

University of São Paulo  
“Luiz de Queiroz” College of Agriculture

Rock powders in the soil-plant system: mineralogy and microbiome response

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Dissertation presented to obtain the degree of Master in  
Science. Area: Soil and Plant Nutrition

Piracicaba  
2021

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**Rock powders in the soil-plant system: mineralogy and microbiome response**  
versão revisada de acordo com a resolução CoPGr 6018 de 2011

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Science. Area: Soil and Plant Nutrition

Piracicaba  
2021

**Dados Internacionais de Catalogação na Publicação**  
**DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP**

Reis, Betania Roqueto dos

Rock powders in the soil-plant system: mineralogy and microbiome response / Betania Roqueto dos Reis - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2021.

42 p.

Dissertação (Mestrado) - USP / Escola Superior de Agricultura "Luiz de Queiroz".

1. Intemperismo acelerado 2. Geomicrobiologia 3. Remineralizador 4. Agromineral 5. Potássio 6. T-RFLP 7. Comunidade bacteriana 8. Lixiviado I.  
Título

To Maria Catarina Roqueto - mother, teacher and the main “factor of life formation”.

**I dedicate.**

## ACKNOWLEDGMENTS

To the National Council for Scientific and Technological Development - CNPq and the Coordination for the Improvement of Higher Education Personnel - CAPES for financial support.

To the "Luiz de Queiroz" College of Agriculture (ESALQ/USP), the Soil and Plant Nutrition Program and the GPEMSO group.

To my advisor, prof. Antonio Azevedo, who since 2013 dedicates his time to my professional and personal growth.

To prof. Fernando Andreote for co-guidance.

The team Rossi, Leandro, Denise, Fernando, Sonia, Armando, Marina C., Eleusa, Kelly, Ana Luisa, Caio D., Caio, Felipe B., Felipe, Catia for their help and teachings.

To the friends brought to me by the graduate program and which made this journey lighter: Jacque, Thairis, Jean, Bia, Miriam, Clênia, Julio, Geraldo and Thayana.

The women and Julio (Mega) from the republic Gaiola das Lokas who have been accompanying and supporting me since 2011.

My family, especially my brothers Barbara and Reinaldo and those who are no longer present in body, but in my heart, José Reinaldo, João Lucio (Uncle Lu) and Franscisco (Uncle Kiko).

To my partner, Pedro.

Thank you.

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## RESUMO

**Pós de rocha no sistema solo-planta: resposta da mineralogia e microbioma**

Pós de rocha (mais corretamente denominados agrominerais silicatados) são rochas apenas moídas e peneiradas, geralmente co-produtos da indústria de mineração e britagem, que podem ser utilizados na agricultura de acordo com a legislação brasileira (Lei 12.890) desde que atendam os critérios da IN 5/2016. Observações e experimentos de campo têm apresentado resultados incompatíveis com a quantidade e cinética de liberação de nutrientes pelos pós de rocha. Estes resultados sugerem que a abordagem da remineralização por um viés exclusivamente químico-inorgânico não é suficiente para entender e prever o funcionamento do sistema. A dissolução mineral é fundamental para regular teores de CO<sub>2</sub>, características químicas e físicas do solo e fonte de energia para micro-organismos. Sabe-se que a interação de rochas/minerais e micro-organismos ocorre desde antes da formação de solos e que a colonização do ambiente terrestre pelas plantas foi possível devido a interação de micro-organismos e raízes primitivas para obtenção de nutrientes de fontes não solúveis. Essas interações são responsáveis por inúmeros processos no solo e dentre eles, a interação com rochas em processos como intemperismo e pedogênese é fundamental para formação de solos. O objetivo deste trabalho foi compreender a interação dos pós de rocha com a comunidade microbiana no sistema (solo + pó de rocha)-planta, em função da introdução de diferentes rochas moídas. O experimento foi desenvolvido com cinco fontes distintas de pós de rocha (um Fonolito, três tipos de Basalto e um Granito) e uma fonte solúvel de fertilizante potássico (KCl) em vasos cultivados com *Brachiaria brizantha* cv. Marandu. O experimento foi montado em cópias para monitoramento das alterações no sistema ao longo do tempo. Foram avaliadas: atividade enzimática e alteração da comunidade microbiana do solo, colonização por fungos micorrízicos arbusculares (FMA), alterações mineralógicas, quantificação da dissolução e liberação de nutrientes dos pós de rocha. Nesta dissertação, apresentamos os resultados da estruturação da comunidade bacteriana ao longo de oito meses pela técnica de fingerprinting T-RFLP e como os elementos lixiviados auxiliam nessa modificação. De modo geral, a comunidade bacteriana se altera lentamente nos tratamentos com pós de rocha, diferenciando em oito meses, e se estrutura de acordo com a rocha aplicada. A comunidade bacteriana para o tratamento com fonte solúvel já se reestrutura após 1 mês da aplicação, influenciada pela liberação dos íons K e Ca.

Palavras-chave: Intemperismo acelerado, Geomicrobiologia, Remineralizador, Agromineral, Potássio, T-RFLP, Comunidade bacteriana, Lixiviado

## ABSTRACT

### **Rock powders in the soil-plant system: mineralogy and microbiome response**

Rock powders are only ground and sieved rocks, usually co-products of the mining and crushing industry, which can be used in agriculture in accordance with Federal Law 12,890. Field observations and experiments have shown results incompatible with the amount and kinetics of nutrient release by rock powders. These results suggest that the approach to remineralization by an exclusively chemical-inorganic perspective is not sufficient to understand and predict the functioning of the system. Mineral dissolution is fundamental to regulate CO<sub>2</sub> contents, soil chemical and physical characteristics and energy source for microorganisms. It is known that the interaction of rocks/minerals and microorganisms occurs since before the formation of soils and that the colonization of the terrestrial Earth by plants was possible due to the interaction of microorganisms and primitive roots to obtain nutrients from non-soluble sources. These interactions are responsible for numerous processes among soil and them, including weathering and pedogenesis, fundamental for soil formation. The objective of this project was to understand the interaction of rock powders with the microbial community in the system (soil + RM)-plant. The experiment was developed with five distinct sources of rock powders (one Fonolith, three types of Basalt and one Granite) and one soluble source of potassium fertilizer (KCl) in pots cultivated with *Brachiaria brizantha* cv. Marandu. The experiment was assembled into copies to monitor system changes over time. The following were evaluated: enzymatic activity and alteration of the soil microbial community, colonization by arbuscular mycorrhizal fungi (AMF), mineralogical alterations, quantification of dissolution and release of nutrients from rock powders. In this chapter we present the results of the structuring of the bacterial community over eight months by the T-RFLP fingerprinting technique and how the leached elements aid in this modification. In general, the bacterial community changes slowly in treatments with rock powders, differentiating in eight months, and is structured according to the applied rock. The bacterial community for treatment with soluble source is already restructured after 1 month of application, influenced by the release of K and Ca ions.

Keywords: Enhanced weathering, Geomicrobiology, Remineralizer, Agromineral, Potassium, T-RFLP, Bacterial community, Leachate





## 1. GENERAL INTRODUCTION

Since the formation of the Earth's current atmosphere, mineral dissolution has played a central role in the concentration of atmospheric CO<sub>2</sub> levels, in addition to being a source of energy and nutrients for organisms, modeling the landscape and the chemical and physical characteristics of the soil (Brantley, 2008).

The rate of dissolution of a mineral depends on several factors such as size, specific surface area (SSA), structure, chemical composition (solubility), crystallinity, type of dissolution, solvent (water or otherwise) and time. In general, a mineral can have two types of dissolution: congruent and incongruent. While the former is stoichiometric and predominates in ionic solids with a high dissolution rate, the latter, typical of silicates, produces temporal variations in ions for not being stoichiometric.

Incongruent or non-stoichiometric dissolution are caused by three main factors: (1) dissolution of specific phases or zones of impurity into the mineral, (2) concurrent dissolution-precipitation of minerals or (3) preferential leaching of surface elements (Brantley, 2008).

In nature, mineral dissolution is usually associated with weathering and pedogenesis processes. The dissolution of rock dust in superficial agricultural soil horizons is a peculiar, “non-natural” condition because primary minerals are subject to an environment with already stabilized organic matter, established microbial populations, already developed soil structure (and porosity), among many other factors that differentiate the A horizon from the saprolitic zone, where these minerals are found in their unaltered or slightly altered state. In addition, rock powders have a specific area much larger than their counterparts in the saprolitic zone.

According to Smith et al. (2019) the use of crushed rocks in agricultural and forest soils is a strategy for greenhouse gas mitigation and sustainable development required by the United Nations. In fact, minerals and rocks are essential in the dynamics of terrestrial ecosystems (Landeweert et al.(2001) and Ward et al. (2013)), which suggests that these interactions must also be significant during soil remineralization.

During the mining companies' comminution process, it is estimated that 15% to 35% of the rock volume is inevitably transformed into a fine powder not marketed as a main product (Mitchell et al., 2008). Therefore, safely applying this mining and crushing co-product to soils would promote the minimization of the impacts caused by this industry (Edwards et al., 2017), in addition of being a source of macro and micronutrients for plants in the medium and long term (Beerling et al., 2018). In addition, the application of rock powder is a enhanced weathering (Terrestrial Enhanced Weathering - TEW) practice (Hartmann et al., 2013; Schuiling and Krijgsman, 2006), consuming atmospheric CO<sub>2</sub> during the dissolution of minerals. With the increasing world population, there is need for more efficient food production and alternative sources of nutrients to supply this demand. Rock powders are a significant contribution to meet these demands (Manning, 2015).

