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Alterations in the rhizosphere of conifer plantations on Ferralsol within the Araucaria Forest domain

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Passo Fundo

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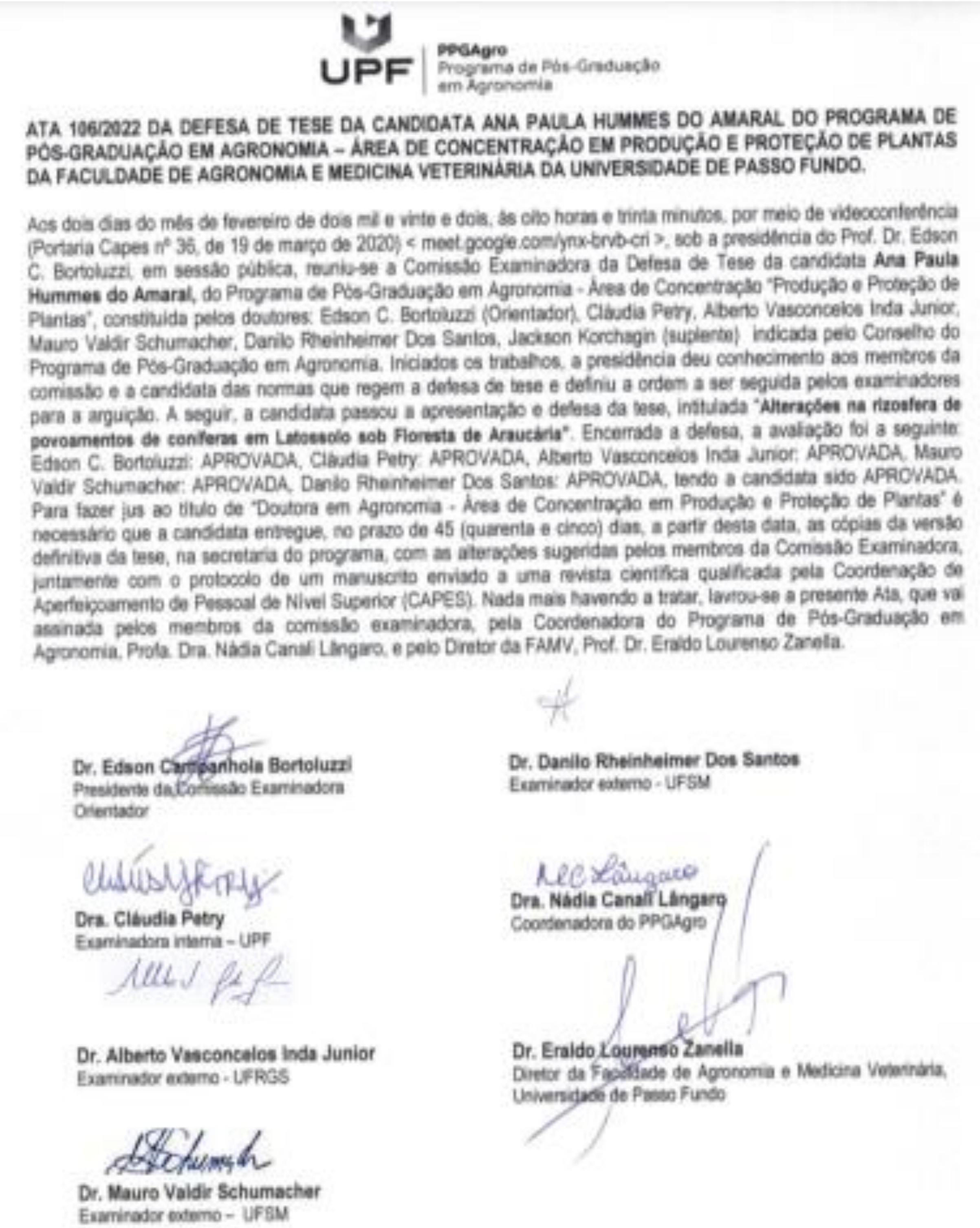

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ATA 106/2022 DA DEFESA DE TESE DA CANDIDATA ANA PAULA HUMMES DO AMARAL DO PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA – ÁREA DE CONCENTRAÇÃO EM PRODUÇÃO E PROTEÇÃO DE PLANTAS DA FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA DA UNIVERSIDADE DE PASSO FUNDO.

Aos dois dias do mês de fevereiro de dois mil e vinte e dois, às oito horas e trinta minutos, por meio de videoconferência (Portaria Capes nº 36, de 19 de março de 2020) < meet.google.com/ynx-brvb-cri >, sob a presidência do Prof. Dr. Edson C. Bortoluzzi, em sessão pública, reuniu-se a Comissão Examinadora da Defesa de Tese da candidata **Ana Paula Hummes do Amaral**, do Programa de Pós-Graduação em Agronomia - Área de Concentração "Produção e Proteção de Plantas", constituída pelos doutores: Edson C. Bortoluzzi (Orientador), Cláudia Petry, Alberto Vasconcelos Inda Junior, Mauro Valdir Schumacher, Danilo Rheinheimer Dos Santos, Jackson Korchagin (suplente) indicada pelo Conselho do Programa de Pós-Graduação em Agronomia. Iniciados os trabalhos, a presidência deu conhecimento aos membros da comissão e a candidata das normas que regem a defesa de tese e definiu a ordem a ser seguida pelos examinadores para a arguição. A seguir, a candidata passou a apresentação e defesa da tese, intitulada "**Alterações na rizosfera de povoamentos de coníferas em Latossolo sob Floresta de Araucária**". Encerrada a defesa, a avaliação foi a seguinte: Edson C. Bortoluzzi: APROVADA, Cláudia Petry: APROVADA, Alberto Vasconcelos Inda Junior: APROVADA, Mauro Valdir Schumacher: APROVADA, Danilo Rheinheimer Dos Santos: APROVADA, tendo a candidata sido APROVADA. Para fazer jus ao título de "Doutora em Agronomia - Área de Concentração em Produção e Proteção de Plantas" é necessário que a candidata entregue, no prazo de 45 (quarenta e cinco) dias, a partir desta data, as cópias da versão definitiva da tese, na secretaria do programa, com as alterações sugeridas pelos membros da Comissão Examinadora, juntamente com o protocolo de um manuscrito enviado a uma revista científica qualificada pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Nada mais havendo a tratar, lavrou-se a presente Ata, que vai assinada pelos membros da comissão examinadora, pela Coordenadora do Programa de Pós-Graduação em Agronomia, Profa. Dra. Nádia Canali Lângaro, e pelo Diretor da FAMV, Prof. Dr. Eraldo Lourenso Zanella.

