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BASALT ROCK IN SUGARCANE GROWN IN FERRALSOLS: CHANGES IN SOIL CHEMISTRY, MINERALOGY, AND MICROBIOLOGY AND IN CROP YIELD

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Thesis submitted to College of Agricultural Sciences, Unesp, Botucatu Campus to obtain the degree of Doctor of Philosophy in Agronomy (Energy in Agriculture).

Supervisor: Prof. Dr. Carlos Alexandre Costa Crusciol

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TÍTULO DA TESE: BASALT ROCK IN SUGARCANE GROWN IN FERRALSOLS: CHANGES IN SOIL CHEMISTRY, MINERALOGY AND MICROBIOLOGY AND IN CROP YIELD

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RESUMO

Visto o cultivo de cana-de-açúcar principalmente em solos brasileiros altamente intemperizados, é necessária uma alternativa para aumentar essa produção, renovando os solos. A remineralização consiste em adicionar rocha moída nos solos, como um condicionador de solo, fornecendo alguns minerais e elementos, além do baixo custo, as consequências desta aplicação não são totalmente elucidadas. Portanto, a hipótese deste estudo inclui o tratamento do pó de rocha basáltica, que pode melhorar o rendimento da cana-de-açúcar, os atributos químicos do solo e da planta; o pó de rocha de basalto aumenta o índice microbiano de qualidade do solo; a aplicação da rocha altera as comunidades microbianas no solo; e o intemperismo das rochas altera a mineralogia do solo. O objetivo deste trabalho foi avaliar o efeito da aplicação de pó de rocha de basalto na cultura da cana-de-açúcar, suas consequências na mineralogia e na microbiologia do solo. Apesar de não ser consistente com as quatro áreas, o tratamento de pó de rocha de basalto pode melhorar o rendimento da cana-de-açúcar, os atributos químicos do solo e o índice microbiano de qualidade do solo, mas pouco é notado nos atributos químicos da planta. A diversidade microbiana não foi a mesma para as quatro áreas, mas pode estar mais relacionada aos padrões geográficos do que à aplicação da rocha, mesmo com uma pequena mudança ocorrendo, não pode ser atribuída ao tratamento. Sinais de intemperismo foram notados, mas há dois pontos questionáveis: o tempo para ocorrer este intemperismo, pode ser mais rápido do que se pensava, e a quantidade de minerais intemperizados nesse tempo. A aplicação de pó de rocha de basalto melhora o rendimento da cana-de-açúcar, destacando-se suas mudanças mineralógicas no solo e não causa danos à diversidade microbiana do solo. A atividade microbiana e as pistas da microbiologia do solo nessas condições poderiam elucidar o motivo da melhoria do rendimento.

Palavras-chave: *Saccharum* spp., Remineralização, rochagem, produtividade de colmos, microbiologia do solo, minerais do solo

ABSTRACT

Since the sugarcane production mostly in highly weathered Brazilian soils, an alternative to increasing its yields, renewing these soils is required. Remineralization consists in add milled rock into the soils, as a soil conditioner, providing some minerals and elements. Besides the low cost, the consequences of their application are not totally elucidated. Therefore, the hypothesis of this study include the basalt rock dust treatment can improve the sugarcane yield, soil and plant chemical attributes; basalt rock dust increases the microbial index of soil quality; the rock application change microbial communities in the soil; and the rock weathering change the soil mineralogy. The aim of this study was evaluate the effects basalt rock dust application on sugarcane crops its consequences in soil mineralogy and microbiology. Despite not consistent to the four areas, basalt rock dust treatment can improve the sugarcane yield, soil chemical attributes, and microbial index of soil quality but a little is noticed in plant chemical attributes. The microbial diversity was not the same to the four areas, but it can be more related to geographical patterns than rock application, even with a little shift occurring, it cannot be attributed to the treatment. Weathering signals were noticed but there are two questionable points: the time to occur this weathering, may be quicker than it was thought, and the amount of weathered minerals. Basalt rock dust application improves sugarcane yield, it was notable its mineralogical changes in the soil and it does not cause damages to the soil microbial diversity. The microbial activity and footprints of soil microbiology in these conditions could elucidate the reason why occurred the yield improvement.

Keywords: *Saccharum* spp., remineralization, rocks for crops, stalk yield, soil microbiology, soil minerals

SUMMARY

GENERAL INTRODUCTION

Sugarcane is the base of one of the major export commodities in Brazil with ethanol and sugar as products, and energy as a principal by-product. Its production in 2018/2019 harvest reached 616 million of tonnes harvested from 8.4 million hectares (CONAB, 2019), resulting in a yield average of 73 tonnes per hectare, and this is the main concern of sugarcane production in Brazil, since there are reports the potential of production is around 300 tonnes per hectare.

In general Brazilian soils are highly weathered, then they are commonly composed by quartz [SiO_2], kaolinite [$Si_2Al_2O_5(OH)_4$], gibbsite [$Al(OH)_3$], hematite [Fe_2O_3], and Goethite [$FeO(OH)$], among others, varying depending on the concentration and the fraction. Specifically Ferralsols contain low availability of Si content, compared to newer soils (TISDALE et al., 1985).

One of the ways to replace those minerals in highly weathered soils is to add milled rocks from diverse sources and components. In soil remineralization, rocks chemical components need to weathering acting to be released from the minerals to soil replacement and to become available to plants and soil organisms. Temperature, water, wind and microorganisms activity are some chemical, physical and biological weathering agents' examples. These rocks contain Si, Ca, Mg, Fe, some trace elements, which can work as growth factor to microorganisms, and heavy metals which can shift the soil chemistry and consequently, biology.

The biogeochemical activities of the microorganisms in the soil are governed by indigenous microbial communities, temperature, pH, soil water capacity, among other environmental conditions, and the soil nutrients availability is the result of biological transformations (WAKSMAN; GERRETSEN, 1931; SCHMIDT et al., 2011).

The nutritional requirement of sugarcane encompass the sixteen nutrients, essential for growth of all plants, as C, H, and O (non-mineral nutrients), primary nutrients (N, P, and K), secondary nutrients (Ca, Mg, S), trace elements (Zn, Cu, Fe, Mn, Cl, B, and Mo), and the beneficial element: silicon (Si) (CALCINO et al., 2018).

Silicon function in plants is related to protection against abiotic and biotic stresses, including reports about its positive effects in chemical and physical soil attributes and it can reach, consequently 17 to 30 % yield improvement (MATICHENKOV; CALVERT, 2002). In the soil, silicate ion neutralize the toxic Al and

compete with phosphate ion for the adsorption sites in ion exchange complex in the soil, dislocating P to the soil solution (PRABAGAR et al., 2011; CASTRO; CRUSCIOL, 2013; EPSTEIN; BLOOM, 2005; POZZA et al., 2007).

Therefore, the hypothesis of this study include the basalt rock dust treatment can improve the sugarcane yield, soil and plant chemical attributes; basalt rock dust increase the microbial index of soil quality; the rock application change microbial communities in the soil; and the rock weathering change the soil mineralogy.

The aim of this study was evaluate the effects basalt rock dust application on sugarcane crops its consequences in soil mineralogy and microbiology.

CHAPTER 1

BASALT ROCK DUST AS A SUPPLEMENTARY FERTILIZER IN SUGARCANE

Abstract

Sugarcane is the main bioenergetic grass crop used in the production of ethanol, sugar, and bioenergy, its high capacity for sequestration of atmospheric CO_2 during development through the production of refractory carbon forms such as phytoliths. However, to reach satisfactory yields, a large amount of synthetic fertilizer is typically used during the crop cycle, causing an increase in agricultural budget and negatively impacting the crops appeal as a source of clean and renewable energy. For this reason, sustainable alternatives such as the addition of rock dusts are being studied to supply the nutritional demands of crops. The aim of this research was to evaluate the effect of basalt rock dust application on the microbial activity and chemical attributes of soil from four sugarcane growing areas, and its impact on the development, yield, nutritional and technological aspects of the crop. The study was carried out in two plant cane areas and two ratoon areas, in São Paulo State, Brazil. A significant increase on stalk yield was observed in all experimental areas submitted to treatment with basalt rock dust, but inconsistent differences for other variables suggest new studies are required to further clarify the mechanistic effects of this practice in agricultural soils.

Key-words: *Saccharum* spp.; remineralizer; soil microbial activity.

Resumo

A cultura da cana-de-açúcar é considerada a principal cultura bioenergética utilizada na produção de etanol, açúcar e bioenergia, seu grande diferencial como gramínea é atribuído ao alto desempenho no sequestro de CO_2 atmosférico durante o desenvolvimento, além da capacidade de acumular energia na forma de sacarose no colmo. Entretanto, para alcançar produtividades satisfatórias, uma grande quantidade de fertilizantes sintéticos é utilizada durante o ciclo da cultura, refletindo assim, no aumento dos custos de produção e impactando negativamente o grande apelo que coloca a cultura em destaque como fonte de energia limpa e renovável. Sendo assim, o objetivo desta pesquisa foi avaliar o efeito da aplicação de pó de rocha basáltica na atividade microbiana e nos atributos químicos do solo, de quatro áreas comerciais de cana-de-açúcar, bem como o impacto no desenvolvimento, produtividade e aspectos nutricionais e tecnológicos da cultura. O estudo foi conduzido em duas áreas de cana-planta e duas áreas de cana-soca, no Estado de São Paulo. Observou-se aumento significativo na produtividade de colmos em todas as áreas experimentais submetidas ao tratamento com pó de rocha basáltica, mas alterações inconsistentes nas demais variáveis sugerem novos estudos para esclarecer melhor os efeitos dessa prática em solos agrícolas.

Palavras-chave: *Saccharum* spp.; remineralizador; atividade microbiana do solo

1.1 INTRODUCTION

Brazil is the world's largest sugar producer, producing 34.6 million tons. Brazil is also the largest producer of ethanol from sugarcane in the world, with 29 billion litres of ethanol, due to the large-scale use of ethanol as a renewable and alternative fuel to oil in Brazil (AGRIANUAL, 2015). However, cane crop yield can be influenced by many factors; one factor is balanced nutrition, which is important in Brazil given the poor fertility in highly weathered Brazilian soils and the observed gradual yield reduction for each harvest (Rossi & Bernardes, 2012).

Despite the importance of the sugarcane crop in Brazil, national income from this crop low, with an average stalk yield of 71 Mg ha^{-1} (AGRIANUAL, 2015), and the main strategy to increase productivity, in addition to the development of improved varieties, is the optimization of the use of resources. Therefore, the importance of investigating renewable alternatives to provide plant nutrition in a sustainable and intelligent way depends on demand.

The mineral nutrition of sugarcane influences its growth and establishment, resistance to climatic changes, photosynthetic processes, control of pests and diseases and crop yield because soil fertility is one of the main limiting factors to greater sugarcane production (Watanabe et al., 2016; Silva et al., 2017; Oliveira et al., 2017). Therefore, it is of fundamental importance to understand the quantity of each nutrient required by the crop, when these nutrients are required in greater amounts, the form of application and availability to the plant to optimize the use of the resources in search of greater crop yields.

To meet the nutritional demand of the crop, a great amount of synthetic fertilizers is used, resulting in high production costs and environmental damage. Thus, it is important to use a multielement fertilizer that releases nutrients slowly because the plant is kept in the field for an average of five years before replanting.

In terms of multielement fertilizer, mining companies have been investing in studying and developing products that can meet the demands of local and national agriculture, prioritizing aspects of quality and standardization of materials that aim to ensure the positive results that are documented in literature.

'Rocks for crops' consists of remineralizing a soil by adding rocks (usually silicate) with a suitable composition that are milled to a desired particle size (Pádua,

2012). This process works like a fertilizer-dependent weathering process that enables its component elements to become available to the soil and plants (Melamed et al., 2009). Particle size distribution can influence weathering, as mineral dissolution is surface-area dependent, which is increased by milling.

There are several rocks that compose as a by-product from quarries and generate benefits to soil and plants, such as Dunite, Phonolite, Sienite, Thermalmagnesium, Micaschist, however, Basalt is the most common rock type in the Earth's crust. Its mineralogical composition is basically pyroxenes (augite), plagioclases (labradorite), and magnetite, and the chemical is SiO2 (43-47%), Al2O3 (11-13%), CaO (10-12%), MgO (8-10%), and many trace elements, constituted by almost all periodic table (Potsch, 1954; Jung & Subramanian, 1993; Militký et al., 2002).

Rock dust has recently been accepted within the Brazilian regulatory system as a ‘remineralizer’ (Ramos et al., 2015), and there is increasing interest in its use. The effect of rock dust on crops depends on the release of plant root exudates, common organic acids that contribute to the weathering process, and their character may change depending on the genetic material (Harley & Gilkes, 2000). Bioweathering promotes an increase in microbial activity following the addition of rock dust to soil; higher microbial activity makes nutrients rapidly available (Hensel, 1894). Therefore, there are challenges to define the recommendations for the application of remineralizer and to achieving success using these newly registered products.

The hypothesis that led to this research is that the supply of basalt rock dust to sugarcane crops promotes increased foliar nutrient concentration and plant development, improved sugarcane technological parameters and crop yield, higher microbial activity in the soil and greater amounts of available nutrients in the soil for the plants.

Thus, the aim of this study was to evaluate the effects of the application of basalt rock dust and its impacts on sugarcane crop yield as well as its interaction with soil microbial activity and fertility.

1.2 MATERIAL AND METHODS

Experimental sites description

Experiments were performed for two consecutive years (2016 – application year and 2017 - residual effects), in four experimental sites named hereafter A, B, C and D, two on plant cane (A and B) and two on ratoon crop (C and D - 1st cut performed on 03/05/2016), located in São Paulo State - Brazil, according to the management described in Table 1.

The predominant climate in the region is Cfa (Köppen), which is mainly temperate humid winter with a hot summer. Mean annual temperatures are 19.1-21.1°C, with 1214-1324 mm range of averages rainfall. The soil texture and chemical attributes within each experimental site were previously characterized and described in Table 2.

Table 1. Site management description, São Paulo State, Brazil, 2016

	Sites			
	A	B	C	D
Coordinates reference[1]				
Latitude*, S*	22°53'13"	22°35'22"	22°23'45"	22°41'10"
Longitude*, W*	48°46'11"	48°43'58"	48°49'45"	48°46'58"
Altitude, *m above sea level*	790	604	563	658
Application, *Crop cycle*	Plant cane	Plant cane	3rd ratoon	3rd ratoon
Variety	RB867515	CV077107	RB966928	RB855156
Row spacement, *m*	1.5	1.5	1.6 x 0.9	1.4 x 0.5
Limestone, *kg ha-1*	2,075	2,000	-	-
Gypsum, *kg ha-1*	-	1,500	-	-
Vinasse, *m³*	-	-	-	90
Formulate fertiliser, *N-P-K*	18-00-27 00-28-00 14-25-13	06-30-16	00-00-60 45-00-00	45-00-00
Fertilizer rates, *kg ha-1*	248 457.5 289	550	180 130	300
Production environment[2]	D1/D2	A2	B2/C1	E2
Soil classification[3]	Rhodic Ferralsols dystric	Rhodic Ferralsols eutroferric	Rhodic Ferralsols dystric	Rhodic Ferralsols dystric
Location, *São Paulo State*	Botucatu	Lençóis Paulista	Pederneiras	Lençóis Paulista

[1] Geographic Coordinate System, SIRGAS 2000 Datum UTM zone 22S
[2] Prado, 2008
[3] IUSS working group WRB, 2014

Table 2. Characterization of soil chemical properties and texture in four experimental sites, São Paulo State, Brazil, 2016.

Sites	pH	OM	P	S	Al^{+3}	$H+Al^{+3}$	K	Ca	Mg	SB	CEC
	$CaCl_2$	$g\ dm^{-3}$	$mg\ dm^{-3}$		$mmol_c\ dm^{-3}$						
					Soil Depth 0.00-0.20 m						
A	6.0	17	15	12	0	14	1.10	25	8	35	48
B	6.0	34	21	5	0	18	1.25	54	21	77	95
C	5.6	16	9	8	0	15	3.20	27	10	39	55
D	5.8	18	6	2	0	14	3.87	19	12	35	49
					Soil Depth 0.20-0.40 m						
A	5.3	14	5	10	0	16	0.48	18	4	22	38
B	5.7	30	22	18	0	24	0.91	44	19	64	88
C	5.3	10	34	2	0	18	0.54	21	7	28	45
D	6.0	10	8	4	0	12	4.96	16	10	31	43

Sites	V%	m%	Fe	Cu	Mn	Zn	B	Si	Sand	Silt	Clay	S/C *ratio*
			$mg\ dm^{-3}$									
					Soil Depth 0.00-0.20 m							
A	72	0	36	4.5	1.2	1.1	0.20	4.20	758	74	168	0.44
B	81	0	11	2.4	3.2	0.6	0.09	8.35	332	197	471	0.42
C	72	0	13	0.5	7.0	0.2	0.06	5.77	705	246	48	5.13
D	71	0	26	0.6	2.8	0.4	0.13	2.40	843	22	135	0.16
					Soil Depth 0.20-0.40 m							
A	58	0	29	2.7	0.6	0.6	0.19	3.33	742	58	200	0.29
B	73	0	12	2.7	2.9	0.9	0.52	8.85	274	163	563	0.29
C	61	0	6	0.5	2.6	0.1	0.09	5.05	702	256	41	6.24
D	72	0	8	0.4	0.6	0.1	0.05	3.37	839	22	139	0.16

OM – organic matter; $H+Al^{+3}$– potential acidity; SB – sum of bases; CEC – cation exchange capacity; V% – base saturation; m% – aluminium saturation.

Experimental design and treatment application

The experiment was arranged in a randomized block design with treatments composed of 1- Control (Rock dust free) and 2- Basalt rock dust application (4 Mg ha^{-1}, respectively), with 12 replicate plots per treatment. Experimental plots consisted of 8 rows (10 m long) disregarding 0.5 m edges at each end. On sites A and B, the incorporation of basalt rock dust was performed with a light harrow during the soil tillage before planting. On sites C and D basalt rock dust was applied over 10 Mg ha^{-1} of sugarcane crop residue (straw and leaves), without soil incorporation.

Basalt rock dust properties

The basalt rock dust was obtained after the crushing of a quarry located in Lençóis Paulista, State of São Paulo, Middle Tietê region. The rocks are derived from the basaltic outcrop of the Serra Geral formation, São Bento Group. The rock dust is composed of 55% plagioclase, 35% pyroxene, 5% magnetite, 5% volcanic glass and 1% apatite, and its chemical composition is presented in Table 3.

Table 3. Basalt rock dust chemical composition. Vancouver, Canada, 2016

SiO_2	**Al_2O_3**	**Fe_2O_3**	**MgO**	**CaO**	**Na_2O**	**K_2O**	**TiO_2**	**MnO**	**P_2O_5**	**LOI**
%	%	%	%	%	%	%	%	%	%	%
48.95	12.26	15.80	4.60	8.44	2.55	1.37	3.79	0.22	0.47	1.2
Ba	**Ce**	**Cr_2O_3**	**La**	**Nb**	**Nd**	**Ni**	**Pb**	**Rb**		
ppm	ppm	%	ppm	ppm	ppm	ppm	ppm	ppm		
441	65.4	0.003	30.3	20.4	34.0	27	1.3	28.2		
Sc	**Sr**	**Th**	**U**	**V**	**Y**	**Zn**	**Cu**	**Zr**		
ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm		
31	483.3	2.7	0.5	475	34.9	66	188.5	215.9		
Eu	**Er**	**Lu**	**Be**	**Dy**	**Co**	**Cs**	**Ga**	**Gd**	**Hf**	**Ho**
ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	Ppm
2.51	3.22	0.43	2	6.42	37.6	0.2	19.7	7.99	5.6	1.18
Pr	**Sm**	**Sn**	**Sum**	**Ta**	**Tb**	**Tm**	**W**	**Yb**	**Ag**	**As**
ppm	ppm	ppm	%	ppm	ppm	ppm	ppm	ppm	ppm	Ppm
8.22	7.44	2	99.68	1.2	1.21	0.46	<0.5	2.90	<0.1	<0.5
Au	**Bi**	**Cd**	**Hg**	**Mo**	**Sb**	**Se**	**Tl**	**B**	**TOT/C**	**TOT/S**
ppb	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	%	%
0.7	<0.1	0.2	<0.01	0.6	<0.1	<0.5	<0.1	8	0.09	<0.02

Source: Acme Analytical Laboratories (Vancouver)

Basalt rock dust particle size distribution were classified according to Table 4.

Table 4. Basalt rock dust particle size analysis, São Paulo State, Brazil, 2015.

	Particle diameter	BRD
	mm	*%*
Very course sand	2.00	0.54
Course sand	1.00	0.13
Medium sand	0.50	10.9
Fine sand	0.25	23.3
Very fine sand	0.10	28.3
Silt	0.05	35.2

Sampling and evaluations

Leaf nutrient concentration

During the period of full vegetative growth of sugarcane leaf nutrient concentration was analysed using the methods of Malavolta et al. (1997) for macronutrients, micronutrients and Si.

Sugarcane biometric and technological parameters

Before harvest, the stalk number m^{-1} was calculated by assessment of a 5 m metal quadrat, counting the stalks located in the two central rows (10 meters total counting) within the useful area of each plot and then converted to number of stalks m^{-1}. Stalk weight, diameter, plant height, internodes number and length were calculated as the means for ten stems randomly collected from each plot, clipped at apical bud height, defoliated and measured with a digital scale, caliper and a ruler marked in meters from the soil surface up to the auricle region of the $^{+}1$.

The technological evaluations of sucrose, purity, fiber, reducing sugars (RS) and total reducing sugars (TRS) concentrations as described by Fernandes (2003). On site A the evaluation of variables including stalk diameter, plant height, internodes number and length, sucrose, purity, fiber, RS and TRS were not performed and results not shown.

Stalk and sugar yield

At the time of harvesting, the four central rows of each plot were mechanically harvested, and stalks weighed. Then, the stalk yield was estimated extrapolating the values to stalk yield ha^{-1}, disregarding planting holes (gaps greater than 0.5 m). Sugar yield ha^{-1} was estimated as the product of the multiplication of sucrose concentration (%) by stalk yield (Mg ha^{-1}) at harvest. This evaluation was not performed on site A.

Soil chemical attributes

After harvest, five subsamples were taken at random from each plot and combined into one composite sample and chemical attributes were determined following the method of Raij et al. (2001).

Microbial Activity

The analysis of the microbial activity was performed using a portion of each 0-20 cm sample submitted to storage in the freezer for further evaluation of basal respiration by the method of Öhlinger (1993). The remaining portion was oven dried at 30 ° C for 24 hours and then stored in the refrigerator before determining dehydrogenase activity, following the method of Alef (1995) and carbon assessment of microbial biomass by the irradiation method of Islam and Weil, 1998. The metabolic quotient (qCO_2) was calculated as the ratio between the microbial respiration and the microbial biomass

carbon (mg C-CO_2 mg C mic^{-1} day^{-1}), representing the amount of carbon released as CO_2 by microbial biomass over time; if high, this means that the microorganisms are under stress.

Statistical analysis

Data were subjected to ANOVA using Sisvar Software (FERREIRA, 2008) and means compared by LSD test at the 90% probability level ($p<0.10$).

1.3 RESULTS

Leaf nutrient concentrations

Foliar nutrient concentrations were influenced by the treatments, but this effect was not observed at all four sites (Table 5). Among the four sites and two years, there were found 8 higher nutrients concentration results with BRD treatment in 2016 (1st year) and 12 in 2017 (residual effects).

Leaves from site A showed greater foliar Mn concentration in the 1st year evaluations and greater Mg, S, Cu and Mn concentrations in the 2nd year with the basalt rock dust treatment than in control (Table 5). At site B, leaves of the same treatment presented lower foliar Si concentration in the 1st year than the 2nd year, but average foliar P, Mg, B and Cu concentrations were higher in the 2nd year than the 1st year for treatment with basalt rock dust (Table 5).

At site C, increased foliar B concentration was observed for the treatment with basalt rock dust in the 1st year, but the opposite occurred for foliar K, B, Fe, Mn, Zn and Si concentrations in the 2nd year within the same treatment. The BRD treatment at site D increased foliar N, Ca, Mg, Cu, Mn and Si in the 1st year and K, B and Cu in the 2nd year (Table 5).

Table 5. Leaf macro, micronutrient, and silicon concentrations in four commercial areas due to the application of basalt rock dust in plant cane (1st year - 2016), and residual effect in the 1st ratoon (2nd year - 2017), and in 3rd ratoon (1st year - 2016) and residual effect in 4th ratoon (2nd year - 2017), São Paulo State, Brazil, 2016/17.

Treatments	N		P		K		Ca		Mg		S	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Site A						g kg^{-1}						
Control[2]	19 a	19 a	2,4 a	2,2 a	8,0 a	9,3 a	6,8 a	5,7 a	2,0 a	1,8 b	1,4 a	1,2 b
BRD[3]	18 a	20 a	2,1 b	2,0 b	8,6 a	9,1 a	6,0 b	5,4 a	1,9 a	2,0 a	1,3 a	1,5 a
p[1]	0,126	0,281	<0,001	0,018	0,188	0,709	0,001	0,145	0,142	0,096	0,308	<0,001
Site B												
Control[2]	16 a	17 a	1,8 a	1,7 b	11 a	13 a	3,1 a	6,2 a	1,3 a	1,1 b	1,3 a	1,2 a
BRD[3]	17 a	18 a	1,8 a	1,8 a	11 a	13 a	2,9 a	5,5 b	1,2 a	1,2 a	1,4 a	1,3 a
p[1]	0,594	0,229	0,285	0,056	0,407	0,137	0,164	0,017	0,258	0,096	0,391	0,336
Site C												
Control[2]	18 a	18 a	1,9 a	1,8 a	11 a	14 a	6,5 a	6,9 a	1,5 a	1,4 a	1,5 a	1,4 a
BRD[3]	17 a	18 a	1,9 a	1,7 a	11 a	13 b	6,8 a	6,8 a	1,5 a	1,4 a	1,4 a	1,4 a
p[1]	0,655	0,955	0,937	0,375	0,635	0,079	0,098	0,204	0,664	0,759	0,191	0,372
Site D												
Control[2]	17 b	18 a	2,2 a	1,8 a	11 a	9,9 b	2,8 b	6,2 a	1,6 b	1,9 a	1,2 a	1,0 a
BRD[3]	18 a	18 a	2,2 a	1,8 a	12 a	11 a	3,5 a	6,5 a	1,8 a	2,0 a	1,3 a	1,0 a
p[1]	0,097	0,238	0,576	0,797	0,160	0,016	<0,001	0,126	<0,001	0,175	0,216	0,752
	B		Cu		Fe		Mn		Zn		Si	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Site A						mg kg-1						g kg-1
Control[2]	13 a	17 a	9,9 a	6,6 b	71 a	94 a	25 b	32 b	20 a	20 a	11 a	10 a
BRD[3]	11 a	12 b	6,7 b	7,5 a	64 a	98 a	31 a	43 a	18 b	20 a	5,3 b	6,0 b
p[1]	0,147	<0,001	<0,001	0,001	0,096	0,321	0,010	<0,001	<0,001	0,943	<0,001	<0,001
Site B												
Control[2]	15 a	14 b	9,6 a	5,6 b	64 a	97 a	31 a	43 a	16 a	14 a	7,6 a	3,4 b
BRD[3]	16 a	17 a	9,7 a	6,1 a	62 a	99 a	30 a	37 b	15 a	15 a	5,5 b	8,3 a
p[1]	0,310	<0,001	0,831	0,019	0,620	0,298	0,537	<0,001	0,119	0,130	0,048	<0,001
Site C												
Control[2]	14 b	16 a	7,0 a	5,8 a	74 a	78 a	65 a	72 a	18 a	18 a	5,8 a	7,2 a
BRD[3]	17 a	13 b	6,7 a	5,9 a	69 a	65 b	69 a	66 b	18 a	15 b	4,0 a	6,3 b
p[1]	0,023	<0,001	0,292	0,558	0,128	<0,001	0,424	<0,001	0,940	<0,001	0,079	0,018
Site D												
Control[2]	20 a	12 b	9,7 b	3,5 b	58 a	84 a	22 b	45 a	17 a	22 a	3,7 b	14 a
BRD[3]	18 a	15 a	11 a	4,1 a	63 a	76 b	25 a	32 b	18 a	19 b	5,3 a	13 b
p[1]	0,248	<0,001	<0,001	0,003	0,168	<0,001	0,021	<0,001	0,226	<0,001	0,049	0,052

[1]*F probability:* Values followed by different letters, presented vertically, differ significantly by the LSD test, at 10% probability. [2]Control (0 Mg ha^{-1}); [3]Application of 4 Mg ha^{-1} of basalt rock dust.

