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ORIGINAL PAPER

Liming in the sugarcane burnt system and the green harvest practice affect soil bacterial community in northeastern São Paulo, Brazil

Silvana Pompeia Val-Moraes · Helena Suleiman de Macedo · Luciano Takeshi Kishi · Rodrigo Matheus Pereira · Acacio Aparecido Navarrete · Lucas William Mendes · Eduardo Barretto de Figueiredo · Newton La Scala Jr. · Siu Mui Tsai · Eliana Gertrudes de Macedo Lemos · Lúcia Maria Carareto Alves

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Abstract Here we show that both liming the burnt sugarcane and the green harvest practice alter bacterial community structure, diversity and composition in sugarcane fields in northeastern São Paulo state, Brazil. Terminal restriction fragment length polymorphism fingerprinting and 16S rRNA gene cloning and sequencing were used to analyze changes in soil bacterial communities. The field experiment consisted of sugarcane-cultivated soils under different regimes: green sugarcane (GS), burnt sugarcane (BS), BS in soil amended with lime applied to increase soil pH (BSL), and native forest (NF) as control soil. The bacterial community structures revealed disparate patterns in sugarcane-cultivated soils and forest soil ($R = 0.786$, $P = 0.002$), and overlapping patterns were shown for the bacterial community structure among the different management regimes applied to sugarcane ($R = 0.194$, $P = 0.002$). The numbers of operational taxonomic units (OTUs) found in the libraries were 117, 185, 173 and 166 for NF, BS, BSL and GS, respectively. Sugarcane-cultivated soils revealed higher bacterial diversity than NF soil, with BS soil accounting for a higher richness of unique OTUs (101 unique OTUs) than NF soil (23 unique OTUs). Cluster analysis based on OTUs revealed similar bacterial communities in NF and GS soils, while the bacterial community from BS soil was most distinct from the others. Acidobacteria and Alphaproteobacteria were the most abundant bacterial phyla across the different

Silvana Pompeia Val-Moraes and Helena Suleiman de Macedo have contributed equally to this work.

S. P. Val-Moraes (✉) · H. S. de Macedo · L. T. Kishi · E. B. de Figueiredo · N. La Scala Jr. · E. G. de Macedo Lemos · L. M. C. Alves (✉)
Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinária FCAV, Univ. Estadual Paulista UNESP, Acesso Prof. Dr. Paulo Donato Castellane, S/N, Jaboticabal, SP 14884-900, Brazil
e-mail: valmoraes.silvana@gmail.com

L. M. C. Alves
e-mail: lmcalves@fcav.unesp.br

R. M. Pereira
Faculdade de Ciências Biológicas e Ambientais FCBA, Universidade Federal da Grande Dourados UFGD, Rodovia Dourados, Itahum, Km 12 - Unidade II, Caixa Postal: 364, Dourados, MS CEP 79804-970, Brazil

A. A. Navarrete · L. W. Mendes · S. M. Tsai
Laboratório de Biologia Celular e Molecular, Centro de Energia Nuclear na Agricultura CENA, Universidade de São Paulo USP, Av. Centenário, 303, Caixa Postal 96, Piracicaba, SP CEP 13400-970, Brazil

soils with Acidobacteria Gp1 accounting for a higher abundance in NF and GS soils than burnt sugarcane-cultivated soils (BS and BSL). In turn, Acidobacteria Gp4 abundance was higher in BS soils than in other soils. These differential responses in soil bacterial community structure, diversity and composition can be associated with the agricultural management, mainly liming practices, and harvest methods in the sugarcane-cultivated soils, and they can be detected shortly after harvest.

Keywords T-RFLP fingerprinting · 16S rRNA gene · Agricultural soil management · Soil microbial ecology

Introduction

Sugarcane (*Saccharum* spp.) is cultivated in more than 70 countries and it is considered one of the most important crops in terms of energy value, with a total cultivation area of more than 20 million hectares (FAO 2016). In Brazil, the area of land used for cropping sugarcane is constantly expanding (CONAB 2013). In these areas under sugarcane cultivation the soil is subjected to traditional management processes, such as tillage, fertilization and liming, and different sugarcane harvest systems are commonly applied such as manual handling with burnt sugarcane and mechanically harvested sugarcane. However, sugarcane harvest practices are undergoing a change with the increasing introduction of mechanical harvesting according to the State Law No.11.241/02.

Microbial responses have been shown for agricultural management and harvest practices for sugarcane production on soil. Navarrete et al. (2015a) used a multi-analytical approach in sugarcane-cultivated soils in a short-term greenhouse experiment and they showed that organic and inorganic amendments and straw retention used as a surface 'blanket' affect the microbial biomass carbon and nitrogen, bacterial community composition and chemical characteristics of sugarcane soils. Souza et al. (2012) showed a decrease in soil fertility and microbial biomass carbon after sugarcane harvesting with burning. In turn, Rachid et al. (2013) showed that sugarcane burning on the Brazilian Cerrado also affects soil bacterial community structure. The results of these studies have assisted in defining management indicators to evaluate the impact of soil agricultural management and harvest practices for sugarcane production on soil microbial communities. It could also be beneficial to define biologically relevant assays to examine the potential impact from the sugarcane production processes on soil health.