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DEDICATION

This thesis is dedicated to the science.

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To God who kept me firm in faith!

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Do not decrease the size of your dreams, increase the size of your faith.

Unknown author

ABSTRACT

AMARAL, Ana Paula Hummes do. Alterations in the rhizosphere of conifer plantations on ferralsol within the araucaria forest domain 145 f. Thesis (Doctorate in Agronomy) – University of Passo Fundo, Passo Fundo, 2022.

The introduction of extensive tree plantations brings recognized economic benefits at the expenses of natural environments. The native *Araucaria angustifolia* (araucaria) and the fast-growing exotic *Pinus elliottii* var. *elliottii* (slash pine), when cultivated in monoculture, may affect differently the soil related to araucaria grown in its native environment, the Mixed Ombrophilous Forest, also called Araucaria Forest. The differences are influenced by environmental conditions, plant species and their associate microorganism, and soil mineral composition. Understanding how forest plantations affect the soil may ensure the sustainability of timber production. In order to investigate the impact of these conifers on soil geochemical and mineral evolution, especially in the rhizosphere where processes are more intense and changes occur in shorter periods of time, the present study was conducted in three instances. Chapter I: **A meta-analysis of physical and chemical changes in the rhizosphere and bulk soil under woodlands**, which consists of a systematic review on physical and chemical changes in rhizosphere and bulk soil promoted by conifers and broadleaved trees, established as monoculture or grown in mixed forest fragments. The soil attributes pH, phosphorus, potassium, calcium, aluminum, effective cation exchange capacity, total organic carbon and particle-size distribution were gathered from thirty-two manuscripts (170 studies from 8 countries) published in peer-reviewed journals indexed to *Science Direct Core Collection* from January 2000 to July 2020. The conclusion was that the influence of conifers and monocultures on soil properties is greater than broadleaved trees and establishment in mixed forests. Chapter II: **Higher mineral weathering of a Ferralsol evidenced in the rhizosphere of conifer plantations grown in a subtropical climate** seeks to apprise the rhizosphere effect on geochemical changes and mineralogical evolution of a deep and high weathered Ferralsol under two conifer plantations, *Araucaria angustifolia* and *Pinus elliottii* var. *elliottii*. It was studied the rhizosphere effect related to bulk soil, and how monoculture of the two conifers affected the soil physical, chemical and mineralogical properties. Araucaria did not affect chemical attributes, but slash pine affected total organic carbon and available phosphorus. No rhizosphere effect was observed for silt and clay content, but slash pine promoted silt decrease followed by clay increase, while in araucaria the opposite was observed. The soil did not show a broad mineral assemblage, but presented variations in the proportions of clay minerals, comprising kaolinite, illite and hydroxy-interlayered minerals (HIM), with kaolinite-enrichment in the rhizosphere and in pinus, with illite absence in slash pine soil. The proportion of crystalline iron oxides was higher in slash pine. This study evidences acceleration of soil weathering by monoculture of conifers, with higher rates in slash pine followed by araucaria, increasing the risk of soil quality loss when compared with the native Araucaria soil. Chapter III: **Edaphic changes in sustainable use conifer plantations within the Mixed Ombrophylous Forest domain** shows how chemical, physical and microbiological soil attributes are affected when monocultures of *Araucaria angustifolia* and *Pinus elliottii* var. *elliottii* are established in a high weathered Latossol within the Mixed Ombrophilous Forest ecosystem, and the importance of the sustainable managed Brazilian National Forests in protecting the natural ecosystems and hosting biodiversity. Soil was characterized in its physical (sand, silt, clay, bulk density, soil particle density and total soil porosity), chemical (pH, Al^{3+}, H^{+}+Al^{3+}, total organic carbon, K^{+}, $Ca^{2+,}$ Mg^{2+}, available P, total cation exchange capacity and base saturation) and microbiological (soil basal respiration, microbial biomass carbon, metabolic and microbial quotients) attributes, with slight changes in soil under the two conifers detected by univariate statistical analysis. However, multivariate analysis highlighted different impact of coniferous on soil by discriminating the clusters. The claying process promoted by slash pine suggests higher weathering rate by the activity of this conifer. This doctoral research shows that studies on the rhizosphere of trees are feasible to detect and monitor short-term impact of long-lived woody species on soil, which can be further investigated especially on the interactions between microorganisms and clay minerals.

Palavras-chave: 1. *Araucaria angustifolia*. 2. *Pinus elliottii*. 3. Clay mineral weathering. 4. Monoculture. 5. Conifer. 6. Ferralsol

RESUMO

AMARAL, Ana Paula Hummes do. Alterações na rizosfera de plantações de coníferas em latossolo no domínio da floresta de araucárias. 145 f. Tese (Doutorado em Agronomia) – Universidade de Passo Fundo, Passo Fundo, 2022.