Sugarcane biometric and technological parameters

In the 1st year, the stalk number m^{-1} was influenced by treatments only at site C, with a 26% increase for the treatment with basalt rock dust in comparison to that with the control. Similarly, in the 2nd year, this stalk number was influenced by treatments on sites A and D, and in comparison to the control, the treatment with basalt rock dust increased the stalk number m^{-1} by 18 and 10%, respectively. Stalk diameter was not influenced by treatments in both years at all sites. Plant height was influenced by the treatments at sites B and D in the 1st year, and at these sites, in comparison to the control, the basalt rock dust treatment increased this variable by 4 and 10%, respectively (Table 6).

The number of internodes was influenced by the treatments only in the 1st year. At site C, in comparison to the control, the basalt rock dust treatment showed a 4% increase in the number of internodes. Similarly, increases of 8, 7 and 10% in internode

length at sites B and D in the first year and at site B in the second year, respectively, were found for the basalt rock dust treatment (Table 6).

Table 6. Sugarcane biometric parameters, stalk yield, technological quality (sucrose concentration, purity, RS, TRS e fiber) and sugar yield (TSH) in four commercial areas due to the application of basalt rock dust in plant cane (1st year - 2016), and residual effect in the 1st ratoon (2nd year - 2017), and in 3rd ratoon (1st year - 2016) and residual effect in 4th ratoon (2nd year - 2017) and F probability, 2016/17.

Treatments	Plant cane application sites				3rd ratoon application sites			
	2016		2017		2016		2017	
	A	B	A	B	C	D	C	D
				Stalk number, m^{-1}				
Control[2]	8.4a	11a	6.2b	20a	8.1b	8.4a	17a	19b
BRD[3]	6.6a	11a	7.5a	21a	11a	8.3a	17a	21a
p[1]	0.131	0.320	0.003	0.253	<0.001	0.716	0.104	0.002
				Stalk diameter, mm				
Control	-	25a	-	25a	22a	22a	23a	22a
BRD	-	25a	-	25a	22a	22a	24a	21a
p	-	0.949	-	0.327	0.988	0.424	0.736	0.115
				Plant height, m				
Control	-	2.6b	-	2.4a	2.9a	1.8b	3.0a	1.9a
BRD	-	2.7a	-	2.4a	2.9a	2.0a	3.0a	1.9a
P	-	0.001	-	0.370	0.762	<0.001	0.552	0.448
				Internodes number, plant^{-1}				
Control	-	21a	-	18a	23b	18a	26a	17a
BRD	-	21a	-	18a	24a	18a	25a	17a
P	-	0.274	-	0.157	0.079	0.791	0.136	0.630
				Internodes length, cm				
Control	-	12b	-	13b	13a	10b	12a	11a
BRD	-	13a	-	14a	12a	11a	12a	11a
P	-	0.020	-	0.049	0.171	<0.001	0.116	0.313
				Stalk weight, kg				
Control	1.0b	1.5b	1.7b	1.4a	1.8a	1.6b	1.7a	1.8a
BRD	1.2a	1.6a	2.0a	1.4a	1.7a	1.8a	1.8a	1.8a
P	<0.001	0.024	0.028	0.998	0.573	0.007	0.220	0.779
				Stalk Yield, Mg ha^{-1}				
Control	82b	106a	68b	190b	113b	145b	125a	178b
BRD	92[a]	110a	106a	197a	155a	158a	128a	196a
P	<0.001	0.149	<0.001	0.006	<0.001	0.003	0.270	<0.001
				Fiber, %				
Control	-	12a	-	11 a	12a	13a	12a	12a
BRD	-	12a	-	11 a	11b	12b	12a	12a
p	-	0.711	-	0.810	0.016	0.042	0.151	0.535
				Sucrose concentration, %				
Control	-	14a	-	13b	14a	14a	15a	17a
BRD	-	14a	-	14a	13a	14a	14b	17a
p	-	0.741	-	0.009	0.266	0.579	0.041	0.696
				Purity, %				
Control	-	86a	-	84b	86a	87a	88a	91a
BRD	-	86a	-	85a	86a	87a	87b	91a
p	-	0.558	-	0.028	0.218	0.928	0.069	0.502
				Reducing sugars, %				
Control	-	0.58a	-	0.64a	0.58a	0.55a	0.5a	0.4a
BRD	-	0.57a	-	0.62b	0.61a	0.55a	0.6a	0.5a
p	-	0.528	-	0.028	0.167	0.765	0.116	0.445
				Total reducing sugars, %				
Control	-	142a	-	131b	139a	140a	145a	168a
BRD	-	141a	-	136a	135a	141a	140b	167a
p	-	0.620	-	0.009	0.273	0.539	0.040	0.707
				Sugar yield, Mg ha^{-1}				
Control	-	15a	-	25b	16b	20b	18a	30b
BRD	-	16a	-	27a	21a	22a	18a	33a
p	-	0.180	-	<0.001	<0.001	0.007	0.865	0.005

[1]*F probability:* Values followed by different letters, presented vertically, differ significantly by the LSD test, at 10% probability. [2]Control (0 Mg ha^{-1}); [3]Application of 4 Mg ha^{-1} of basalt rock dust.

The highest stalk weight means were found for the treatment with basalt rock dust at site A in both years, with increases of 17 and 15%, respectively. At sites B and D, in comparison to the control, the basalt rock dust treatment influenced stalk weight only in the 1st year with increases of approximately 7 and 12%, respectively (Table 6).

Fiber percentage was only influenced by treatments in the first year, and at sites C and D, in comparison to the control, the treatment with basalt rock dust showed an 8% decrease in fiber concentration (Table 6).

Sucrose, purity, RS and TRS concentrations were influenced by treatments only in the 2nd year at sites B and C (except RS). The treatment with basalt rock dust increased the concentration of sucrose, purity and TRS by 8, 2 and 4%, respectively, at site B. However, the same treatment at site C proportionally decreased the concentration of these variables. The concentration of RS at site B also decreased by 4% in the treatment with basalt rock dust (Table 6).

Stalk and sugar yield

Stalk and sugar yields were influenced by treatments, and the highest means were found for treatment with basalt rock dust in both years at all sites, except for site B in the 1st year and site C in the 2nd year, where no significant difference was observed.

Soil chemical attributes

For the soil chemical properties, among the four sites, two years, and both depths, there were found 16 higher results means with BRD treatment in 2016 (1st year) and 8 in 2017 (residual effects).

At site A, samples from a depth range of 0.0-0.20 m showed higher soil pH, K, and B in the 1st year than the 2nd year and only higher K in the 2nd year than in the 1st year. For the treatment with basalt rock dust, S, K, B, and Si were higher the 1st year than in the 2nd year, and K and Fe were higher in the 2nd year than in the 1st year at a depth of 0.20-0.40 m (Table 7).

Table 7. Chemical soil properties in two commercial areas of sugarcane due to the application of basalt rock dust (BRD) in plant cane (1st year – 2016) and residual effect in the 1st ratoon crop (2nd year - 2017) and F probability. Lençóis Paulista, São Paulo State, Brazil, 2016/17.

Treatments	pH		P		S		Al^{+3}		K		Ca		Mg	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
	--$CaCl_2$--		*----------mg dm^{-3}----------*						*---------- $mmol_c$ dm^{-3}----------*					
Site A							*Soil depth 0.0 - 0.20 m*							
Control[2]	5.2 b	5.5 a	12 a	37 a	3.5 a	8.4 a	0.00 b	0.00 b	0.31 b	0.27 b	24 a	29 a	6.2 a	7.9 a
BRD[3]	5.4 a	5.0 b	6.0 b	15 b	3.7 a	4.4 b	0.28 a	0.73 a	0.46 a	0.48 a	19 b	12 b	6.1 a	4.6 b
p[1]	0.002	<0.001	<0.001	<0.001	0.562	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.608	<0.001
							Soil depth 0.20 - 0.40 m							
Control	5.2 a	5.4 a	8.6 a	17 a	3.6 b	6.8 a	0.21 b	0.10 b	0.30 b	0.15 b	21 a	21 a	4.8 a	6.5 a
BRD	5.1 a	4.9 b	6.1 b	8.5 b	4.3 a	4.2 b	0.68 a	1.8 a	0.40 a	0.29 a	18 b	6.7 b	5.1 a	3.4 b
p	0.852	0.001	<0.001	<0.001	0.035	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	0.183	<0.001
Site B							*Soil depth 0.0 - 0.20 m*							
Control	5.7 a	5.5 a	33 a	26 a	6.8 b	27 a	0.00	0.00	0.57 a	3.7 a	57 a	39 a	16 a	17 a
BRD	5.6 a	5.5 a	19 b	19 b	13 a	29 a	0.00	0.00	0.57 a	3.9 a	54 a	36 a	15 b	15 a
p	0.331	0.883	<0.001	<0.001	<0.001	0.141	-	-	0.948	0.620	0.339	0.150	0.024	0.327
							Soil depth 0.20 - 0.40 m							
Control	5.0 a	5.2 a	15 a	35 a	25 a	41 a	1.8 a	0.01 b	0.30 b	2.2 a	30 a	22 a	10 a	11 a
BRD	5.1 a	4.9 b	16 a	11 b	24 a	51 a	0.83 b	0.48 a	0.34 a	2.1 a	30 a	15 b	8.9 b	8.2 b
p	0.892	0.008	0.206	<0.001	0.800	0.160	<0.001	<0.001	<0.001	0.454	0.936	<0.001	0.002	<0.001

Treatments	V%		Cu		Mn		Zn		B		Si	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
	------%------		*----------mg dm^{-3}----------*									
Site A							*Soil depth 0.0 - 0.20 m*					
Control	65 a	72 a	5.7 a	4.5 a	3.8 a	3.3 a	1.3 a	0.98 a	0.18 b	0.18 a	4.3 a	4.3 a
BRD	57 b	54 b	1.2 b	1.4 b	2.8 b	1.7 b	0.73 b	0.58 b	0.27 a	0.17 b	4.4 a	3.1 b
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.051	0.042	0.675	<0.001
							Soil depth 0.20 - 0.40 m					
Control	60 a	65 a	4.4 a	3.0 a	2.4 a	1.9 a	0.88 a	0.73 a	0.16 b	0.18 a	3.7 b	3.9 a
BRD	53 b	44 b	1.0 b	0.92 b	2.3 a	1.2 b	0.43 b	0.39 b	0.25 a	0.17 a	4.4 a	3.0 b
p	0.059	<0.001	<0.001	<0.001	0.158	<0.001	<0.001	<0.001	<0.001	0.168	0.002	<0.001
Site B							*Soil depth 0.0 - 0.20 m*					
Control	75 a	74 a	1.7 b	1.9 a	2.0 a	2.5 a	0.43 b	0.48 a	0.23 a	0.21 b	9.8 a	7.4 a
BRD	74 a	71 a	1.9 a	1.4 b	1.7 b	2.0 b	0.50 a	0.38 a	0.11 b	0.27 a	9.7 a	6.6 b
p	0.512	0.111	0.014	<0.001	<0.001	0.038	0.077	0.147	<0.001	0.034	0.732	0.007
							Soil depth 0.20 - 0.40 m					
Control	52 b	58 a	1.8 b	1.9 a	1.1 a	1.5 a	0.38 b	0.33 a	0.23 a	0.20 b	7.5 a	6.7 a
BRD	55 a	47 b	2.1 a	1.4 b	1.1 a	1.2 b	0.52 a	0.16 b	0.10 b	0.27 a	7.8 a	5.7 b
p	0.019	<0.001	<0.001	<0.001	0.659	0.015	<0.001	<0.001	<0.001	<0.001	0.304	0.002

[1]*F probability*: Values followed by different letters, presented vertically, differ significantly by the LSD test, at 10% probability. [2]Control (0 Mg ha^{-1}); [3]Application of 4 Mg ha^{-1} of basalt rock dust. V% – base saturation

Increases in soil S, Fe, Cu and Zn concentrations in the 1st year and B concentration in the 2nd year were found at the 0.0-0.20 m depth on site B for treatment with basalt rock dust. Similarly, soil K, Cu and Zn concentrations decreased and soil V% increased. However, in the 2nd year, only the soil B concentration increased at the 0.20-0.40 m depth at the same site and treatment (Table 7).

Treatment with basalt rock dust increased soil Ca and S in the 1st year at site D at the 0.0-0.20 m depth, while at the 0.20-0.40 m depth, increased P and Ca concentrations were found. In the 2nd year, no significant difference was observed (Table 8).

Table 8. Chemical soil properties in two commercial areas of sugarcane due to the application of basalt rock dust in 3rd ratoon (1st year - 2016) and residual effect on 4th ratoon crop (2nd year - 2017) and F probability. Lençóis Paulista, São Paulo State, Brazil, 2016/17.

Treatments	pH		P		S		Al^{+3}		K		Ca		Mg	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
	--$CaCl_2$--		*----------mg dm^{-3}----------*				*---------- $mmol_c$ dm^{-3}----------*							
Site C							*Soil depth 0.0 - 0.20 m*							
Control[2]	5.1 a	5.0 a	4.0 b	8.8 b	14 b	10 a	1.2 a	1.1 b	1.7 b	1.5 b	16 a	12 a	5.1 a	4.8 a
BRD[3]	4.9 a	4.8 a	4.6 a	20 a	20 a	8.8 a	1.3 a	1.9 a	2.2 a	1.8 a	15 b	11 b	4.7 a	4.5 b
p[1]	0.308	0.115	<0.001	<0.001	<0.001	0.009	0.388	<0.001	<0.001	0.009	<0.001	<0.001	0.218	0.052
							Soil depth 0.20 - 0.40 m							
Control	4.8 a	5.1 a	4.3 b	10 b	11 b	13 a	2.1 a	0.78 b	0.87 b	2.0 a	10 a	12 a	3.0 a	4.8 a
BRD	4.7 a	4.6 b	4.6 a	23 a	13 a	7.8 b	2.0 a	3.7 a	0.97 a	1.2 b	10 a	7.6 b	3.2 a	2.8 b
p	0.329	0.005	0.062	<0.001	<0.001	<0.001	0.290	<0.001	0.002	<0.001	0.768	<0.001	0.481	<0.001
Site D							*Soil depth 0.0 - 0.20 m*							
Control	5.5 a	5.2 a	6.8 a	4.7 a	3.2 a	8.0 b	0.00 a	0.46 a	1.7 a	1.5 a	16 b	9.8 a	6.4 a	4.4 b
BRD	5.6 a	5.2 a	5.6 b	6.5 a	3.5 a	10 a	0.00 a	0.58 a	1.6 a	1.3 b	18 a	11 a	6.9 a	4.9 a
p	0.719	0.476	<0.001	<0.001	0.200	<0.001	-	0.002	0.687	0.079	0.056	0.115	0.163	0.012
							Soil depth 0.20 - 0.40 m							
Control	5.4 a	5.0 a	5.2 b	5.1 a	3.8 a	8.6 a	0.00 a	1.0 a	2.2 a	1.4 a	12 b	6.4 b	6.0 a	3.3 b
BRD	5.4 a	5.1 a	6.2 a	5.3 a	4.2 a	11 a	0.00 a	1.0 a	2.2 a	1.4 a	13 a	7.0 a	6.1 a	3.6 a
p	0.618	0.567	<0.001	0.302	0.110	<0.001	-	0.670	0.788	0.630	0.016	0.089	0.818	0.004

Treatments	V%		Cu		Mn		Zn		B		Si	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
	------%-----		*----------mg dm^{-3}----------*									
Site C							*Soil depth 0.0 - 0.20 m*					
Control[2]	51 a	53 a	0.56 a	0.77 a	6.5 b	6.6 a	0.33 b	0.63 a	0.35 a	0.28 a	5.7 a	5.2 a
BRD[3]	50 b	44 b	0.59 a	0.70 a	9.1 a	5.0 b	0.37 a	0.63 a	0.24 b	0.23 b	5.3 b	4.1 b
p[1]	0.015	<0.001	0.401	0.231	<0.001	<0.001	<0.001	0.828	<0.001	<0.001	<0.001	<0.001
							Soil depth 0.20 - 0.40 m					
Control	36 b	53 a	0.52 a	0.78 a	4.4 b	6.5 a	0.15 b	0.63 a	0.29 a	0.30 a	4.7 a	5.0 a
BRD	38 a	37 b	0.41 b	0.73 a	5.3 a	4.6 b	0.19 a	0.77 a	0.19 b	0.22 b	5.2 a	4.2 b
p	0.055	0.002	<0.001	0597	<0.001	0.020	<0.001	0.597	0.021	0.026	0.162	<0.001
Site D							*Soil depth 0.0 - 0.20 m*					
Control	66 a	55 b	0.68 a	0.42 a	5.1 a	1.5 a	1.4 a	0.36 a	0.26 a	0.20 b	4.5 a	2.6 b
BRD	66 a	57 a	0.64 a	0.40 a	4.0 b	1.3 b	0.54 b	0.25 b	0.27 a	0.27 a	4.4 a	2.8 a
p	0.929	0.049	0.303	0.496	<0.001	0.003	<0.001	<0.001	0.582	<0.001	0.520	0.003
							Soil depth 0.20 - 0.40 m					
Control	59 a	48 b	0.70 a	0.42 a	3.2 a	0.85 a	0.97 a	0.23 a	0.31 a	0.20 b	4.4 a	2.4 a
BRD	61 a	50 a	0.68 a	0.37 b	2.4 b	0.75 b	0.34 b	0.18 a	0.30 a	0.26 a	4.0 b	2.5 a
p	0.502	0.032	0.201	0.033	<0.001	0.006	<0.001	0.139	0.429	<0.001	0.011	0.516

[1]*F probability:* Values followed by different letters, presented vertically, differ significantly by the LSD test, at 10% probability. [2]Control (0 Mg ha^{-1}); [3]Application of 4 Mg ha^{-1} of basalt rock dust. V% – base saturation

At site C, the soil P, S, K, Ca, V%, Mn, and Zn concentrations were influenced by the treatments the 1st year, at the 0.0-0.20 m depth, with the highest means observed for soil P, S, K, Mn and Zn concentrations, but decreased means for soil Ca

concentration and V% were found for the treatment with basalt rock dust. In the 2nd year at the same site and depth, beneficial effects of soil P and K concentrations were found for the treatment with basalt rock dust. For the 2nd year, only an increase in soil P concentration was observed within the same site and treatment (Table 8).

Soil microbial activity

Dehydrogenase enzyme activity showed an increase at sites A, B and C for the treatment with basalt rock dust (Table 9).

Table 9. Dehydrogenase, basal respiration, microbial biomass carbon (C) and metabolic quotient (Q), in four commercial areas of sugarcane crop due to the application of basalt rock dust in plant cane and 3rd ratoon and F probability. Presidente Prudente, São Paulo State, Brazil, 2016.

Treatments	Dehydrogenase *µg TTF g^{-1} soil*		Basal Respiration *mg CO_2 g^{-1} soil*		Biomass C *mg C kg^{-1} soil*		Metabolic Q *mg C-CO_2 mg C mic day^{-1}*	
Site	A	B	A	B	A	B	A	B
Control[2]	2.03b	1.59b	2.54a	1.38b	65.7a	63.3b	0.04a	0.01b
BRD[3]	3.08a	2.17a	3.08a	2.25a	69.5a	124a	0.05a	0.04a
p[1]	0.007	0.004	0.130	0.012	0.706	<0.001	0.579	<0.001
Site	C	D	C	D	C	D	C	D
Control	3.25a	1.54b	1.65a	3.17a	64.7b	75.7a	0.03a	0.04a
BRD	3.46a	3.08a	1.75a	2.98a	164a	56.6b	0.01b	0.06a
p	0.414	<0.001	0.748	0.842	<0.001	0.024	0.002	0.376

[1]*F probability:* Values followed by different letters, presented vertically, differ significantly by the LSD test, at 10% probability. [2]Control (0 Mg ha^{-1}); [3]Application of 4 Mg ha^{-1} of basalt rock dust.

The basal respiration rate increased at site B with basalt rock dust. The highest means of carbon of microbial biomass were found for treatment with basalt rock dust at sites B and C, but at site D, the carbon of microbial biomass improved without basalt rock dust application (Table 9).

The metabolic quotient increased at site B for the treatment with basalt rock dust and increased for the control at site C (Table 9).

1.4 DISCUSSION

Leaf nutrient concentration

Regarding foliar nutrient concentrations, the plants showed adequate levels of N, P, Ca, Mg, B, Cu, Fe, Mn and Zn at site A. The same was observed for the nutrients P, K, Ca, Mg, B, Cu, Fe, Mn and Zn at site B; P, K, Ca, Mg, B, Cu, Fe, Mn and Zn at site C; and P, K, Ca, Mg, B, Cu, Fe and Zn at site D (Raij et al., 1997).

The reduced foliar nutrient concentrations that were observed for basalt rock dust treatment, beyond the microbial activity changes, were likely accompanied by hormonal effects in the plants and consequently nutrient demand as well, which could also have been related to higher biomass production stalk yield. This result could have occurred from the ‘dilution effect’ of the nutrients in the plants (Malavolta et al., 1997).

Therefore, considering that the nutrients available to plants can influence their development, low nutritional availability can result in less biomass production, with a low dilution effect, and as a consequence, foliar nutrient concentrations can show the same means or higher means than those found in plants that had high or adequate nutrient availability. Therefore, improved biomass production and expression of the ‘dilution effect’ means that plant growth speed (in this case, it was observed for the stalks) is proportionally higher than the uptake speed of some or all nutrients (Malavolta et al., 1997).

Consequently, the leaf nutrient concentration alone is conclusive enough to explain the nutrition status of plants, so other alternative parameters are needed, such as nutrient accumulation (Silva et al., 2012).

Leaf nutrient concentration could also be associated with competitive inhibition between Mn and other metallic cationic micronutrients, which is influenced by environmental conditions (soil and water availability), species and varieties of the same species (Malavolta, 2004).

The greater plant height (sites B and D) and the heavier stalks (sites A, B and D) were due to higher nutrient availability by the basalt rock dust, which improved plant development it was not verified by Souza et al. (2013) and Cordido et al. (2015) applied rock dust, in manioc and maize plants.

Biometric and technological parameters

Rock dust influenced the stalk weight in this study, a result that was also observed by Mafra et al. (2016) increases in beans plant weight using conventional inorganic fertilization.

Silverol and Machado Filho (2007), using rock dust instead of synthetic fertilization for maize, found that rock dust treatments provided adequate crop development results compared to that provided by the control, but rock dust was

inferior to inorganic fertilization, probably due to higher fertilizer solubility, thus highlighting the importance of long-term rock dust use.

The stalk yield increase found in the basalt rock dust treatment is consistent with results from studies by Theodoro et al. (2012) who reported increases in maize yield with rock dust application, in addition to increases in soil water holding capacity, higher crop biomass and increases in biometric parameters, including greater tillering (Theodoro, 2013).

According to Theodoro et al. (2012), maize and bean crops showed improvements in yield of approximately 20% in both grain crops and in tuber yield by approximately 30%. In this paper, the basalt rock dust application in the sugarcane experimental sites A and B showed increases in stalk yields of 12 and 4%, respectively. In the ratoon crop sites C and D, the improvements were approximately 37 and 10%, respectively. Therefore, 6 in 8 harvests had the yield increased.

Soil chemical properties

In terms of soil chemical properties, more changes were observed at the ratoon sites probably because the basalt rock dust superficial application. However, the sugarcane roots frequently reach deeper layers and the yield may be improved if basalt rock is in touch within the range of the activity of the whole root.

Large changes in soil Si concentration were not observed, and higher values were found at site B, which is the site with higher clay content, than at the other sites, thus showing the relation between both factors, a result that is in agreement with the result of Raij and Camargo (1973). Soil P values at site B remained the same over the 2 years at the 0.0-0.20 m depth, and there was a slight increase in site D. In contrast, a large increase in soil P values at site C, mainly in the 2nd year, was observed at both soil depths. This increase could have been an effect of the adopted synthetic fertilizer management, but this approach was applied in both treatments. In addition, 2-fold increases were found for the treatment with basalt rock dust in comparison to the control.

Therefore, a different scenario must be occurring; thus a question arises as to whether the available P from the retained pool was already in the soil, and whether the Si took its place in the Fe and Al oxides. Alternatively, two other questions arise, which are the following: was the available P provided by the rock dust that contained just

0.47% of this nutrient in 4 ton, and could this result in a total of approximately 20 kg of P ha^{-1}. The answer to these questions is that the available P from the retained pool was already in the soil because 2 years had already elapsed after the basalt rock dust application, which could be enough time for this soil reaction to happen. Large differences in pH and the Si and P interaction were not noticed in this study, and P desorption was not necessarily influenced by pH changes, results that were in agreement with the results of Carvalho et al. (2000) who studied the silicate acting in the desorption of phosphate in eucalyptus grown in Rhodic Ferralsols and Cambisols in greenhouse conditions. According to Melo (2005), studying a similar soil, high doses of silicate can allow the use of lower doses of phosphate to supply P to Marandu grass crops. Therefore, we hypothesize that basalt rock dust provided an increase in the P available in soil with soil chemical reactions from Si available, in one of four studied areas, for both years.

The highest initial soil chemical properties were shown at site B, in general, despite not being in balance; in the same area, no significant difference in the stalk yield was observed in the 1st year. Therefore, in areas with good soil fertility, basalt rock dust supply might not be as efficient as in areas with sandy and low fertility soils.

Soil microbial activity

Soil microbial activity showed slight stress with the basalt rock dust application only in site B, as indicated by an increase in basal respiration and metabolic quotient; however, soil microbial activity showed high dehydrogenase activity. Site C showed a higher metabolic quotient in the control treatment than in the basalt rock dust treatment, indicating stress under this condition, and showed high C microbial biomass with the basalt rock dust application, indicting better soil quality and fertility when this product was applied.

According to Carvalho (2012) and Doumer (2011) the use of rock dust on soil does not restrict microbial activity in soil. In this study, the analysis showed a trend of increasing soil microbial activity with the basalt rock dust supply, reflecting increased soil biological function. This increased function could be related to the rock of origin of these soils and their characteristics, which is consistent with the conclusions of Paz et al. (2008) that noticed the great homogeneousness of Ferralsols, and given the

extensive period of soil formation, it is assumable the soil microbial activity influencing these soils formation.

1.5 CONCLUSION

Basalt rock dust application promoted better plant development, observed as increased plant height, length and number of internodes besides greater number and weight of sugarcane stalks.

Basalt rock dust applied to plant cane increased the stalk yield up to 12% in the 1st year (application) and 36% in the 2nd year (residual) and when applied in ratoon sites, the stalk and sugar yield improved by up to 37 and 31%, respectively in the 1st year (application) and 9.2 and 9.1% in the 2nd year (residual effect).

Basalt rock dust results application in an overall trend of increasing soil microbial activity.

Basalt rock dust use resulted in changes in both soil chemical properties and microbial activity, but due to the inconstancy of these results, more detailed research about microbial activity is required in order to further clarify the improvement to crop yield.

Considering these results, there is a possibility to find the microorganisms with high affinity with this rock dust to promote higher weathering rates, enabling the nutrients to become more quickly available to the soil and plants.

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CHAPTER 2

CROP CYCLES AND BASALT ROCK DUST INFLUENCES ON THE MICROBIAL DIVERSITY (rRNA) IN BRAZILIAN SUGARCANE-CULTIVATED RHODIC FERRASOLS

Abstract

Bioweathering of minerals by microbes is an important process for the provision of nutrients from rocks for crop growth but some practices of soil management can change the microbial diversity, potentially influencing this process efficiency. This study aimed to analyse the microbial diversity associated with sugarcane-cultured soils previously treated with basalt rock dust, and comparative controls, to correlate measured soil chemical attributes with microbial diversity index. Experiments were performed in four experimental sites located in São Paulo State – Brazil with treatments composed of 1- Control (rock dust free) and 2- basalt rock dust application (4 Mg ha^{-1}), with 6 bulk soil samples per treatment. Soil chemical attributes were evaluated and the bacterial/archaeal (16S rRNA) and fungal (18S rRNA ITS) diversities were analysed by next-generation sequencing (Illumina) in addition to quantification of 16S rRNA genes by real-time PCR. The overall analysis of microbial diversity presented shows low influence of cropping cycles and rock dust treatment on the microbial diversity; diversity shows more relation with geological and pedological differences. Cane-plant has higher 16S proportional sequences to genus *Massilia* compared to ratoon. There was no obvious taxa involved in bioweathering but also no negative impact on community composition either, that means the basalt rock dust appears benign in terms of effects on community diversity and function. OM, Si, SB, CEC, Fe, and Zn soil concentrations relate to the 16S microbial diversity's index in cane-plant and ratoon. Soil chemical attributes have no correlation with ITS microbial diversity index. Si soil concentration correlates with microbial diversity index positively in cane-plant and negatively in ratoon. It is not, however, possible to identify a specific group of bacteria or fungal taxa involved explicitly in bio-weathering which on the basis that rock dust does have an impact of crop yields, implies that the indigenous community in all soils if mediating basalt weathering are doing so as part of their normal function.

Key-words: Soil microbiology. *Saccharum* spp.. Remineralization. Bacteria/Archaea (16S). Fungi (ITS).

