abundance mean values of 77.4% and the SSA-ASD showed 61.2%. This difference can be explained by the

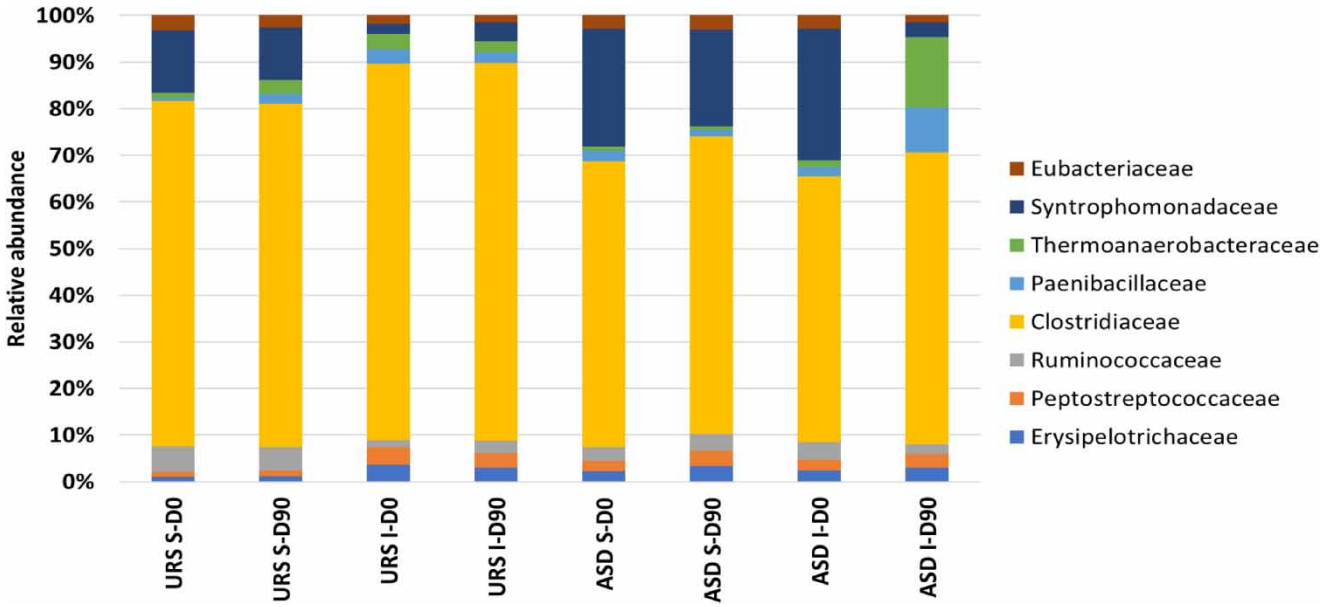

Figure 5 | Relative abundance of *Firmicutes* families in SSA after URS and ASD disposal by surface and incorporated method.

Clostridiaceae higher relative abundance in URS sludges, as shown in Figure 2(a). The family *Clostridiaceae* comprises 13 genera (De Vos 2009) and, according to Pepper *et al.* (2006), *Clostridium* organisms are important heterotrophic soil bacteria able to use the carbon present in the soil as a source for the fermentation process and toxin production.

Studies concerning the *Syntrophomonadaceae* family in the soil are scarce. The results show that ASD sludge can increase the relative abundance in soil and the incorporation sludge disposal method can negatively affect this population. According to Liu *et al.* (2011), members of this family can degrade important intermediates during the degradation of organic residues in paddy field soils, such as butyrate.

The high relative abundance of *Chloroflexi* in ASD sludges (Figure 2(a)) increased their presence in the soil after SSA (Figure 3). According to Madigan *et al.* (2019), this phylum contains metabolically diverse organisms, including aerobic and anaerobic chemoorganotrophs but is still poorly characterized.

The *Actinobacteria* include the actinomycetes, a large group of primarily filamentous soil bacteria that mainly includes filamentous bacteria and is intimately involved in the nitrogen cycle (Madigan *et al.* 2019). In this study, the relative abundance of the *Actinobacteria* phylum increased in SSA after sludge disposal (Figure 3). This result suggests that SSA could improve these phylum members and could contribute to enhancing the nitrogen cycle. This enrichment result is also in agreement with previous studies (Wolters *et al.* 2018; Bai *et al.* 2019). According to El Azhari *et al.* (2012), particular emphasis has been given to soil *Actinobacteria*, intimately involved in the cycling of nutrients and organic matter decomposition. The change in composition is generally considered an indicator of environmental impact.

Meanwhile, the members of the phylum *Acidobacteria* were predominant in soil control compared with sludge samples (Figure 2(b)) and increased after SSA (Figure 3) independent of the sludge type or application methods. *Acidobacteria* are abundant in soils, particularly acid soils (pH 6.0) (Madigan *et al.* 2019), suggesting that this phylum probably plays an ecologically important role in soil ecosystems (van Elsas 2019). According to Kielak *et al.* (2009), relatively little is known about their distribution, diversity, and function in soils despite their dominant presence.