A new perspective in soil microbial ecology has emerged due to the current advances in molecular biology methods, allowing the interpretation of ecological aspects by culture-independent approaches. Microbial community structure including ecological traits such as the number of phylotypes and the relative abundance comprising a community has been assessed by molecular fingerprinting techniques (Burlage 1998). Microbial community composition, an ecological concept referring to the members of a community in a particular ecosystem, can be studied using DNA sequencing approaches (Venter et al. 2004; Val-Moraes et al. 2009; Uroz et al. 2010). These ecological features of microbial communities can be statistically related to environmental parameters, such as soil chemical factors (Jesus et al. 2009; Kuramae et al. 2012) and the liming effects on soil pH along with covariant chemical factors (Navarrete et al. 2013).

Because of the substantial effects that different types of soil agricultural management and harvest methods may have on the chemical and microbiological characteristics of sugarcane-cultivated soils, and the unknown effects of liming burnt cane, we aim to obtain better insight into ecological characteristics of soil bacterial communities in sugarcane croplands. For this purpose, we applied terminal restriction fragment polymorphism (T-RFLP) analysis and 16S rRNA gene cloning and sequencing to analyze the effects of liming in sugarcane burnt system and of the green harvest practice on soil bacterial communities in northeastern São Paulo, Brazil. The explicit relationship between the bacterial community structure and soil physicochemical properties was examined by multivariate statistical analyses.

Materials and methods

Experimental design and soil sampling

The field experiment was conducted in the northeastern region of São Paulo state, Mococa municipality,

Brazil (Fig. 1). The average altitude of the area is 645 m and it has an average annual rainfall of 2500 mm, which is concentrated between the months of October and March, and a relatively dry period occurs between April and September This region has a humid tropical climate, with an average annual temperature of about 21 °C. The experimental area cultivated with sugarcane has been used for about 20 years (Fig. 1) and was originally converted from tropical semi-deciduous forest. The field was divided into four different areas (split-plot) in which three different regimes were applied: Green Sugarcane (GS), the harvest was performed using a machine and the leaves remained in the soil cover, practiced in some areas of this region since 2007 (21°25′28.95″S and 47°05′34.75″W); Burnt Sugarcane (BS), the sugarcane was set on fire to remove the leaves before harvest, the stem was then manually harvested, and the soil remained without straw after harvest (21°25′31.95″S and 47°05′25.95″W); Burnt Sugarcane with dolomite (BSL), burnt cane with conventional treatment and application of dolomite, an agent to cut soil acidity (raise pH) (21°25′48.95″S and 47°05′45.95″W); and Native Forest (NF), a fragment of native Atlantic Forest was used as control (21°25′27.05″S and 47°05′50.05″W).

Sugarcane cultivation was conducted in a crop cycle of 3 years, including 1 year of cane planting and 2 years of root drying. The variety SP81-3250 of *Saccharum* spp. was cultivated in BS area, and the variety SP91-3011 was cultivated in GS area. Approximately 5 months after harvest, the soil was conventionally prepared in the following ways: (1) heavy grade, with a cutting depth of 25 cm; (2) average grade, with a cutting depth of 20 cm; (3) with a subsoil depth of 40 cm and (4) harrowing grade, with a depth of 10 cm (twice) of ultisol soil. After mechanical treatment, the BSL area was chemically treated with 400 kg ha^{-1} $CaMg(CO_3)_2$ (dolomite) in order to increase soil pH (De Figueiredo and La Scala 2011).

Soil sampling was performed in Feb 2010, 9 days after soil tillage and application of dolomite. Triplicate soil cores were collected from the 0 to 20 cm topsoil layer in each of the four areas. A total of 12 soil samples were collected in field (4 sampling areas ×3 soil cores per area). Samples were transported to the laboratory on ice and stored at 20 °C until processing, which was carried out within 72 h of sampling. The chemical measurements of each of the 12 soil samples were determined as described in Raij et al. (1987).

Soil DNA isolation and bacterial 16S rDNA T-RFLP fingerprinting analysis

Total DNA was extracted from 250 mg of soil using the Power Soil® DNA kit according to the manufacturer's protocol (MoBIO, Carlsbad, CA). DNA extraction was performed for each soil sample. To verify quality, 5 µl of total extracted DNA was analyzed on a 1 % (w/v) agarose gel in TSB buffer (100 mM Tris–

Fig. 1 The sampling areas are localized in the northeastern region of São Paulo state, Mococa municipality, Brazil. Sampling areas: *GS* Green Sugarcane, *BS* burnt sugarcane, *BSL* burnt sugarcane with liming application, and NF soil from a native forest as a control Source: Google Earth (http://www.google.com.br/intl/pt-BR/earth/)