Resumo

O biointemperismo é importante por fornecer nutrientes das rochas para as plantas, porém algumas práticas de cultivo podem alterar a diversidade microbiana, influenciando nesse processo. Esse estudo teve como objetivo analisar a diversidade microbiana nos solos cultivados com cana-de-açúcar previamente tratados com pó de rocha basáltica, e o controle comparativo, e correlacionar os atributos químicos do solo com os índices de diversidade microbiana. Os experimentos foram realizados em quatro locais experimentais localizados no Estado de São Paulo com tratamentos compostos por 1- Controle (sem pó de rocha) e 2 - aplicação de pó de rocha basáltica (4 Mg ha^{-1}), com 6 amostras de solo por tratamento. Os atributos químicos do solo foram avaliados e a atividade e diversidade bacteriana/arqueia (16S) e o fungica (ITS) foram analisadas por PCR em tempo real e sequenciamento de nova geração (Illumina), respectivamente. A análise global da diversidade microbiana apresentada mostra baixa influência dos ciclos de cultivo e do tratamento de pó de rocha na diversidade microbiana; a diversidade mostra mais relação com as diferenças geológicas e pedológicas. A cana-planta apresenta maiores sequências proporcionais 16S ao gênero *Massilia* em relação à soca. Não houve taxa óbvia envolvida em biointemperismo, mas também nenhum impacto negativo na composição da comunidade, o que significa que o pó de rocha de basalto parece benigno em termos de efeitos sobre a diversidade e função da comunidade. As concentrações de OM, Si, SB, CEC, Fe e Zn no solo estão relacionadas ao índice de diversidade microbiana do 16S na cana-planta e na soqueira. Os atributos químicos do solo não apresentam correlação com o índice de diversidade microbiana fúngica (ITS). A concentração de Si no solo correlaciona-se positivamente com o índice de diversidade microbiana na cana-planta e negativamente na soca. Não é, no entanto, possível identificar um grupo específico de bactérias ou taxa de fungos envolvidos explicitamente em biointemperismo, visto que o pó de basalto tem um impacto no rendimento das culturas, o que implica que a comunidade indígena em todos os solos é mediadora do intemperismo. fazendo isso como parte de sua função normal.

Palavras-chave: Microbiologia de solo. *Saccharum* spp.. Remineralização. Bacteria/Archaea (16S). Fungi (ITS).

2.1 INTRODUCTION

Soil fauna contribute in the environment to carbon, energy fluxes and nutrients cycles (Pavao-Zuckerman, 2008). The soil health, maintenance and quality is governed by the magnitude of soil microorganism diversity as a wide range of microorganisms related in key soil functions (Garbeva et al., 2004).

Geology formations (ie the soil parent materials; Mitchell et al., 2013), climate (Ladau et al., 2018), sugarcane varieties (indirectly in the bulk soil; Dong et al., 2018), crop age (perennial crops, Hargreaves and Hofmockel, 2014), and fertilization (Zhen et al., 2014; Luo et al., 2015) can influence in the microbial composition. On the other hand, soil microbial communities can influence plant communities and soil properties, including the availability of nutrients in the soil, soil physical characteristics, and nutrient cycling (Pavao-Zuckerman, 2008).

Microorganisms play specific roles in nutrient biogeochemical cycles, such as *Rhizobium* and *Azospirillum* in Biological Nitrogen Fixation, *Bacillus* and *Aspergillus* in phosphate solubilisation, *Glomus* as arbuscular mycorrhizas, *Thiobacillus* in sulphur oxidation, among others influencing composing more than one cycle such as *Arthrobacter*.

Investigations on the role of microorganisms in remineralization have been reported since the XIX century (Hensel, 1898). Remineralization involves using pulverized milled stone in the soil, produced as a by-product of the mining industry, allowing farmers, near these mining sites, to substantially improve the physical, chemical and biological quality of the soil and reduce fertilizer input costs and increase yields (D'Hotman de Villiers,1961; Straaten, 2006). This dust is able to deliver some crop nutrients, like silicon (Si), potassium (K), magnesium (Mg), and calcium (Ca), among other elements, with weathering's help, which is partly mediated by microorganisms through a process called bioweathering.

However, some processes could influence the microbial diversity of agricultural systems, such as soil management, agrochemicals applied, synthetic fertilisers, among other practices.

This work provides an assessment of changes in overall bacterial diversity, and indicator taxa of sugarcane crop cycles in soils that have been remineralized. Secondly, it considers a mechanistic understanding of the reason for differences in

microbial diversity due to cropping regimen or rock dust. The study aimed to analyse the microbial diversity in sugarcane-cultured soils previously treated with basalt rock dust, and the comparative controls, and to correlate the soil chemical attributes with microbial diversity index.

2.2 MATERIAL AND METHODS

Sample Collection

Experiments were performed in four experimental sites named hereafter A, B, C and D, two on plant cane (A and B) and two on advanced sugarcane crop cycles (ratoon, C and D), located in São Paulo State - Brazil, according to the management described in Table 1.

Table 1. Site management description, São Paulo State, Brazil, 2016

	Sites			
	A	B	C	D
Coordinates reference[1]				
Latitude*, S*	22°53'13"	22°35'22"	22°23'45"	22°41'10"
Longitude*, W*	48°46'11"	48°43'58"	48°49'45"	48°46'58"
Altitude, *m above sea level*	790	604	563	658
Application, *Crop cycle*	Plant cane	Plant cane	3rd ratoon	3rd ratoon
Variety	RB867515	CV077107	RB966928	RB855156
Row spacement, *m*	1.5	1.5	1.6 x 0.9	1.4 x 0.5
Limestone, *kg ha^{-1}*	2,075	2,000	-	-
Gypsum, *kg ha^{-1}*	-	1,500	-	-
Vinasse, *m³*	-	-	-	90
Formulate fertiliser, *N-P-K*	18-00-27 00-28-00 14-25-13	06-30-16	00-00-60 45-00-00	45-00-00
Fertilizer rates, *kg ha^{-1}*	248 457.5 289	550	180 130	300
Production environment[2]	D1/D2	A2	B2/C1	E2
Soil classification[3]	Rhodic Ferralsols dystric	Rhodic Ferralsols eutroferric	Rhodic Ferralsols dystric	Rhodic Ferralsols dystric
Location, *São Paulo State*	Botucatu	Lençóis Paulista	Pederneiras	Lençóis Paulista

[1] Geographic Coordinate System, SIRGAS 2000 Datum UTM zone 22S
[2] Prado, 2008
[3] IUSS working group WRB, 2014

The predominant climate in the region is Cfa (Köppen), which is mainly temperate humid with a hot summer. Mean annual temperatures are 19.1-21.1°C, with 1214-1324 mm range of average rainfall. The soil texture and chemical attributes within each experimental site were described in Table 2.

Table 2. Characterization of soil chemical properties and texture in four experimental sites, São Paulo State, Brazil, 2016.

Sites	**pH**	**OM**	**P**	**S**	**Al^{+3}**	**$H^{+}Al^{+3}$**	**K**	**Ca**	**Mg**	**SB**	**CEC**
	$CaCl_2$	*g dm^{-3}*	*mg dm^{-3}*		*$mmol_c$ dm^{-3}*						
					Soil Depth 0.00-0.20 m						
A	6.0	17	15	12	0	14	1.10	25	8	35	48
B	6.0	34	21	5	0	18	1.25	54	21	77	95
C	5.6	16	9	8	0	15	3.20	27	10	39	55
D	5.8	18	6	2	0	14	3.87	19	12	35	49
					Soil Depth 0.20-0.40 m						
A	5.3	14	5	10	0	16	0.48	18	4	22	38
B	5.7	30	22	18	0	24	0.91	44	19	64	88
C	5.3	10	34	2	0	18	0.54	21	7	28	45
D	6.0	10	8	4	0	12	4.96	16	10	31	43

Sites	**V%**	**m%**	**Fe**	**Cu**	**Mn**	**Zn**	**B**	**Si**	**Sand**	**Silt**	**Clay**	**S/C** *ratio*
			mg dm^{-3}									
					Soil Depth 0.00-0.20 m							
A	72	0	36	4.5	1.2	1.1	0.20	4.20	758	74	168	0.44
B	81	0	11	2.4	3.2	0.6	0.09	8.35	332	197	471	0.42
C	72	0	13	0.5	7.0	0.2	0.06	5.77	705	246	48	5.13
D	71	0	26	0.6	2.8	0.4	0.13	2.40	843	22	135	0.16
					Soil Depth 0.20-0.40 m							
A	58	0	29	2.7	0.6	0.6	0.19	3.33	742	58	200	0.29
B	73	0	12	2.7	2.9	0.9	0.52	8.85	274	163	563	0.29
C	61	0	6	0.5	2.6	0.1	0.09	5.05	702	256	41	6.24
D	72	0	8	0.4	0.6	0.1	0.05	3.37	839	22	139	0.16

OM – organic matter; $H^{+}Al^{+3}$– potential acidity; SB – sum of bases; CEC – cation exchange capacity; V% – base saturation; m% – aluminium saturation.

Experimental design and treatment application

The experiment was arranged in a randomized block design with treatments composed of 1- Control (Rock dust free) and 2- Basalt rock dust (BRD) application (4 Mg ha^{-1}, respectively), with 12 replicate plots per treatment. Experimental plots consisted of 8 rows (10 m long) disregarding 0.5 m edges at each end. On sites A and B, the incorporation of basalt rock dust was performed with a light harrow during the soil tillage before planting. On sites C and D basalt rock dust was applied over 10 Mg ha^{-1} of sugarcane crop residue (straw and leaves), without soil incorporation.

Basalt rock dust properties

The basalt rock dust was obtained after crushing from a quarry located in Lençóis Paulista, State of São Paulo, Middle Tietê region. The rocks are derived from the basaltic outcrop of the Serra Geral formation, São Bento Group. The rock dust is composed of 55% plagioclase, 35% pyroxene, 5% magnetite, 5% volcanic glass and 1% apatite, and its chemical composition is presented in Table 3.

Table 3. Basalt rock dust chemical composition. Vancouver, Canada, 2016

SiO_2	**Al_2O_3**	**Fe_2O_3**	**MgO**	**CaO**	**Na_2O**	**K_2O**	**TiO_2**	**MnO**	**P_2O_5**	**LOI**
%	%	%	%	%	%	%	%	%	%	%
48.95	12.26	15.80	4.60	8.44	2.55	1.37	3.79	0.22	0.47	1.2
Ba	**Ce**	**Cr_2O_3**	**La**	**Nb**	**Nd**	**Ni**	**Pb**	**Rb**		
ppm	ppm	%	ppm	ppm	ppm	ppm	ppm	ppm		
441	65.4	0.003	30.3	20.4	34.0	27	1.3	28.2		
Sc	**Sr**	**Th**	**U**	**V**	**Y**	**Zn**	**Cu**	**Zr**		
ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm		
31	483.3	2.7	0.5	475	34.9	66	188.5	215.9		
Eu	**Er**	**Lu**	**Be**	**Dy**	**Co**	**Cs**	**Ga**	**Gd**	**Hf**	**Ho**
ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
2.51	3.22	0.43	2	6.42	37.6	0.2	19.7	7.99	5.6	1.18
Pr	**Sm**	**Sn**	**Sum**	**Ta**	**Tb**	**Tm**	**W**	**Yb**	**Ag**	**As**
ppm	ppm	ppm	%	ppm	ppm	ppm	ppm	ppm	ppm	ppm
8.22	7.44	2	99.68	1.2	1.21	0.46	<0.5	2.90	<0.1	<0.5
Au	**Bi**	**Cd**	**Hg**	**Mo**	**Sb**	**Se**	**Tl**	**B**	**TOT/C**	**TOT/S**
ppb	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	%	%
0.7	<0.1	0.2	<0.01	0.6	<0.1	<0.5	<0.1	8	0.09	<0.02

Source: Acme Analytical Laboratories (Vancouver)

Basalt rock dust particle size distribution was classified according to Table 4.

Table 4. Basalt rock dust particle size analysis, São Paulo State, Brazil, 2015.

	Particle diameter	BRD
	mm	*%*
Very course sand	2.00	0.54
Course sand	1.00	0.13
Medium sand	0.50	10.9
Fine sand	0.25	23.3
Very fine sand	0.10	28.3
Silt	0.05	35.2

Sampling and evaluations

The analysis of the microbial diversity was performed using 6 from the 12 replicate plot samples, for a 0.0-0.20 m depth bulk soil sample, submitted to storage in the freezer before further evaluation.

Soil chemical attributes

After harvest, five subsamples were taken at random from each plot and combined into one composite sample and chemical attributes were determined following the method of Raij et al. (2001).

Gene Sequencing Preparation

Genomic DNA was extracted from 0.5 g of thawed field-moist soil using the BIO 101 FastDNA Spin Kit for soils (Q-BioGene, UK), and a Fast Prep Ribolyser (MP Biomedicals, USA) in triplicate, according to the manufacturer's protocol. DNA extracts were quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Q33231). A procedural blank was carried out using 250ll of microbiological grade filter sterilised water (Sigma, UK) in place of soil to check kits were clear of contaminants. All PCRs were performed on a Techne 512 thermocycler. The primers used were 16s rRNA 515F (GTGNCAGCMGCCGCGGTAA, Muyzer et al., 1993) and 806R (CCGYCAATTYMTTTRAGTTT, Muyzer et al., 1993) primers, which target the V4 region of the 16S rRNA gene (*Escherichia coli* numbering, Brosius et al., 1978), and the primers ITS3 (5` – GCATCGATGAAGAACGCAGC, White et al., 1990) and ITS4 (5` – TCCTCCGCTTATTGATATGC; White et al., 1990). 16S rRNA amplifications underwent the following PCR conditions: initial denaturation at 95°C for 2 minutes, followed by 30 cycles of 95°C for 20 seconds, 55°C for 15 seconds, and 72°C for 5 minutes, and a final elongation step at 72°C for 10 minutes. For ITS amplification initial denaturation was 95°C for 10 minutes, followed by 35 cycles of 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and a final elongation step of 72°C for 10 minutes. Completed PCR reactions were held at 4°C. The presence of extracted DNA was checked by agarose gel electrophoresis. The DNA extracted was stored at -20°C until further use.

Real-time PCR (qPCR)

Quantitative PCR of 16S rRNA genes was performed on an iQ5 thermocycler (Bio-RadLaboratories, Inc., Hercules, USA). Each reaction contained iQSupermix (10 µl; Bio-Rad Laboratories, Inc., Hercules, USA), PCRprimers (1 µl containing 10 pmoles μl^{-1}each), sterile water (6 µl), SYBR® Green (0.2 µl per reaction of 100× diluted from 10,000× concentrate) and DNA template (3 µl) added to a final volume of 20µl. In

addition, we selected primers 1055f (ATGGCTGTCGTCAGCT) and 1392r (ACGGGCGGTGTGTAC) to quantify bacterial 16S rRNA genes. The qPCR reactions comprised an initial denaturation (7 min at 95 °C), followed by 40 cycles (for the bacterial primer pair) of 30 s at 95 °C, 30 s at the specific primer annealing temp and 40 s at 72 °C. Optimal annealing temperatures were empirically determined by performing a temperature gradient PCR with annealing temperatures ranging from 57 °C to 70 °C. The qPCR assays were calibrated using known amounts of PCR amplified and cloned 16S rRNA gene fragments from corresponding taxa (pure standard) and mixed standards. DNA templates for qPCR analyses were extracted from 3 biological replicates and prior to analysis were diluted 400 and 1000-fold. The mean PCR efficiency based on the standard curve produced from plotting threshold cycle versus gene abundance of standards was calculated using BioRad iQ5 software. This was based on the relationship $E = 10^{(-1/\text{slope})}$ and percentage efficiencies were calculated as $\%\ \text{efficiency} = (E-1)*100$. Thus E = 2 is equivalent to 100% efficiency, E = 1.9 is equivalent to 90% efficiency, E = 1.5 is equivalent to 50% efficiency and so on. All raw threshold cycle data were collected using the iQ5 baseline subtraction analysis mode. In addition, no-template controls were included in every qPCR run. Melt curve analysis was conducted at the end of each run to identify non-specific amplification of DNA.

Illumina MiSeq Sequencing

Illumina sequencing was performed by using a MiSeq platform (Illumina, USA) at NUOMICs group (Northumbria University). For sequencing, library pools were denatured by adding 5 µL of 0.2 N NaOH to 10 µL of library in a microcentrifuge tube and incubated at room temperature for 5 minutes. 990 µL of HT1 buffer was added to the mixture to create a 4 pM denatured library. A PhiX positive control was prepared by adding 2 µL of the PhiX control, 3 µL of molecular grade water, and 5 µL of 0.2 N NaOH, which was vortexed and spun for 1 minute at 400 x g, and 990 µL of HT1 added to create a 4 pM PhiX control. 900 µL of 4 pM library and 100 µL of 4 pM PhiX were combined. 600 µL of the combined solution was loaded on a MiSeq V2 500 bp reagent cartridge for sequencing (Illumina, USA). Demultiplexed paired-end FASTQ files were returned from sequencing by Northumbria University for subsequent pipeline analysis.

Sequenced data processing and statistical analysis

Raw sequencing data (FastQ files) obtained from the Illumina sequencing platform were de-multiplexed and quality filtered using dada2 (Callahan et al., 2016) within the QIIME2 analysis pipeline (Caporaso et al., 2010). Closed-reference operational taxonomic unit (OTU) picking was performed using VSEARCH (Rognes et al., 2016) using the 'cluster-features-closed-reference' plugin in QIIME2, using the SILVA119 reference database to produce a table detailing the frequencies of taxonomically assigned representative sequences within individual sample libraries.

Evolutionary analyses were conducted in MEGA7, using the Neighbour-Joining method (Kumar et al., 2016; Saitou and Nei, 1987). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2011) and the percentage of replicate trees in which the associated taxa clustered together was determined by bootstrap analysis of 1000 replicates (Westerlund and Edgerton, 2007).

PCA analysis and Post-hoc tests (Tukey-Kramer; Bluman, 2007) was processed with relative abundance data in Statistical Analysis of Metagenomic Profiles (STAMP) Biostatistical software (Parks et al., 2014).

Gene abundance data (qPCR) were processed in SISVAR (Ferreira, 2008) and the graphic was generated in Microsoft Excel.

The biplots (Loading and PCA) and Pearson correlation ($p<0.05$) were generate with soil chemical attributes and Evenness (Pielou's index, J) and Richness (Shannon's index, H') (from QIIME2) using Minitab® 16 Statistical software (Minitab, 2013).

2.3. RESULTS AND DISCUSSION

Rarefaction analysis were performed from 1,982,713 sequences, 14,833 for 16S and from 2,563,428 sequences, 18,173 for ITS were applied. Furthermore, Faith - Phylogenetic distance, Pielou – evenness, and Shannon – richness diversity index were determined for the same OTU (Table 5).

Table 5. Diversity index Faith – PD, Pielou – eveness, and Shannon – richness in the four experimental sites, treated with basalt rock dust (BRD) and control means.

Sites	A	B	C	D
		Faith PD		
		16S		
BRD[2]	90.30 a	78.77 a	86.75 a	104.70 a
Control[1]	81.63 b	71.60 a	83.64 a	111.30 a
		Pielou - e		
		16S		
BRD	0.81 a	0.87 a	0.87 a	0.90 a
Control	0.84 a	0.86 a	0.83 a	0.91 a
		ITS		
BRD	0.64 a	0.68 a	0.60 a	0.61 a
Control	0.66 a	0.64 a	0.59 a	0.64 a
		Shannon - r		
		16S		
BRD	8.22 a	8.73 a	8.85 a	9.48 a
Control	8.40 a	8.40 a	8.38 a	9.51 a
		ITS		
BRD	4.71 a	5.05 a	4.29 a	4.37 a
Control	4.74 a	4.60 a	4.18 a	4.87 a

Values followed by different letters, presented vertically (between treatments), differ significantly by the LSD test, at 10% probability. [1]Control (0 Mg ha^{-1}), [2] Application of 4 Mg ha^{-1} of basalt rock dust.

Faith PD was higher in BRD treatment just in site A. Other sites did not present any shifts in diversity index (Table 5).

Brazilian sugarcane-cultivated soils showed dominant bacterial phyla consistent with the most abundant bacterial phyla observed in global meta-analysis of soils (Janssen, 2006), and with sugarcane soil studies (Dini-Andreote et al, 2010; Costa et al., 2014), except the phylum *Rokubacteria* was not reported by these studies and it is presented here. Note presence of Archaeal *Thaumarchaeota*, *Nitrososphaeria* which is an ammonia oxidizer (Figure 1).

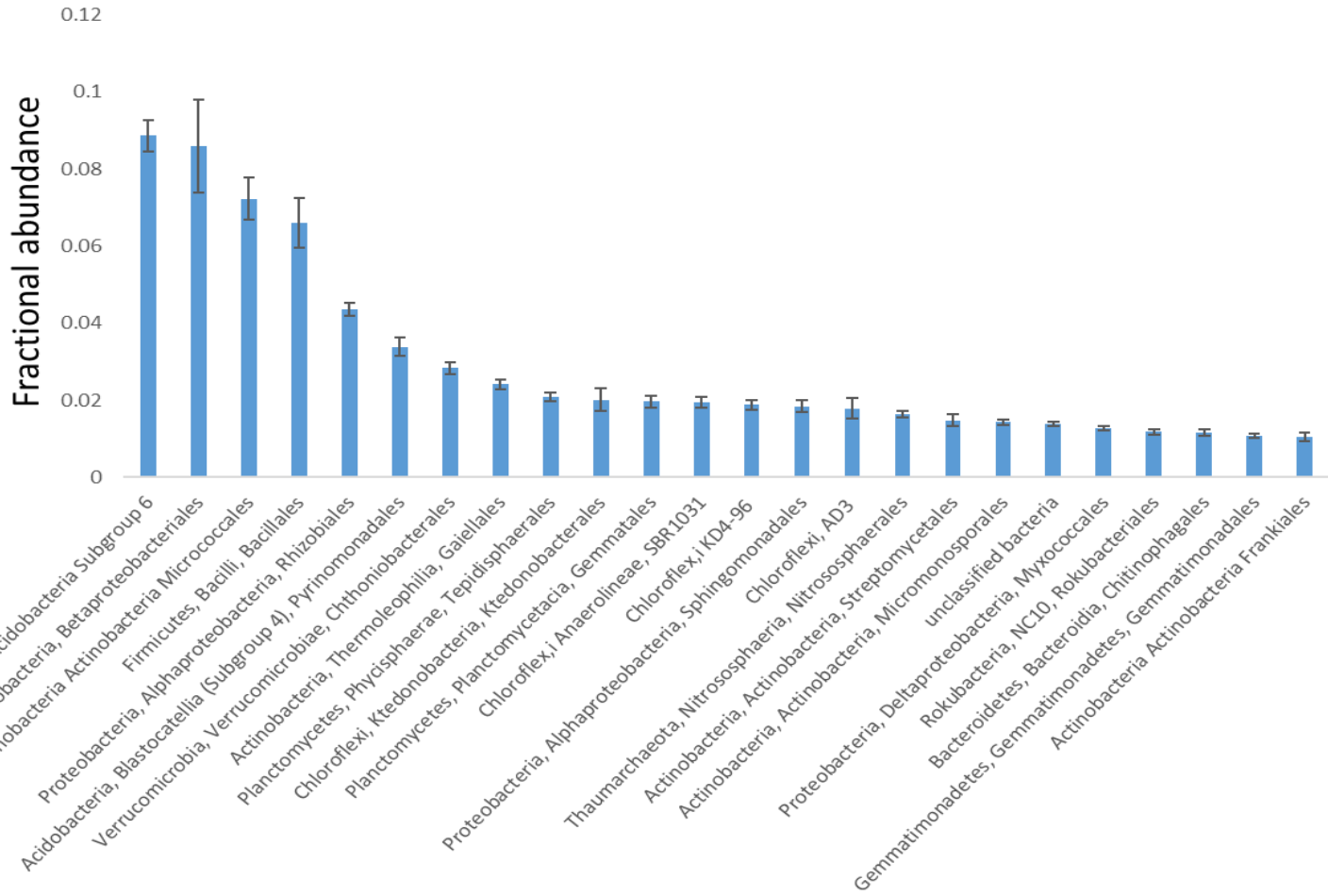


Figure 1. General composition of the Brazilian soil bacterial and archaeal (16S rRNA) abundances averages calculated from all the libraries. Error bars represent standard errors of the averages.

The biogeochemical nutrient cycle involves other participants listed in Figure 1, as well. *Rhizobiales*, *Sphingomonadales*, *Gemmatimonadales* and *Frankiales* relate to the N cycle, *Bacillales* compounding N and P cycles and *Micrococcales* and *Streptomycetales* are often linked to the P cycle (Vieira, 2017; Mendes and Reis Junior, 2003).

The phylogenetic affiliations of the 16S bacterial/archaeal phylotypes represented by these sequences is shown in Figure 2. *Massilia*, *Burkholderia*, *Bradyrhizobium*, *Firmicutes*, *Arthrobacter*, and *Bacillus* had been reported relating to the N cycle (Vieira, 2017), and some of these taxa have been reported in Brazilian sugarcane soil (Figure 2).

Figure 2. Evolutionary relationships of 16S taxa The evolutionary history was inferred using the Neighbor-Joining method (saitou and Nei, 187). The optimal tree with the sum of branch length = 2.15485245 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2011) and are in the units of the number of base substitutions per site. The analysis involved 62 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 227 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

The dominant fungi found in the Brazilian sugarcane-cultivated soils (Figure 3) are broadly consistent with the most abundant fungi observed in a global meta-analysis of soil fungi (Tedersoo et al., 2014).