Brazil is a global hotspot for the use of rock dust (Strefler et al., 2018). Manning and Theodoro (2018) showed positive results from the use of rock dust in Brazil. A case study carried out in the State of São Paulo (Brazil) by Lefebvre et al. (2019) evaluated the life cycle of basalt powder considering the production and consumption of carbon in all stages of the process, from transportation to the final agricultural product. The results showed that the transport of the material to the agricultural area is the main factor that determines the potential for CO<sub>2</sub> sequestration of the practice, being necessary to use local sources of rock powders (550 ± 65 km) for carbonation and that it could be captured 1.3 to 2.4 Mt CO<sub>2</sub>eq through the application rate of 1 t/ha applied on all its 12 million hectares.

However, field observations and experiments have shown results incompatible with the amount and kinetics of nutrient release from rock powders. For example, Souza et al. (2017) used biotite gneiss powder as a source of

potassium in the millet culture and found that increasing doses of rock powder increased the availability of K, CTC and soil pH, with the treatment of 6 Mg ha<sup>-1</sup> of rock powder compatible with KCl treatment. Resende et al. (Resende et al., 2006) evaluated three rocks as a source of potassium in different cultures and found that the release of nutrients is different between the rocks, releasing other nutrients besides potassium and the residual effect of remineralizers is beneficial when compared to soluble fertilizers.

These results suggest that remineralization approach through an exclusively chemical-inorganic perspective is not sufficient to understand and predict the functioning of the system.

Lithotrophic microorganisms have existed since the beginning of life on Earth, therefore it is logical to accept that soil microorganisms and terrestrial plants co-evolved since at least the Devonian, 500 million years ago (Brundrett, 2002; Lambers et al., 2009; Verma et al., 2017). Microorganisms are fundamental for mineral dissolution and there is a diversity of mechanisms that vary both in specificity and effectiveness according to the geological material (Samuels et al., 2020).

Lu and Hedin (2019) described how three symbiosis strategies, arbuscular mycorrhiza, ectomycorrhiza and nitrogen-fixing bacteria, were fundamental for the colonization of the land by plants, from the aquatic environment. These three symbiosis allowed the acquisition of unavailable nutrients, maintenance of different biogeochemical cycles and the expansion into new areas. Shortly, within this evolutionary scale, after the appearance of vascular roots, the first form of symbiosis between plants and fungi (Mycorrhiza arbuscular) began, which indicates a high degree of evolution of this symbiosis.

Turner et al. (2017) studied the Chronosequence of Franz Josef (120,000 years), and correlated the abundance and composition of the soil microbial community with the development of the soil (depth, soil age and mineralogical changes). The study revealed that with the increase of the depth and age of the soil there is a predominance of the archaea community, which can be correlated with the soil mineralogical alteration. On the other hand, a greater presence of fungi and bacteria was found in more organic soils (superficial horizons). In general, the study indicates that archaea communities are greatly affected by the age of the soil and mineralogical changes, being predominant in subsoil, mineral regions with less nutrient availability.

The soil-plant system undergoes selective pressure during its agricultural use, generally decreasing the microbial diversity of the soil (Lupatini et al., 2013; Mendes et al., 2014; Rodrigues et al., 2013).

The solubilization and absorption of nutrients by microorganisms is well known. For example, there is a greater dissolution of silicate minerals by both continuous and discontinuous flow dissolution mechanisms, when compared to the control without biofilm (Seiffert et al., 2014). Barker and Banfield (1998) evaluated the weathering front in silicate minerals under an electron microscope and concluded that the action of microorganisms forms zones of alteration in the mineral, with formation of smectites and goethite.

Among plant-microorganism relationships, rhizosphere activity and symbiosis between arbuscular mycorrhizal fungi and plant roots are extremely important for the system. Mycorrhizae increase the absorption of water and nutrients. Burford et al. (2003) described the importance of fungi in different biogeochemical processes such as transformation and redistribution of inorganic and organic nutrients, symbiosis with plants through mycorrhiza, biodeterioration of wood, cement and other materials, mineral dissolution of rocks and formation of secondary minerals.

Several studies, such as the one carried out by Xu et al. (2018), found that the low availability of P favors the action of AMF and affects both the abundance and diversity of the bacterial community. The opposite was also observed, that is, high availability of P decreases the action of AMF. If the use of fertilizers affects microbial

communities, their interactions and functions in the soil, we must be aware of these changes to manage the soil fertilization methodology (Feng et al., 2017).

The number of research projects that includes rock powders and microorganisms is growing. Basak and Biswas (2010) studied the use of mica with nitrogen-fixing bacteria and obtained greater accumulation of biomass, availability of nutrients, mainly in the maintenance of K and N throughout the development of the culture, suggesting an alternative source of nutrient with rocks. Florentino et al. (2017) also concluded that strains of nitrogen-fixing bacteria favored the release of K when compared to control.

In Brazil, with the exception of P-solubilizing microorganisms, research on rock powders and microorganisms is still scarce (Doumer et al., 2011; Silva et al., 2011; Tavares et al., 2018). Little is known about how the use of rock powders affects the microbial community already existing in the soil.

In an attempt to understand how the dissolution of minerals occurs according to the mineralogical composition and its effect on the soil microbiome, an experiment was carried out over 12 months using five different rock powders applied to the surface horizon of a soil cultivated with *Brachiaria brizantha* cv. Marandu. In this work, we present the changes in the structure of the bacterial community during eight months as a function of addition of KCl and different types of powdered rocks into the system.

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## 2. THE ROLE OF ROCK POWDERS INTO THE SOIL-PLANT SYSTEM AND BACTERIAL COMMUNITIES

### Abstract

Consumption of atmospheric carbon by bioweathering are central in the global cycles. Enhancing the weathering by applying powdered rock to soils (remineralization) on a worldwide scale is claimed as Negative Emission Technology (NET). Brazil is among the global hotspots for remineralization. Among the challenges to establish such NET is the lack of information on how bacterial soil communities (BSC) interact with rocks during enhanced weathering. Considering such scenario, this greenhouse experiment was established with five types of rocks (T3-Phonolite; T4-Basalt 1; T5-Basalt 2; T6-Basalt 3; T7-Granite) and comparative controls (T1-Control; T2-KCl) added to a soil and cultivated with *Urochloa brizantha*. The diversity of the BSC was monitored at 1 (1M), 4 (4M) and 8 (8M) months using the molecular technique Terminal Restriction Fragment Length Polymorphism (T-RFLP). The Non-Metric Multidimensional Dimensioning (NMDS) and constrained ordination analysis (RDA) were used to relate the changes in BSC with the type of amendment used. At 1M, the BSC in T2-KCl differed from all the other treatments. The BSC changes in rock-amended soils were only detected at 4M and defined groups at 8M. At the end of the experiment, the basalt rocks grouped together, and T3-Phonolite was not significantly different from the amygdaloidal basalt (T6). The BSC in T7-Granite was isolated from the other treatments. In 1M, the amount of Fe, K, Al and Ca in the leachate was significantly related to the change in BSC (as measured by the RDA) and in 8M the same was observed for Fe, K and Na. Results showed that rock powders were suitable sources of K for plants and also diminished the K and Ca losses by leaching, as compared to KCl source. The soil bacterial community changed after 1 month for KCl and 8 months (and was modulated by) the type of rock.

**Keywords:** Potassium; Enhanced weathering; Geomicrobiology; T-RFLP; Remineralizer

### 2.1. Introduction

The interaction between microorganisms and minerals has been occurring since the early stages of life on Earth (Nealson and Stahl, 1997). Today this interaction plays a major role in biogeochemical cycles including atmospheric CO<sub>2</sub> (Samuels et al., 2020). The complex interplay between microorganisms and minerals occurs mainly on the surface of mineral particles and therefore may be enhanced by increasing the surface area of the rock grains applied onto the soil. The increase in CO<sub>2</sub> consumption during bioweathering is claimed as a negative emission technology (NET) if used on a worldwide scale (Beerling et al., 2018; Renforth et al., 2009). Brazil is one of the global hotspots for the use of rock powder in agricultural fields (Strefler et al., 2018), a practice already regulated by Federal Laws since 2013 (Manning and Theodoro, 2018). However, the variables usually measured in experimental trials, such as elemental composition of soil and rock, are not sufficient to fully understand and model the weathering of rock powders in a tropical climate (Dalmora et al., 2020; Korchagin et al., 2019; Mancuso et al., 2014; Santos et al., 2016). Filling this gap is paramount to provide the basis for the formulation of reliable quantitative models of carbon capture and biomass increase that could be expected using rock powders. Among the challenges to establish such models is the better understanding of the role of soil microbial communities, since biological weathering is probably the main driver of the enhanced weathering present in soils from tropical regions (Krahl et al., 2020).

Several mechanisms of rock-microorganism interactions are known (Samuels et al., 2020) such as biofilm formation, Fe reduction and oxidation, release of organic acids and metal complexing molecules (Barker and Banfield, 1998a; Basak and Biswas, 2010; Flemming and Wuertz, 2019; Florentino et al., 2017; Seiffert et al., 2014). These interactions have important roles in terrestrial ecosystems (e.g., Landeweert et al. (2001) and Ward et al. (2013)). In agroecosystems, Beerling et al. (2018) predict that, in the long term, the addition of powdered rocks will change the



functioning of soil systems with respect to biomass production, consumption and production of gases by the soil and the dynamics of soil organisms.