HCl, 1 % sodium dodecyl sulfate, and 2 % 2-mercaptoethanol) at 80 V for about 60 min, and then visualized. The DNA was then quantified using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, USA). T-RFLP analysis was used to characterize the bacterial community structure in the triplicate soil samples from each of the four sampling areas (GS, BS, BSL and NF). Bacterial 16S rRNA gene fragments were amplified using the primers fD1, 5′-CGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3′ and rD1, 5′-CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC-3′ (Lane et al. 1985). The forward primer was labeled with hexacarboxifluorescein at the end. PCR reactions were performed according to Mendes et al. (2012). The hexacarboxifluorescein-labeled PCR products for each sample were purified using GFX^{TM} PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech, NJ, USA) after analysis by gel electrophoresis. After purification, 5 µl of amplification products (nearly 60 ng) were digested with the endonucleases *Msp*I and *Hha*I in separate reactions (Invitrogen, Carlsbad, CA, USA). The reactions (15 µl) were performed at 37 °C for 3 h. The resulting fragments were purified by sodium acetate/EDTA precipitation, and then mixed with 0.25 µl of the Genescan 500 ROX size standard (Applied Biosystems, Foster City, CA) and 9.75 µl of deionized formamide. Fluorescently labeled terminal restriction fragments (TRF) were separated and detected using an ABI PRISM 3500 Genetic Analyzer capillary sequencer (Applied Biosystems, Foster City, CA, USA). TRF patterns were first analyzed by the GeneMapper program v 4.1 (Applied Biosystems, Foster City, CA, USA) for the assessment of the run, quality in the sequencer, and a spreadsheet was exported to Excel (Microsoft Corporation, Redmond, DC, USA). The data sheets were then subjected to multivariate statistical analysis with the software Canoco 4.5 (Biometrics, Wageningen, NL) and Primer5 (Plymounth Marine Laboratory, Primer E, UK). Firstly, Principal Component Analysis (PCA) was used to evaluate the microbial community structure. Later, the statistical differences between groups were tested by similarity analysis (ANOSIM), and similarity percentage analysis (SIMPER) based on the calculated Bray-Curtis similarity and dissimilarity coefficients (Clarke 1993), respectively. The explicit relationship between the bacterial community structure and soil chemical factors was examined by constrained ordination generated by a redundancy analysis (RDA) performed using CANOCO 4.5 (Braak and Šmilauer 2002).

Amplification and sequencing of 16S rRNA gene fragments

Aliquots of the same triplicate DNA samples used for T-RFLP fingerprinting analysis were pooled for each sampling area and bacterial 16S rRNA gene fragments (1500 bp) were amplified using the universal primers 27f (5′AGA GTT TGA TCC TGG CTC AG 3′) and 1492r (5′ACC TTG TTA CGA CTT 3′) (Brody and Kern 2004). Amplification of 16S rRNA gene fragments was performed according to Pereira et al. (2006). The PCR products were purified with Kit GFX^{TM} PCR DNA and Gel Band Purification (GE Healthcare, Little Chalfont, United Kingdom), inserted into the pGEM® T Easy vector (Promega, Madison, WI, USA), and transformed into *E. coli* DH5α cells. Sequencing of the 16S rRNA gene fragments was performed using an Applied Biosystems 3730 DNA Analyzer sequencer (Applied Biosystems, Foster City, CA, USA). Libraries were constructed for the samples from GS (GenBank accession no. KU883818–KU883929), BS (GenBank accession no. KU884043–KU884212), BSL (GenBank accession no. KU883930–KU884042), and NF (GenBank accession no. KU883686–KU883817), and DECIPHER V was used to locate any chimeras (Wright et al. 2012).

Sequence and diversity analysis

The electropherograms obtained from sequencing were analyzed through the Ribosomal Database Project (Wang et al. 2007) web-based taxonomy assignment tool (http://rdp.cme.msu.edu/) version 10 against the RDP 16S rRNA training set 10. Sequences were removed from the analysis if they did not have the primer sequence or were less than 400 nt in length, and bases with a quality score lower than 20 were trimmed (1 error per 100 bases). A comparative analysis of the libraries was performed using the RDP Library Compare tool from the RDP website (95 % confidence), which has a system of taxonomic classification (RDP Hierarchy) that follows the proposal of Bergey's manual (Cole et al. 2009), where the main taxonomic levels are: Domain, Phylum, Class, Order, Family,

Genus and Species. For diversity analysis, a distance matrix was generated using MOTHUR software (Schloss et al. 2009), and the sequences were assigned operational taxonomic units (OTUs) for an evolutionary distance of 0.03 (97 % similarity) using the algorithm "furthest neighbor", in which all sequences within each OTU are no more than 3 % evolutionarily distant from each other. The MOTHUR software was also used to calculate nonparametric richness estimators, such as Chao 1 (Schloss et al. 2009) and Abundance-based Coverage Estimator (ACE) (Chao 1987), and diversity indices, such as Shannon and Simpson, which use different mathematical approaches to estimate diversity, thereby providing quantitative measures of the diversity found in the samples. Another method used to estimate the richness of OTUs within the community was the Jackknife estimator (Chao and Lee 1992), which is based on the frequency of rare OTUs. Estimated coverage index (ECI), a cluster analysis based on 1000 replicates was performed to comp Soil organic matter (SOM) content are composition of libraries based on the OTUs found in different samples. A Venn diagram was prepared to compare the unique and shared OTUs between libraries, and rarefaction curves were constructed to verify whether the sampling effort was sufficient (Colwell and Coddington 1994). Finally, we performed S-Libshuff analysis (Roesch et al. 2007) to detect statistically significant differences among the four libraries.