A introdução de extensos plantios florestais traz reconhecidos benefícios econômicos às custas dos ecossistemas naturais. A nativa *Araucaria angustifolia* e o exótico, de rápido crescimento, *Pinus elliottii* var. *elliottii*, quando cultivados em monocultura, podem alterar diferentemente o solo em relação à araucária cultivada no seu ambiente natural, a Floresta Ombrófila Mista, também chamada Floresta de Araucária. As diferenças são influenciadas pelas condições ambientais, pela espécie vegetal e microrganismos a elas associados, e pela composição mineral do solo. O entendimento de como as plantações florestais alteram o solo pode assegurar a sustentabilidade da produção madeireira. Com o objetivo de investigar o impacto dessas coníferas na evolução geoquímica e mineralógica do solo, especialmente na rizosfera onde os processos são mais intensos e as mudanças ocorrem em curtos períodos de tempo, o presente estudo foi conduzido em três estágios. Capítulo I: **Meta-análise das alterações físicas e químicas na rizosfera e na matriz do solo sob florestas**, que consiste em uma revisão sistemática das alterações físicas e químicas na rizosfera e na matriz do solo promovidas por coníferas e folhosas, cultivadas em monocultura ou estabelecidas em fragmentos florestais mistos. Os atributos do solo pH, fósforo, potássio, cálcio, alumínio, capacidade de troca de cátions, carbono orgânico total e distribuição granulométrica foram extraídos de trinta e dois artigos científicos (170 estudos de 8 países) publicados em periódicos revisados por pares e indexados à *Science Direct Core Collection*, de janeiro de 2000 a julho de 2020. Concluiu-se que coníferas e monoculturas alteram mais as propriedades do solo do que folhosas e quando estabelecidas em fragmentos mistos. Capítulo II: **Aumento do intemperismo de um Latossolo evidenciado na rizosfera de plantios de coníferas cultivadas em clima subtropical**, em que foi avaliado o efeito da rizosfera nas alterações geoquímicas e na evolução mineralógica de um Latossolo altamente intemperizado, sob plantações das coníferas *Araucaria angustifolia* e *Pinus elliottii* var. *elliottii*. Foi estudado o efeito da rizosfera em relação à matriz do solo, e se monocultura afetou diferentemente as propriedades físicas, químicas e mineralógicas desse solo. A araucária não alterou os atributos químicos, mas o pinus afetou o carbono orgânico total e o fósforo disponível. Não foi observado qualquer efeito da rizosfera no teor de silte e argila, mas o pinus promoveu a diminuição do silte seguido de aumento da argila, enquanto que na araucária foi observado o oposto. O solo não apresentou uma assembleia de minerais diversificada, mas variações nas proporções dos argilominerais, compreendendo caulinita, ilita e minerais hidroxi-entrecamadas, com enriquecimento de caulinita na rizosfera e no pinus, e ausência de ilita no solo sob pinus. A proporção de óxidos de ferro bem cristalizados foi superior no pinus. O estudo evidencia uma aceleração do intemperismo do solo por monocultura de coníferas, com taxas mais elevadas no pinus seguido da araucária, aumentando o risco de perda de qualidade do solo. O capítulo III, **Alterações edáficas em plantações de coníferas de uso sustentável no domínio da Floresta Ombrófila Mista,** mostra como os atributos químicos, físicos e microbiológicos do solo são afetados quando monoculturas de *Araucaria angustifolia* e *Pinus elliottii* var. *elliottii* são estabelecidas em Latossolo altamente intemperizado no ecossistema da

Floresta Ombrófila Mista, e a importância das práticas de manejo sustentáveis adotados pelas Florestas Nacionais brasileiras para proteção dos ecossistemas naturais e da biodiversidade. O solo foi caracterizado por seus atributos físicos (areia, silte, argila, densidade do solo, densidade de partículas do solo e porosidade total), químicos (pH, Al^{3+}, $H^{+}+Al^{3+}$, carbono orgânico total, K^{+}, Ca^{2+}, Mg^{2+}, P disponível, capacidade de troca de cátions total e saturação por bases) e microbiológicos (respiração basal do solo, carbono da biomassa microbiana, quocientes metabólico e microbiano), com poucas alterações no solo sob as duas coníferas detectadas pela análise estatística univariada. No entanto, a análise multivariada demonstrou o impacto diferenciado das coníferas no solo através da discriminação dos agrupamentos. O processo de argilização detectado no solo com pinus sugere um maior intemperismo pela atividade dessa conífera. Esta pesquisa de doutorado mostra que estudos sobre a rizosfera das árvores são viáveis para detectar e monitorizar o impacto, a curto prazo, das espécies lenhosas perenes no solo, o qual sugere-se ser melhor investigado especialmente em relação às interações entre microrganismos e argilominerais.

Palavras-chave: 1. *Araucaria angustifolia*. 2. *Pinus elliottii*. 3. Intemperismo de argilominerais. 4. Monocultura . 5. Coníferas. 6. Latossolo.

SUMMARY

1 INTRODUCTION

In southern Brazil, *Araucaria angustifolia* (Bert. O. Ktze.) is a long-lived pioneer conifer of the Araucariaceae family that occurs in the highland plateaus of Rio Grande do Sul state. Also called Paraná-pine, it is characterized by its imposing size (up to 50 m in height and trunk diameter reaching 2 m), high wood value and appreciable edible seeds. It is an important tree component of the Mixed Ombrophilous Forest (MOF), also known as Araucaria Forest, an ecosystem of social, cultural and economic relevance mainly for smallholder farmers who seeks for the conservation-by-use since the edible seeds of Araucaria provide revenue, and important commercial species such as yerba mate (*Ilex paraguariesis*) are grown in the understorey.

The cylindrical and uniform trunk of the *Araucaria angustifolia* (araucaria) have made the wood very appreciable by the industry, which is the reason for its intense exploitation and the drastic reduction of its population from the beginning of the twentieth century, leading it to figure on The IUCN Red List of Threatened Species^{TM} as ‘critically endangered’ (THOMAS, 2013). Despite the efforts to recompose the population of araucaria as monoculture, its relatively slow growth resulted in a movement toward the exploitation of exotic, fast-growing and low-density woody species such as *Pinus* spp. and *Eucalyptus* spp. that initiated in the early 1970s. In Brazil, the total planted area with these exotic species reached 7.3 million ha in 2018, of which *Pinus* spp. occupy 1,6 million ha. Contrastingly, only around 13 thousand hectares were planted with araucaria (IBA, 2019). The decline of Araucaria Forests has been resulting in loss of diversity due to fragmentation and habitat reduction (HARTLEY, 2002; FONSECA et al., 2009; SILVA; SCHMITT, 2015) with disruption on soil ecosystem, drawing attention to the need of assessing the impacts on soil quality (DORAN; PARKIN, 1994; ISLAM; WEIL, 2000; USHARANI; ROOPASHREE; NAIK, 2019).

Exotic woody species such as *Pinus* spp. and *Eucalyptus* spp. are much appreciated by the industry for their high productivity, as they escape from their natural enemies and quickly reach large populations and high growth rates when resources are available. However, the introduction of exotic species in an ecosystem, as well as the management practices, should be preceded by environmental impact studies at different scales to understand the potential of environmental change. Previous studies showed that the soil of monocultures with araucaria presents more similarity with the native forest soil than the exotics *Pinus* spp. or *Eucalyptus* spp. (FAGOTTI et al., 2012; BINI et al., 2013a; PAZ et al., 2015), besides hosting Araucaria Forest biodiversity (FONSECA et al., 2009; MALYSZ; OVERBECK, 2018). Firn, Erskine and Lamb (2007) found that highly productive woody species, as the above exotics, are associated with both lower soil pH and soil nutrient availability when compared to the native, and that tree plantations which allow the recolonization of native plants ensure the soil heterogeneity and the maintenance of original properties (MALYSZ; OVERBECK, 2018). Studies with monocultures with these conifers on soil geochemical and mineralogical evolution are uncommon in tropical and subtropical regions, but of great relevance in ensuring the sustainability of both ecosystems and timber production, especially in areas considered the new agricultural frontier.