There is a great amount of knowledge regarding the transformation of rock into soil, in the soil-saprolite zone. In contrast, remineralization happens in the top soil (where the powdered rock is added) and therefore has several significant differences: there is a massive increase in reactive surface area of the rock due to comminution, the high organic matter content, the fluxes of water and gases, the temperature regimes, the microbiome, the light patterns, etc., in the soil surface are quite different from those in the soil-saprolite zone.

In the Brazilian context, most of the research on the interactions of microorganisms with rock powder in soil surface of agroecosystems has concentrated on P solubilizers (Busato et al., 2017; Coutinho et al., 2012; De Cássia Silva et al., 2014; do Carmo et al., 2019; Giro et al., 2016; Oliveira et al., 2009; Rosatto-Moda et al., 2014; Silva Filho et al., 2002; Souchie et al., 2010, 2005; Edson L. Souchie et al., 2006; Edson Luiz Souchie et al., 2006; Souchie and Abboud, 2007; Yagi et al., 2020). Only during the last decade results with other rocks and processes have been published (de Souza et al., 2013; Doumer et al., 2011; Machado et al., 2016; Silva and Lana, 2015; Silva et al., 2011; Tavares et al., 2018).

The objective of this paper was to evaluate how the structure of the native bacterial soil community (BSC) changed after the application of five different powdered rocks and KCl, during an 8-month experimental period.

## 2.2. Material and Methods

### 2.2.1. Soil, rocks and plants

The soil used in the experiment was classified as a Latossolo Vermelho Amarelo Distrófico típico according to the Brazilian Soil Classification System (Embrapa, 2018a), as a Haplustox in Soil Taxonomy (USDA, 2014) and a Haplic Ferralsol (Loamic, Aric) in the WRB (WRB and IUSS, 2014). The 0.00 -0.20 m soil layer was collected at 21°42'32.0"S, 46°51'42.0"W site, in the state of São Paulo, Brazil, under a humid subtropical climate (Cwa) (Köppen climate classification, Alvares et al. (2013)).

Five rocks were chosen according to their occurrence in São Paulo State (Brazil southeast) and to their content of K-bearing minerals. Rocks were passed through a jaw rock crusher and then into a motorized mortar and pestle mill (Marconi, model MA890). After sieving, all the rock powder's particle size fractions were prepared to have similar particle size distribution (mass basis): 50% very fine sand (VFS, from 0.10 mm to 0.05 mm), 25% medium sand (MS, from 0.5 mm to 0.25 mm) and 25% very coarse sand (VCS, from 2 mm to 1 mm). These fractions were chosen to concentrate rock grains into particle size fractions of the soil that had small amount of material, in such a way that we could easily recover these "rock powder rich" fractions for chemical and mineralogical analyses after the experiment. All treatments except the T1-Control and T2-KCl (Figure 1) used a single dose of rock powder of 21 Mg ha<sup>-1</sup> (10 grams VFS, 5 grams MS and 5 grams VCS per pot). In the T2-KCl, it was applied at 0.3 Mg ha<sup>-1</sup> KCl (reagent grade, Merck) as recommended by Novais et al. (1991) for Brazilian soils. The soil used in the experiment was chosen based mainly on its mineralogy that was as different as possible from the rocks to be tested. This soil was developed from a granite parent material, with muscovite, while the granite rock used as amendment has biotite. Soil (2.5 kg) and rock powders or KCl were thoroughly mixed inside plastic bags before being dispensed into 3 L plastic pots.

The pots were cultivated with the tropical grass *Urochloa brizantha* (Hochst. Ex A. Rich.) STAPF. (syn. *Brachiaria brizantha* cv. Marandu). A few seeds (approx. 15) were sown and after the first month, the most vigorous

plant was selected, and the rest were removed. When plants reach approximately 40 cm high, they were cut 15 cm above the soil surface (de Gimenes et al., 2011). Totalling 2 cuts in 8 months of experiment.

The soil in the pots were maintained at 20% moisture by dry weight (Embrapa, 2018b). Once a month an extra 1 L of water was added, leading to a flow of excess water from the bottom of the pots. These leached solutions were collected and analyzed as described below (item 2.2.5.)

The seven treatments were applied in 4 blocks, resulting in 28 experimental units (28 pots). As the soil analysis were destructive, it was necessary to make three copies of the 28 experimental units totalizing 84 pots, so that one set of 28 could be disassembled at each time point. We named each of these copies “replica”. Therefore, one replica was disassembled after 1 month, the second replica after 4 months and the last one after 8 months (Figure 1). Samples were designated by the month of disassembling of each replica followed by the capital M letter (“M” for month”), therefore 1M, 4M and 8M (Figure 1). After disassembling each replica, aliquots of the soils were immediately stored at -80°C for conservation of the microbial DNA.

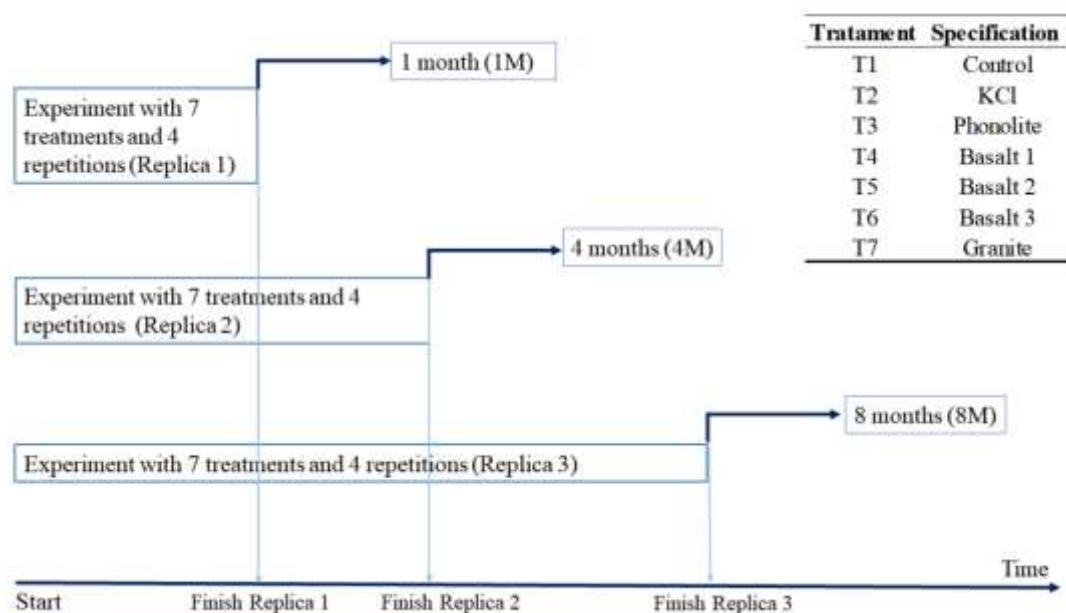


Figure 1. Experimental design and replicas.

## 2.2.2. Chemistry and mineralogy of the soil and the rocks

The soil chemical fertility indexes were measured according to the standard methods for Sao Paulo State (De Camargo et al., 2009). Briefly, soil exchangeable bases (Ca, Mg, K, P) were extracted using the resin method, Al using KCl 1 mol L<sup>-1</sup> solution, S using Ca- phosphate, B using hot water; Cu, Fe, Mn and Zn using DTPA; Na using Mehlich 1; and Si using CaCl<sub>2</sub> 0.01 mol L<sup>-1</sup>.

Mineralogical analysis of the soil (clay and sand) and the rocks were carried out in a Rigaku Miniflex II XRD benchtop system, using CuK $\alpha$  radiation, at 30 kV and 15 mA, coupled to a graphite monochromator and a spinning sample holder. Samples were irradiated from 3 to 60 °2 $\theta$ , at 0.01 °2 $\theta$  s<sup>-1</sup>. XRD scans were manipulated utilizing the “Match!” software (Cristal Impact, BRANDENBURG and PUTZ, 2003) and mineral spacings were analyzed using

the COD reference database (“Crystallographic open Database”), and mineralogical tables in Chen (1977) and Brindley and Brown (1980).

The soil had an intermediate to advanced degree of weathering. Quartz dominated the sand fraction and kaolinite plus gibbsite the clay fraction, therefore the degree of desilication of the soil is between monosialitization and allitization (Figure 2). No easily bioweatherable primary minerals that could function as a nutrient source for living microbial or plant cells were identified in the soil. Despite the scarcity of Fe-rich primary minerals, the soil has some amount of goethite, but has kaolinite as the main clay mineral. The following soil particle size classes (Table 1) showed smaller mass among all: very fine sand (VF), medium sand (M) and very coarse sand (VC). As mentioned, rock powders were prepared in such a way to enrich these size fractions aiming to increase the (rock powder)/(soil) ratio and yield a better signal/noise ratio in the XRD analysis (not discussed in this paper). Even not using these variable in the multivariate analysis, they were essential to characterize the soil and rock powders and integrate the results.

Table 1. Physical and chemical soil characteristics.