Results and discussion

Soil physicochemical properties

The soils under investigation were found to be acidic, with pH values between 4.0 and 6.1 (Table 1). The soil sample from NF showed lower pH (pH 4.0) in comparison with sugarcane-cultivated soils. In general, forest soils have a lower pH in comparison with agriculture soils (Val-Moraes et al. 2013). The area that had been treated with dolomite had a pH adjusted to 6.1. A general method for increasing soil pH is to lime soils with $CaCO_3$, CaO, or $Ca(OH)_2$. The liming material reacts with carbon dioxide and water in the soil to yield bicarbonate, which is able to take H^+ and Al_3^+ (acid-forming cations) out of solution, thereby raising the soil pH (Rehm et al. 1992). Soil pH, phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were higher in sugarcane-cultivated soils compared to native forest probably reflecting the regular liming and fertilization to support crop productions in BSL area (Val-Moraes et al. 2013). Similar results to those for soil pH were observed when analyzing the soil potential acidity (H + Al) (Souza et al. 2012).

Table 1 Soil chemical and physical factors of the 0–20 cm topsoil layer from areas in which three different regimes were applied for sugarcane production (*GS* Green Sugarcane, *BS* Burnt Sugarcane, *BSL* Burnt Sugarcane with Dolomite) and control area (*NF* Native Forest)

Parameters	GS	BS	BSL	NF
pH ($CaCl_2$)	4.9	4.6	6.1	4.0
SOM (g dm^{-3})	13	11	11	20
P (mg dm^{-3})	5	7	9	6
K ($mmol_c$ dm^{-3})	0.5	1.1	0.9	1.6
Ca ($mmol_c$ dm^{-3})	19	12	40	6
Mg ($mmol_c$ dm^{-3})	5	3	12	4
H + AL ($mmol_c$ dm^{-3})	25	25	11	64
SB	24.5	16.1	52.9	11.6
CEC	49.5	41.1	63.9	75.6
V	49	39	83	15
Clay (g kg^{-1})	179	150	146	140
Silt (g kg^{-1})	170	175	179	158
Fine Sand (g kg^{-1})	422	438	437	356
Coarse Sand (g kg^{-1})	229	237	238	346
Texture	Medium	Sandy	Sandy	Sandy

Average followed by the same letter did not differ by Tukey's test with 5 % probability

SOM soil organic matter, *SB* sum of bases, *CEC* cation exchange capacity, *V* base saturation

Soil organic matter (SOM) content was higher in NF and GS soils compared to other soils. Management with burning before harvesting reduces the organic matter content, which affects soil structure and stability (Filho et al. 2010). According to Razafimbelo et al. (2006), burning sugarcane may increase soil temperature from 160 to 200 °C in the superficial layers, oxidizing a lot of the organic matter in these layers, leaving them exposed to rain and causing the loss of soil nutrients essential for plant growth. The GS soil was classified as medium texture and is distinct from the other three soils, which have a sandy texture (NF, BS and BSL).

Soil bacterial community structure

Principal component analysis (PCA) ordination based on bacterial T-RFLP data from restriction profiles generated by enzymes *Msp*I and *Hha*I analysis revealed distinct groups for each management regime in sugarcane-cultivated soils and forest soil (Fig. 2). This grouping pattern was confirmed by significant *R*-values with ANOSIM based on the presence and absence of terminal restriction fragments in T-RFLP profiles (Table 2). ANOSIM showed significant differences ($R = 0.786$, $P = 0.002$) between bacterial community structure of the NF soil and those from sugarcane-cultivated soils. NF soil samples were not grouped closer together in the ordination; however, heterogeneity between replicas was observed. However, overlapping patterns were shown for the bacterial community structure among the different management regimes applied to sugarcane ($R = 0.194$, $P = 0.002$). Results obtained with the SIMPER test confirmed these findings, and showed lowest dissimilarity between BSL and GS soils (Table 2).

Redundancy analysis (RDA) was performed between the T-RFLP data, and the chemical-physical properties of the soil (Fig. 3). RDA was efficient to display relationships between soil bacterial community structure and soil physicochemical characteristics for the different regimes. The structure of soil bacterial community in NF soil was closely related to SOM, potential acidity and coarse sand. In turn, the bacterial community structure was related to fine sand in BS soil, while Mg and P were more related to bacterial community structure in BSL soil. The clay content of the GS soil was closely related to the soil bacterial community structure revealed in this soil. Soil organic matter, mainly of plant origin, is the main energy source for microorganisms, especially those involved in terrestrial biogeochemical cycles (Rasche et al. 2011). It has been observed that GS soil has a higher correlation with clay content, and BS and BSL soils are correlated with bioavailable phosphorus (P) and the sum of bases (SB) (Panosso et al. 2011).