The acquisition of nutrients by plants requires an efficient root system, especially with fine roots and high colonization by microorganisms. This soil-root-microorganism combination consists of a small portion, usually between 1% and 5% of the total soil volume, defined as rhizosphere zone, where processes take place at higher rates than in the surrounding bulk soil (KUZYAKOV; BLAGODATSKAYA, 2015). The rhizosphere characteristics vary greatly in relation to bulk soil (HINSINGER, 1998; DIEFFENBACH; MATZNER, 2000; SÉGUIN et al., 2005; CHEN et al., 2006; CALVARUSO; N’DIRA; TURPAULT, 2011; COLLIGNON; CALVARUSO; TURPAULT, 2011; TOBERMAN; CHEN; XU, 2011; BORTOLUZZI et al., 2019; HUMMES et al., 2019; LIU et al., 2019) and are related to intrinsic characteristics of each species, soil parental material and environmental conditions (UROZ et al., 2016; RONDINA et al., 2019).

It is already known that araucaria, oppositely to *Pinus elliottii* var. *elliottii* (slash pine), presents a diversity of organic residues of easy degradation, thus promoting the restoration of soil properties at levels similar to native forests, being more sustainable, while slash pine makes the soil more similar to agricultural soils (BINI et al., 2013). It is also known the ability of plant species in altering the soil constituent proportion (AGNELLI et al., 2016; BORTOLUZZI et al., 2019; KORCHAGIN et al., 2019; KORCHAGIN et al., 2020) and the mineral stability (APRIL; KELLER, 1990; ALEKSEEVA et al., 2011), especially in the rhizosphere related to bulk soil (CALVARUSO et al., 2009; TURPAULT; NYS; CALVARUSO, 2009; MCGAHAN; SOUTHARD; ZASOSKI, 2014). These processes are defined as the “rhizosphere effect” (HINSINGER et al., 2009). However, in humid subtropical regions, the impact of conifers growth, particularly of the native araucaria, on mineralogical changes of a highly weathered soil is lacking in the literature. The hypothesis that trees established as monoculture present a greater potential of soil physicochemical and mineralogical alterations guide the present study. The approach was to compare the soil properties of the rhizosphere and bulk soil that supports mature araucaria (*Araucaria angustifolia*) and slash pine (*Pinus elliottii* var. *elliottii*) forests, grown in a basalt-derived soil within the Mixed Ombrophilous Forest domain, with emphasis on physical, chemical and mineralogical changes.

In forest ecosystems, the availability of nutrients regulates the primary productivity (FERNÁNDEZ-MARTÍNEZ et al., 2014), where the demand of trees for plant-essential mineral nutrients such as calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K) often exceed the supply fluxes from mineral weathering (CLEVELAND et al., 2013). The organic mineral nutrient turnover, which are the nutrients continuously returned from trees to forest floor through litterfall, is the largest source of plant nutrition especially in forests that have already reached their climax (UHLIG; VON BLANCKENBURG, 2019; UHLIG; AMELUNG; BLANCKENBURG, 2020). However, this organic nutrient source will be depleted within centuries (UHLIG; AMELUNG; BLANCKENBURG, 2020) since there is continuous losses through plant litter dissolution and erosion, and the great nutrient inventory is of geogenic origin, in the upper (<3 m) and deep (>3 m) regolith, which replenishes the reservoir through chemical weathering and will sustain forest ecosystems nutrition for centuries to millennia (UHLIG; VON BLANCKENBURG, 2019; DAWSON; HAHM; CRUTCHFIELD-PETERS, 2020).

Atmospheric deposition is another nutrient source, although some authors consider it negligible (CHADWICK et al., 1999; UHLIG; AMELUNG; BLANCKENBURG, 2020). However, Mg inputs via deposition were found to be 2-fold higher than via weathering inputs (ROSENSTOCK et al., 2019), while sulphate (xSO_4) and inorganic nitrogen compounds (NO_3, NH_4) are continually deposited and are sources of sulfur and nitrogen, respectivelly (VUORENMAA et al., 2018), to such an extent that N deposition alter N status of forests (ABER et al., 2003). However, in a naturally regenerated, even-aged Norway spruce forest minimally affected by forest activities, Rosenstock et al. (2019) found that for a 65-year rotation, inputs of Na, K and Ca via weathering were 1.3-, 1.5-, 1.8-fold higher, respectively, than atmospheric deposition inputs.

The objective of this study was to investigate, in a high weathered basalt-derived soil (Ferralsol), the results of processes occurring at the root-soil interface of two conifers grown in monoculture, the native araucaria and the fast-growing exotic slash pine. The study is focused on soil physical, chemical, biological and mineralogical changes due to conifer species in monoculture whose hypothesis is that the exotic will promote greater changes than the native. Thus, the present research was divided into three stages. The first, presented in Chapter I, consists of a systematic review, also known as meta-analysis, of physical and chemical changes in rhizosphere and bulk soil promoted by conifers and broadleaved trees established as monoculture and grown in mixed forest stands. The second stage, presented in Chapter II, seeks to apprise the rhizosphere effect on geochemical changes and mineralogical evolution of a high weathered Ferralsol under two conifer plantations in subtropical climate, the native araucaria and the exotic slash pine. The Chapter III shows how chemical, physical and microbiological soil attributes are affected when monocultures of araucaria and slash pine are established in a high weathered Ferralsol within the Mixed Ombrophilous Forest ecosystem, and the importance of the management practice to achieve sustainability.

2 LITERATURE REVIEW

The implantation of monocultures with forest species encourages the search for elucidation on edaphic issues that have been poorly addressed in the literature so far and that can be used as a support for decision making on the species adoption and management practices in order to ensure the sustainability of the forestry activity. This review will address topics involving soil, plant nutrition and changes occurring in the rhizosphere, soil weathering processes with its byproducts, and methodological aspects of studies on rhizosphere of tree species.