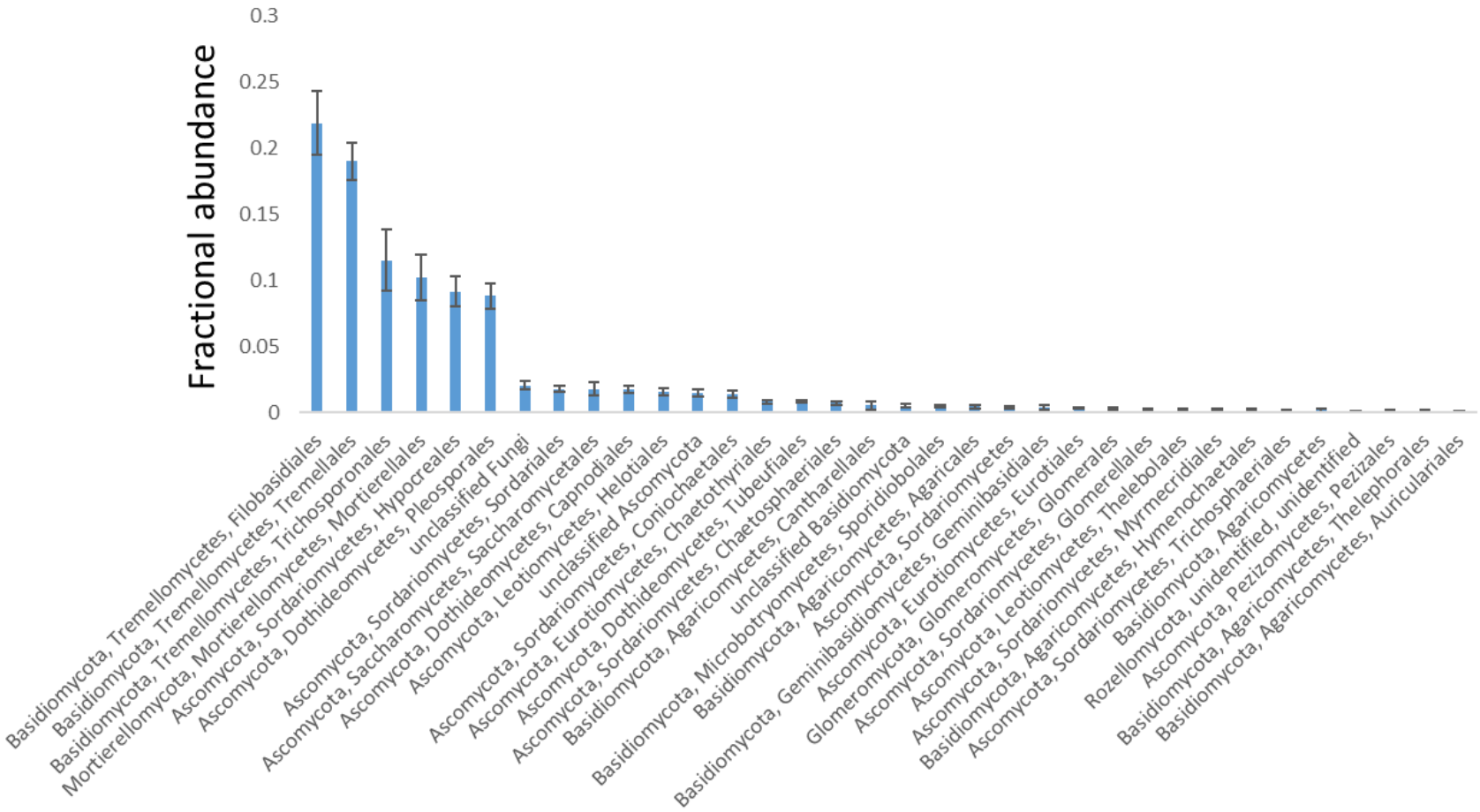


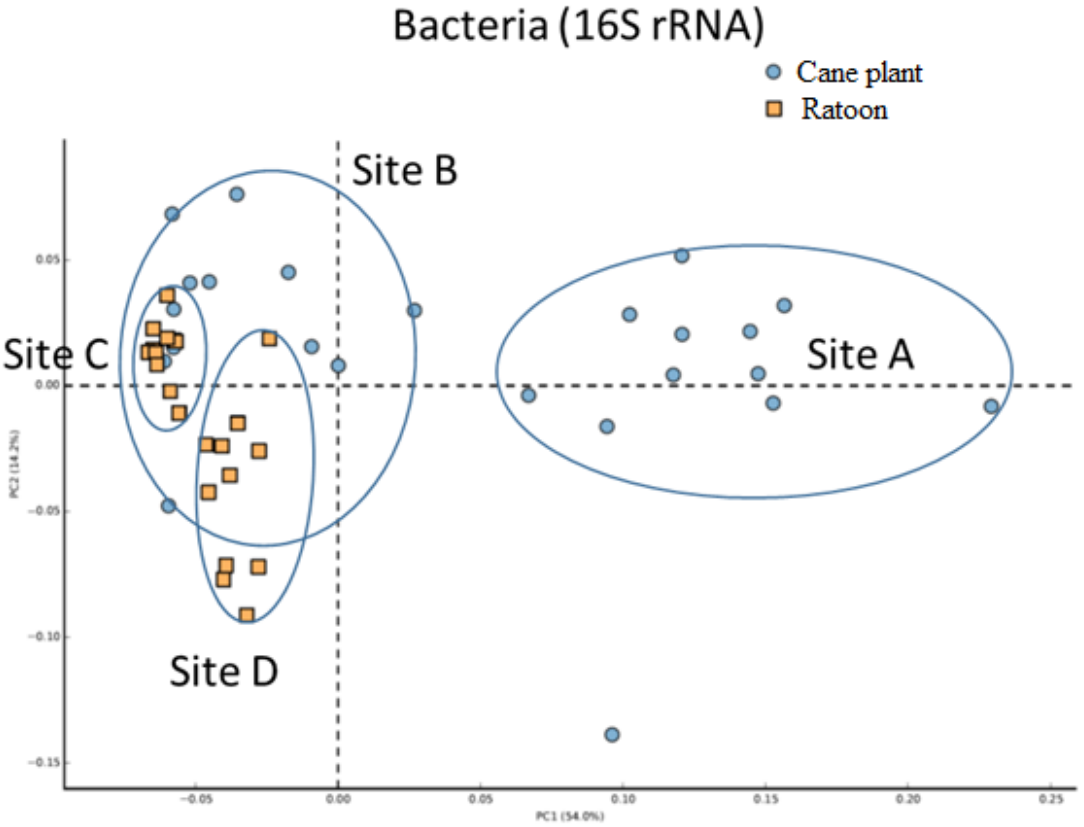
Figure 3. General composition of the Brazilian soil fungal (ITS rRNA) abundances averages calculated from all the libraries. Error bars represent standard errors of the averages.

However, note that usually dominant *Basidiomycota*, *Agaricomycetes* is a minor component relative to the *Basidiomycota*, *Tremellomycetes* (Figure 2).

The phylogenetic affiliation of the ITS fungal phylotypes represented by these sequences is shown in Figure 4. Note that the *Ascomycota*, *Dokmaia* sp. was reported in sugarcane rhizosphere. Futhermore, some species of *Mortierella* genus have been reported as maize growth promoter and phosphate solubilizer (Tayyab et al., 2019; Li et al., 2018).

Figure 4. Evolutionary relationships of ITS taxa The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 2.47585602 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2011) and are in the units of the number of base substitutions per site. The analysis involved 29 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 75 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

Based on the PCA results it appears that both bacterial/archaeal communities are structured by plant cropping cycle (Figure 5A). However, the microbial diversity of site A totally differs from the other sites. Ratoon sites have the microbial diversity completely covered by site B, except four samples from Site D. ITS PCA results did not show such clearly divergent groups, but it shown that fungal communities are completely different between Ratoon sites (C and D) and Site A. However, Site B has a part in common with site C and another part with site A (Figure 5B).



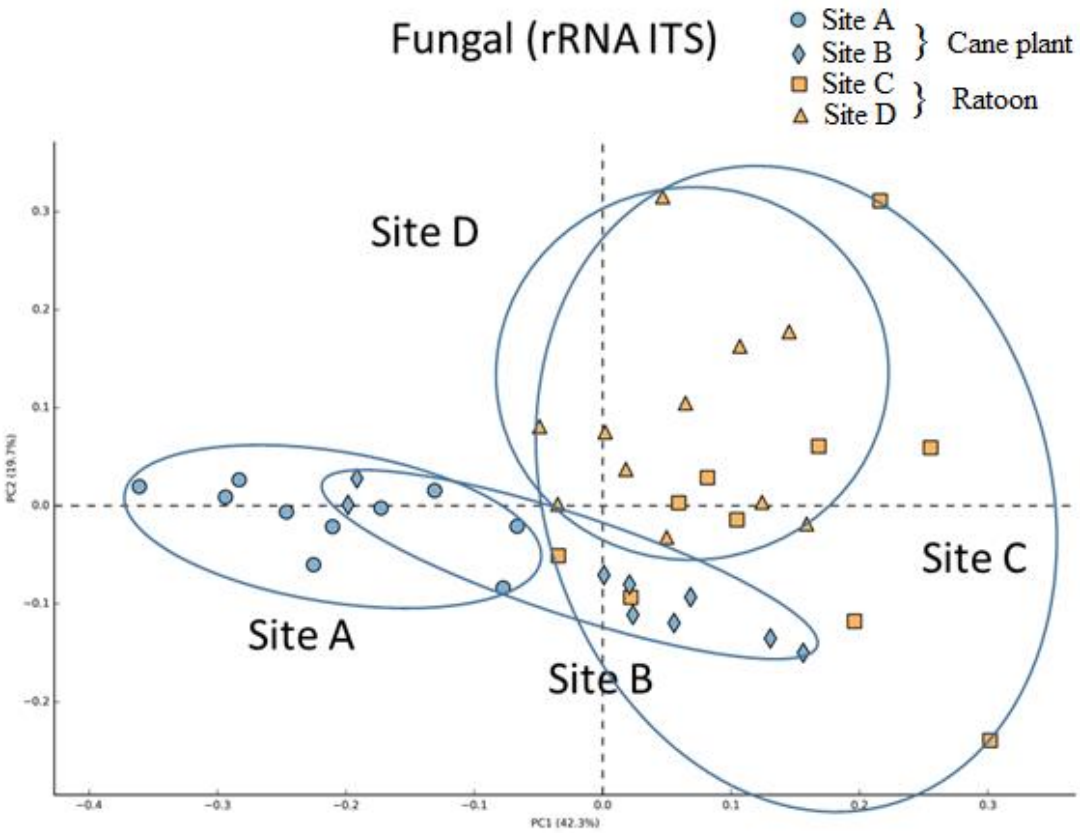


Figure 5. Bacterial/archaeal and fungal community diversity comparisons across the Brazilian sugar cane cropping soils

Most fungi have to interact with plants to live in the soil, however, the bacteria can survive among soil particles; that means fungi need the time of plant growth to start or continue their activity (Martins, 2019; Garcia et al., 2015). Therefore, probably this is the reason why fungi shown a clearer group in ratoon than in cane-plants.

Post-hoc tests show differences in some agriculturally important taxa, such as *Massilia* (NBF), reported as a plant endophytic bacteria; *Sphingomonas*, *Noviherbaspirillum* (NBF), *Paenibacillus*, and *Burkholderiaceae* (NBF) that influence biogeochemical nutrient cycles, mainly the N cycle (Zhang et al., 2006; Zul et al., 2008; Wang et al., 2012; Du et al., 2012; Altankhuu and Kim 2016; Vieira, 2017; Conley, 1999; Madigan et al., 2015) (Figure 6). Cane-plant sites shown a higher sequences proportion than ratoon sites in *Massilia*, *RB41*, *Sphingomonas*, *Subgroup* 7, *Noviherbaspirillum*, *Chthnomonadales*, *Paenibacillus*, *Blastococcus*, *Flavisolibacter*, *Gitt-GS-136*, and *Bukholdariaceae* (Figure 6).

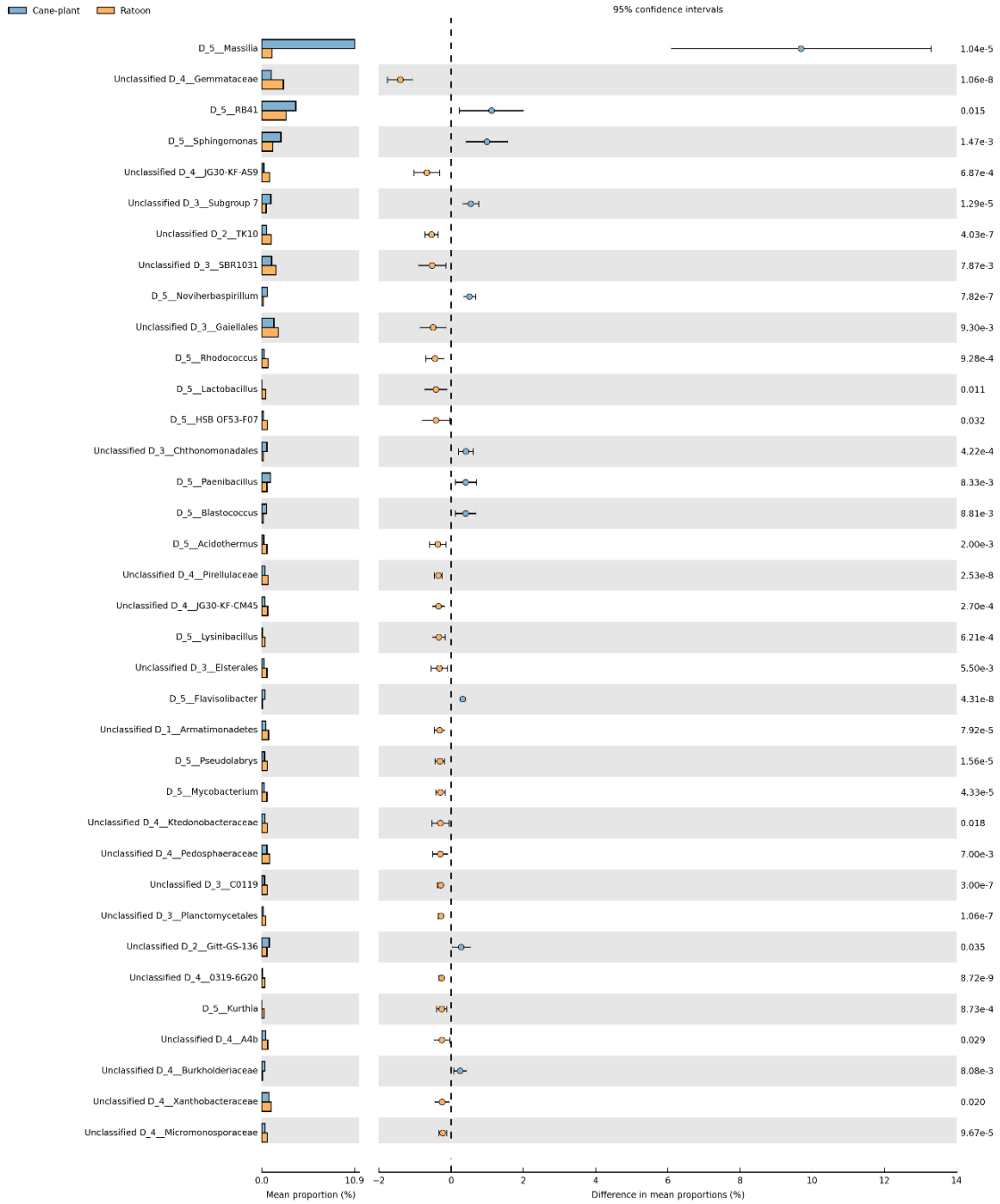


Figure 6. Post hoc tests generated (STAMP) for 16S rRNA gene sequence analysis comparing plant cropping cycle ratoon and cane-plant for all four sites. The number on the right hand side represents to p-value (<0.05) for the significance in the difference between the two groups being compared.

Despite these results, it appears that both bacterial/archaeal communities are structured by plant cropping cycle (Figure 6) a different interpretation can be considered. For instance PCA (Figure 7) shows clearer evidence that site specific selection for both bacterial/archaeal and fungal communities supersedes the effect of

cropping cycle. Furthermore, there is no evidence of rock dust selection within any of these site communities (Figure 7).

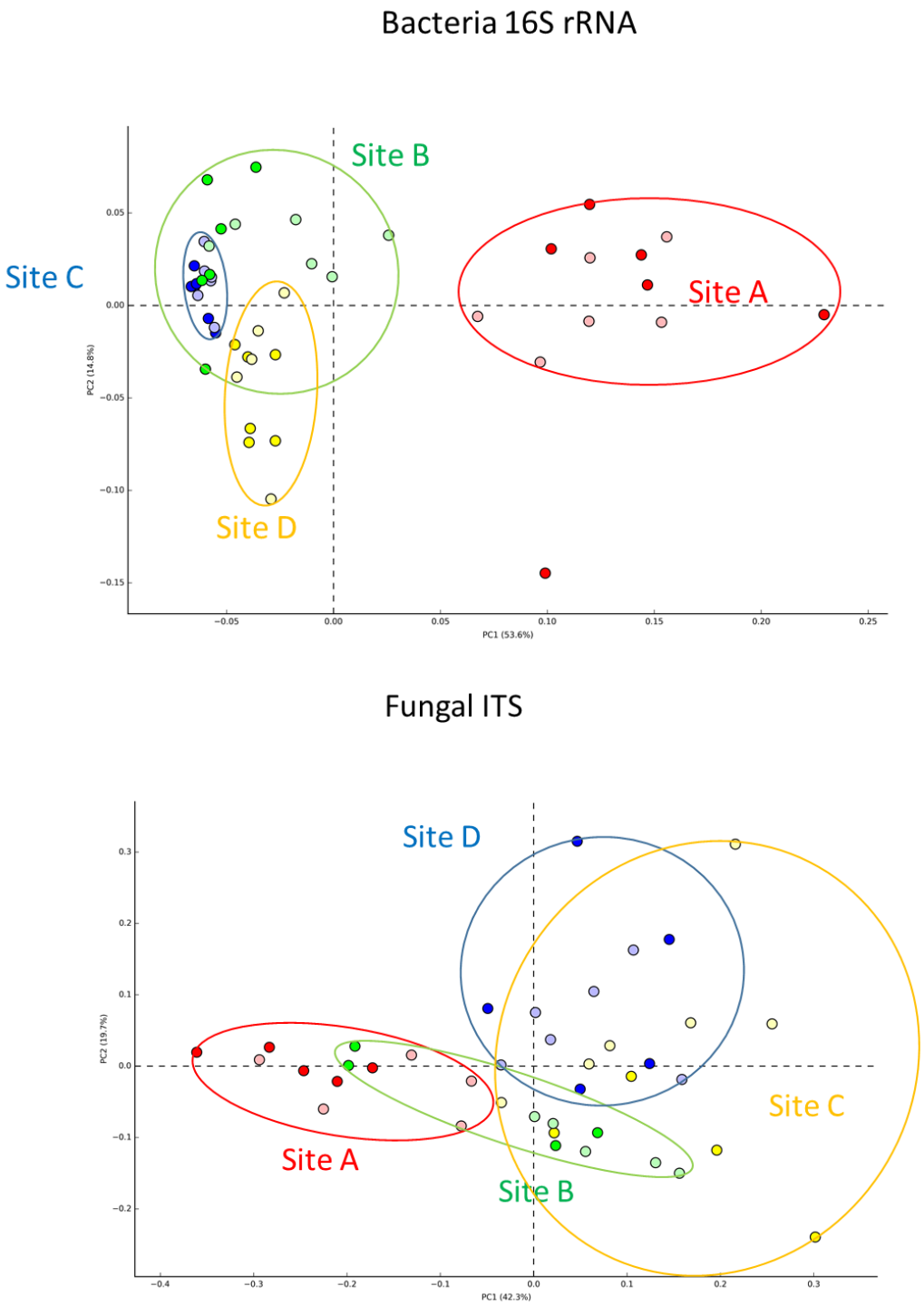


Figure 7. PCA Bacterial/archaeal (16S rRNA) and fungal (ITS) community diversity comparisons across the Brazilian sugarcane cropping soils. Light colours spots are control samples, and dark are basalt rock dust treated samples.

Although it is assumed that microbial communities' spatial variation is reduced with agriculture management, mainly in soil, the reason why biogeographical patterns

should be weak or non-existent (Gumiere et al., 2016), probably these differences among the sites, in this study, could be related to different plant genetic characteristics, or geographic or geologic influences, as Figure 8 shows.

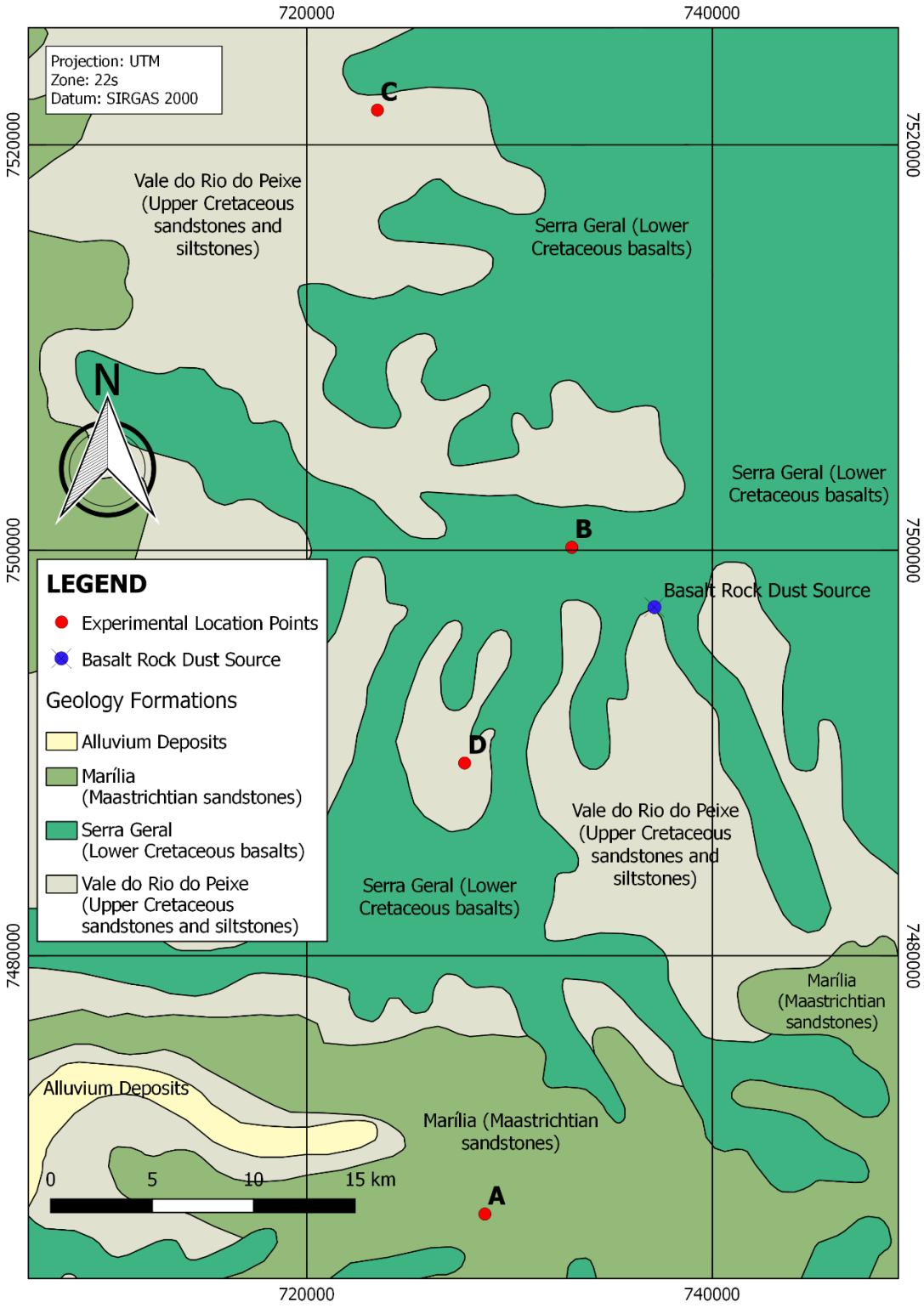


Figure 8. Geological map covering all four sites and the Basalt rock dust source.

Geologically (Fernandes and Coimbra, 2000), Site A is located on the Marília Formation (Maastrichtian sandstones), that is characteristically distant from sites C and D. These are both located on the Vale do Rio do Peixe Formation (Upper Cretaceous sandstones and siltstones), which underlies the Marilia Formation and overlies the older Serra Geral Formation (Lower Cretaceous basalts), where Site B is located.

Despite the different parent rocks among the sites, the proximity of sites B, C and D, and the distance of Site A, is evident from PCA, as well (Figure 8). Biogeographical patterns for fungi in sugarcane-cultured soil have been reported by Gumiere et al. (2010), and they were best explained by geographical distance.

Regarding gene abundance, confirming PCA results, there are no substantial effects of BRD in the sites, except for Site A, where the Control shows higher log gene abundance than BRD treatment (Figure 9).

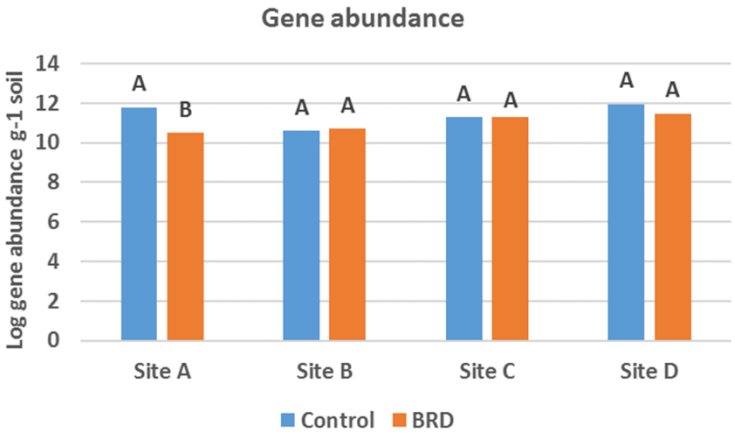


Figure 9. Log gene abundance g^{-1} soil four sites with BRD previous and control.

In agreement to gene abundance, post-hoc test shown a notorious difference between treatments with and without BRD in Site A (Figure 10).

Among the most pronounced differeces in proportional sequences at site A, the sequences from treatment with BRD show lower means than the control, including some N cycle important taxa such as *Nitrososphaeraceae*, *Rhizobiales*, and *Azospirillales*; and P cycle such as *Streptomyces* (Vieira, 2017; Mendes and Reis Junior, 2003) (Figure 10).

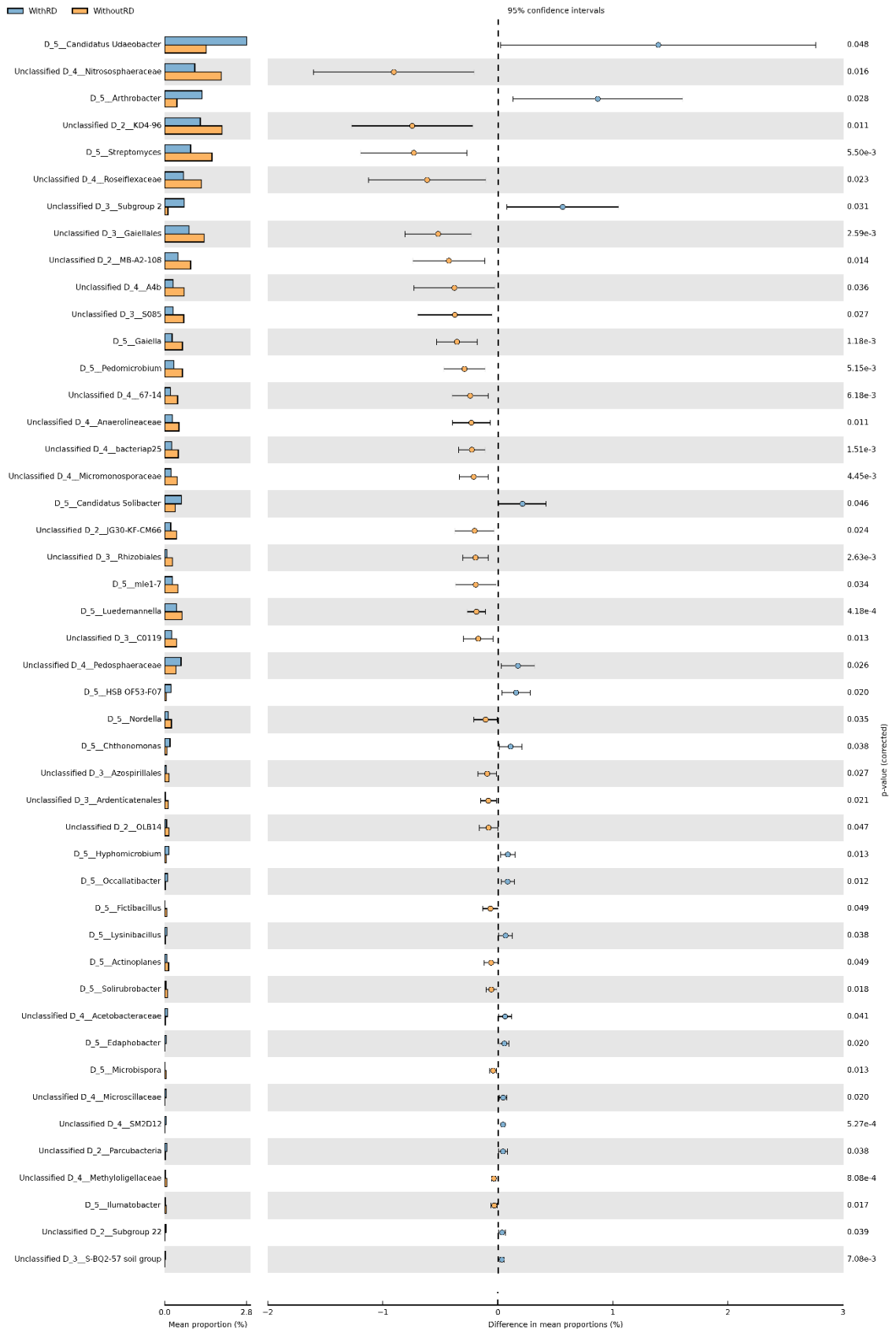


Figure 10. Post hoc tests generated (STAMP) for 16S rRNA gene sequence analysis for Site A comparing BRD and Control communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

Arthrobacter is an important genus associated with both N and P biogeochemical cycles, and it was observed to have higher means for BRD treatment when compared to control (Figure 10).

The post-hoc test in Site B treated with BRD presented higher proportional sequences for the following taxa: *Reyranella* (nitrate reducer; Pagnier et al., 2011), *Methanomassillicoccales* (Methylotrophs), mle1-27, *Cellulomonas* (hydrocarbon-utilizing bacteria; Rivas et al., 2004), AKYG587, *Nitrolancea* (nitrite-oxidizing; Sorokin et al., 2014), *SM2D12*, and *Micavibrionales* (Figure 11).