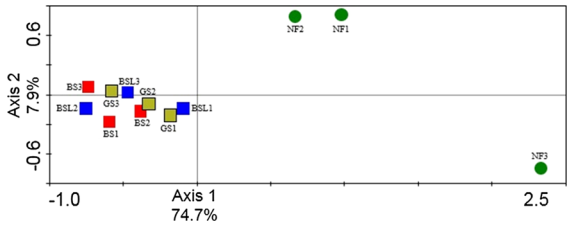

Fig. 2 Principal component analysis (PCA) plot based on the structure of soil bacterial communities as determined by T-RFLP analysis. *GS* Green Sugarcane, *BS* Burnt Sugarcane, *BSL* Burnt Sugarcane with Dolomite, *NF* Native Forest. The numbers 1, 2 and 3 indicate replicate soil core

Table 2 Similarity analysis (ANOSIM) and dissimilarity index (SIMPER) based on bacterial community structure as revealed by T-RFLP fingerprinting

	ANOSIM[a]		SIMPER[b]	
	*Msp*I	*Hha*I	*Msp*I (%)	*Hha*I (%)
NF × BS	0.623	0.851	18.97	40.74
NF × GS	0.627	0.949	20.11	44.28
NF × BSL	0.574	0.925	19.14	42.63
BS × BSL	0.161	0.228	15.22	17.70
BS × GS	0.235	0.251	15.15	17.89
BSL × GS	0.016	0	13.27	14.14

[a] *R*-values are expressed at the significance level of $P < 0.005$. *R*-values >0.75 are statistically different; >0.5 have overlap but are still clearly different; and <0.5 not statistically different

[b] Percentage of the dissimilarity between samples

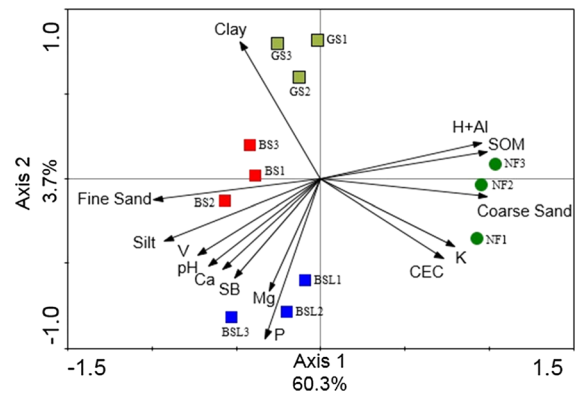

Fig. 3 Constrained ordination diagram for sample plots (*GS* Green Sugarcane, *BS* Burnt Sugarcane, *BSL*, Burnt Sugarcane with Dolomite, *NF* Native Forest) in the first two redundancy analysis (RDA) axes based on the soil chemical characteristics of the different sampling sites and their relationship with the structure of soil bacterial communities as determined by T-RFLP analysis. The numbers *1*, *2* and *3* indicate replicate soil core

Soil bacterial community diversity and composition

For diversity analysis based on partial sequences of the bacterial 16S rRNA genes, 252, 254, 226 and 217

high-quality sequences were obtained for NF, BS, BSL and GS libraries, respectively. The species diversity consists of two components: richness and equitability of species (Magurran 2004). The OTUs richness and diversity found in NF, BS, BSL and GS libraries are shown in Table 3. OTUs richness estimators ACE, CHAO1 and Jackknife showed the greatest richness of OTUs in sugarcane-cultivated soils. However, Shannon and Simpson diversity indices revealed a lower diversity in NF soil compared with sugarcane-cultivated soils. The ECI from 16S rRNA gene libraries (Table 3) indicates the percentage of diversity within the sequences. The NF soils had a large ECI (91.6 %) followed by the BS, GS and BSL soils.

Bacterial diversity in sugarcane-cultivated soils was higher than in forest soil. This may be due to seasonal variation in crop plant communities that may generate a more dynamic environment and, therefore, greater diversity in the bacterial community. The cultivated area is also more exposed than the forest; therefore, bacterial community members may often be introduced through wind or animals. In addition, tillage of the soil can break the existing community structure and increase the likelihood of successful bacterial migration into the area; common weeds in crops may also influence diversity (Torsvik et al. 1998; Togawa et al. 2012).

Venn diagram analyses showed a greater richness of unique OTUs in the BS soil (101 unique OTUs) and lower richness in NF soil (23 unique OTUs). BSL soil shared more OTUs with other soils than any of the other soils did with each other (Fig. 4a). The NF soil shared 55 % OTUs with other soils. The analysis of overlapping OTUs through Venn diagrams showed that only six OTUs were shared among all soils, and six OTUs were common among sugarcane-cultivated soils. Cluster analysis of libraries based on OTUs revealed that the bacterial community in the NF soil was more similar to GS soil; however, the bacterial community from BS soil was most different from the other soils (Fig. 4b).

To check the differences among the clone libraries, statistical analysis was performed using S-Libshuff (Schloss et al. 2009). A comparison of the four libraries revealed that the bacterial communities are different ($P < 0.05$) for each management regime applied, suggesting that different treatments can influence their composition. The phylogenetic classification of the 949 sequences of the bacterial 16S rRNA gene revealed the presence of 12 different bacterial phyla across of the four different soils: Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria and Verrucomicrobia. Members of the Acidobacteria and Proteobacteria phyla were the most abundant bacteria across all libraries (Fig. 5).