2.1 Soils

Soil is the unconsolidated material on the Earth's surface that supports plants and animal life (COTTER-HOWELLS; PATERSON, 2000). According to World Reference Base for Soil Resource 2014 (IUSS WORKING GROUP WRB, 2015), soil is defined as "any material within 2 m of the Earth's surface that is in contact with the atmosphere, excluding living organisms, areas with continuous ice not covered by other material, and water bodies deeper than 2 m" (VANMAEKELBERGH, 2009). However, in a broader concept, soil can be defined as the natural body of the Earth's surface, consisting of mineral and organic materials resulting from the interactions of the formation factors (climate, living organisms, parent material and relief) over time, containing living matter and partly modified by human action, capable of supporting plants, hold water, store and transform waste, and support buildings.

Soil formation, or pedogenesis, occurs through the breakdown of geological deposits by physical, chemical and biochemical processes called "weathering" (BUOL et al., 2003). Minerals formed at the same time that the rock containing them are called "primary minerals", mostly occurring in the coarser sand and silt-size particles; however, when they result from the alteration of primary minerals or mineraloids, they are named "secondary minerals", which are the most reactive inorganic material presents in soil (CHURCHMAN; LOWE, 2012).

The decomposition of rocks and minerals, both primary and secondary, promotes the formation of the smallest and most reactive soil particles, the clays (< 2 µm), in a process known as "claying" (BUOL et al., 2003). When the material results from the break-down of antecedent minerals originating new clays and other secondary minerals, the process is known as "neogenesis" (CHURCHMAN; LOWE, 2012). Thus, six main types of minerals can be found in the soil clay fraction: primary minerals such as quartz, feldspars, and others; phyllosilicates, known as clay minerals; metallic oxides and hydroxides, among them goethite, hematite, ferrihydrite; carbonates; gypsum; and short-range order alumina-silicate minerals (allophane, imogolite) (BARRÉ et al., 2014). In tropical and subtropical soils, the main minerals are 1:1 clay type (kaolinite), iron oxides (hematite, goethite, ferrihydrite and maghemite) and aluminum oxides (gibbsite), and 2:1 clay minerals with Al-hydroxy interlayers (hydroxy-interlayered smectite HIS and hydroxy-interlayered vermiculite HIV) (SCHAEFER; FABRIS; KER, 2008).

Changes in the soil environment may alter the clay minerals and their functions (soil organic matter, water retention, nutrient supply, physical stability, etc.) on a time scale ranging from a few months to hundreds of years, depending on the environment where processes occur in the soil (CORNU et al., 2012). In the rhizosphere, these changes can occur very fast (HINSINGER; JAILLARD, 1993; HOUBEN; SONNET, 2012), affecting the soil as a whole over pedogenic time. The impact of root-soil contact time on mineral weathering and nutrient release in pots was investigated by many authors. Naderizadeh, Khademi and Arocena (2010) studied the transformation of phlogopite and muscovite in the rhizosphere of alfalfa (*Medicago sativa* L.) under influence of added organic matter (OM). They observed an increase of acidity, K release and mineral transformation due to OM addition with dependence on the type of mineral. Houben and Sonnet (2012) observed the weathering of smithsonite, a Zn mineral, as consequence of dissolved organic carbon produced by the rhizosphere activity of ryegrass (*Lolium multiflorum* Lam). The effect of a monocot (fescue, *Vulpia myuros*) and a dicot (tomato, *Solanum lycopersicum*) on mineral stability and soil solution composition was investigated by Mcgahan, Southard and Zasoski (2014). They demonstrated a greater influence of the dicot then the monocot rhizosphere, with rhizosphere solution extracts farther from equilibrium than bulk soil ones. These studies were conducted on annual plants and in laboratory, where the conditions are very different from those existing in natural ecosystems since the environment is controlled, resources are limited, and the surface of contact between roots and minerals is larger, which maximizes the impact on mineral weathering.

Several *in situ* studies that evaluate the rhizosphere effect of tree species have been conducted to understand the ecosystem dynamics in temperate climate regions, while in subtropical and tropical regions this type of study is scarce so far. Turpault, Gobran and Bonnaud (2007) assessed the differences and the evolution of chemical properties for bulk soil, rhizosphere and rhizosphere interface in a Douglas-fir (*Pseudotsuga menziesii*) ecosystem. Calvaruso, N'dira and Turpault (2011) compared physicochemical properties of rhizosphere and bulk soil of five tree species in Breuil-Chenue forest (France). The soil phosphorus (P) supply due to rhizosphere organic P of two tree species (*Populus simonii* and *Pinus sylvestris*) in semiarid forests of Northeast China was investigated by Zhao et al. (2015), who detected variations in rhizosphere recalcitrant organic P due to tree species. Temporal variations in phosphatase activity and P fractions in bulk soil and rhizosphere of *Larix olgensis* plantations with different developmental stages (16-, 23-, 34- and 49-y-old stands) were examined by Chen, Zhang and Duan (2016). The study indicated that the species development affects P bioavailability through changes in P fractions dynamic and phosphatase activities. Studying the mixture effect of Chinese fir (*Cunninghamia lanceolate*) with broadleaved species, Bu et al. (2020) compared the rhizospheres of pure plantations of Chinese fir with mixed plantations of the species. The rhizosphere effect of Chinese fir grown in mixed plantations was greater, increasing soil P availability.

In hot and humid regions, with intense rainfall, mineral weathering is more intense (XU et al., 2016) since most of weathering processes occur due to the reaction of minerals with water or aqueous solution (CHURCHMAN; LOWE, 2012). Most aqueous solutions have an acidic character as the dissolved CO_2, originated from the biota respiration and the atmosphere, reacts with water, forms carbonic acid (H_2CO_3) and releases H^+ ions into solution (CHURCHMAN; LOWE, 2012). Also, the permanent exudation of organic acid compounds (RONDINA et al., 2019) accompanied by pH decrease due to H^+ released for balance charge (HINSINGER et al., 2001; CALVARUSO et al., 2009) is a great soil weathering source. In this sense, the important role of plants and microorganisms in soil weathering is highlighted, whose intensity is greater in the upper layers of the profile since plant nutrition occurs mostly through fine roots rhizosphere (CÉSPEDES-PAYRET et al., 2012; GERENDÁS; RATCLIFFE, 2013).

The interaction between plants and soil happens in two ways: the first occurs on the soil surface by litter deposition, and the second in the rhizosphere zone (COTTER-HOWELS; PATERSON, 2000). However, the acquisition of nutrients through mineral weathering is related to intrinsic characteristics of plant species, rhizosphere biological activities and environmental conditions (TURPAULT; NYS; CALVARUSO, 2009; UROZ et al., 2016; RONDINA et al., 2019) and is regulated by the nutrient availability in the ecosystem (TURPAULT; NYS; CALVARUSO, 2009).