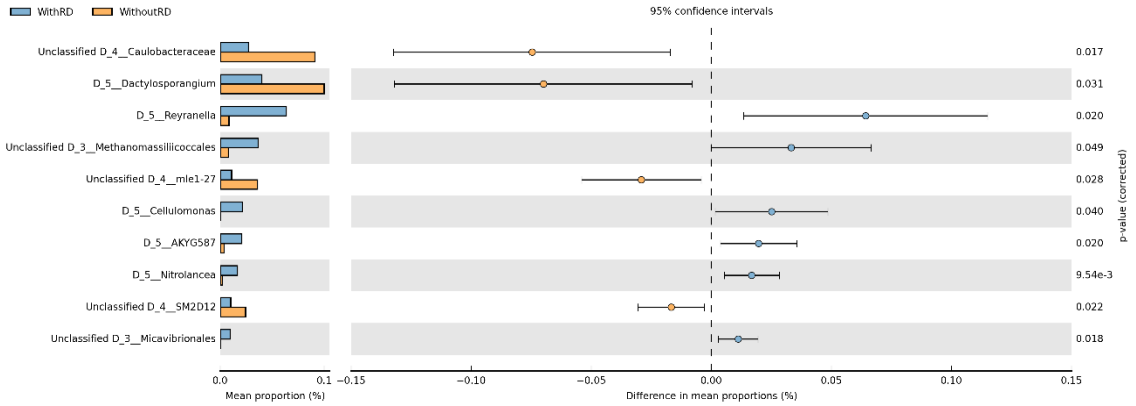


Figure 11. Post hoc tests generated (STAMP) for 16S rRNA gene sequence analysis for Site B comparing BRD and Control communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

The BRD treatment in Site C is associated with increased proportional sequences in the following taxa: *Tumebacillus* (nitrate reducer; Baek et al., 2011), *Conexibacter* (forest soil; Monciardini et al., 2003), *Mycobacterium* (plant growth stimulatory; Egamberdiyeva, 2007), *Sollirubrobacteraceae*, *Babeliales* (sugarcane field; Yeoh et al.2015), and *Turicibacter* (maize rhizosphere, thermal spring; Yang et al., 2017; Filippidou et al., 2016) (Figure 12).

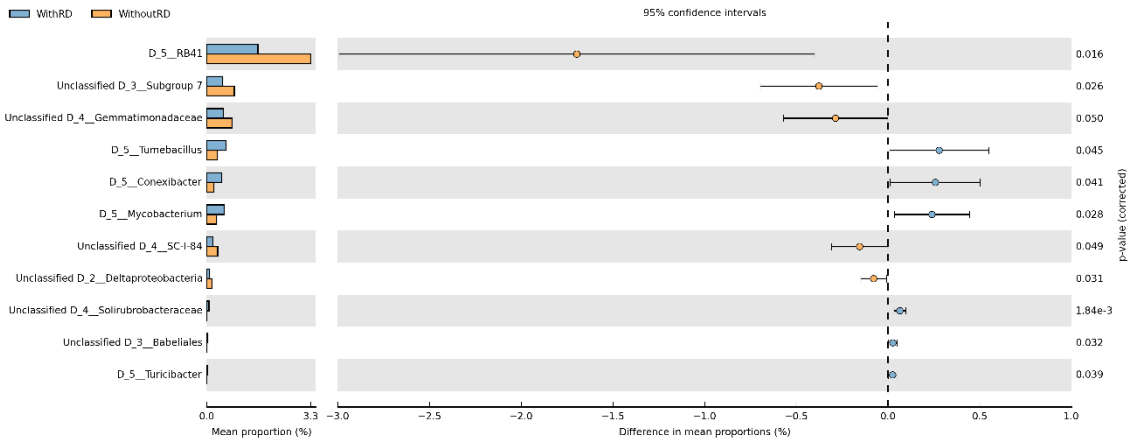


Figure 12. Post hoc tests generated (STAMP) for 16S rRNA gene sequence analysis for Site C comparing BRD and Control communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

In site D, the soil treated with basalt rock dust shown higher proportional sequences in the following taxa: *Candidatus udaeobacter* (grasslands, low-resource conditions; Brewer et al., 2016); *Pirellulaceae* and *Planctomycetales* (correlated with soil attributes, mainly pH, Buckley et al., 2006; Hermans et al., 2017); *Candidatus nitrocosmicus* (coal tar contaminated sediment; Jung et al.2016); *Isosphaeracea* (tundra soils; Ivanova et al., 2016); *Sporosarcina*, *Rhodoplanes*, *Rhizobiales*, *Candidatus koribacter*, and *Sporomusa* (N, Fe, C, and O biogeochemical cycles; Claus et al., 2006; Santana et al., 2016; Ma et al., 2017; Ward et al., 2009; Boga et al., 2003); *Thermosporothrix* (fallen leaves on geothermal soil; Yabe et al., 2016) (Figure 13).

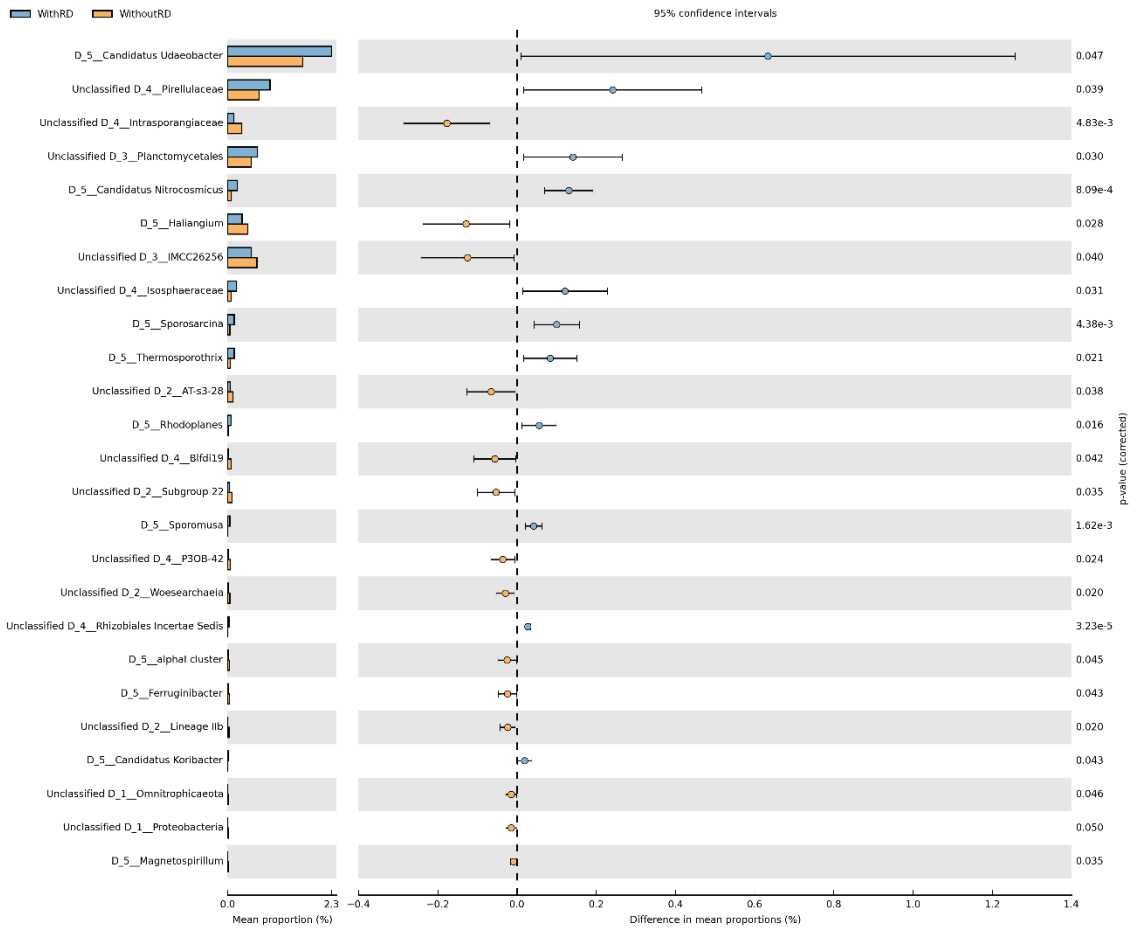


Figure 13. Post hoc tests generated (STAMP) for 16S rRNA gene sequence analysis for Site D comparing BRD and Control communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

Overall, from PCA analysis, 16S rRNA did not show much effect of Basalt Rock Dust, even looking at the small differences shown here in post-hoc tests. The differences based on plant cropping cycles or site location were much bigger.

Regarding fungal (ITS) evaluations, PCA presented a clear separation between ratoon sites (C and D) and Site A, but Site B was shown to overlap with both of these (Figure 5). The BRD treatment did not show selection within any of site communities (Figure 7).

The cane-plant sites showed higher proportional sequences in the following fugal taxa:

Mortierella, *Rhodosporidiobolus*, and *Staphylotrichum* (beneficial for plants; Li et al., 2018; Tayyab et al., 2019; Limtong et al., 2014; Tan et al., 2017);

Saitozyma (acidic soils, correlated to presence of Al; Grzadziel and Galazka, 2019; Fell and Statzell-Tallman, 1998; Moreira and Vale, 2018);

Purpureocillium, *Epicoccum*, *Metarhizium*, and *Nigrospora* (biological control promoter; Hotaka et al., 2015; Barra, Etcheverry and Nesci, 2015; Brown et al., 1987; Humic acids producer; Martin et al., 1967; Mochi et al., 2005; Dutta et al., 2014; Coombs et al., 1963);

Didymellaceae, *Nectriaceae*, *Cylindrocarpon*, and *Capnodiales* (phytopathogen; Tsuneda et al., 2011; Lombard et al., 2015; Chung, 1975; Crous et al., 2009);

Chaetomiaceae, *Myrmecridium*, and *Spegazzinia* (cellulolytic; Wilhelm et al., 2017; Powell et al., 2012; Bezerra et al., 2015; Coupland, 1979);

Chaetothyriales, and *Bionectriaceae* (saprotrophic on plants; Badali et al. 2011; Schroers, 2001) (Figure 14).

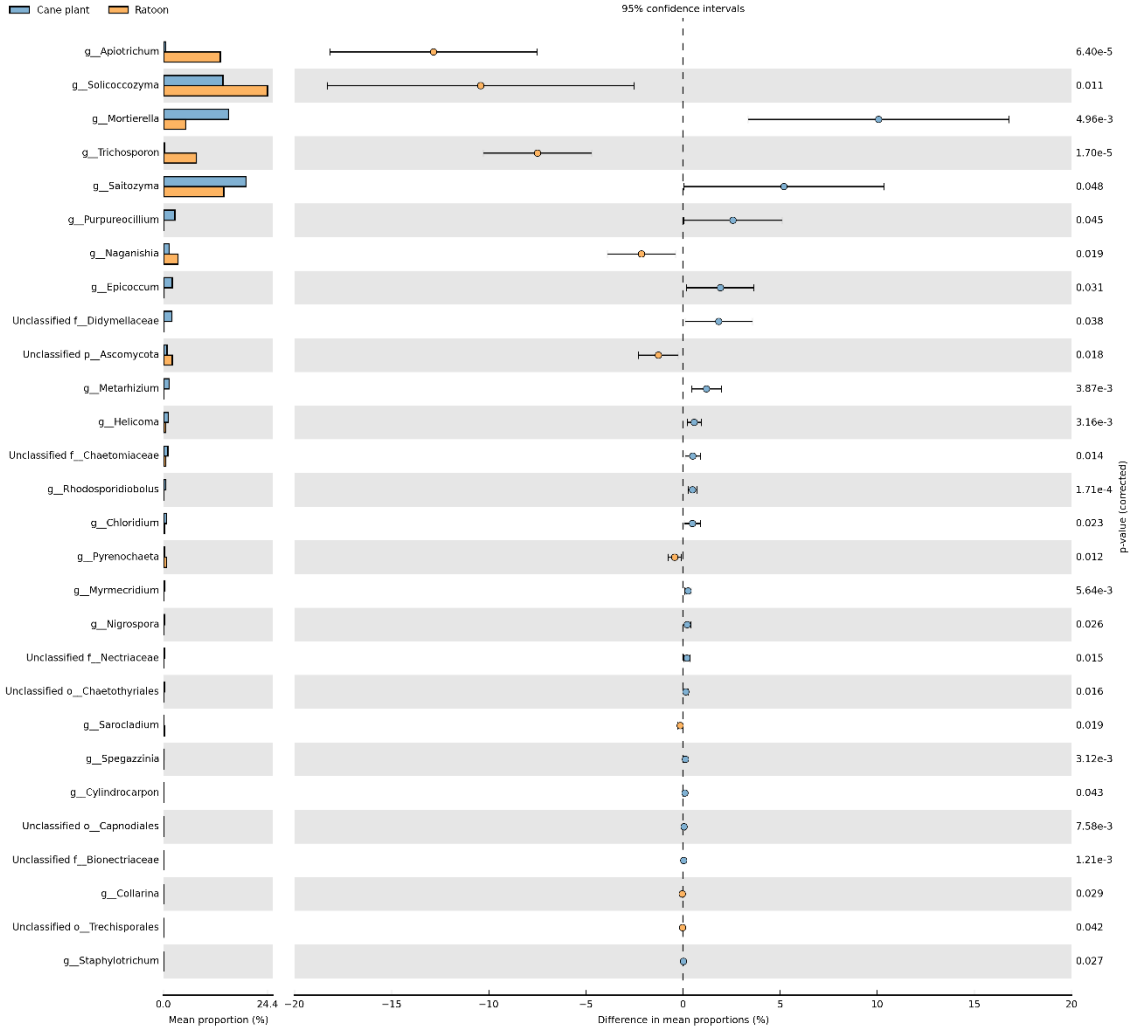


Figure 14. Post hoc tests generated (STAMP) for ITS rRNA gene sequence analysis comparing cane plant and ratoon communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

BRD treatment shown higher proportional sequences for *Capnodiaceae* (physical barrier to plant pathogens; Petelle, 1982); *Westerdykella* (bioremediation and plant growth promoter; Srivastava et al., 2012); and *Codinaea* (plant pathogen; Waipara, 1996). In contrast, *Thielavia* (plant pathogen; Gilbert, 1909) and *Rozellamycota* (correlated to extreme pH conditions; Tedersoo et al., 2017) presented the higher means in Control (Figure 15).

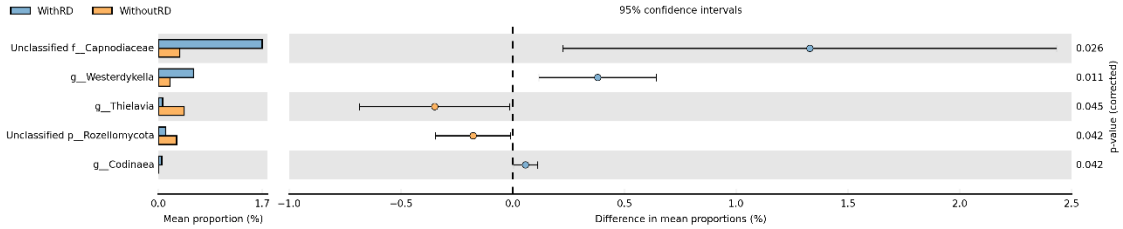


Figure 15. Post hoc tests generated (STAMP) for ITS rRNA gene sequence analysis for Site A comparing BRD and Control communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

The proportional sequences in Sites B, C, and D presented higher ITS proportional sequences in Control, when compare with BRD treatment (Figures 16, 17, and 18).

The Control, site B, showed higher proportional sequences in the following taxa: *Candida* (Plant growth promoter; Amprayn et al., 2012) and *Umbelopsis* (soil and plants residues; Wang et al., 2013) (Figure 16).

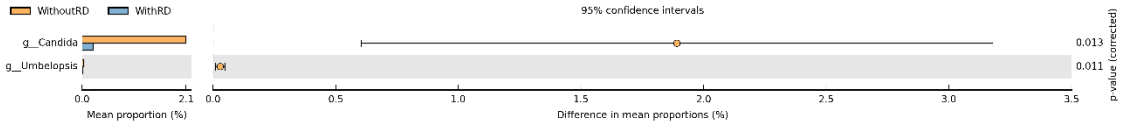


Figure 16. Post hoc tests generated (STAMP) for ITS rRNA gene sequence analysis for Site B comparing BRD and Control communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

Papiliotrema (yeast; Surussawadee et al., 2014) and *Westerdykella* (bioremediation and plant growth promoter; Srivastava et al., 2012) had presented higher means to Control in Site C (Figure 17).

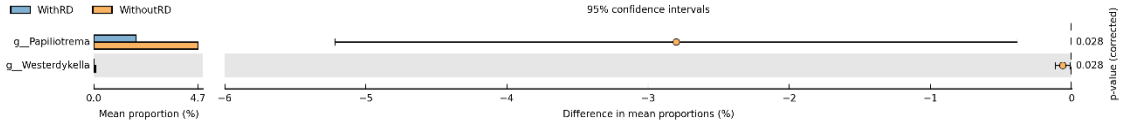


Figure 17. Post hoc tests generated (STAMP) for ITS rRNA gene sequence analysis for Site C comparing BRD and Control communities. The number on the right hand

side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

Furthermore, Site D presented the higher means to control in the following taxa: *Chaetomiaceae*, and *Myrmecridium* (cellulolytic; Wilhelm et al., 2017; Powell et al., 2012; Bezerra et al., 2015); *Podospora* (suppressor to Verticillium wilt on tomato; Tayyab et al., 2019); *Dictyosporium* (dead wood and plant litter, Pinruan et al., 2007); and *Acremonium* (saprobic; Domsch et al., 2007) (Figure 18).

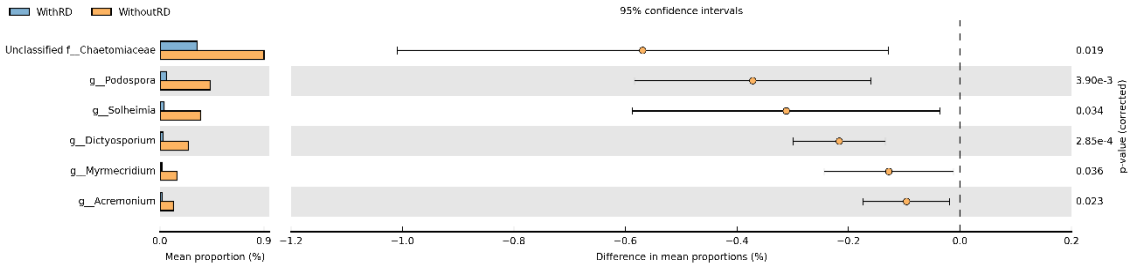


Figure 18. Post hoc tests generated (STAMP) for ITS rRNA gene sequence analysis for Site D comparing BRD and Control communities. The number on the right hand side represents to p-value p-value (<0.05) for the significance in the difference between the two groups being compared.

Thereby, the cropping cycles' post-hoc plots indicate microorganisms more related to plants and with specific known functions. However, the post-hoc plots related to rock dust treatments differences appears not to be frequently reported in soil and plants.

Possibly, the reason for differences between the proportional sequences in basalt rock dust treatment and control, may be related to rock composition and not about the differences in microorganisms communities. According to Table 3, trace elements added to soil by basalt rock dust such as B, Co, Cu Fe, Mn, Mo, Ni, Se, W, V, and Zn, work as a growth factor for microorganisms (MADIGAN et al., 2015) and they are not necessarily essential to plants, so they are not noticed very often. However they are very important to activate and increase population rate of microorganisms, performing an important role in enzymatic, respiration and photosynthesis processes (MADIGAN et al., 2015).

Nevertheless, there is no obvious and consistent pattern of selection of fungal taxa associated with BRD. In any comparison by post-hoc tests were observed differences between the treatments. However, this not means that rock dust made these differences; they are just an indication that they possibly might, on the basis that none of these fungi or bacteria can be associated from other studies with BRD

treatments, and a similar pattern across all four sites was not seen for apparent selection.

Responses of microorganisms associated with plants depend on genetic and functional compatibility between vegetal species and fungi lineage, also the environmental conditions such as pH, soil, and nutrients availability mainly phosphorus (P) (BERBARA et al., 2006).

In PCA, the first component (50.0%) was influenced by Mg, CEC, SB, Si, OM, and Ca, and the second (18.9%) was influenced by $H+Al^{+3}$ (Figure 19).

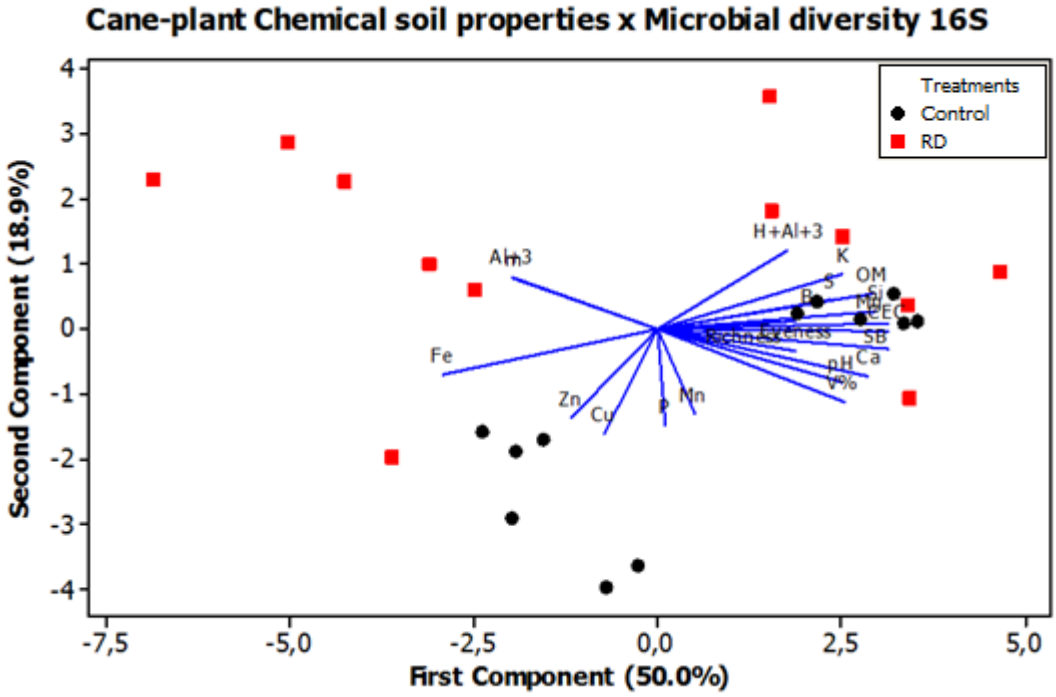


Figure 19. Biplot 16S rRNA based microbial diversity versus soil chemical attributes in Cane-plant sites. OM – organic matter; $H+Al^{+3}$– potential acidity; SB – sum of bases; CEC – cation exchange capacity; V% – base saturation; m% – aluminium saturation.

Evenness (Pielou's index, J) presented a positive Pearson correlation with pH (R= 0.544, p=0.006), OM (R= 0.479, p= 0.018), Ca (R= 0.515, p= 0.010), Mg (R=0.525, p= 0.008), SB (R= 0.516, p=0.010), CEC (R=0.4, p= 0.021), Si (R= 0.499, p= 0.013), and V% (R=0.565, p= 0.004). Furthermore, the evenness was negatively correlated with Fe (R= -0.517, p= 0.010) and Zn (R= -0.426, p= 0.038) (Figure 19).

Richness (Shannon's index, H') shown positive correlation with pH (R= 0.424, p= 0.039), and Si (R= 0.425, p=0.038) (Figure 19).

Those results shown the evenness increased with pH, O.M., Ca, Mg, SB, CEC, Si, and V%. Moreover, the richness is improved with pH and Si. And, when the evenness is high, the Fe and Zn concentration will be lower (Figure 19).

Ca, Mg, SB, Cu, and Zn contents influenced the first component (38.6%), and the second component (26.5%) was influenced by pH and V% (Figure 20).

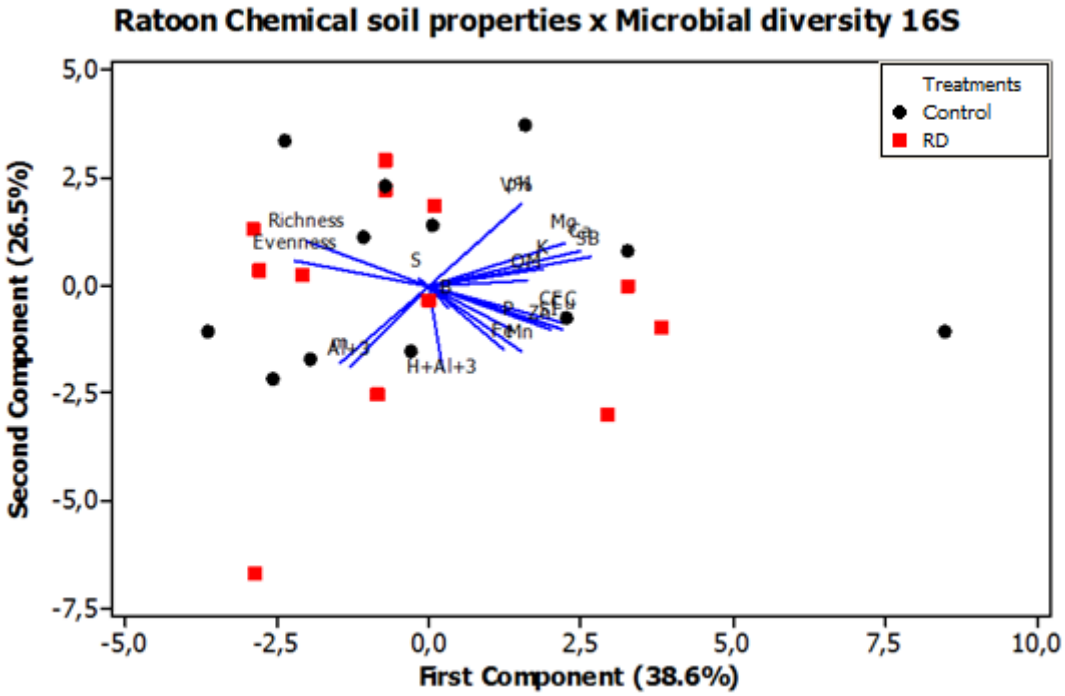


Figure 20. Biplot 16S rRNA based microbial diversity versus soil chemical attributes in ratoon sites. OM – organic matter; $H+Al^{+3}$– potential acidity; SB – sum of bases; CEC – cation exchange capacity; V% – base saturation; m% – aluminium saturation.

16S microbial diversity in ratoon sites showed for Evenness (J) a negative correlation with O.M. (R= -0.464, p= 0.023), K (R= -0.432, p=0.035), and Ca (R= -0.504, p=0.012). Evenness and Richness correlated negatively with SB (J: R= -0.577, p= 0.003; H': R= -0.480, p= 0.018), CEC (J: R= -0.461, p= 0.023; H': R= -0.504, p= 0.012), Si (J: R= -0.729, p= 0.000; H': R= -0.825, p= 0.000), Fe (J: R= -0.496, p= 0.014; H': R=0.528, p= 0.008), Cu (J: R= -0.662, p= 0.000; H': R= -0.689, p= 0.000), Mn (J: R= -0.552, p= 0.005; H': R= -0.693, p= 0.000), and Zn (J: R= -0.735, p= 0.000; H': R= -0.788, p= 0.000).

OM, Si, SB, CEC, Fe, and Zn soil concentrations influence the 16S microbial diversity's index in cane-plant and ratoon. Although most of these parameters are related with soil pH, the pH does not often influence microbial diversity as much as the

other soil attributes. However, according to Fierer and Jackson, 2006, soil microbial diversity can be predicted by pH.

Cane-plant ITS microbial diversity first component (48.8%) was influenced by CEC, SB, Mg, Si, Ca, and O.M. and the second (22.8%) was influenced by Zn, and Cu (Figure 21).

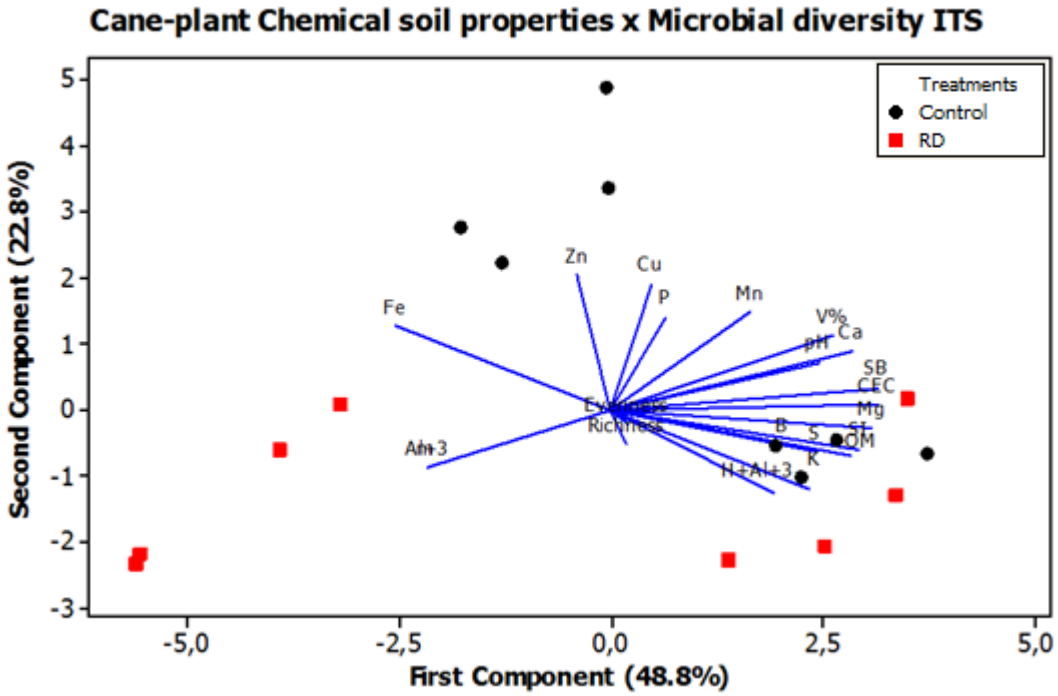


Figure 21. Biplot ITS rRNA based microbial diversity versus soil chemical attributes in Cane-plant sites. OM – organic matter; $H+Al^{+3}$– potential acidity; SB – sum of bases; CEC – cation exchange capacity; V% – base saturation; m% – aluminium saturation.