The most abundant phyla found across all soils are presented in Fig. 5, with ranking order as following: Acidobacteria (34 %), Proteobacteria (25 %), Firmicutes (10 %), Verrucomicrobia (8 %), Gemmatimonadetes (2 %), Actinobacteria (1.3 %), Chloroflexi (2 %) and Planctomycetes (1 %). Four other bacterial phyla were found in at least one sample: Armatimonadetes, Nitrospira, Cyanobacteria, and Bacteroidetes. The relative frequencies of the different bacterial groups found in the studied soils by clone libraries of the 16S rRNA bacterial gene were correlated with the chemical constituents of the soil, organized according to the redundancy analysis (RDA). The spatial coordinates of the bacterial community and the chemical properties of the soil are shown in Fig. 6.

The relative abundance of Acidobacteria decreased in the cultivated areas, mainly in the BS soil (Fig. 5). The amount of Acidobacteria in BSL soil was higher

Table 3 OTUs richness and diversity indices calculated based on partial sequences of the bacterial 16S rRNA genes

Parameters	GS	BS	BSL	NF
Chao1	358.38	463.08	420.02	151.07
Jackknife	381.10	497.00	455.40	171.00
Simpson	0.0027	0.0045	0.0028	0.0127
Shannon	5.02	5.06	5.05	4.46
Number of sequences	217	254	226	252
Number of operational taxonomics units	166	185	173	117
Estimated Coverage Index (ECI)	64.9 %	71.2 %	58.8 %	91.6 %

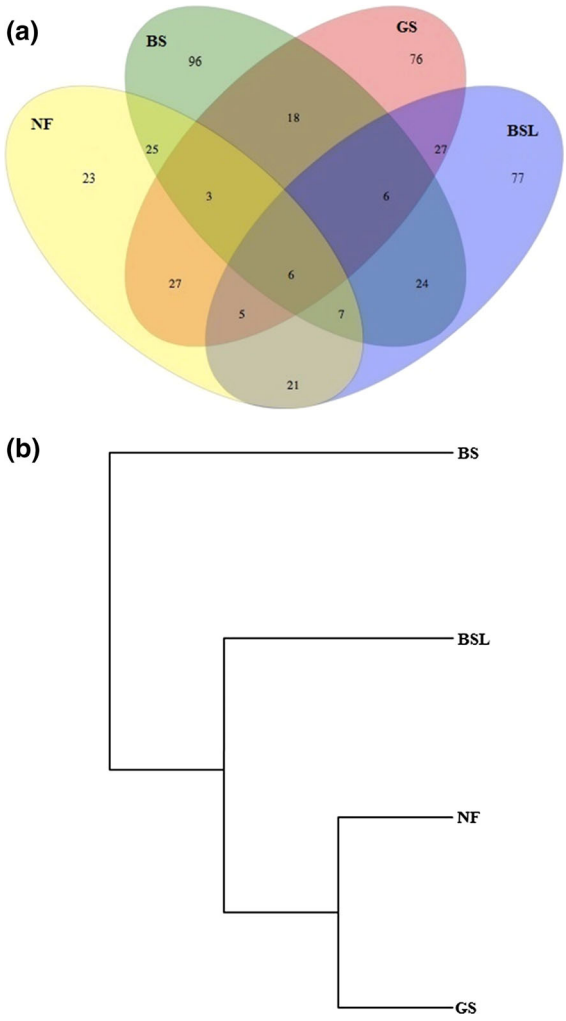

Fig. 4 **a** Venn diagrams based on bacterial OTUs clustered with a 3 % distance threshold showing the number of unique and shared OTUs for the different soils (*GS* Green Sugarcane, *BS* Burnt Sugarcane, *BSL* Burnt Sugarcane with Dolomite, *NF* Native Forest). **b** Cluster analysis of clone libraries of the 16S rRNA sequences in the soil areas studied (*GS* Green Sugarcane; *BS*, Burnt Sugarcane, *BSL* Burnt Sugarcane with Dolomite, *NF* Native Forest). DNA sequences were classified into bacterial phyla using the Ribosomal Database Project II classifier tool

when compared to other sugarcane-cultivated soils. Acidobacteria are among the most common bacteria in soils worldwide (Janssen 2006; Jones et al. 2009; Rachid et al. 2013; Navarrete et al. 2015b). The analysis of 16S rRNA gene sequences has demonstrated that acidobacteria abundance within a community may be regulated by soil pH (Fierer et al. 2007; Lauber et al. 2008; Jones et al. 2009; Rousk et al. 2010; Kuramae et al. 2011) and nutrient availability (Zhao et al. 2014).