Parameters	pH	K	Ca	Mg	Al	H+Al	EB <sup>a</sup>	CEC <sup>b</sup>	BS <sup>c</sup>	m <sup>d</sup>
	CaCl <sub>2</sub>	mmolc.dm <sup>-3</sup>					.... % ....			
Soil	5.8	2.6	41.3	11.7	<0.02	22	55.6	77.6	71.7	0
	OM <sup>e</sup>	P	B	Cu	Fe	Mn	Zn	Na	Si	FC <sup>f</sup>
	g.dm <sup>-3</sup>	mg.dm <sup>-3</sup>					mg.Kg <sup>-1</sup>		g.g	
Soil	28.3	46.3	0.3	1.8	49	5.9	2.3	14.3	10	0.2
	Sand					Silt		Clay		BD <sup>i</sup>
	VF <sup>g</sup>	F	M <sup>h</sup>	C	VC <sup>i</sup>	Total	..	H <sub>2</sub> O	Total	..
	g.cm <sup>-3</sup>									
Soil	37	137	125	127	85	511	178	227	311	1.32

<sup>a</sup> Sum of exchangeable base; <sup>b</sup> potential cation exchange capacity; <sup>c</sup> base saturation; <sup>d</sup> aluminium saturation; <sup>e</sup> organic matter; <sup>f</sup> field capacity; <sup>g</sup> very fine sand – 0.05 mm to 0.10 mm; <sup>h</sup> medium sand – 0.25 mm to 0.5 mm; <sup>i</sup> very coarse sand - 1.0 mm to 2.0 mm; <sup>j</sup> bulk density.

The mineralogy of the rocks (Figure 2a) was in agreement with their total chemical analysis (Table 2).

For the total chemical analysis soil and rock samples were air dried, homogenized and 10 g milled to a particle size of < 200 µm. Subsamples (0.25 g for rock; 0.1 g for soil samples) were subjected to two different analyses. Firstly, determination of the samples after Fusion with Lithium Metaborate in a muffle furnace (LiBO<sub>2</sub>) and dissolving with an acid solution (C<sub>4</sub>H<sub>6</sub>O<sub>6</sub> and HNO<sub>3</sub>). The elements were determined by optical emission spectrometry (ICP-

OS). The second analysis, after Multi-Acid Digestion (HCl, HNO<sub>3</sub>, HF and HClO<sub>4</sub>), the elements were determined by mass spectrometry with inductively coupled plasma (ICP-MS).

The high values of Na and K in phonolite relate to the presence of alkali feldspar (sanidine) and feldspathoids (leucite – Lct and nepheline - Nph), and mica (Mca) (Figure 2b). The basalts were similar to each other, with the dominance of plagioclase and pyroxene (augite), as expected (Gill, 2011) (Figure 2b) and their variation was used to test the sensitiveness of the BSC to small changes in rock characteristics. All the basalts came from sites on the Serra Geral Formation in São Paulo State. There, basalts are usually tholeiitic and present small amounts of 2:1 phyllosilicate in their structure (Peate, 1997). The T4 basalt was aphanitic and the main minerals were plagioclase (Plg) and mafic minerals. The T5 basalt had a coarser texture and presented plagioclase, pyroxene, olivine and magnetite, while the T6 basalt was amygdaloidal, containing a certain amount of zeolite (heulandite) (Figure 2b). The T7 granite was the only acid rock used (SiO<sub>2</sub> greater than 65 i.e., Si greater than 30.4%) (Streckeisen, 1980) (Table 2), which explains the expressive quartz peak in the XRD scan (Figure 2).

Table 2. Total chemistry of soil initial and rocks ICP-OS.

Element	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	MgO	Na <sub>2</sub> O	K <sub>2</sub> O	MnO	P <sub>2</sub> O <sub>5</sub>	LOI
	%	%	%	%	%	%	%	%	%	%
Soil	69.36	14.10	5.14	0.34	0.09	0.03	0.33	0.05	0.09	8.47
Phonolite	56.4	20.9	3.87	1.76	0.32	6.74	8.05	0.25	0.07	2.83
Basalt 1	50.6	12.15	15.1	7.59	3.82	2.66	1.5	0.21	0.61	1.45
Basalt 2	47.7	11.8	16.1	9.03	5.77	2.41	1.09	0.22	0.45	1.61
Basalt 3	48.8	11.9	13.25	8.99	4.64	2.43	1.15	0.19	0.21	5.44
Granite	76.7	13.8	1.14	0.43	<0.01	4.68	3.87	0.16	<0.01	0.51

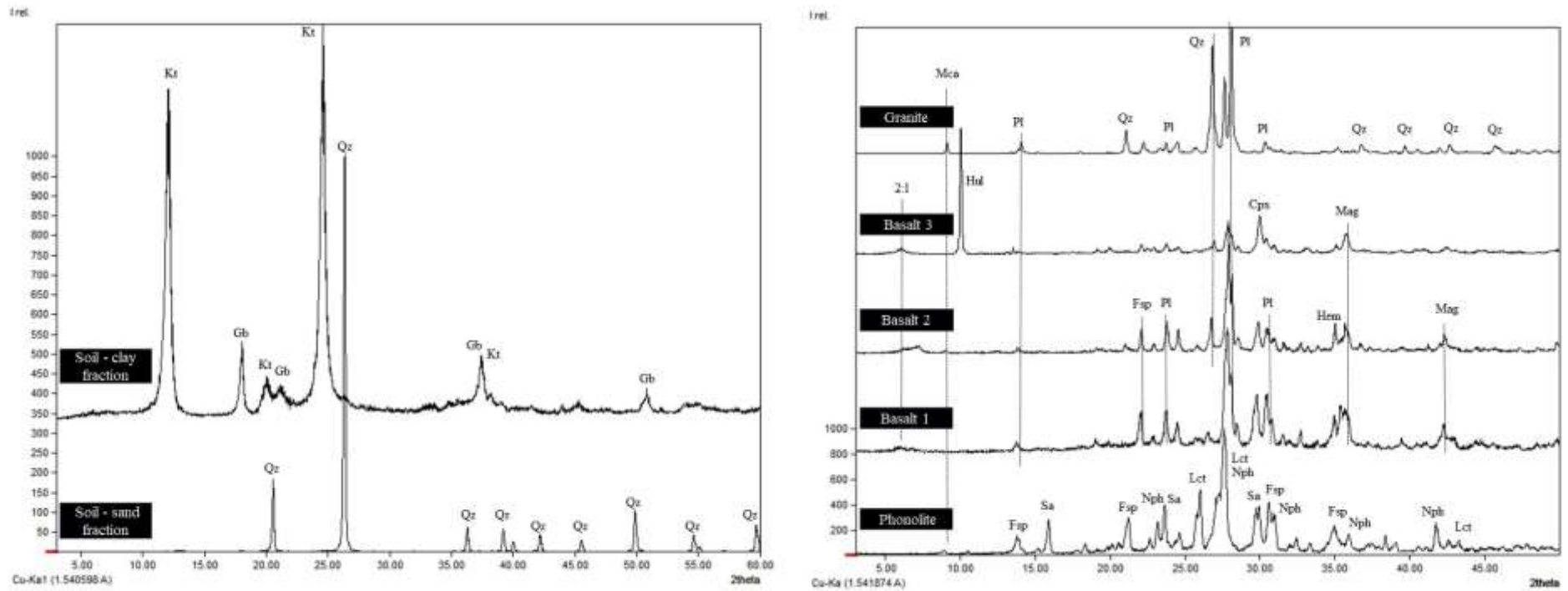


Figura 2. XRD scans depicting the mineralogy of the soil used (Kt: kaolinite, Gb: Gibbsite, Qz: Quartz) and of the rocks used in the experiment (Fsp: Feldspar, Sa: Sanidine, Nph: Nepheline, Lct: Leucite, Pl: Plagioclase, Hem: Hematite, Mag: Magnetite, Hul: Heulandite, Mca: Mica, Cpx: Clinopyroxene, Qz: Quartz).

### 2.2.3. Bacterial Soil Community (BSC) analyses

BSC were approached by the use of molecular analyses, performed on samples taken from the 1M, 4M and 8M replicas (Figure 1). Total DNA was extracted from 0.4 g of soil using a commercial kit (PowerSoil DNA Isolation, MoBio, Inc. Solana Beach, USA), resulting in 84 DNA samples with four replicates. The DNA integrity was tested using gel electrophoresis (1% agarose). Terminal Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting was used to characterize the structure of the Bacterial Soil Community (BSC). The bacterial gene 16S rRNA was amplified using the primers 8-FM (5'-6AGAGTTTGATCMTGGCTCAG-3') and 926r (5'-CCGTCAATTTCCTTTTRAGTTT-3') (Schütte et al., 2009), the primer 8-FM is labelled with 6-FAM (6-carboxy fluorescein). The reaction mix consisted of 1 µl of DNA template (ca. 50 ng), 4 µl of dNTP (0.2 mM), 0.1 µl of each primer, 5 µl of PCR Buffer X10, 6 µl of MgCl<sub>2</sub> (50 mM) and 0.2 µl (1U) of platinum Taq DNA polymerase (Sinapse Inc), in a final volume of 50 µl. The amplifications were carried out with the following cycling conditions: 95 °C for 4 min, followed by 30 cycles of 95 °C for 30 s, 53 °C for 30 s and 75 °C for 45 s and a final step at 72 °C for 10 min, modified from Durrer et al. (2017) and Pimentel et al. (2019).