No correlations between ITS microbial diversity index and soil chemical attributes in cane-plant were observed.

pH, V%, Mg, Ca, and SB influenced the first component (38.1%) in ratoon. The second component (23.8%) was influenced by CEC, and Zn (Figure 22).

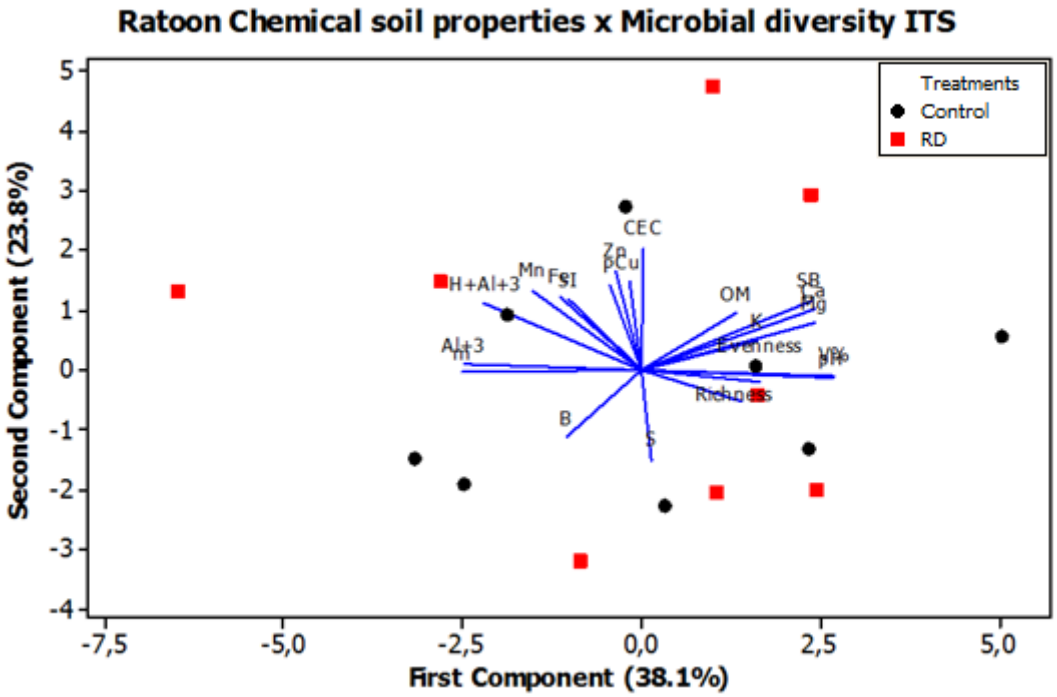


Figure 22. Biplot ITS rRNA based microbial diversity versus soil chemical attributes in ratoon sites. OM – organic matter; $H+Al^{+3}$– potential acidity; SB – sum of bases; CEC – cation exchange capacity; V% – base saturation; m% – aluminium saturation.

The BRD treatment appears to be more related to PC1, and control probably has low relation with PC2 (Figure 22). Nevertheless, the soil chemical attributes do not correlate with ITS microbial diversity index. According to Gumiere, et al., 2016, soil characteristics explain only 3.6% of the variance in fungal community composition in sugarcane-cultivated soil.

In the present study, an ectomycorrhizal *Scleroderma citrinum* (relative abundance = 0.000859152) was found in Site A control plot 2, in sugarcane-cultivated soil.

2.4 CONCLUSION

The overall analysis of microbial diversity presented shows low influence of cropping cycles and rock dust treatment on the microbial diversity; diversity shows more relation with geological and pedological differences. Cane-plant has higher 16S proportional sequences to genus *Massilia* compared to ratoon.

There was no obvious taxa involved in bioweathering but also no negative impact on community composition either, that means the basalt rock dust appears benign in terms of effects on community diversity and function.

OM, Si, SB, CEC, Fe, and Zn soil concentrations relate to the 16S microbial diversity's index in cane-plant and ratoon. Soil chemical attributes have no correlation with ITS microbial diversity index. Si soil concentration correlates with microbial diversity index positively in cane-plant and negatively in ratoon.

It is not, however, possible to identify a specific group of bacteria or fungal taxa involved explicitly in bioweathering which on the basis that BRD does have an impact of crop yields, implies that the indigenous community in all soils if mediating basalt weathering are doing so as part of their normal function.

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CHAPTER 3

WEATHERING OF BASALT ROCK DUST IN SUGARCANE-CULTIVATED RHODIC FERRALSOLS

Abstract

Due to the slow release of elements from rock minerals and the low rate of rock dust usually applied to the soils, it is difficult to obtain the rate of weathering of rock dust in the soil environment. This paper aimed to evaluate the weathering of basalt rock dust applied to Rhodic Ferralsols after two years of cultivation of sugarcane. Experiments were performed in pairs on plant cane (A and B) and two on advanced sugarcane crop cycles (ratoon, C and D), located in São Paulo State – Brazil. The soil samples were characterized in chemical, textural and mineralogical (XRD) attributes, and then submitted to Thermogravimetry and its derivative (DTG). Most evident basalt rock dust components are 2:1 phyllosilicate, plagioclase, pyroxen. Ferralsols are composed by quartz, gibbsite, and kaolinite basically, but Site C showed Goethite signals and site D, Hematite. Site B presented the highest mass loss among the four sites, due to its geological formation and parental rock, and consequently clays concentration. Basalt rock dust treatment increased the mass loss in Ratoon sites C, and D. Basalt rock dust application changed mineral amount estimated, increasing Kaolinite in soil and clay compared to control, but this difference is not evident to Gibbsite.

Key-words: Thermogravimetry. Soil mineralogy. Reminealization. *Saccharum* spp..

Resumo

Devido à liberação lenta de elementos da rocha e a baixa taxa de pó de rocha normalmente aplicada ao solo, este trabalho teve como objetivo avaliar a ação do pó de rocha basáltica aplicado aos Latossolos Vermelhos após dois anos de cultivo da cana-de-açúcar por técnicas térmicas (TG e DTG). Os experimentos foram conduzidos em dois sites experimentais de cana-planta (A e B) e dois em soqueira (C e D), localizados no Estado de São Paulo. As amostras de solo foram caracterizadas em atributos químicos, texturais e mineralógicos (DRX) e depois submetidas à termogravimetria e sua derivada (TG e DTG). Os componentes do pó de basalto mais evidentes são filossilicato 2:1, plagioclásio e piroxênio. Latossolos são compostos basicamente por quartzo, gibbsita e caulinita, mas o site C mostra sinais de Goetita e o site D, hematita. O site B apresentou a maior perda de massa entre os quatro locais, devido a sua formação geológica e rocha mãe, e consequentemente a concentração de argilas. O tratamento de pó de basalto aumentou a perda de massa nos sítios de soqueira (C, e D). A aplicação de pó de basalto alterou a quantidade mineral estimada, aumentando a caulinita no solo e argila comparada ao controle, mas esta diferença não é evidente em gibbsita.

Palavras-chave: Termogravimetria. Mineralogia de solo. Remineralização. *Saccharum* spp..

3.1 INTRODUCTION

Sugarcane is a major export commodity in Brazil with ethanol and sugar as products and energy as a principal by-product. This crop requires the application of a large amount of fertilisers to its growth, and to supply the highly weathered Brazilian soils.

The sugarcane-cultivated soils are much worn due to the monoculture, and intensively used, needing around 5 years to renovate, and the renovation is very heavy for physical, chemical and biological soil structure.

Nowadays the fertilisers used in crops have an almost instantaneous release, but the sugarcane spends as much as 5 years in the field, and the application over the crop, even in initial phases, can cause damage to regrowth. Then, ideally, a product with slow nutrients release would be useful.

Remineralization, or rocks for crops, consists of applying milled rocks to the soil, as a multi-element source, replacing some and adding other elements to the soil, and consequently to crops. Some elements are not constantly evaluated, as routine agronomic analysis, and they are not consider as essential to plants growth (as far as we know), but they work as growth factor to microbiome and encourage the microbial community to act as a glue to soil aggregates (Pavao-Zuckerman, 2008), as an example for soil modifications.

Rock dust needs some weathering agents to release the elements from its components since the only ‘weathering’ received by these rocks was mechanical. For this reason, the release of nutrients from this material is very slow, as it needs temperature, water, biological activity, among other agents.

In spite of studies for some specific minerals (Manning et al., 2017), the releasing and weathering of rock dust and its consequence in the soil is particular for each compound mineral, so it is poorly understood yet.

Geosciences has a long history in thermal analysis, as an investigative method, that was primarily used in the materials science (Fernández et al., 2010). Thermal analyses methods are relatively low-cost compared to other methods, involve few sample preparation, are fast, give reproducible results, and are information rich (Plante et al., 2009). Furthermore, their results have been reported to be strongly correlated to some fundamental soil properties (Siewert, 2004) and offer the unique opportunity to directly measure changes as a complement to structural–chemical investigations (Maharaj et al., 2007; Plante et al., 2009).

This paper aims to evaluate the weathering of basalt rock dust applied to Rhodic Ferralsols after two years of cultivation of sugarcane using thermal (TG and DSC) techniques.

3.2 MATERIAL AND METHODS

Sample Collection

Experiments were performed in four experimental sites named hereafter A, B, C and D. The treatment was applied before planting in first cycle (plant cane, Sites A and B) and two on advanced sugarcane crop cycles (ratoon, C and D), located in São Paulo State - Brazil, according to the management described in Table 1.

Table 1. Site management description, São Paulo State, Brazil, 2016

	Sites			
	A	B	C	D
Coordinates reference[1]				
Latitude, *S*	22°53'13"	22°35'22"	22°23'45"	22°41'10"
Longitude, *W*	48°46'11"	48°43'58"	48°49'45"	48°46'58"
Altitude, *m above sea level*	790	604	563	658
Application, *Crop cycle*	Plant cane	Plant cane	3rd ratoon	3rd ratoon
Variety	RB867515	CV077107	RB966928	RB855156
Row spacement, *m*	1.5	1.5	1.6 x 0.9	1.4 x 0.5
Limestone, *kg ha^{-1}*	2,075	2,000	-	-
Gypsum, *kg ha^{-1}*	-	1,500	-	-
Vinasse, *m³*	-	-	-	90
Formulate fertiliser, *N-P-K*	18-00-27 00-28-00 14-25-13	06-30-16	00-00-60 45-00-00	45-00-00
Fertilizer rates, *kg ha^{-1}*	248 457.5 289	550	180 130	300
Production environment[2]	D1/D2	A2	B2/C1	E2
Soil classification[3]	Rhodic Ferralsols dystric	Rhodic Ferralsols eutroferric	Rhodic Ferralsols dystric	Rhodic Ferralsols dystric
Location, *São Paulo State*	Botucatu	Lençóis Paulista	Pederneiras	Lençóis Paulista

[1] Geographic Coordinate System, SIRGAS 2000 Datum UTM zone 22S
[2] Prado, 2008
[3] IUSS working group WRB, 2014

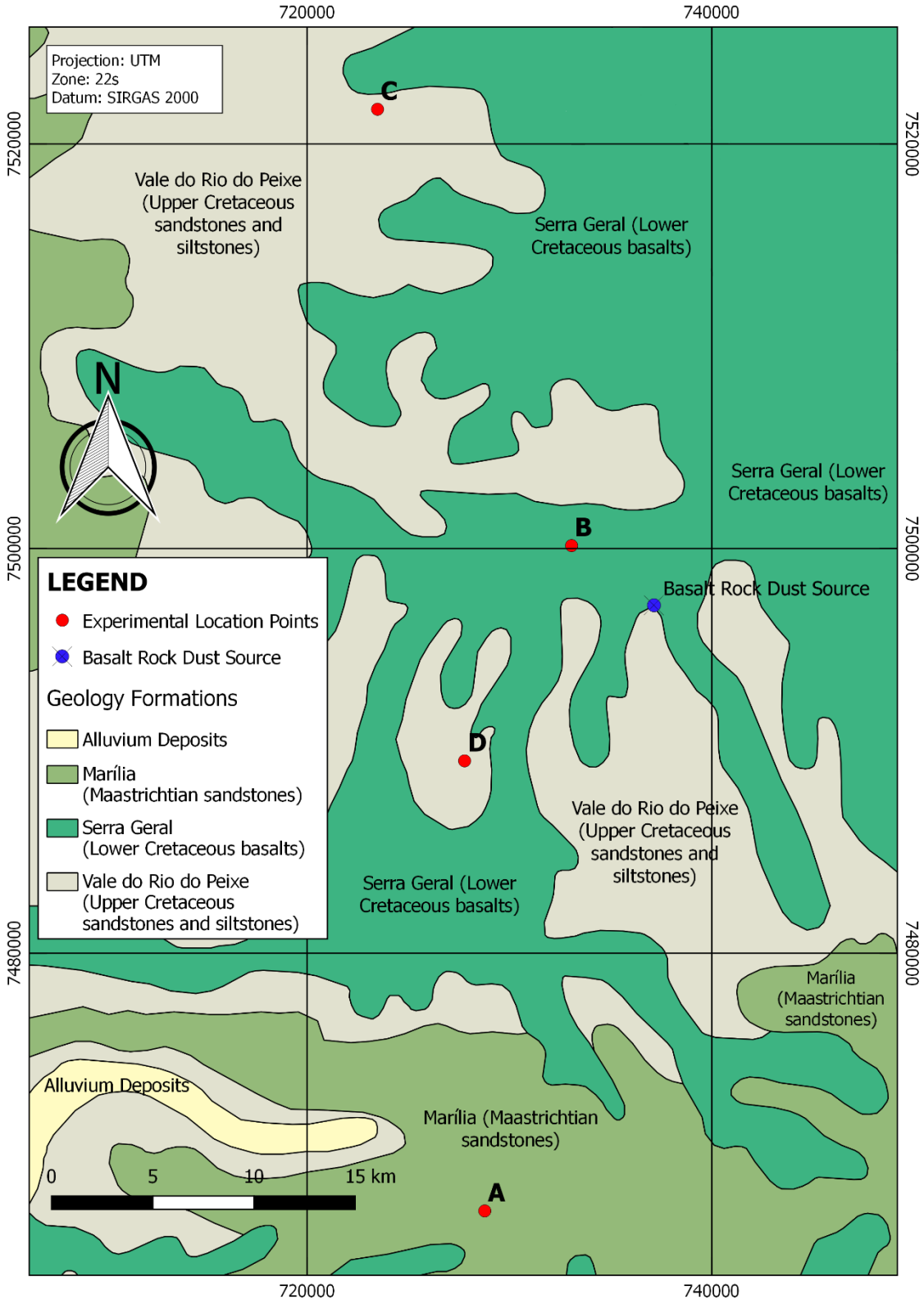

Figure 1. Sites location and geological formation according to Table 2 coordinates (Source: CPRM – Geological Survey of Brazil).

The predominant climate in the region is Cfa (Köppen), which is mainly temperate humid with a hot summer. Mean annual temperatures are 19.1-21.1°C, with 1214-1324 mm range of average annual rainfall (Figure 2).

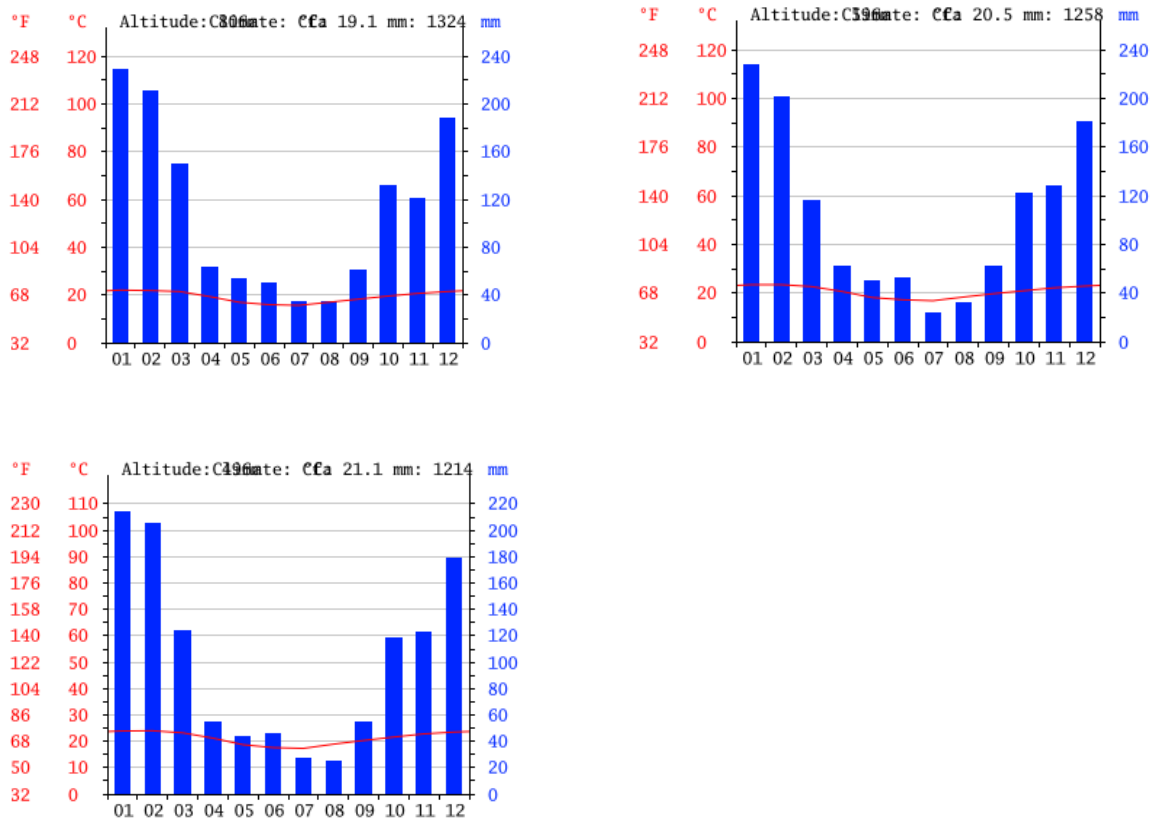

Figure 2. Climogram of sites municipality A) Botucatu B) Lençóis Paulista C) Pederneiras according to Table 2 location (Source: climate-data.org).

The soil texture and chemical attributes within each experimental site were described in Table 2.

Table 2. Characterization of soil chemical properties and texture in four experimental sites, São Paulo State, Brazil, 2016.

Sites	pH	OM	P	S	Al^{+3}	$H^{+}Al^{+3}$	K	Ca	Mg	SB	CEC
	$CaCl_2$	$g\ dm^{-3}$	$mg\ dm^{-3}$		$mmol_c\ dm^{-3}$						
					Soil Depth 0.00-0.20 m						
A	6.0	17	15	12	0	14	1.10	25	8	35	48
B	6.0	34	21	5	0	18	1.25	54	21	77	95
C	5.6	16	9	8	0	15	3.20	27	10	39	55
D	5.8	18	6	2	0	14	3.87	19	12	35	49
					Soil Depth 0.20-0.40 m						
A	5.3	14	5	10	0	16	0.48	18	4	22	38
B	5.7	30	22	18	0	24	0.91	44	19	64	88
C	5.3	10	34	2	0	18	0.54	21	7	28	45
D	6.0	10	8	4	0	12	4.96	16	10	31	43

Sites	V%	m%	Fe	Cu	Mn	Zn	B	Si	Sand	Silt	Clay	S/C *ratio*
			$mg\ dm^{-3}$									
			Soil Depth 0.00-0.20 m									
A	72	0	36	4.5	1.2	1.1	0.20	4.20	758	74	168	0.44
B	81	0	11	2.4	3.2	0.6	0.09	8.35	332	197	471	0.42
C	72	0	13	0.5	7.0	0.2	0.06	5.77	705	246	48	5.13
D	71	0	26	0.6	2.8	0.4	0.13	2.40	843	22	135	0.16
			Soil Depth 0.20-0.40 m									
A	58	0	29	2.7	0.6	0.6	0.19	3.33	742	58	200	0.29
B	73	0	12	2.7	2.9	0.9	0.52	8.85	274	163	563	0.29
C	61	0	6	0.5	2.6	0.1	0.09	5.05	702	256	41	6.24
D	72	0	8	0.4	0.6	0.1	0.05	3.37	839	22	139	0.16

OM – organic matter; $H^{+}Al^{+3}$– potential acidity; SB – sum of bases; CEC – cation exchange capacity; V% – base saturation; m% – aluminium saturation.

Experimental design and treatment application

The experiment was arranged in a randomized block design with treatments composed of 1- Control (Rock dust free) and 2- Basalt rock dust application (4 Mg ha^{-1}, respectively), with 12 replicate plots per treatment. Experimental plots consisted of 8 rows (10 m long) disregarding 0.5 m edges at each end. On sites A and B, the incorporation of basalt rock dust was performed with a light harrow during the soil tillage before planting. On sites C and D basalt rock dust was applied over 10 Mg ha^{-1} of sugarcane crop residue (straw and leaves), without soil incorporation.

Basalt rock dust properties

The basalt rock dust was obtained after crushing from a quarry located in Lençóis Paulista, State of São Paulo, Middle Tietê region. The rocks are derived from the basaltic outcrop of the Serra Geral formation, São Bento Group.

The total chemical analysis was made in Acme Analytical Laboratories (Vancouver) Ltd. following the methods below:

LF 200:

The prepared sample was mixed with $LiBO_2/Li_2B_4O_7$ flux. Crucibles were fused in a furnace. The cooled bead was dissolved in ACS grade nitric acid and analysed by ICP and/or ICP-MS. Loss on ignition (LOI) was determined by igniting a sample split then measuring the weight loss. Total Carbon and Sulphur may be included and was determined by the Leco method (TC003).

PF100: Peroxide Fusion

An aliquot of sample was fused with sodium peroxide in either a zirconia crucible or alumina crucible. The melt was dissolved in dilute hydrochloric acid and the solution was analysed. This process provides complete dissolution of most minerals including silicates. Volatile elements were lost at the high fusion temperatures.

The mining company shared the petrographical analysis of the rock, which is resumed below.

Table 3. Basalt rock mineralogy microscopic characteristics estimated.

Total mineralogy			
	%		%
Labradorite (Plagioclase)	55	Apatite	<1
Augite (Pyroxen)	35	Iron oxides	Traces
Magnetite	5	Carbonate	Traces
Volcanic glass	5	Chalcedony	Traces

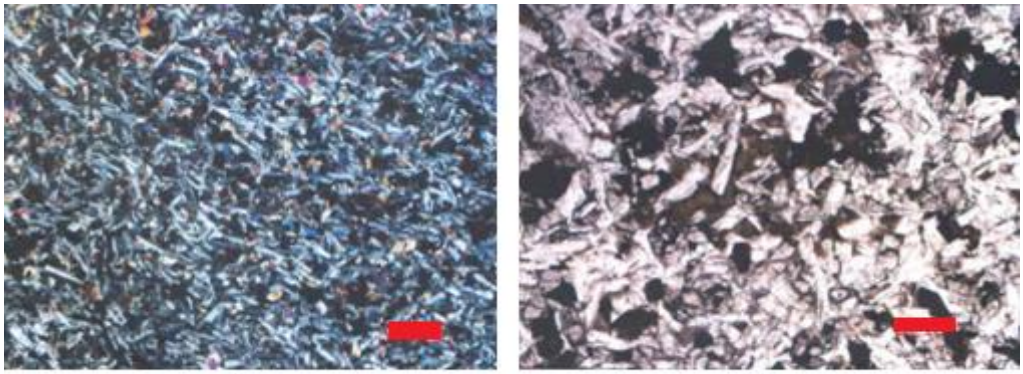

Figure 3. Basalt rock photomicrography. (A) Zoom 50x/Polarized light – Flow structure defined by preferred crystal orientation of labradorite matrix (red line scale: 0.5mm) (B) Zoom 100x/Natural light – Volcanic glass detail (brown) interstitial to crystals of matrix labradorite (red line scale: 0.2mm).

The minerals identified by optical analysis (Table 3 , Figure 3) are in accord with the standard composition of basalt rocks, that is, dominance of plagioclases, presence of augite, accompanied by several other accessory minerals (Gill, 2010).

Sampling and evaluations

The analysis was performed two years after the BRD application, using a 0.0-0.20m depth bulk soil sample. Samples were dried and sieved before further evaluation.

Mineralogical analyses

X-ray Diffraction

The basalt rock dust was sieved to give the following fractions: very coarse sand, coarse sand, medium sand, fine sand, very fine sand, and silt+clay. Each fraction was milled using an agate mortar and analysed by X-ray diffraction (XRD).

Crystalline minerals in soils were identified using a Rigaku Miniflex II X–ray diffractometer with Cu K–alpha radiation, a Ni filter and a graphite monochromator. The sand fraction was wet sieved out, and the silt fraction was obtained by extracting the clay fraction from the clay+silt suspension based on settling velocity estimated by the Stokes equation. Sand and silt fractions were random mounted in the diffractometer sample holder. Clay powder mounts were irradiated after elimination of iron oxides with CBD (citrate-bicarbonate-dithionate treated, Mehra and Jackson, 1958) and organic matter (treated with hydrogen peroxide 30%). Diffraction patterns were interpreted according to Jackson (1975), Brown and Brindley (1980) and Moore and Reynolds (1997) as well as the COD database (Gražulis et al., 2012).

Thermal analysis

The samples were analysed using thermogravimetry (TG) and differential scanning calorimetry (DSC), combined with quadrupole mass spectrometry (QMS) analysis of the gas evolved during thermal decomposition.

Samples were supplied for analysis as finely ground powders and dried overnight at room temperature where appropriate. A subsample (ca. 65 mg) was accurately weighed into an alumina crucible and analysed using a Netzsch Jupiter STA 449C TG-DSC (thermogravimetry-differential scanning calorimetry). The evolved gas stream was continuously sampled using a fused silica capillary transfer line connected to a Netzsch Aeolos 403C quadrupole mass spectrometer (QMS).

Samples were heated from 25°C to 1000°C at a rate of 10°C min-1 in an atmosphere of 20% oxygen in helium (purge gas, flow rate 30 ml min-1). The protective gas was helium (flow rate 20 ml min-1). Adapter heads and transfer lines (between the Jupiter and Aeolos) were held at 150°C.

TG and DSC data were acquired and processed using Netzsch Proteus 61 software. The QMS was operated in full scan mode over the range m/z 10-160 and mass spectrometric data were acquired and processed using Aeolos software. The main ions of interest in the QMS analysis were: m/z 12, carbon; m/z 18, water; m/z 44, carbon dioxide.

Additional ions were selected/monitored where they showed significant deviation from baseline values. Quantitative data for the abundance of selected ions in the evolved gas during heating were converted into ASCII format and then into Excel format for further processing.The samples were analysed using thermogravimetry (TG). Samples were supplied for analysis as finely ground powders and dried overnight at room temperature where appropriate. A subsample (ca. 65 mg) was accurately weighed into an alumina crucible and analysed using a Netzsch Jupiter STA 449C TG-DSC (thermogravimetry-differential scanning calorimetry).

Samples were heated from 25°C to 1000°C at a rate of 10°C min^{-1} in an atmosphere of 20% oxygen in helium (purge gas, flow rate 30 ml min^{-1}). The protective gas was helium (flow rate 20 ml min^{-1}). Adapter heads and transfer lines (between the Jupiter and Aeolos) were held at 150°C.

TG and DSC data were acquired and processed using Netzsch Proteus 61 software.

The estimates amount of gibbsite (Gb) and kaolinite (Kt) were calculated by TG-DTG weight loss values. Since the Gb main temperature range loss is around 146.0-285.6°C (Steps 1-2), and Kt around 356.9-600.3°C (Steps 3-4), the calculation consists on the difference between weight loss in step 2-1 to Gb and 4-3 to Kt, and using the proportion OH molecular mass of each mineral to calculate the %mass (Amonette and Zelazny,1994).

Data analysis

XRD data were processed in Match! Software, and the TG and DTG graphics were made using Microsoft Excel.

3.3 RESULTS AND DISCUSSION

X-Ray Diffraction

Mineralogy of Basalt

The basalt rock dust presented 2:1 Phyllosilicates (2:1Ph, 6.09°2th), Plagioclase (Pl, 12.39, 20.36, 24.87, 36.06, 38.46, and 45.10°2th), Pyroxen (Py, 29.88, 30.29, 34.91, and 35.62°2th) (Figure 4). Halite was used as a standard, (hl, 45.48 and 56.52°2th).