Recently, increased attention has been paid to the response of Acidobacteria to environmental changes (George et al. 2009; Naether et al. 2012; Catão et al. 2014). Despite this appreciation for the phylum Acidobacteria, little is still known about the differential response at the subgroup level to alterations in soil chemical properties and fertility, and how their community similarity changes with distance in mosaic landscapes. Navarrete et al. (2013) reported the impact of agricultural management of soybean in Amazon forest soils on the composition of the Acidobacteria community, and they revealed that the abundance of Acidobacteria subgroups was related to soil chemical properties, which were clearly affected by agricultural management. These findings opened the possibility that subgroups of Acidobacteria could be used as management indicators for the consequences of agricultural practices in the Amazon region. Navarrete et al. (2015a) showed that Acidobacteria and their subgroups can act as management indicators in sugarcane-cultivated soils. The present study identified 11 of the current 26 Acidobacteria subgroups, with the subgroups Gp1 and Gp2 being the most frequent across the studied soils (Table 3). Acidobacteria Gp1, Gp2, Gp3, Gp4 and Gp6 were present in all studied soils, and are considered ecologically important due to their high abundance in the total Acidobacteria community together with their ability to use carbon sources that span from simple sugars to more complex substrates such as hemicellulose, cellulose, and chitin, the reduction of nitrate, nitrite, and possibly nitric oxide, iron scavenging and production of antimicrobial compounds (Ward et al. 2009; Rawat et al. 2012). Furthermore, plenty of members of the Acidobacteria Gp1 seem to be controlled by soil pH (Sait et al. 2006) Acidobacteria Gp4 was present only in sugarcane-cultivated soils, while Acidobacteria Gp15 was detected in NF soil only and the Acidobacteria Gp17 and Gp18 were detected in the BSL soils. Navarrete et al. (2015b) revealed that responses of Acidobacteria Gp1, Gp3, Gp4, Gp5, and Gp6 are at least in part through alterations in acidity- and nutrient-related properties of the soil in the Amazon region.

The second most abundant phylum was Proteobacteria, representing 24.3 % for GS soil, 26.5 % for BS soil, 22.6 % for BSL soil and 24.5 % for NF soil. This

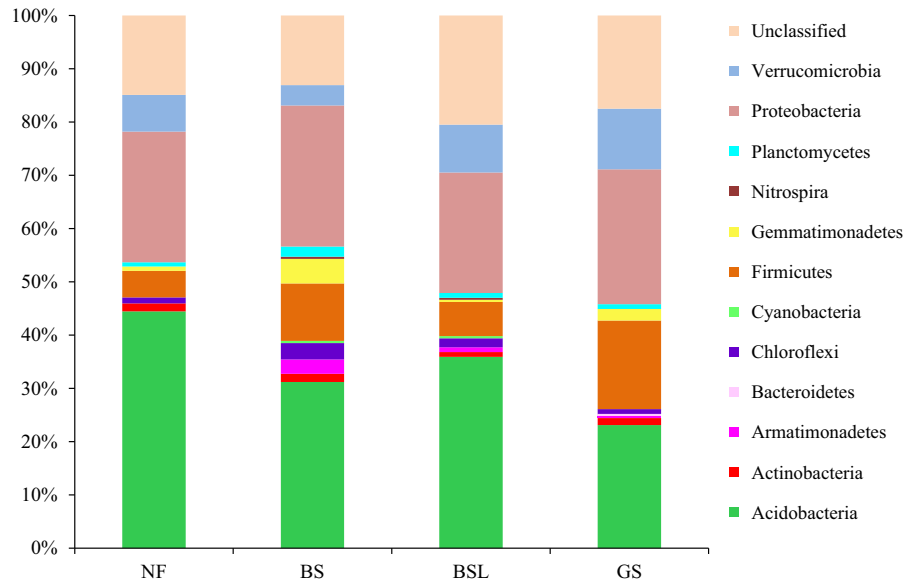

Fig. 5 The relative frequencies of the different bacterial phyla found in the studied soils (*GS* Green Sugarcane, *BS* Burnt Sugarcane, *BSL* Burnt Sugarcane with Dolomite, *NF* Native Forest). DNA sequences were classified into bacterial phyla using the Ribosomal Database Project II classifier tool

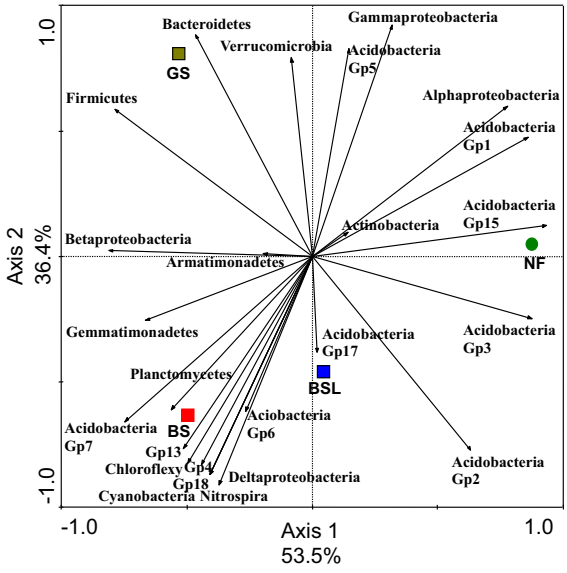

Fig. 6 Constrained ordination diagram for studied soils (*GS* Green Sugarcane, *BS* Burnt Sugarcane, *BSL* Burnt Sugarcane with Dolomite, *NF* Native Forest) in the first two redundancy analysis (RDA) axes based on the relative frequencies of the different taxonomic groups of *Bacteria* found in the studied soils by clone libraries of the 16S rRNA bacterial gene and soil chemical characteristics of the different sampling areas