Aliquots of PCR amplicons (approx. 200ng) was digested with the restriction enzyme HhaI (10 U/µL) (Thermo Scientific) at 37 °C for 3 h. The digested material was then precipitated with 2 µl Na-acetate 3 M, 2 µl EDTA 125 mM and 50 µl absolute ethanol and centrifuged at 4000 rpm for 30 minutes. The precipitated DNA was washed with ethanol 70% and dried by centrifugation. The DNA was suspended in formamide Hi-Di™ (Applied Biosciences, Foster City, CA) and analyzed on an automatic sequencer ABI Prism 3500 (Applied Biosystems, Life Technologies).

### 2.2.4. Leachate and plant tissues analysis

Leaching solutions were collected (see 2.2.1.) and the volume, pH, electrical conductivity (EC) and temperature were immediately measured. Then, the pH of the leachate was adjusted to pH 3 with 3% nitric acid to avoid precipitation and microbial growth and stored in 15 ml plastic tubes at 5 °C until ICP-OES analysis (Laxen and Harrison, 1981).

The aerial plant tissue was collected, dried at 60°C and aliquots (0.25 g) were put into Teflon vessels and subjected to microwave acid digestion in a Mars Xpress - CEM with 2 mL hydrogen peroxide and 4 mL of nitric acid. The heating routine was 80°C for 3 minutes, 150°C for 10 minutes, 180°C for 10 minutes and finally 180 °C for 5 minutes. The extract volume was adjusted to 35 mL with ultrapure water and analyzed by ICP-OES (Araújo et al., 2002).

### 2.2.5. Statistics

The “area of distinct peaks of T-RF” obtained from T-RFLP was used in the analysis to characterize BSC structure, as well as any correlations with other variables. For each replicate the BSC structure was subjected to ordination analysis, Non-Metric Multidimensional Scaling (NMDS), adjusted by the Bray-Curtis similarity index. The One-way ANOSIM (Similarity Analyze) test with the Bray-Curtis Index was carried out to verify the significance of any differences among the NMDS groups, using the software Past 3 (Hammer et al., 2001).

The redundancy analysis was carried out using the constrained ordination analysis (RDA) method using the Bray-Curtis Index by the vegan routine in the R software package (version 3.6.3) to determine which soil chemical variables (leached elements) had driven the changes in BSC structure, with a significance test performed by the nonparametric Monte Carlo permutation test (with 999 random permutations). The ANOVA test was used for the K concentration in the leachate and plant tissue to verify their significance.

## 2.3. Results

### 2.3.1. Element concentration into leaching solutions and plant tissue

Leaching events were used to accelerate the rock powder weathering as well as to investigate how chemical elements were released from the rock minerals. Figure 3a and Table 3 shows the amount of K exported from the soil (plant + leachate), where only the T2-KCl presented a significant difference (ANOVA,  $p < 0.001$ ) from the other treatments and control. After 8 months (8M), the K in the plant tissue was similar in all the treatments (Figure 3a), but the total K leached from pots in T2-KCl was about 30 times greater than the amount leached from the T3-Phonolite, which is the rock with greatest concentration of K (Table 3). Figure 3a shows how much more soluble KCl is as compared to the rock powders. In addition, the higher K concentration in the T2-KCl solution affected Ca leaching (Figure 3b), contributing to the strong impact this treatment had on the BSC, as discussed below.

Table 3. Cumulative of K leached at 1M, 4M and 8M replicas, total K in aboveground plant tissue and dry biomass.

Tratament	K leachate (mg)			K plant (mg)	Dry biomass (mg)
	1M	1M+4M	1M+4M+8M		
T1-Control	20.80 b	20.26 b	36.96 b	96.89 a	5996.7 a
T2 - KCl	774.45 a	1020.16 a	1105.59 a	101.79 a	4790.0 a
T3-Phonolite	18.49 b	28.92 b	36.19 b	94.13 a	6243.3 a
T4-Basalt1	20.96 b	26.36 b	34.92 b	71.19 a	4020.0 a
T5-Basalt2	17.49 b	24.72 b	33.34 b	70.54 a	3960.0 a
T6-Basalt3	17.36 b	22.47 b	32.35 bc	78.25 a	4720.0 a
T7-Granite	14.79 b	21.78 b	28.96 c	92.57 a	5530.0 a

Different letters refers to significant statistical difference ( $p < 0.05$ ).

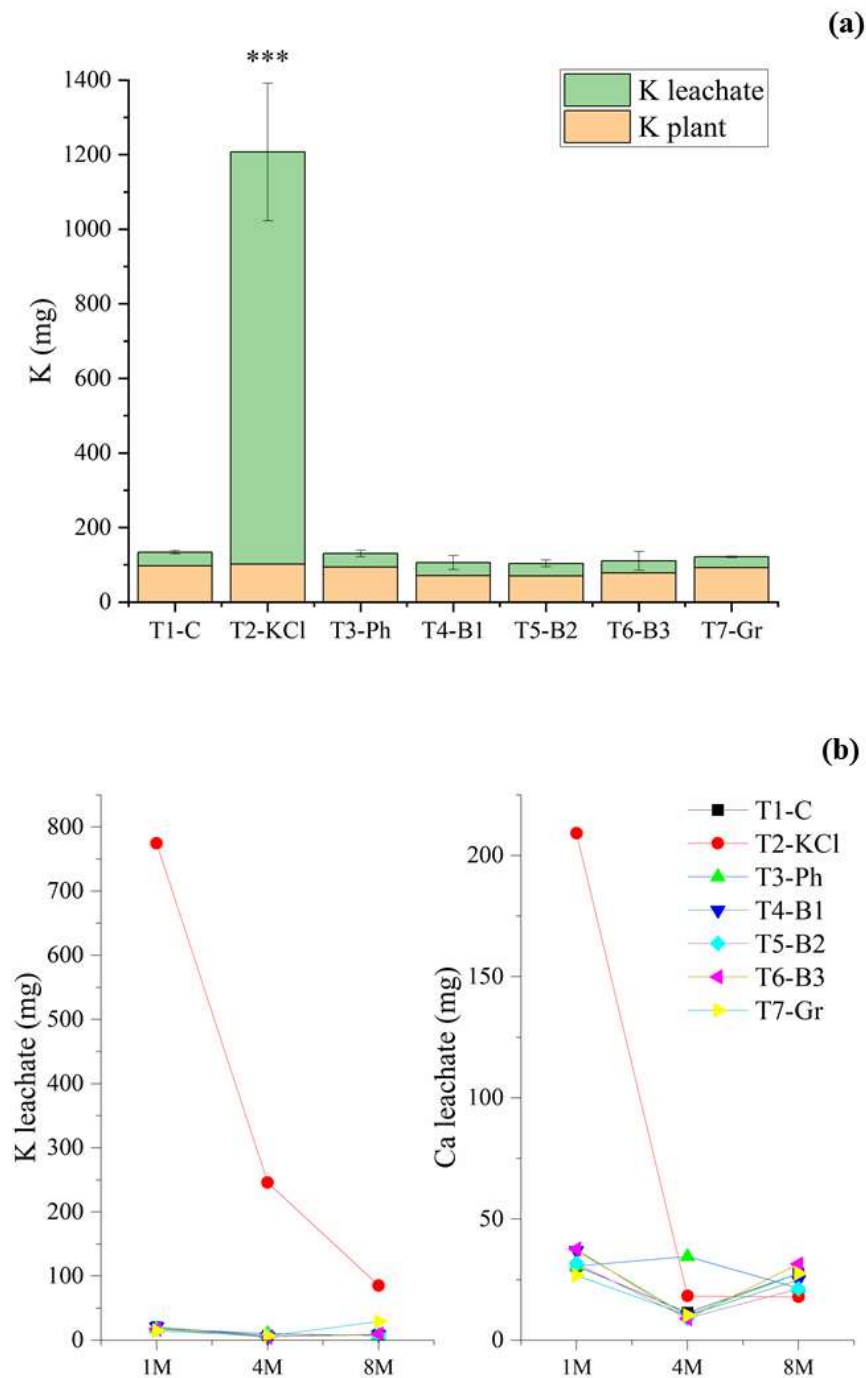


Figure 3. (a) Total loss of K (leachate + plant) up to 8M and (b) evolution of K and Ca leached (mg) from 1 month (1M) to 8 months (8M).

### 2.3.2. The Bacterial Soil Community (BSC) structure

Based on the NMDS analysis, only the T2-KCl treatment had a significant impact on BSC structure after 1M (Figure 4a). The treatments with rock powder (T3 to T7) clustered around the control (T1) suggesting small or no impact of these amendments after the first month. Such a contrast was also observed during the similarity analysis (ANOSIM) in which T2-KCl was the only treatment that differ from the others ( $p < 0.05$ ) (Table 4a).