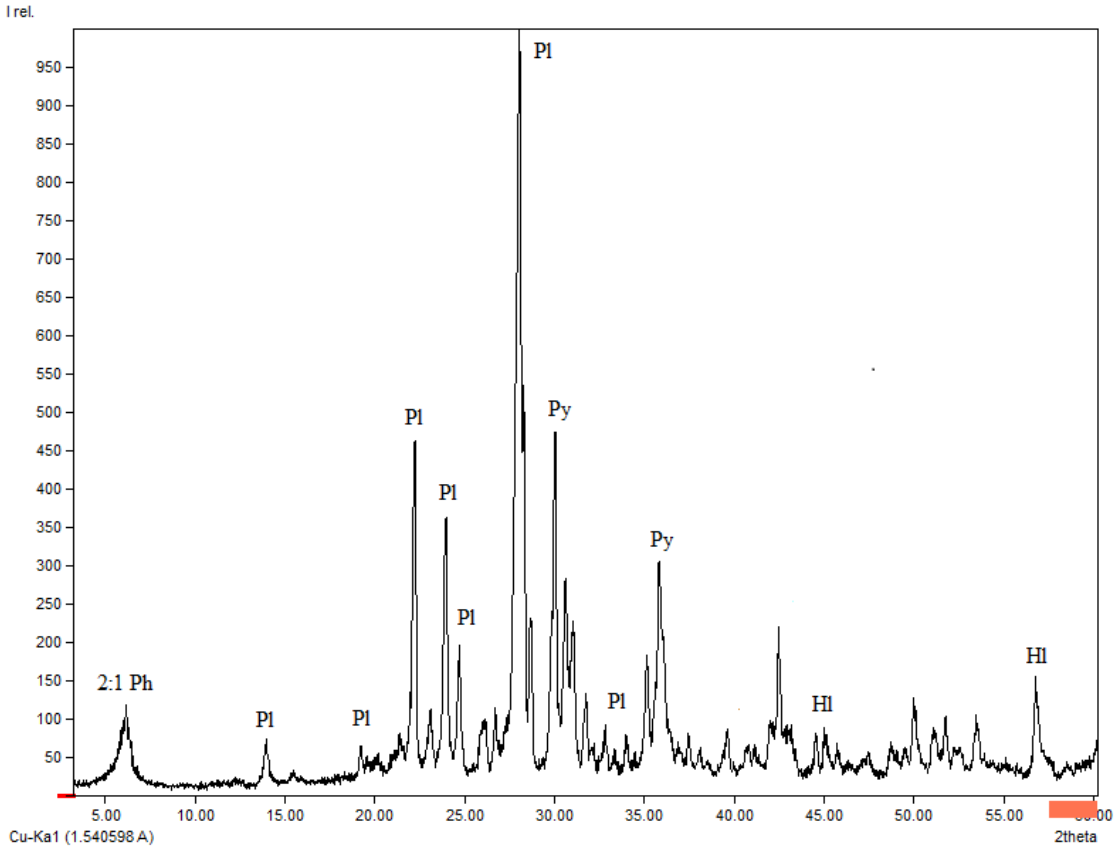

Figure 4. X-Ray diffractogram of Basalt rock dust. 2:1 Ph: 2:1 Phyllosilicates; Pl: Plagioclase; Py: Pyroxen; Hl: Halite.

The XRD pattern is in accordance with the petrological analysis (Table 3, material and methods) and with the chemical composition. The pattern show dominance of plagioclases and presence of pyroxens and accessory phases such as ilmenite and rutile. The presence of a 2:1 phyllosilicate at 6º 2theta is common in these toleitic basalts of the Serra Geral Formation and is not easy to identify (Faria, 2008).

The rock dust chemical is composed mainly by SiO_2, Fe_2O_3, and CaO and the total composition is presented in Table 4.

Table 4. Basalt rock dust chemical composition.

SiO_2	**Al_2O_3**	**Fe_2O_3**	**MgO**	**CaO**	**Na_2O**	**K_2O**	**TiO_2**	**MnO**	**P_2O_5**	**LOI**	**total**
%	%	%	%	%	%	%	%	%	%	%	%
48.95	12.26	15.80	4.60	8.44	2.55	1.37	3.79	0.22	0.47	1.2	99.65
Ba	**Ce**	**Cr_2O_3**	**La**	**Nb**	**Nd**	**Ni**	**Pb**	**Rb**	**Sc**	**Sr**	**Th**
Ppm	ppm	%	ppm	ppm	ppm	ppm	ppm	ppm	Ppm	ppm	ppm
441	65.4	0.003	30.3	20.4	34.0	27	1.3	28.2	31	483.3	2.7
U	**V**	**Y**	**Zn**	**Cu**	**Zr**	**Eu**	**Er**	**Lu**	**Be**	**Dy**	**Co**
ppm	ppm	ppm	ppm	ppm	ppm	Ppm	ppm	ppm	ppm	ppm	ppm
0.5	475	34.9	66	188.5	215.9	2.51	3.22	0.43	2	6.42	37.6
Cs	**Ga**	**Gd**	**Hf**	**Ho**	**Pr**	**Sm**	**Sn**	**Sum**	**Ta**	**Tb**	**Tm**
ppm	ppm	ppm	ppm	ppm	Ppm	ppm	ppm	%	ppm	ppm	ppm
0.2	19.7	7.99	5.6	1.18	8.22	7.44	2	99.68	1.2	1.21	0.46
W	**Yb**	**Ag**	**As**	**Au**	**Bi**	**Cd**	**Hg**	**Mo**	**Sb**	**Se**	**Tl**
ppm	ppm	ppm	ppm	Ppb	ppm	ppm	ppm	ppm	ppm	ppm	ppm
<0.5	2.90	<0.1	<0.5	0.7	<0.1	0.2	<0.01	0.6	<0.1	<0.5	<0.1
B	**TOT/C**	**TOT/S**									
ppm	%	%									
8	0.09	<0.02									

Source: Acme Analytical Laboratories (Vancouver, Canada, 2016)

Basalt rock dust particle size distribution was classified according to Table 5.

Table 5. Basalt rock dust particle size analysis, São Paulo State, Brazil, 2015.

	Particle diameter	BRD
	mm	*%*
Very course sand	2.00	0.54
Course sand	1.00	0.13
Medium sand	0.50	10.9
Fine sand	0.25	23.3
Very fine sand	0.10	28.3
Silt	0.05	35.2

Mineralogy of soils

In all four sites, kt, gibbsite (gb), and qtz presented stronger peaks (Figure 5-8).

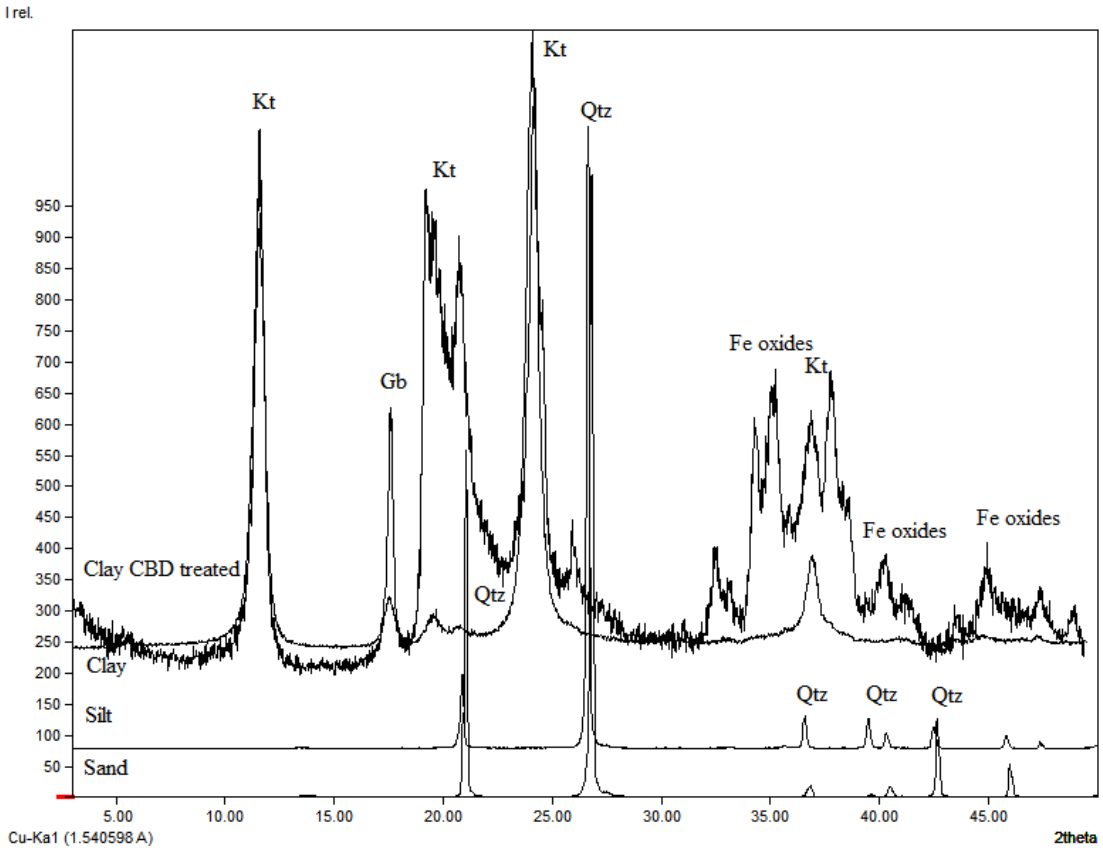

Figure 5. Site A Control XRD, fractions sand, silt, clay, and clay CBD treated.

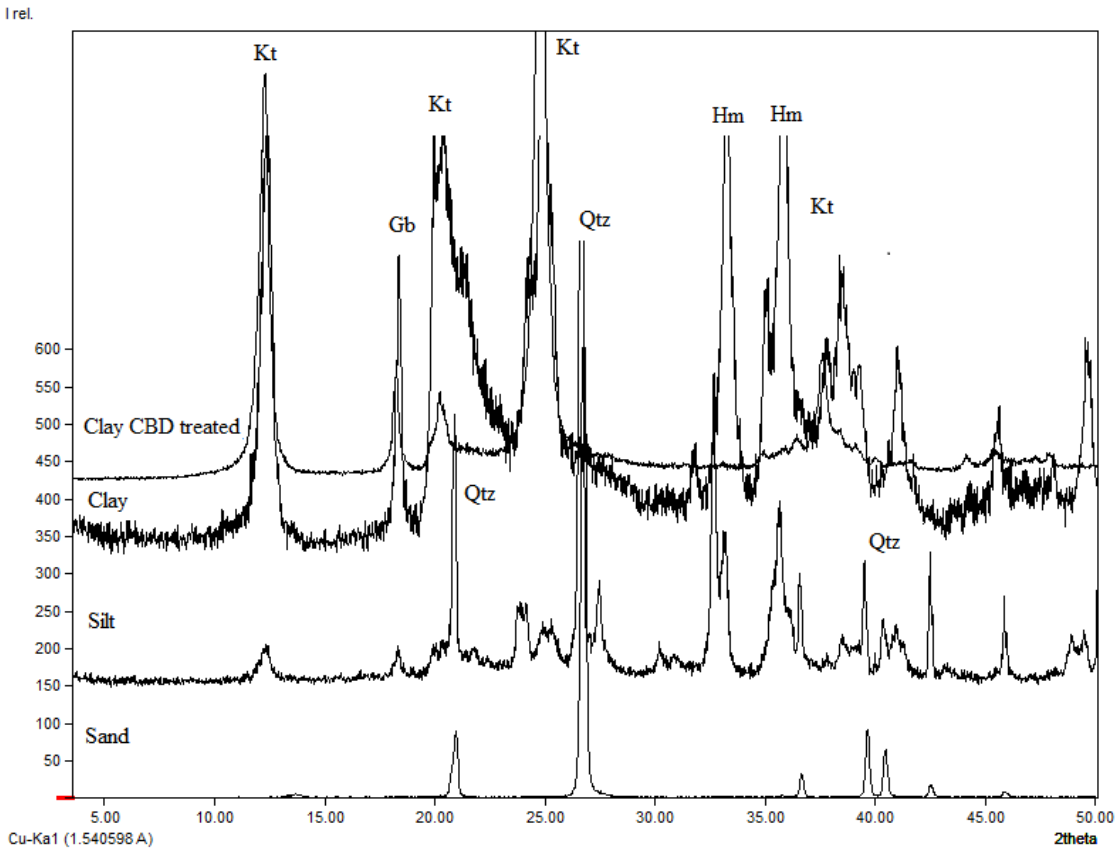

Figure 6. Site B Control XRD, fractions sand, silt, clay, clay CBD treated.

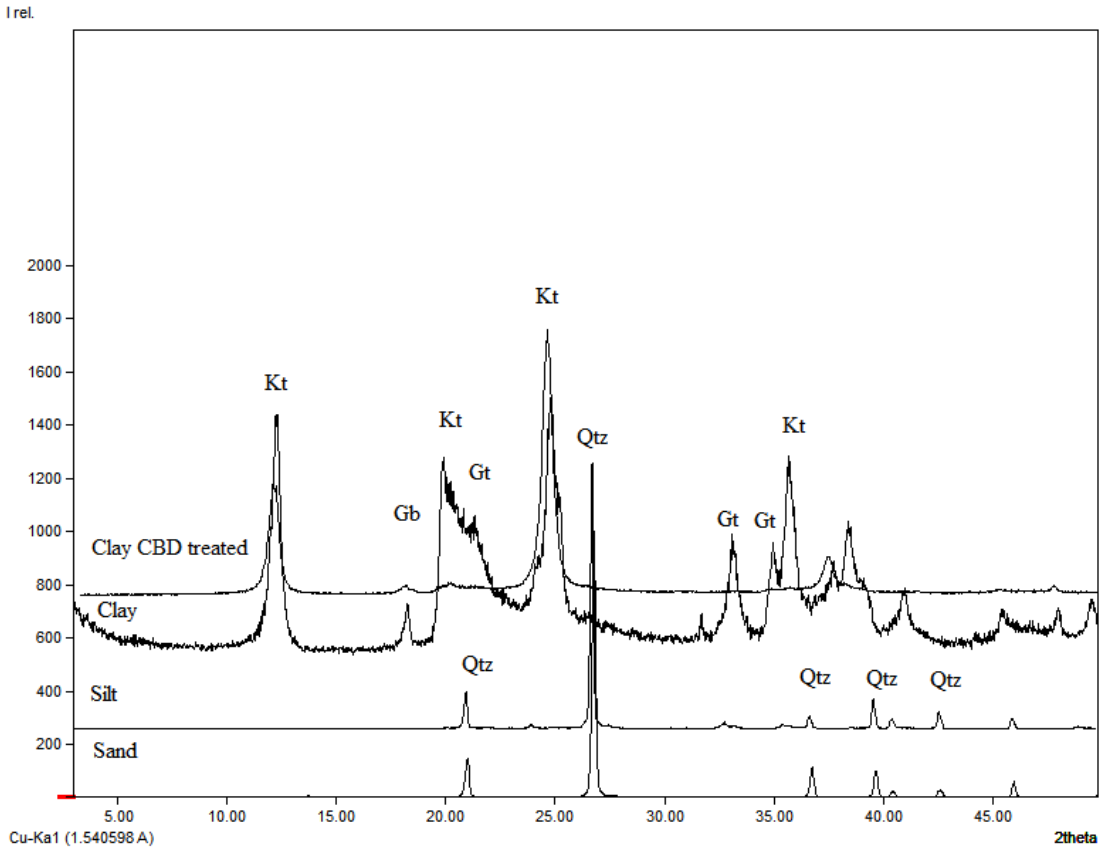

Figure 7. Site C Control XRD, fractions sand, silt, clay, clay CBD treated.

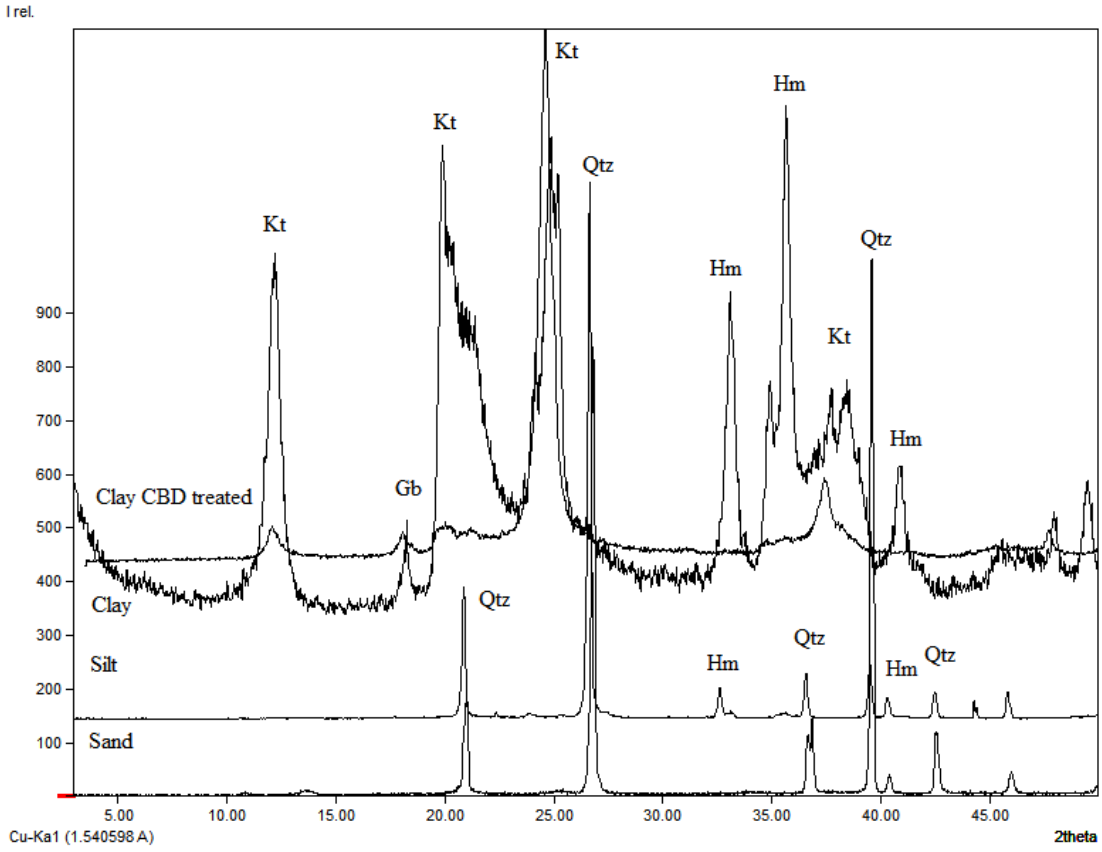

Figure 8. Site D Control XRD, fractions sand, silt, clay, clay CBD treated.

As all soils were Ferralsols, their mineralogy is simple, mainly composed by quartz in the sand and silt fractions, and kaolinite in the clay fractions (Ferreira et al., 1999; Ghidin et al., 2006; Montanari et al., 2010; Silva et al., 2012) (Figure 5-8). In site C (Figure 7) there was Goethite (Gt) and in site B and D (Figure 6 and 8) Hematite (Hm) enough to yield a signal in the XRD pattern.

Differential X-ray Diffraction (DXRD)

The minimum concentration required to reach an appropriate signal ratio to read the X-ray diffractometer, about 5% volume basis, was higher than the rock dust concentration found in the soils (Moore and Reynolds, 1999). This is the reason why we could reach an enough amount to present the DXRD between soils BRD treated and control just in the samples showed below.

The differential X-ray diffraction from Site C shows the difference between BRD and the DXRD from sand fraction (the difference between sand BRD treated and sand control), resulting in the BRD weathered (Figure 9).

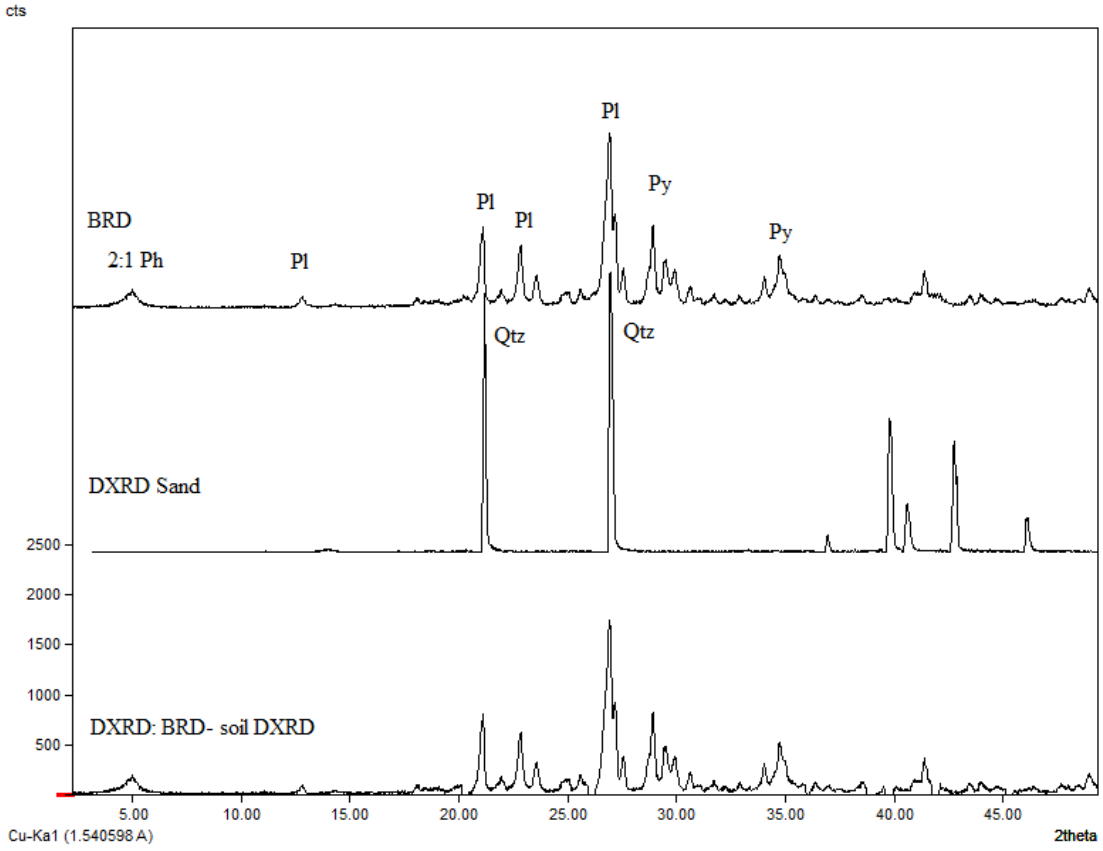

Figure 9. DXRD Site C sand fraction.

In clay samples, the difference between clay fraction BRD treated and clay control was minimum, showing lower detectable BRD in this fraction (Figure 10).

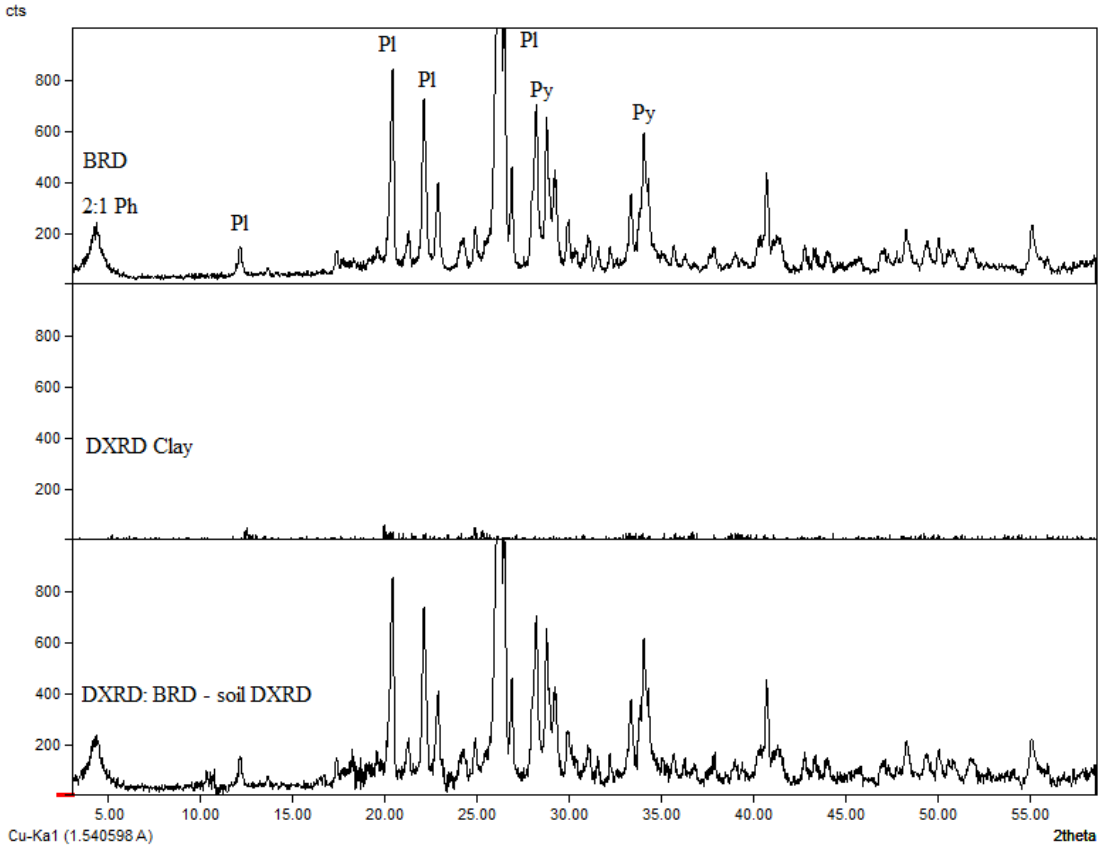

Figure 10. DXRD Site C clay fraction.

Thermogravimetry (TG), and Differential Scanning Calorimetry (DSC)

All four sites presented the same temperature range events, however the mass loss and heat flow occurring in different intensities. Two mass losses are more evident in TG and DSC analysis: the first one in starting after 200°C, representing loss of water because of gibbsite dehydration, an endothermic reaction (DSC curves). The other one in 450°C, 450–550 °C correlates strongly with clay content, and probably overlaps with carbon decomposition (Plante et al., 2009; Kučerík et al., 2018), in an endothermic reaction, more evident to Site B both, BRD and Control, curves (Figure 11), corroborating with ion current analysis in Figures 15, 16, 17 and 18. In DSC curves a smooth endothermic reaction occurred around 600°C, probably representing the carbonates degradation, and between 900-1000°C occurred an exothermic reaction, representing probably calcite degradation forming CO_2 (Todor, 1976; Plante et al., 2009) (Figure 11).

Site B has a highest mass loss, compared to other 3 sites, and the weight loss curves for both control and treated samples overlap (Figure 11). However, Site C Control curve was higher than BRD treatment in 1000°C (Figure 11).