phylum forms the largest and most diverse group of bacteria, with about 528 genera, has a great diversity of morphology and metabolism, and is present in different environments (Sambrook and Russel 2001). In this study, the class Alphaproteobacteria (Table 4) was predominant in all soils: GS (66.10 %), BS (52.2 %), BSL (71.70 %) and NF (75 %). Alphaproteobacteria are abundant in the upper soil layers, possibly due to the high amount of organic matter (Spera et al. 2001). The present study identified sequences from the Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria classes in the four libraries, with the exception of sequences from the Deltaproteobacteria class in the GS soil.

The phylum Firmicutes was the third most abundant group in both soils under sugarcane cultivation and forest soils, and most of the sequences belonged to the classes Bacilli and Clostridia. These bacteria were detected in all soil samples, with frequencies 5 % for NF, 11 % for BS, 7 % for BSL and 16 % for GS. Representatives of the phyla Cyanobacteria and Nitrospira were only found in BS and BSL soils, and member of the phylum Bacteroidetes were found in the GS soil at a frequency of 0.4 %. Unclassified bacteria were detected in all soil and represented 14.9 % of NF sequences, 13.1 % of BS sequences, 20.5 % of BSL sequences and 17.5 % of GS sequences.

The fourth most abundant phylum in the four libraries was Verrucomicrobia. The phylum Verrucomicrobia is a phylum with few cultivated representatives (Hedlund 2011). Members of the phylum Verrucomicrobia have been shown to make up 1 to

Table 4 Percentage of Acidobacteria subgroups relative to all Acidobacteria in the different clone libraries

Bacterial groups	Clone libraries			
	GS	BS	BSL	NF
Acidobacteria subgroups				
Gp1	43.40	13.60	19.00	41.40
Gp2	24.50	39.50	36.90	31.90
Gp3	9.40	13.60	9.50	16.40
Gp4	1.90	9.90	8.30	nd
Gp5	7.50	1.20	3.60	2.60
Gp6	9.40	9.90	13.10	4.30
Gp7	3.80	6.20	3.60	0.90
Gp13	nd*	4.90	1.20	nd
Gp15	nd	nd	nd	0.90
Gp17	nd	nd	1.20	nd
Gp18	nd	nd	1.20	nd
Unclassified	nd	1.20	2.40	1.70
Proteobacteria class	GS	BS	BSL	NF
Alphaproteobacteria	75.00	5.22	71.70	66.10
Betaproteobacteria	7.80	21.00	9.40	15.30
Gamaproteobacteria	3.10	18.80	11.30	0.00
Deltaproteobacteria	12.50	2.90	7.50	13.60
Unclassified	1.60	4.30	0.00	5.10

* nd indicates that sequences of this subgroup were not detected

10 % of the total bacterial 16S rRNA in soils (Buckley and Schmidt 2001, 2003). Navarrete et al. (2015c) showed a clear link between soil Verrucomicrobia community structure and abundance and fertility status of sugarcane-cultivated soils. Members of Verrucomicrobia are distributed in various habitats and are important to the soil environment. This group of bacteria can be classified in five classes and seven subdivisions (Janssen et al. 2011). The class Spartobacteria was observed in our study, which were detected in all soils with values less than 10 % of the total bacterial sequences. Likewise, Bergmann et al. (2011) have shown members of the class Spartobacteria dominating verrucomicrobia communities in grasslands and subsurface soil horizons.

Representatives of the phyla Cyanobacteria and Nitrospira were detected only in GS and BSL soils, and members of the phylum Bacteroidetes was detected in the GS soil with a frequency of 0.4 %. The phylum Cyanobacteria includes bacteria that have the typical structure of gram-negative cells, and they are photoautotrophic, using CO_2 as a carbon source and H_2O as a reducing agent (Woese et al. 1985; Garrity et al. 2004). Microorganisms respond quickly to natural perturbations and environmental stress due to their short generation time and their intimate relationship with their surroundings, attributed to their higher surface to volume ratio (Sharma et al. 2011).

Conclusion

In this study, we showed that liming in the sugarcane burnt system and the green harvest practice affect soil bacterial community structure, diversity and composition in northeastern São Paulo, Brazil. Although each of the four studied regimes revealed a particular ecological pattern for the soil bacterial community, the burnt systems differed more from soil under natural forest condition. The liming practice revealed changes in soil bacterial community composition for the burnt system in sugarcane croplands, which can be detected shortly after harvest. Changes in the soil bacterial community involved preponderant responses of Acidobacteria and its subgroups, Proteobacteria and Firmicutes, at least in part through alterations in soil organic matter content, soil pH, and soil texture as revealed for the community structure.

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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