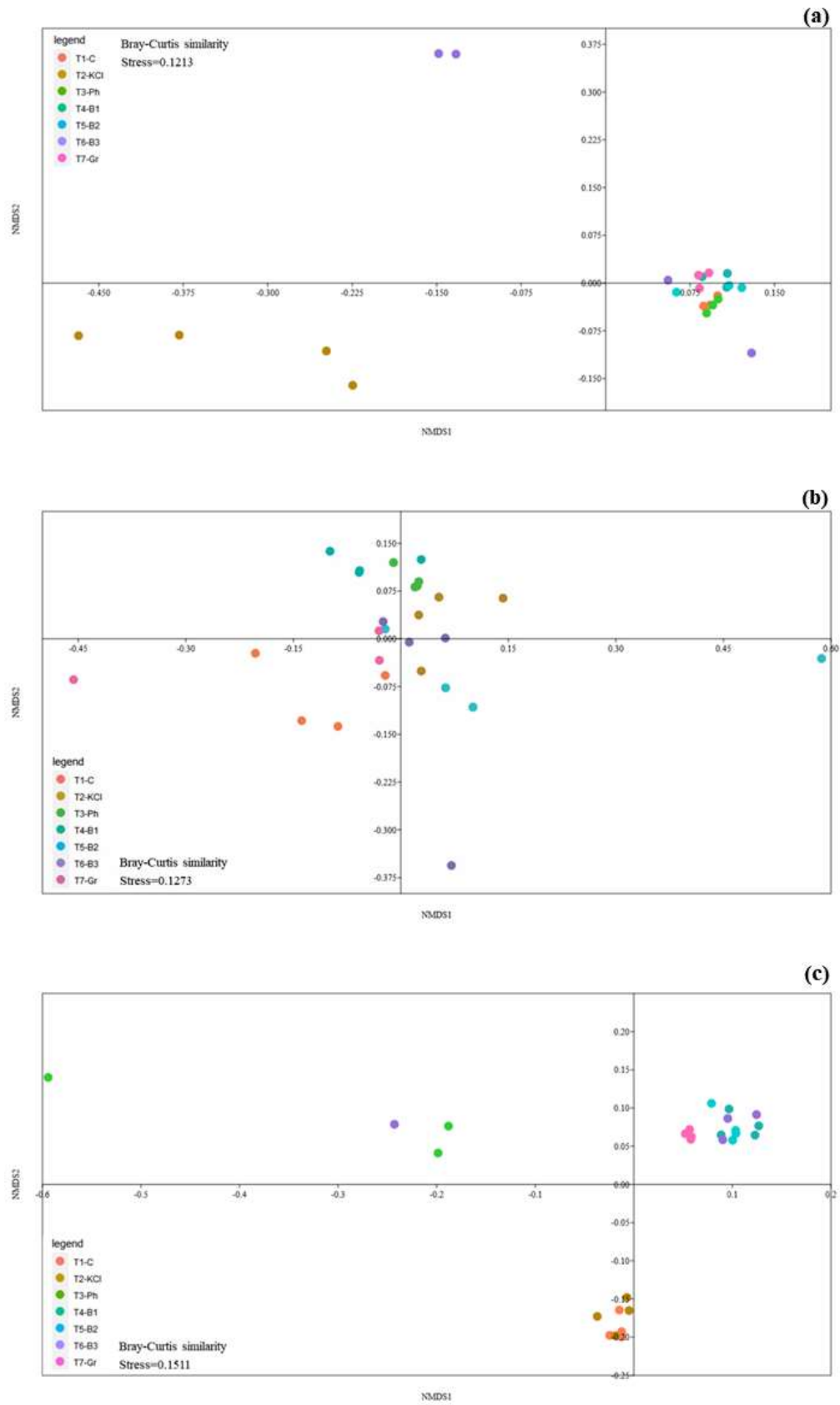


Figura 4. NMDS of the bacterial soil Community (BSC) after 1 month experiment (1M) (a), 4 months (4M) (b) and 8 months (8M) (c).

Table 4. Analysis of similarity (ANOSIM) of microbial community structure among the treatments. (a) 1 month (1M); (b) 4 months (4M); (c) 8 months (8M).

<b>(a)</b>	p Value - 1 month (1M)					
	T1-C	T2-KCl	T3-Ph	T4-B1	T5-B2	T6-B3
T1 - Control	-	-	-	-	-	-
T2 - KCl	0.03*	-	-	-	-	-
T3 - Phonolite	0.80	0.03*	-	-	-	-
T4 - Basalt 1	0.10	0.03*	0.09	-	-	-
T5 - Basalt 2	0.20	0.02*	0.30	0.60	-	-
T6 - Basalt 3	0.23	0.03*	0.23	0.31	0.31	-
T7 - Granite	0.10	0.03*	0.10	0.40	0.30	0.35

<b>(b)</b>	p Value - 4 months (4M)					
	T1-C	T2-KCl	T3-Ph	T4-B1	T5-B2	T6-B3
T1 - Control	-	-	-	-	-	-
T2 - KCl	0.03*	-	-	-	-	-
T3 - Phonolite	0.03*	0.03*	-	-	-	-
T4 - Basalt 1	0.03*	0.03*	0.06	-	-	-
T5 - Basalt 2	0.03*	0.14	0.03*	0.03*	-	-
T6 - Basalt 3	0.09	0.23	0.03*	0.03*	0.77	-
T7 - Granite	0.51	0.17	0.03*	0.03*	0.43	0.23

<b>(c)</b>	p Value - 8 months (8M)					
	T1-C	T2-KCl	T3-Ph	T4-B1	T5-B2	T6-B3
T1 - Control	-	-	-	-	-	-
T2 - KCl	0.03*	-	-	-	-	-
T3 - Phonolite	0.03*	0.03*	-	-	-	-
T4 - Basalt 1	0.03*	0.03*	0.03*	-	-	-
T5 - Basalt 2	0.03*	0.03*	0.03*	0.43	-	-
T6 - Basalt 3	0.03*	0.03*	0.11	0.29	0.46	-
T7 - Granite	0.03*	0.03*	0.03*	0.03*	0.03*	0.03*

After four months (4M), further changes in BSC structure were observed (Figure 4b). The initially strong effect of T2-KCl seemed to be nearly exhausted. The K concentration in the leachate also suggests that the K concentration in the soil solution dropped sharply (see 3.2, Figure 3b). On the other hand, the effects of some of the rock powders started to appear. Although the ANOSIM analysis indicated that the BSC in the T2-KCl did not return to the T1-C condition, it did not differ significantly from T5-B2, T6-B3 and T7-Gr. The T3-Ph and T4-B1 treatments were grouped together ( $p < 0.05$ ) (Table 4b).

Two BSC structural clusters could be identified: one cluster formed by T1-C and T2-KCl and the other formed by all the rock powder treatments.

The ANOSIM analysis (Table 4c) showed that the basalts (T4-B1, T5-B2 and T6-B3) demonstrated no significant differences among them ( $p < 0.05$ ), and no significant difference was also observed between T3-Ph and T6-B3, possibly related to the amygdaloidal nature of the T6-B3 and the minerals in the amygdales, such as the Heulandite (Figure 2b). T7-Gr formed a distinct cluster.

### **2.3.3. RDA – leached elements and the Bacterial Soil Community (BSC)**

The RDA analysis allowed the identification of any correlation between the microbiological data (NMDS) and leached chemical elements from the samples. At 1M (Figure 5a), 38.9% of the variation ( $p < 0.001$ ) was associated with Al, Fe, K and Ca in the leachates. The K and Ca are probably the drivers for the changes in the T2-KCl BSC structure (see also Figure 3b), and Fe and Al for the other treatments. The importance of Fe and Al is highlighted in the T1-Control and in the rock powder treatments there is a relationship with their composition. The basalts (T4-B1, T5-B2 and T6-B3) are rich in Fe, but all rocks are rich in Al, particularly the phonolite (T3-Ph) (Table 2).

At 8M (Figure 5b), the 31.6% of the variation in the correlation was explained by Fe ( $p < 0.01$ ), K ( $p < 0.05$ ) and Na ( $p < 0.1$ ). The dispersion of samples increased, with the T2-KCl points being positioned towards the periphery of the cloud. The leached K, Fe and Na ions are associated with the BSC structural change in T2-KCl.

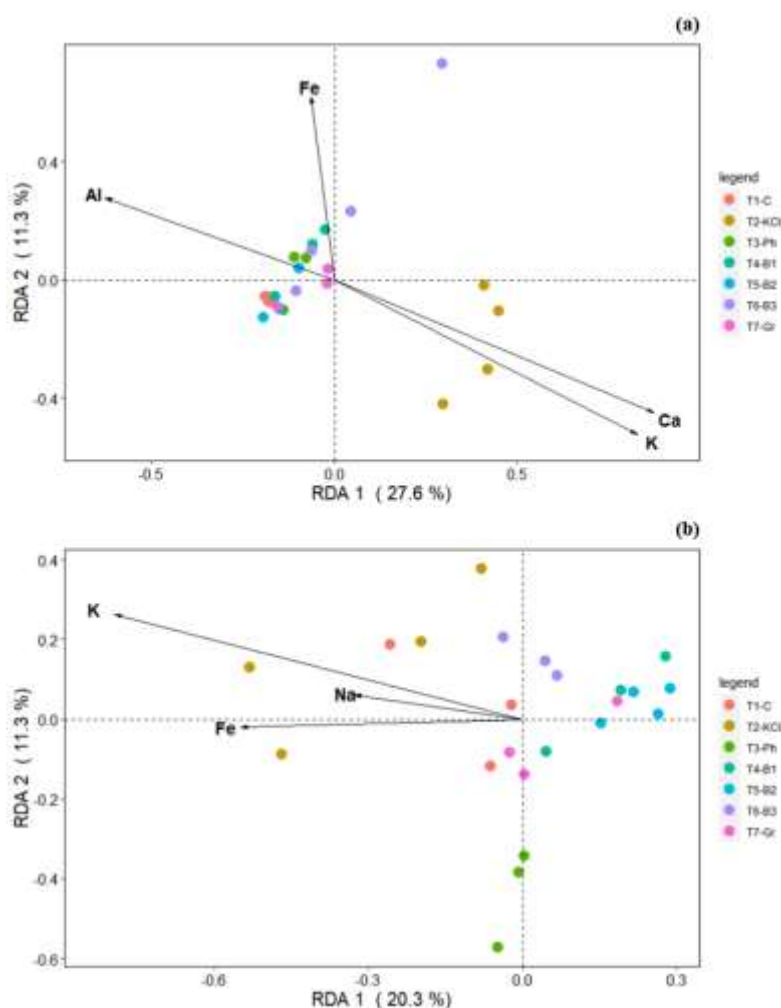


Figure 5. Redundancy analysis (RDA) of the bacterial soil community (BSC) and leached elements after 1 month (1M) (a) and 8 months (8M) (b).