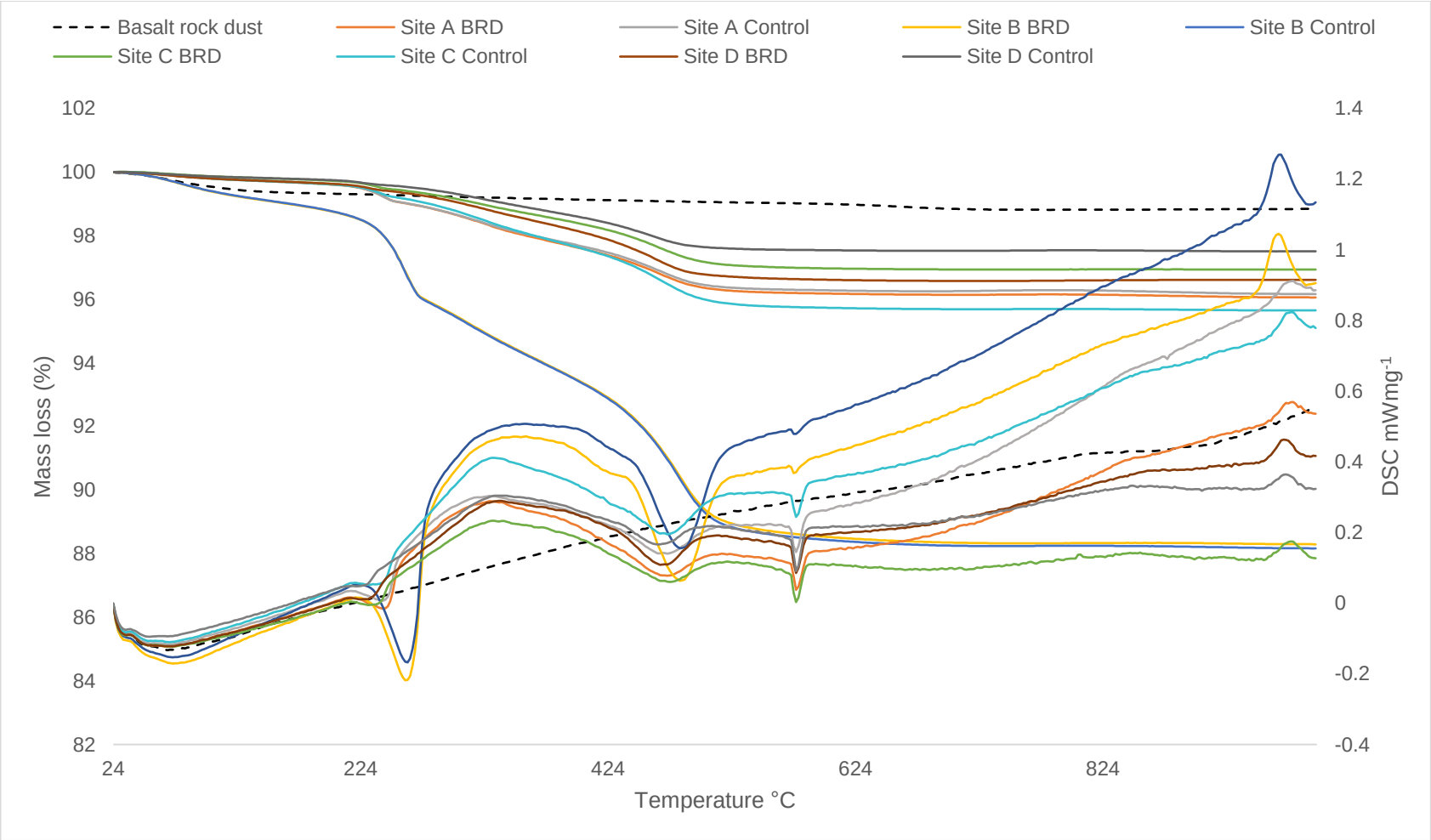

Figure 11. Weight loss (TG) and differential scanning calorimetry (DSC) for four sites with basalt rock dust treatment (BRD) and control comparing to Original basalt rock dust (dashed line) within the temperature range from 24 to 1000°C.

The samples percent loss of mass as a function of temperature increase were very similar (Figure 12).

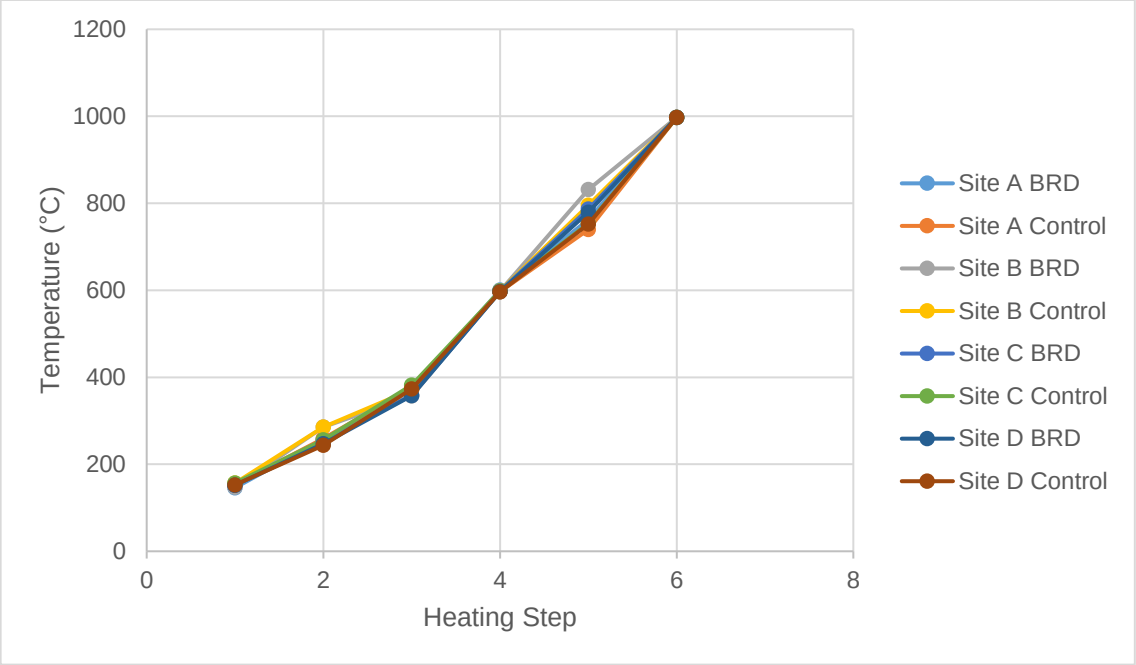

Figure 12. Mass loss (%) as a function of temperature (°C) for four sites BRD treated and control.

Therefore, the curves were segmented into six major steps. The major mass loss occurred at the steps 2, 3 and 4 (Table 6). Soil properties influence the thermogravimetric patterns (Kristensen, 1990; Golebiowska et al., 1996). In the present study, sites A, C, and D have sandy soils, whilst Site B has a clayey soil (Table 2). Moreover, Site B is located in the best production environment classification (A, Table 2), and this site is situated in the same geological formation of the basalt rock dust source (Figure 1).

Table 6. Temperature range (°C) and mass loss (%) range occurring in respective step.

STEP	Temperature range	Mass loss range	Associated event
	°C	%	
1	0 to 157	0.20 to 0.93	
2	146 to 285.6	0.21 to 3.39	Loss of water
3	243.4 to 382.2	0.74 to 1.79	
4	356.9 to 600.3	1.24 to 5.77	Loss of CO_2 Dihydroxylation of kaolinite OM oxidation
5	596.4 to 831.9	0.01 to 0.20	Decomposition of carbonate ions associated
6	997.2 to 831.9	0.00 to 0.11	with Mg and Ca

Source: Hollingbery and Hull, 2010; Ashraf et al., 2009; Plante et al., 2009

The cumulative loss of mass (Figure 13) was similar for all soils (losses between 2 and 4%) except the B soil, which was much greater (almost 12 %).

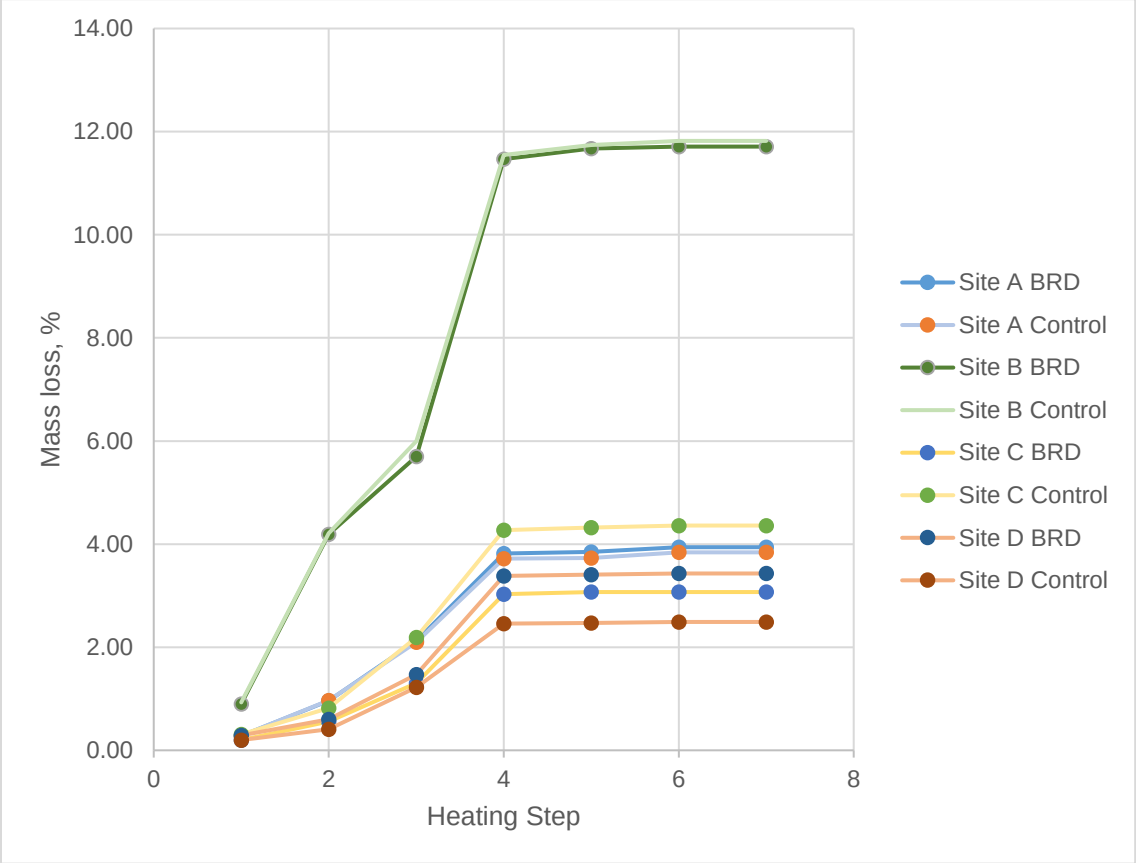

Figure 13. Mass loss of samples, dry mass basis. Heating step as in Table 6

For all soils the mass loss increased up to 600°C (end of step 4) and reached a plateau with very small further loss.

Carbonate as CO_2–C releases occurs at temperatures ranges above 550°C (Liptay, 1974; Gaál et al., 1994), usually up to 1000°C (Table 6). Since lime was applied in sites A and B (see Materials and Methods) it should be related to mass loss at step 5, but apparently, the lime was consumed by soil acidity in the two years from application to sample collecting, since no significant mass loss was detected at this temperature range.

Regarding the amount of mass loss, two contrasting groups of soils can be identified: 1) the A, C and D soils, the plateaus established between 2 and 4% loss, and 2) the B soil, near the 12% loss (Figure 13).

The great content of clay in Site B is the reason for the great loss of mass (Table 6), instead of a different type of clay (for example a 2:1 phyllosilicate). According to

Siewerty (2004), the intensity of weight loss signals depends on the soil material composition, and the clayey samples revealed higher weight losses at each temperature range than the sandy samples.

The weight loss in the temperature range 200-450°C is related to soils management and microbial activity (Chernikov, 1988; Anhehrn-Betti-nazzi et al., 1988; Peschke et al., 1991; Sevcova and Sidorina, 1988), while the temperature range about 500°C depends on geological formation and soil texture, mainly clay content (Chernikov,1988; Leinweber et al., 1993). This corroborates with the present study because probably the other reason why Site B had higher weight loss than other sites is its parental rock (basalt) and geological formation (Serra Geral – showed in Figure 1), while other soils are from sandstones parent rocks (Fernandes and Coimbra, 2000).

Regarding the difference in mass loss between BRD treated and untreated soils, again two groups can be devised: 1) soils that BRD treatment did not change the mass loss (A and B) and 2) the ones with different losses between BRD treated and untreated (C, and D) (Figure 11). This may be related to the application of BRD onto surface in these two sites, as compared to the other two (A and B) where the BRD was incorporated into the soil (see Table 1). Moreover, limestone was applied in both cane-plant sites (A and B), and they were in initial crop-cycles (cane-plant).

The TG analysis allows to estimate the amount of gibbsite [$Al(OH)_3$] in samples, in a low crystallinity (Figure 14).

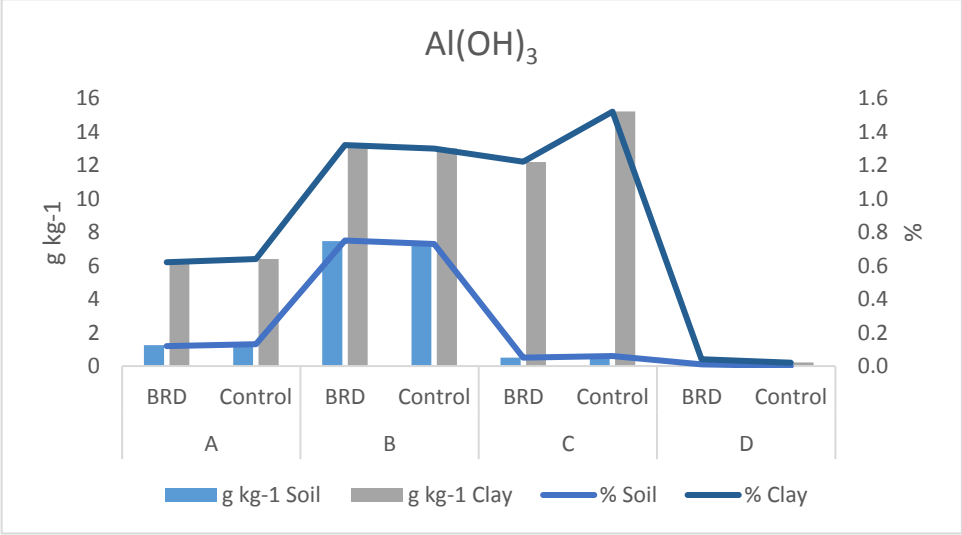

Figure 14. Estimates amount of non-crystalline gibbsite from TG analyses data.

The estimates are in accord with the amount of clay in each soil (Figure 14), as well as with the gibbsite relative intensities in the XRD patterns (Figure 7 and 8). Taking into account for these uncertainties, these results suggest that the secondary phases being precipitated from the BRD are mainly silicate phases, supposedly kaolinite instead of gibbsite (a TG study of the clay fraction alone would give much more reliable information about this issue). This is surprising, since the fast weathering of plagioclases in the saprolite zone (at depth) produces gibbsite and Al-amorphous phases, which are further source of Al for kaolinite formation in the soil, as Clemente and Azevedo (2007) has found in a rhyolite regolith profile nearby the basalt quarry where the rock dust used in this experiment was mined. It is important to take into account that in the temperature range that dehydroxylation of kaolinite is occurring, other losses are in process as well, for example, the OM oxidation, and CO_2 losses. This is the reason why we cannot in this instance, calculate the amount of kaolinite formation.

The mass spectra of the four soils (control and treated with BRD) thermal decomposition showed clearly pronounced signals of m/z = 12, 18, and 44 (Figures 15, 16, 17 and 18). Moreover, m/z = 20, 27, 28, 29, 30, and 34 signals we measured as well, but they were not considered, due to their insignificant levels.

IC derived from m/z = 12 corresponded to TOC was shown at temperature ranges between Step 2 and 4. The main signal from H_2O molecules was observed as m/z = 18 in two peaks: one in temperature range covered by Step 2 and another one covered by Step 4. The intensities recorded as m/z = 44 were related to CO_2 release, observed between step 2 and 4 (Manning et al., 2005; Tudorachi and Chiriac, 2011).

Site A Basalt rock dust treatment showed a slight higher IC derived from m/z = 44 compared to control (Figure 15), in the temperatures between Steps 2 to 4.

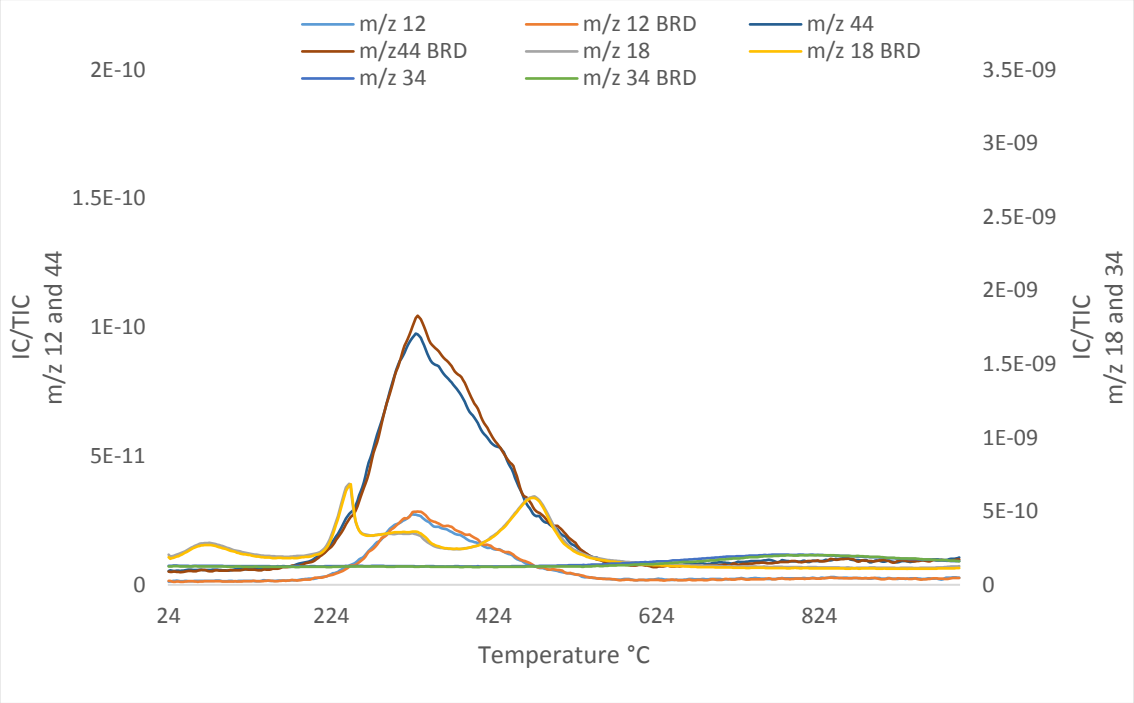

Figure 15. The distribution of the ion current (IC) of the main m/z intensities for Site A

Among the four sites, Site B showed the highest IC values derived from the three m/z studied here. IC derived from m/z = 12 and 44 showed higher values in BRD treatment compared to Control (Figure 16), representing increasing of Carbon (C and CO_2) release.

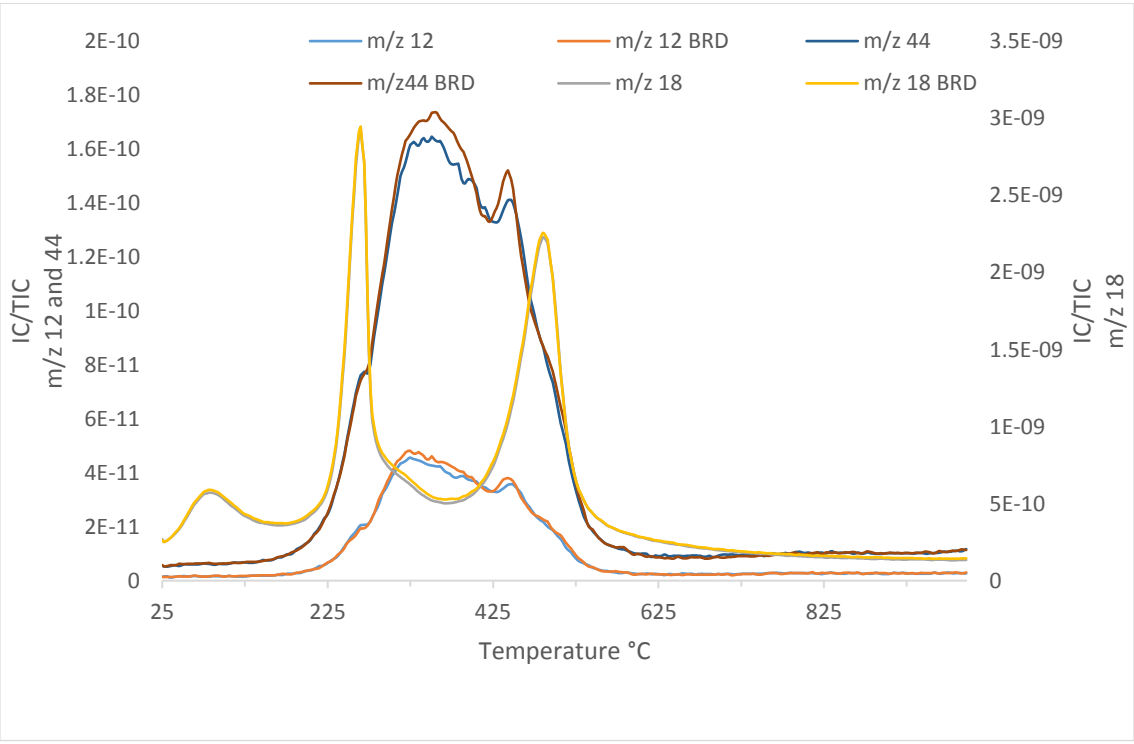

Figure 16. The distribution of the ion current (IC) of the main m/z intensities for Site B.

Ratoon sites (C, and D) presented the highest difference between BRD treatment and control in IC values derived from m/z = 12, and 44 (Figures 17, and 18). In Site C, higher carbon, and carbon dioxide were released by control plots, whilst BRD treatment showed higher water release (Figure 17). The water (H_2O) evaporation above 300°C is resulted from oxygen-containing groups decomposition, mainly hydroxyl groups (Arenillas et al., 1999).

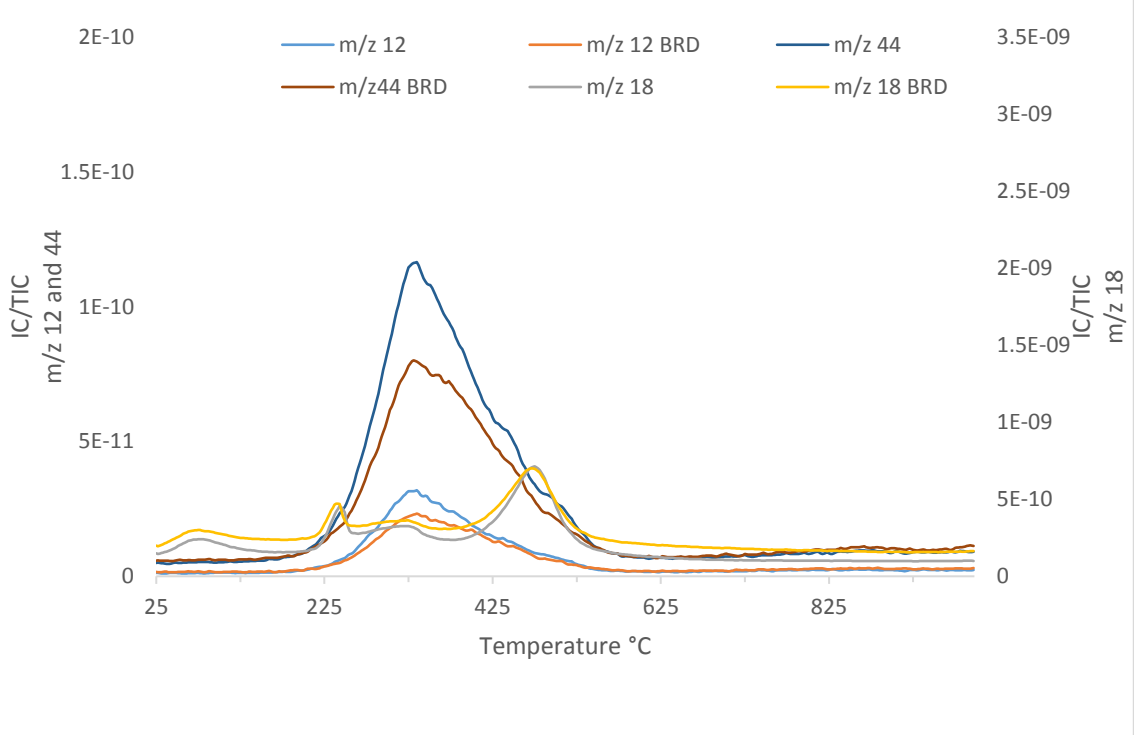

Figure 17. The distribution of the ion current (IC) of the main m/z intensities for Site C.

Regarding Site D, basalt rock dust increased IC values derived from m/z = 12, 18 and 44, representing higher release of Carbon, water and carbon dioxide (Figure 18).

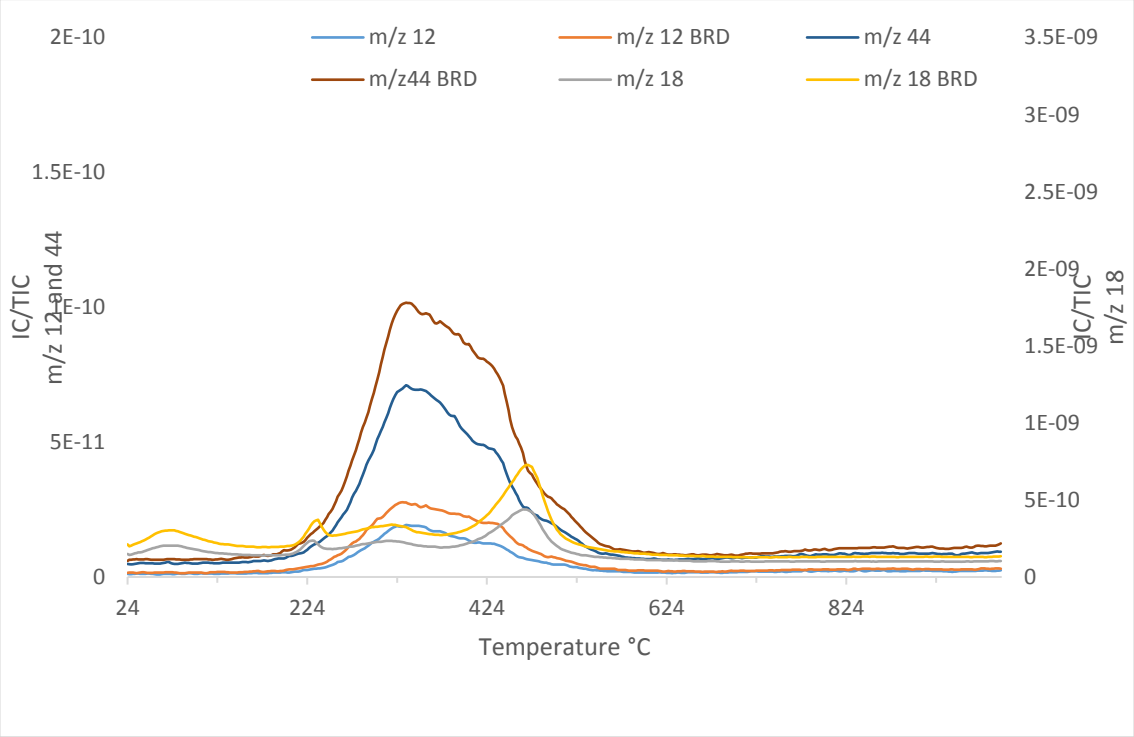

Figure 18. The distribution of the ion current (IC) of the main m/z intensities for Site D.

The QMS allows to confirm the decomposition of gibbsite at around 200°C in ion current derived from m/z = 18 corresponding the water derived from gibbsite dehydration and similarly for kaolinite at around 500°C (Figures 16, and 18). The ion current derived from m/z = 44 confirms the weight loss corresponded to organic matter decomposition, at 300-500°C temperature range (Figures 17, and 18), corroborating with previous results presented above (Figure 14).

Any release was observed in temperature ranges covered by steps 5, and 6 for all sites.

3.4 CONCLUSION

Most evident Basalt rock dust components are plagioclases and pyroxen, which are sources of Ca, Mg, Al, Fe and Si.

Ferralsols are composed by quartz, gibbsite, and kaolinite basically, but Site C showed Goethite signals and sites B and D, Hematite in the XRD patterns, therefore the presence of these oxides should be near or above 5% in these soils.

Site B presented the highest mass loss among the four sites, due to its geological formation and parental rock, and consequently clays concentration.

Basalt rock dust treatment increased the mass loss in ratoon sites C, and D, where it was applied at the soil surface.

Basalt rock dust application changed the estimated amount of mineral, increasing the loss of non-crystalline kaolinite in soil and clay compared to control, but this difference is inverse to gibbsite, purposing the gibbsite (non-crystalline) forming kaolinite (non-crystalline).

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FINAL CONSIDERATIONS

Despite not consistent to the four areas, basalt rock dust treatment can improve the sugarcane yield, soil chemical attributes, and microbial index of soil quality but a little is noticed in plant chemical attributes.

The microbial diversity was not the same to the four areas, but it can be more related to geographical patterns than rock application, even with a little shift occurring, it cannot be attributed to the treatment.

Weathering signals were noticed but there are two questionable points: the time to occur this weathering, may be quicker than it was thought, and the amount of weathered minerals.

Basalt rock dust application improves sugarcane yield, it was notable its mineralogical changes in the soil and it does not cause damages to the soil microbial diversity.

The basalt rock, the soil environment and the plants work as a cycle, that means, the rock release some trace elements that work together to plants exudates compounds as microorganisms growth factor, then the soil microbiota communities and population increase, the rock bioweathering occurs more intensively, providing more elements and plant nutrients, always to reach a balance in the soil.

The microbial activity and footprints of soil microbiology in these conditions could elucidate the reason why occurred the enormous yield improvement.

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