2.2 Rhizosphere vs. bulk soil

Initially conceptualized by the German scientist Lorenz Hiltner, in 1904, as the soil surrounding plant root, the term “rhizosphere” was best defined by Hinsinger (1998) as the volume of soil influenced by root activity. This microenvironment within the soil profile presents characteristics and behaviors that differ from the bulk soil , that is the soil not in direct or intermediate interaction with active roots (HINSINGER, 1998; APRIL; KELLER, 1990; RICHTER et al., 2007; TOBERMAN; CHEN; XU, 2011; MCGAHAN; SOUTHARD; ZASOSKI, 2014; BORTOLUZZI et al., 2019; HUMMES et al., 2019; LIU et al., 2019). Among the factors that can significantly alter the physicochemical properties of the rhizosphere are root respiration and exudation of organic compounds, which are also responsible the intense microbial activity of this portion of the soil (COTTER-HOWELS; PATERSON, 2000; HINSINGER et al., 2001;

CALVARUSO et al., 2009; CHURCHMAN; LOWE, 2012; RONDINA et al., 2019). Over pedogenic time, the interactions occuring in the rhizosphere affect, and even transform, soils on a large scale, from minerals to horizons and complete soil profiles (RICHTER et al., 2007). In unmanaged ecosystems, the differences between rhizosphere and bulk soil tend to be balanced.

Several methods are used to collect the rhizosphere soil, among them is by shaking the plant roots and the volume of soil associated with them. The soil that remains strongly adhered to the roots after shaking is considered the rhizosphere soil, or just rhizosphere, while the portion easily detached is referred to as bulk soil. However, the characteristics of the rhizosphere vary in space and time (MCGAHAN; SOUTHARD; ZASOSKI, 2014) since as roots grow, the soil initially adhered to the root apex becomes associated with zones of elongation, maturation, and ultimately, mature regions. Furthermore, because the roots growth process is very dynamic, the bulk soil will only become rhizosphere for a short period of time before becoming bulk soil again (HINSINGER; PLASSARD; JAILLARD, 2006).

2.3 Nutrition strategies of higher plants

The nutrient acquisition from soil by plants requires distinct strategies. Plants produce morphologically efficient root systems, mostly with fine roots highly colonized by microorganisms in order to improve the soil exploitation potential, especially when the site receives high incidence of light and low input of organic material in the soil (RONDINA et al., 2019). These conditions are observed in early-successional stages and during the initial stages of trees as monoculture, in which plants exhibit a scavenging strategy for nutrient acquisition. In contrast, when plants are already established in shaded environments, such as late-successional stages and adult trees in monoculture, the input of organic matter is greater and the mining strategy is exhibited for nutrition acquisition, with exudation of labile carbon in the rhizosphere (RONDINA et al., 2019). Both strategies are accompanied by pH reduction and acidification of the rhizosphere.

Soil acidification in response to plant growth plays an important role in weathering. The finest roots, being more active and absorbing more nutrients, are mainly responsible for pH reduction in the rhizosphere (CÉSPEDES-PAYRET et al., 2012; GERENDÁS; RATCLIFFE, 2013); however, there are other several sources of soil acidification. Root respiration releases CO_2 in concentrations 30 to 100 times higher than the existent in the atmosphere (HINSINGER et al., 2003), and is responsible for the formation of carbonic acid, a weak acid that significantly alters the soil pH (COTTER-HOWELS; PATERSON, 2000). The greater uptake of cations rather than anions promotes protons release (H^+) into the soil solution for charge balance (HINSINGER et al., 2001; CALVARUSO et al., 2009). The preferential uptake of NH_4^+ rather than NO_3^- by the roots of certain tree species is also a source of soil acidification (CALVARUSO et al., 2009), as far as the exudation of organic acids by roots and fungal hyphae (COURCHESNE; KRUYTS; LEGRAND, 2006).

The rhizosphere dynamics affects its mineralogical composition in relation to the bulk soil (SÉGUIN et al., 2005; TURPAULT; NYS; CALVARUSO, 2009; NADERIZADEH; KHADEMI; AROCENA, 2010; MCGAHAN; SOUTHARD; ZASOSKI, 2014). pH values lower than four can promote clay dissolution by action on the octahedral sheet, release Al^{3+} into the soil solution (WANG; GÖTTLEIN; BARTONEK, 2001; VIENNET et al., 2016; KORCHAGIN et al., 2019), affect clay contents and the ratio of 2:1 clay minerals with Al interlayers (KORCHAGIN et al., 2019), and promote the transformation of illite into vermiculite due to the loss of K^+ from the interlayers (BORTOLUZZI et al., 2012). Furthermore, other soil geochemical properties are also affected by acidification (WANG; GÖTTLEIN; BARTONEK, 2001; ALEKSEEVA et al., 2011; KORCHAGIN et al., 2019).

2.4 Mineral weathering and stability

Mineral weathering transforms rocks into soil and releases nutrients into the soil solution that are used for plant nutrition. It usually involves the reaction of minerals with water or aqueous solution in a process known as hydrolysis (CHURCHMAN;

LOWE, 2012). Through this process occurs the solubilization of primary minerals from the group of phyllosilicates, feldspars, pyroxenes, amphiboles and volcanic glass (mineraloid), and alteration into secondary minerals such as illite, smectite, vermiculite, chlorite and kaolinite (NESBITT; YOUNG, 1989). Also, the mechanical forces exerted by roots growth accelerate mineral degradation, exposing the mineral surface to root-induced chemical gradients and, consequently, increasing soil weathering (APRIL; KELLER, 1990). However, there is a great variation in soil mineralogy and mineral abundance due to parent material and intrinsic characteristics of the minerals, environmental conditions, and human activity (CHURCHMAN; LOWE, 2012).

Silicates are the most common primary minerals in the Earth's crust, although secondary minerals are the most reactive inorganic soil materials, which are generally associated with organic materials (CHURCHMAN; LOWE, 2012). Phyllosilicates have a laminar structure composed of the elements silicon (Si) and oxygen (O) in the ratio 2:5, with the sharing of three oxygen to form tetrahedral sheets, and aluminum (Al), iron (Fe) or magnesium (Mg) coordinated by six hydroxyls (6 x OH) forming octahedral sheets. However, the basic cations potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), and lithium (Li^+) are commonly associated with phyllosilicates, either in the structures of the tetrahedral and octahedral layers or in the interlayers. These laminar structures intercalate and generate different minerals with distinct physical and chemical characteristics, originating soils with varying properties associated with the parent material (MOORE; REYNOLDS, 1997).