## 2.4. Discussion

In this paper we emphasized the change in the K plant, K and Ca leachate and soil bacterial community (SBC) during the 8-month experimental period after the application of several rock powders into the soil in comparison with the control and KCl treatments.

After the first month, the T2-KCl was the only treatment that altered the BSC structure. The effect was greater and faster when compared to the rock powder treatments, as indicated by the NMDS (Non-Metric Multidimensional Scaling) analysis (Figure 4a). Such drastic changes in BSC structure are known to occur when using highly soluble fertilizers (Böhme et al., 2005; Chen et al., 2015; Čuhel et al., 2019; Ding et al., 2016; Liang et al., 2020; Marschner et al., 2003; Wolsing and Priemé, 2004; Zhang et al., 2019). Allison and Martiny (2008) reported such an effect in more than 80% of the studies they reviewed. Obviously, this change was caused by the introduction of a large amount of K and Cl ions into the system. The magnitude of the change in solute concentration in the soil solution of the T2-KCl treatment can be inferred by comparing the amount of K leached at 1M with the amount leached from the other treatments (Table 2). Potassium is a macronutrient, i.e., organisms require large quantities of it (Epstein, 2003). The optimal potassium concentration in bacterial growth media is roughly 60 mg L<sup>-1</sup> (Lester, 1958). If the

concentration in the leachate is taken as an approximation of the  $K^+$  concentration in the soil solution, the  $K^+$  concentration in single leaching event ranged from  $0.0 \text{ mg L}^{-1}$  up to  $31.79 \text{ mg L}^{-1}$  in all but the T2-KCl treatment, which ranged from  $12.34 \text{ mg L}^{-1}$  to  $1022.00 \text{ mg L}^{-1}$  (data not shown). Therefore, the  $K^+$  concentration in the T2-KCl was more than 15 times the optimal concentration for bacterial growth, which occurred at 1M and may have had a strong effect on its BSC. This may explain the large deviation in BSC structure when compared to the other treatments. Although we could not find the toxic threshold for  $K^+$  concentration for bacterial growth in the literature, other effects of this high  $K^+$  concentration may have occurred inducing such fast changes in BSC structure. The great solubility of the KCl is result of the ionic nature of its structural bonds, easily releasing  $Cl^-$  anions. Although the amount of  $Cl^-$  anions was not quantified in this experiment, by the principle of electrostatic neutrality of the solution, the sum of cationic and anionic charges into the solution has to be equal. Therefore, most of the anions in T2-KCl soil solution should be in the  $Cl^-$  form. Both  $K^+$  and  $Cl^-$  ions form large hydration shells (hydration enthalpy:  $K^+ = -321 \text{ J K}^{-1} \text{ mol}^{-1}$ ;  $Cl^- = -363 \text{ J K}^{-1} \text{ mol}^{-1}$ ; (Essington, 2004)) that interferes in the water flux through the cell membrane (Friedman, 2018). Therefore, in addition to the nutritional effects, the change in the BSC in T2-KCl may also have been related to the water flux through cell membrane and its water relations to the soil surroundings. An increase in  $Ca^{+2}$  ions was also observed in the leachate at 1M (Figure 3b) in T2-KCl. Calcium is an element that greatly influences the structure of the BSC (Li et al., 2018; Sridevi et al., 2012; Wehr et al., 2019; Xia et al., 2016; Xue et al., 2017). In addition, the  $Cl^-$ ,  $Na^+$ ,  $K^+$  and  $Ca^{+2}$  ions are among those that markedly turn the osmotic potential of the solution more negative and affect microbial metabolism in order to compensate this condition (Halverson, 2014; Shi and Wang, 2005; Yan et al., 2015). There were significant differences in  $K^+$  and  $Ca^{+2}$  in the RDA (Redundancy Analysis) at 1M (Figure 5a) and  $K^+$  and  $Na^+$  in the RDA at 8M (Figure 5b), both associated with T2-KCl. The  $Na^+$  ion is also important in setting the osmotic potential. Despite the greatest potential sources of  $Na^+$  was Phonolite, T3-Ph, (6.74%  $NaO_2$ , Table 2) and Granite, T7-Gr, (4.68%  $NaO_2$ , Table 2), it seemed that they do not release a high enough concentration to induce a significant change in their BSC structures, possibly due to the covalent nature of the silicate mineral phases that constitute these rocks. These measured and inferred changes in the T2-KCl treatment could result in the observed changes in BSC structure.

The rock powders, on the other hand, are made of solid phases in which most chemical bonds are covalent, leading to a much lower solubility and a greater buffering capacity (Lacroix et al., 2014a, 2014b, 2012). This can be perceived by comparing the two treatments that used sources with highest potassium content: KCl presents 41.5% K (60%  $K_2O$ , m/m) and the Phonolite rock 5.45% K (8.0%  $K_2O$ , m/m). Considering the doses applied in the treatments, T2-KCl presents 2.06 times more K than T3-Phonolite. However, the T2-KCl leaching solution at 1M presents 41.88 times more K than T3-Ph (Table 3).

The great impact of T2-KCl on the BSC structure at 1M decreased at 4M and this treatment clustered with the other treatments (Figure 4b), not significantly differing from T5-B2, T6-B3 and T7-Gr ( $p < 0.05$ ) (Table 4b). Concurrently, the amount of  $K^+$  and  $Ca^{+2}$  (and probably  $Cl^-$ ) in the leachate solution of T2-KCl dropped (Figure 3b).

As the system inside the pots evolved, T2-KCl exhausted its source of ions (Figure 3, Table 1 supplementary) and the BSC gradually returned to (or close to) its original state (Čuhel et al., 2019), but was still influenced by the greater amount of soluble K than in the other treatments (Figure 3b, Table 1 supplementary, RDA 1M, Figure 5a, and RDA 8M, Figure 5b).

Another interesting result from the RDA at 8M (Figure 5b) was the importance of soluble Fe in structuring the bacterial community. At 1M, the vector is associated with the rock powder treatments, mainly with the basalts (T4-

B1, T5-B2 and T6-B3) and T2-KCl, possibly due to the scarcity of this nutrient in the soil (Table 1 and Table 3) and lack of it in the KCl treatment.

The basalt treatments (T4-B1, T5-B2 and T6-B6) possessed several sources of Fe (Table 2) located in the minerals pyroxene, magnetite and hematite (Figure 2b). All rocks are rich in Al (Table 2), which is mainly located in the feldspars (Figure 2b). Pyroxenes and feldspars are silicates with high and intermediate solubility, respectively (hornblend pyroxene  $\log k = -7.00$ ; albite feldspar  $\log k = -9.87$ ; (Palandri and Kharaka, 2004)). The release of Fe and Al from these silicates is high at the beginning but rapidly lowers as the the atoms of these elements located in the outer layers of these minerals were exhausted. This change in the type, amount and rate of release of elements during the weathering is typical of silicate minerals, which has mainly incongruent solubility. This can be seen by the changes in the direction of the Fe vector from 1M to 8M. At 1M there was Fe leaching in all the rock powder treatments (non-null values in at least one replication of the T3 to T7 treatments, Table 1, supplementary) and all null values in T1-Control and T2-KCl (Table 1 Supplementary). At 8M the Fe vectors change direction and was associated with T2-KCl instead of the rock powder treatments. This is possibly related to the displacement of exchangeable Fe by the K ions, and/or to the presence of Fe reducing bacteria in the T2-KCl BSC, since it was different from the BSCs in the rock powder treatments (T3 to T7) and T1-Control (Table 4a and Table 4c).

The amount of leached Al was contrasting at 1M and its vector was associated with the rock powder treatments (T3 - T7) and T1-Control treatment. However, most of the Al readily precipitates into amorphous forms if the soil pH is above 4.5 (Sposito, 1995), as it was in this soil (Table 1). At 8M, the phase of fast and intense release of Al from rocks was already slowing and the amount of Al leached in all treatments tended to level out (Table 1, supplementary).

It seems that the BSC was not sensitive to small variations in rock characteristics (texture, mineralogy and chemistry), as inferred by the similarity of the BSCs among the basalt variations (no significant difference at  $p < 0.05$  among T4-B1, T5-B2, T6-B3 at 8M, Table 4c).

Despite the similar amount of Si in phonolite and the basalts (Table 2), T3-Ph clustered with only T6-B3 (Table 4c), suggesting that the amount of Si leached was not important. This is probably because the soil is well supplied with Si sources, as can be seen by the similar amount of leached Si in the T1-Control when compared to the treatments at both 1M and 8M (Table 1, supplementary). The similarity of the BSC in T3-Ph and T6-B3 (Table 4c) may be related to the peculiarity of the mineralogy of the basalt B3, that is, the presence of zeolites (Heulandite) in the amygdalae (Figure 2b). The zeolites are a group of minerals rich in Ca and Na, with great surface reactivity. Such characteristics are similar to those of Phonolite and may be responsible for the similar BSC structures encountered in the soils amended with these rocks.