Species of *Verrucomicrobia* are aerobic or facultative aerobic bacteria capable of fermenting sugars, are widespread in forest and agricultural soils (Madigan *et al.* 2019), and they are susceptible to changes in chemical factors linked to soil fertility under tropical environmental conditions (Navarrete *et al.* 2015). The SSA practice decreased the relative abundance of the *Verrucomicrobia* phylum compared to the soil control sample, independent of sludge type or application methods.

This study contributed to clarifying the bacteria community changes response after the soil sludge amendment. In general, although there was a decrease of bacterial

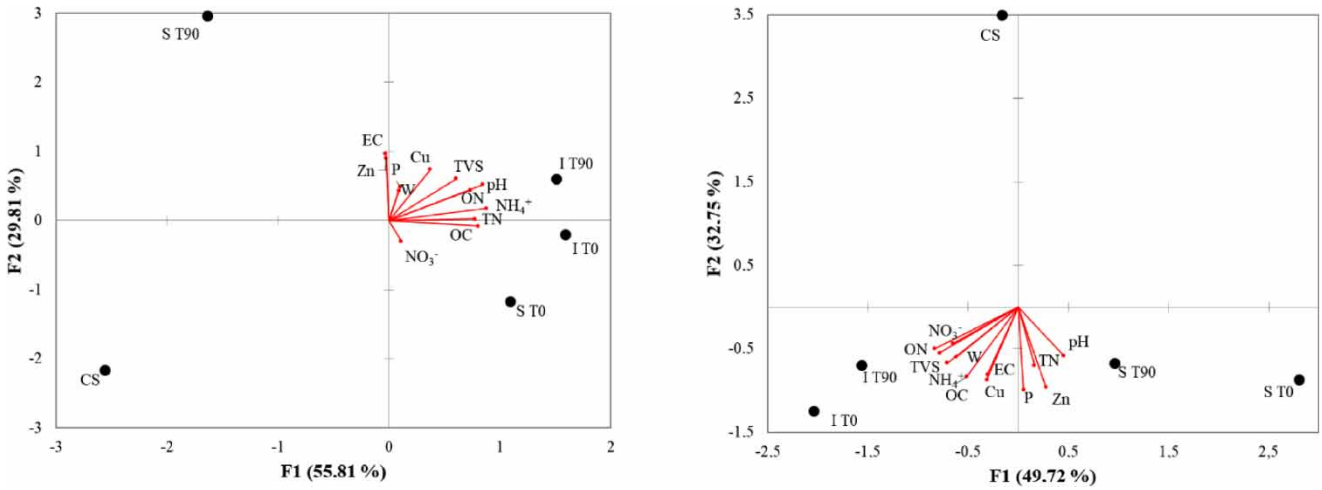

Figure 6 | Redundancy analysis diagram of correlations between physicochemical proprieties and OTUs for SSA with ASD (a) and URS (b) by T0 and T90. TN: total nitrogen; ON: organic nitrogen; NO_3^-: nitrite; NH_4^+ ammonium; pH-soil; TVS: total volatile solids; W: water content; OC: organic carbon; EC: electrical conductivity; available P, Cu, Zn.

community diversity after 90 days of SSA, different phyla groups linked to the biogeochemical process, especially cycling of nutrients and organic matter decomposition, increased their relative abundance in soil. It could be concluded that the bacterial community present in soil responded to SSA, increasing the activity of microorganisms involved in the functional process.

Soil physicochemical properties and bacterial community

In this study, soil bacterial community diversity was changed by SSA. The redundancy analysis diagram (RDA) was used to identify how the soil properties impacted the bacterial community diversity over 90 days (Figure 6).

The RDA indicates that changes in soil chemical parameters over time due to the SSA method of application resulted in changes in bacterial community composition of plots with both soil–sludge mixtures. These results agree with a previous study (Miranda *et al.* 2019) that the bacterial population is significantly affected by environmental variables, such as pH, EC, P, total organic carbon, Ca, and Cr. In this study, the sludge's incorporation into the 0–10 cm soil layer affected the soil bacterial community more compared with the surface application method with both sludges evaluated. It suggested that the application method by incorporation could improve the contact of soil microorganisms and nutrients and bacterial communities present in sludge, creating favorable conditions to improve the bacterial growth, which is reflected by changing soil chemical parameters.

CONCLUSION

The use of sanitary sludges induced a change in the bacterial community in tropical soil and no difference was observed among the two different sludges evaluated. Over 90 days of SSA practice, some microorganisms were introduced, and others were enhanced, leading to a decrease in the bacterial community's diversity and richness. *Synergistetes* and *Cloacimonetes* phyla were added to the soil by SSA practice. Despite the decline observed in the bacterial community in general, some bacteria families were enhanced by SSA, mainly those responsible for cycling of nutrients and organic matter decomposition. The sludge type and application methods impacted the bacterial community in soil. The time evaluated (90 days) was not sufficient to restore the initial diversity from control soil. Also, RDA corroborates the next generation sequencing analysis. Some of the physicochemical parameters were linked to changes in the SSA samples' bacterial community, mostly when the incorporation application method was applied.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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