The stability of silicate minerals is related to their degree of weathering. Goldich (1938) defined a stability series for the common main primary minerals (Goldich’s series) based on the principles of thermodynamics. He affirmed that the higher the temperature of the magma in which a mineral is crystalized, the farther it is from equilibrium with relation to the temperature of Earth's surface and, therefore, the greater its susceptibility to breakdown by weathering agents. However, this is only a generalization, because according to Churchman and Lowe (2012), the minerals stability also depends on (*i*) the nature of the chemical bonds between the tetrahedral and octahedral sheets and the interlayers – the greater the bond strength, the greater the mineral stability; (*ii*) the amount of isomorphic substitutions – the greater the number of isomorphic substitutions, the greater the number of free charges, and the lower the mineral stability; and (*iii*) the extent of the cations balance charge embedded in the laminar structure and the bonding sites. Other additional characteristics that determine the mineral stability are their particular chemical composition and mineralogical structure, the mineral relative solubility in water, and their thermodynamic stability against breakdown, besides environmental factors such as the soil solution composition, the redox conditions, and the flow of water through the minerals (CHURCHMAN; LOWE, 2012).

Soil weathering occurs typically in acidic environments, in which involves a range of organic compounds that can complex ions into silicate structures, enhance their breakdown, thus increasing decomposition and affecting the formation of new minerals (VELDE; BARRÉ, 2010). Also, highly weathered soils with increased moisture and water time residence besides with organic matter accumulation can promote reductive/complexive dissolution of mineral crystalline phases and neoformation of new metastable phases (FINK et al., 2014).

In humid tropical and subtropical regions, high temperatures and intense rainfall accelerate mineral weathering and leaching, resulting in more developed soils, typically deep, and mostly composed of 2:1 clay minerals with hydroxy-Al interlayers (BORTOLUZZI et al., 2008; CANER et al., 2014), low-activity 1:1 clay minerals of kaolinite-group (SCHAEFER; FABRIS; KER, 2008) and accumulation of large amounts of Al and Fe oxi-hydroxides (PADUANI et al., 2009; BORTOLUZZI et al., 2015; XU et al., 2016). In these soils, the level of poorly crystallized iron oxides (Fe_o), as well as the proportion of goethite and gibbsite in the clay fraction, are related to the organic matter physical protection and colloidal stability (INDA JUNIOR et al., 2007).

Several authors have studied weathering and pedogenesis in southern Brazil, establishing relationships between climate, clay minerals formation and iron oxides (BORTOLUZZI et al., 2008; KÄMPF; CURI; MARQUES, 2009; INDA et al., 2010; OLIVEIRA et al., 2020). Soils formed from volcanic rocks, such as basalt, occupy an area of approximately 1.2 million km^2 within the Paraná Basin. The humid climate of the region accelerate the basalt weathering, with rapid loss of cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and relative accumulation of Si^{4+}, Al^{3+} and Fe^{3+} in the form of secondary minerals (CHOROVER; AMISTADI; CHADWICK, 2004) and oxides (BORTOLUZZI et al., 2008; INDA et al., 2010).

2.4.1 Oxides and hydroxides

Deeply weathered Ferralsols (VANMAEKELBERGH, 2009), also known as Latosols (Brazilian Classification System), are the most important Brazilian soils occurring in over 60% of the country area (SCHAEFER; FABRIS; KER, 2008). According to these authors, besides kaolinite, the clay mineralogy is dominated by the pedogenic iron oxides goethite (α-FeOOH) and hematite (α-Fe_2O_3), which can present Al-substitution. They are indicators of the pedogenic processes and pedoenvironment and affect the chemical and physical behavior of tropical and subtropical soils (BIGHAM; FITZPATRICK; SCHULZE, 2002) In soil microsites, the alternation of reducing and oxidizing conditions may induce iron oxide mobilizations and help to explain the redistribution of these minerals in soil with change in land use (INDA et al., 2013). Also, the land use change may promote the selective dissolution of iron oxides (FINK et al., 2014), with goethite being more susceptible to dissolution than hematite under similar drainage conditions area (SCHAEFER; FABRIS; KER, 2008).

In tropical and subtropical soils, Fe- and Al- oxy-hydroxides are the main source of variable charges (XU et al., 2016). Under acidic conditions, the protonation of the hydroxyl groups (OH^-) in Fe- and Al-oxides produces positively charged surfaces, while at high pH occurs deprotonation of hydroxyls of these oxides, generating negatively charged surfaces. As pH increases, the magnitude of the negative charges (OH^-) increases and the positive charges (H^+) decreases. The pH in which the amount of negative charges is equal the amount of positive charges is called zero charge point (ZCP). The ZCP was used by Hendershot and Lavkulich (1978) as an index of soil development, and the higher the ZCP, the more developed and weathered is the soil (HENDERSHOT; LAVKULICH, 1978; ANDA et al., 2008).

The absence of weatherable minerals and the concomitant accumulation of iron and aluminum extractable with acid ammonium oxalate (AAO) (Fe_o and Al_o) in the vicinity of the roots indicate that weathering is stimulated by root activity (COURCHESNE; GOBRAN, 1997). According to Schwertmann, Schulze and Murad (1982), the method of extraction with AAO, or Tamm reagent, was proposed in 1959 by Schwertmann and later, by McKeague and Day (1966) to separate the more active portion of Fe-oxides, of low crystallinity (ferrihydrite), from the less active, the pedogenic Fe-oxides (hematite, goethite and maghemite), since the solubility of the latter in AAO is lower (SCHWERTMANN; SCHULZE; MURAD, 1982; KÄMPF; CURI, 2000; BIGHAM; FITZPATRICK; SCHULZE, 2002). The higher concentration of Al_o and Fe_o in the rhizosphere extracted with AAO can be interpreted as the formation of low crystalline, or amorphous, oxides in response to the higher weathering in the root zone than in the bulk soil (SÉGUIN et al., 2005). The amorphous Al oxi-hydroxides precipitated on the root surface also suggests that pedogenetic processes in the rhizosphere differ from the surrounding bulk soil (APRIL; KELLER, 1990). However, Al exhibits an antagonistic behavior. It can either present a higher concentration in the rhizosphere (COURCHESNE; GOBRAN, 1997), or a higher content in the bulk soil. Because Al is the main component of aluminosilicates, the short-range order minerals imogolite and allophanes are easily weathered in the rhizosphere, and Séguin et al. (2005) attributed the lower Al_o content in rhizosphere to this behavior.