The BSC structure in the granite treatment, although near the basalt group as shown by NMDS analysis at the start of the experiment (Figure 4c), ended the experiment period distanced from this group ( $p < 0.05$ ) (Table 4c). The rock in T7-Gr is the most similar to the parent material of the soil chosen for the experiment. Manning and Theodoro (2018) suggested that the plant biomass response to rock powder application is greater if the rock powder applied is not the same as the parent material of the soil. This statement could not be confirmed in the present study because the biomass production was not significantly changed by the different treatments (dry mass, Table 3).

Microbial life on Earth has existed for at least 3,8 billion years (Cavicchioli et al., 2019) and has evolved during the geological eras, leaving microorganisms very well adapted to extract nutrients from the silicate phases (Arocena et al., 2003; Banfield et al., 1999; Barker and Banfield, 1998b; Cockell et al., 2009; Gadd, 2010, 1992; Jongmans et al., 1997; Rodríguez-Navarro et al., 1997) that drives the Earth's biogeochemical cycles (Lu and Hedin,

2019). On a much shorter time scale, BSC also changes concurrently with the changes in the pedoenvironment as soil develops, especially at the limit between soil and saprolite (Jangid et al., 2013a, 2013b; Nemergut et al., 2007; Schütte et al., 2009b; Turner et al., 2017; Wu et al., 2012; Zumsteg et al., 2011). Even after the soil reaches the advanced stages of development, bacteria related to parent rock weathering can still be found in the soil horizons (Huang et al., 2014).

The present results suggest that, although crop performance was not affected (see K in plant and biomass production in Table 3), the BSC was. The rock powders did not cause an extreme and fast change in the bacterial diversity of the soil and by doing so, would not interrupt abruptly the microbial-plant cooperative processes active in the rhizospheric environment such as nutrient absorption, pathogen protection, etc. (Richter et al., 2007). In fact, Cui et al. (2018) observed that the use of NPK decreased BSC richness and increased abundance of specific groups, decreasing the redundancy and as a consequence, may decrease the resilience of the BSC.

Regarding the rock powder treatments, the BSC started showing significant alterations in the BSC only at 8M.

This paper contributes to a better understanding of the interplay among rock powders and its impact on the structure of the BSC in the soil. It also showed that the magnitude and kinetics of the ionic phases are quite different from the covalent ones.

## 2.5. Conclusion

This paper showed that the response of the bacterial soil community (BSC) structure in the chosen soil was different depending on the type of amendment applied. KCl caused a fast, great and ephemeral change in the BSC, the only treatment to show a significant change at the end of the first month (1M). Although with time, it tended to group closer to the control treatment, it was still statistically different at the end of the experiment, 8 months (8M) later. The change in the structure of the BSC in the rock powder treatments was slow, gradual and different according to the rock types. Basalts tended to cause similar effects on the BSC despite their small variation in mineralogy, chemistry and texture. At the end of the experiment (8M), there were no significant differences between T4-B1 and T5-B2; the amygdaloidal basalt (T6-B3) was similar to the phonolite treatment (T3-Ph); and the BSC in the granite amended soil (T7-Gr) was significantly different from all the other treatments. Using the NMDS analysis, the elements in the leachate with significant effects on the BSCs were Fe, Al, Ca and K in the first month (1M) and Fe, Na and K at 8 months (8M).

The results showed that the same starting BSC structure changes differently depending on the type of powdered rock applied into the soil. It also has shown that such changes were not as fast as those observed in pots with highly soluble fertilizer. Although the BSC structures were reasonably grouped according to rock type after 8 months, there is a possibility that they could continue to change during longer periods of time.

Finally, the results had shown that even in a soil well supplied of nutrients, in which the application of the amendments did not impacted the amount of biomass produced, the amendments changed significantly the Bacterial Soil Community. This might explain why some field experiments had reposted increase in plant production with application of powder rock, even when the soil was well supplied of nutrients, reinforcing the need to consider soil fertility beyond the inorganic chemistry approach.

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## Supplementary information

Table Supplementary 1. Elements leached used in RDA analysis.

Elements leached (mg) - 1M																				
Treatment	Al		Ca		Fe		K		Mg		Mn		Na		P		S		Si	
	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd
T1-Control	0,27	0,03	30,65	3,11	0,00	0,00	20,80	0,84	10,80	1,43	0,00	0,00	10,75	0,39	0,00	0,00	7,69	1,24	3,54	0,36
T2 - KCl	0,05	0,03	215,53	30,46	0,00	0,00	817,69	197,98	75,64	11,59	0,00	0,00	13,74	1,40	0,00	0,00	6,80	1,67	3,11	0,63
T3-Phonolite	0,59	0,11	30,65	8,82	0,09	0,03	18,49	2,92	9,97	3,57	0,00	0,00	21,64	2,55	0,00	0,00	9,27	1,09	4,13	0,21
T4-Basalt1	0,36	0,18	32,11	9,29	0,07	0,05	18,34	4,19	11,08	3,63	0,00	0,00	14,64	0,67	0,00	0,00	8,11	0,60	3,59	0,10
T5-Basalt2	0,31	0,05	28,40	5,32	0,00	0,00	16,53	1,53	9,47	2,11	0,00	0,00	15,22	0,30	0,00	0,00	8,05	0,95	3,32	0,36
T6-Basalt3	0,46	0,10	38,87	6,69	0,13	0,06	17,86	1,93	12,89	2,15	0,00	0,00	17,42	2,21	0,00	0,00	9,23	0,66	4,31	0,37
T7-Granite	0,36	0,08	26,81	4,69	0,08	0,02	14,79	1,91	9,13	1,65	0,00	0,00	11,17	1,93	0,00	0,00	7,42	1,37	2,88	0,58
Elements leached (mg) - 8M																				
Treatment	Al		Ca		Fe		K		Mg		Mn		Na		P		S		Si	
	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd	M	sd
T1-Control	0,43	0,19	10,87	6,00	0,13	0,06	2,35	1,03	3,11	1,84	0,00	0,00	10,71	5,15	0,00	0,00	9,49	3,86	1,32	0,67
T2 - KCl	0,40	0,11	6,21	1,59	0,12	0,04	18,45	5,99	1,51	0,31	0,00	0,00	9,79	3,09	0,00	0,00	8,94	2,30	1,21	0,35
T3-Phonolite	0,31	0,09	6,49	0,50	0,08	0,02	1,42	0,39	1,78	0,21	0,00	0,00	8,05	0,47	0,00	0,00	7,48	0,49	0,93	0,02
T4-Basalt1	0,21	0,02	6,87	0,42	0,02	0,03	1,54	0,12	1,94	0,10	0,00	0,00	7,18	0,38	0,00	0,00	7,19	0,43	0,88	0,06

T5-Basalt2	0,25	0,08	7,09	1,00	0,03	0,03	1,83	0,41	1,88	0,34	0,00	0,00	8,15	1,11	0,00	0,00	7,09	0,29	1,00	0,17
T6-Basalt3	0,26	0,04	7,06	0,54	0,07	0,01	1,49	0,10	1,95	0,20	0,00	0,00	7,06	0,61	0,00	0,00	6,66	0,31	0,87	0,09
T7-Granite	0,63	0,42	10,30	5,43	0,15	0,09	1,80	0,96	2,94	1,55	0,00	0,00	10,95	5,99	0,00	0,00	8,69	2,85	1,31	0,69

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### **3. FINAL CONSIDERATIONS**

This work is the first stage of a larger project that seeks to understand how the application of rock powders acts in the soil system. The initial project results showed that the bacterial community in the soil varied in accordance with the type of rock powder after 8 months of experiment, even when the fertility indexes did not change much.

The next steps aim to answer the following questions: What are these species? How do they correlate with the mineralogy of each rock? What is the influence of rock powders in the soil quality bioindicators? Do significant mineralogical changes happen after a 12-month period? If so, what are they?

This new step will be fundamental to understand how rock powders act on the mineral/microorganisms interface.

**Figura 1.** Experimental design and replicas.

**Figura 2.** XRD scans depicting the mineralogy of the soil used (Kt: kaolinite, Gb: Gibbsite, Qz: Quartz, Gt: Goethite) and of the rocks used in the experiment.

**Figura 3.** (a) Total loss of K (leachate + plant) up to 8M and (b) evolution of K and Ca leached (mg) from 1 month (1M) to 8 months (8M).

**Figura 4.** NMDS of the bacterial soil Community (BSC) after 1 month experiment (1M) (a), 4 months (4M) (b) and 8 months (8M) (c).

**Figura 5.** Redundancy analysis (RDA) of the bacterial soil community (BSC) and leached elements after 1 month (1M) (a) and 8 months (8M) (b).

**Table 1.** Physical and chemical soil characteristics.

**Table 2.** Total chemistry of soil initial and rocks ICP-MS (Si) and ICP-OS (other elements).

**Table 3.** Cumulative of K leached at 1M, 4M and 8M replicas, total K in aboveground plant tissue and dry biomass.

**Table 4.** Analysis of similarity (ANOSIM) for microbial community structure. (a) 1 month (1M); (b) 4 months (4M); (c) 8 months (8M).

**Table Supplementary 1.** Elements leached used in RDA analysis.