2.5 Mechanisms of clay mineral neoformation and transformation

The dynamics of soil formation involve losses, i.e. the leaching of chemical elements and solids from the soil solution. Although the difference between the energetic potential of the soil solution ("attack solution") and the minerals defines the degree of the solids’ alteration. The leaching rate is what exerts the greatest influence on the degree of transformation and neoformation of subsequent minerals (CHURCHMAN; LOWE, 2012). Neoformation is understood as the new mineral formation from solution, and transformation is the alteration of an existing structure in which constituents of the parent mineral are maintained (MOORE; REYNOLDS, 1997).

Soils, whether natural or cultivated, are an open system resulting from the complex interaction between mineral and live worlds (MEUNIER; BORTOLUZZI; MEXIAS, 2016). They are organized in horizons parallel to the surface where chemical and mineralogical reactions propagate from the atmosphere interface, forming and changing the soil clay minerals. In depth, the clay minerals are formed by the dissolution of rocks' primary minerals and accelerated by organic acids; on the surface, minerals are formed by mineralization of the organic matter (MEUNIER; BORTOLUZZI; MEXIAS, 2016).

Clay mineral dissolution kinetics is part of the kinetic theory that has been develop to explain and quantify silicate minerals dissolution, which have been extensively studied since the early 1980s and compiled by Nagy (1995). However, recently Cama and Ganor (2015) have taken up the topic again where they related the formation and alteration processes of clay minerals to two dissolution mechanisms: stoichiometric dissolution, which occurs under equilibrium conditions, and non-stoichiometric dissolution, which under equilibrium conditions results mainly from the precipitation of secondary phases. Non-stoichiometric dissolution encompasses five specific mechanisms: (*i*) clay dissolution due to pH, which refers to dissolution due to three processes that occur, preferentially, on the surface of the crystals: driven by protons, by hydroxyls, and by water, whose reactions predominate in acidic, basic, or neutral media, respectively (WHITE; BRANTLEY, 1995); (*ii*) clay dissolution rate dependent on the deviation from equilibrium, expressed by Gibbs free energy reaction; (*iii*) catalytic/inhibitory effect of aluminum (Al^{3+}) on mineral dissolution, which consists of the fast adsorption of Al^{3+} on the mineral surface followed by a slow hydrolysis; (*iv*) effect of ionic strength on the mineral dissolution rate; and (*v*) reactive surface area of the clay minerals. The sixth mechanism refers to *(vi*) stoichiometric dissolution, which consists on the release of cations from the clay minerals interlayers, induced by the exchange reaction between clay minerals and the soil solution.

2.6 Methodological aspects in the study of the rhizosphere

The methods used in the rhizosphere soil analyses do not differ much from those practiced in the assessment of general soil properties (JAILLARD, 2006); however, sampling methodologies and rhizosphere soil preparation should be observed (TURPAULT, 2006; BARILLOT et al., 2013).

There are several established procedures for obtaining samples of the rhizosphere fractions, most of them based on manually shaking and washing the roots with adhered soil particles after their detachment from the soil block. In the manual shaking method, it is assumed that mucilage and exudates will keep the rhizosphere soil attached to the roots. However, the outcome of this technique depends on the nature of the roots (e.g. main or secondary), the type of soil (e.g. sandy or clayey), and the way the operator conducts the procedure (e.g. agitation time and applied force) (BARILLOT et al., 2013). Thereafter, soil moisture and texture also have a great influence on the amount of soil adhered to the roots (LUSTER et al., 2009). In methods that include the step of root washing to collect rhizosphere soil, there is variation in the immersion time and the solution used (LUSTER et al., 2009; BARILLOT et al., 2013). All these factors may affect the determination of the chemical and physical properties of the rhizosphere.

Turpault (2006) proposed a physical method to separate the rhizosphere from the bulk soil for physical, chemical and mineralogical analyses. The method consists in physical separation that results in the bulk soil fraction, which is the soil free of roots, and the rhizosphere, which is the soil aggregates < 1 cm attached to the living roots that spontaneously detaches after drying. However, its main disadvantage is that not all soil adhering to the roots is removed after drying and it is necessary a great amount of soil to obtain small rhizosphere samples. This method is suitable for field sampling, especially for forest soils cultivated with any species in monoculture (TURPAULT, 2006).

2.7 Total elemental and mineralogical analyses

The basic description of a soil sample primarily consists of the total chemical composition and the mineralogical nature of the solid phase (JAILLARD, 2006). The classical methods for determining the chemical composition of a soil sample involve dissolving the solid phase in acidic solutions followed by analysis by atomic absorption spectrometry or inductively coupled plasma. However, these techniques are time consuming and dangerous because the handling of strong acids. More modern techniques consider the use of fluorescence and X-ray diffraction for total characterization of soil samples, which are detailed by Weindorf, Bakr and Zhu (2014).

X-ray fluorescence spectroscopy (XRF) is an analytical technique based on the analysis of the energy-dispersive X-rays fluorescence by the sample that allows the detection of the total concentration of elements in soil, from magnesium (Mg) to uranium (U) (WEINDORF; BAKR; ZHU, 2014), with the advantage of being a non-destructive, fast and accurate method.

X-ray diffractometry (XRD) is a laboratory technique for studying the behavior of the crystalline structure of materials based on the analysis of the spatial distribution of X-rays diffracted by ordered atomic structures that allows the identification of mineral assemblage in soils and sediments (BORTOLUZZI; POLETO, 2013). Recent advances in XRD patterns combined with sequential soil studies have shown that changes in the mineral assemblage of the clay fraction occur faster than previously thought (CORNU et al., 2012).

2.8 Meta-analysis studies

Meta-analysis is a systematic review used to deal with the massive volume and increased complexity of information existent mainly in the technical literature and scientific databases (SCHERM et al., 2014). It that has been developed for statistical analysis of multiple independent experiments which combines the results from two or more separate quantitative studies (KOUTSOS; MENEXES; DORDAS, 2019), estimates more precisely the effect of treatments adjusting for experimental heterogeneity (LOVATTO et al., 2007), and provides objective, transparent and replicable summaries of certain subjects covered in scientific researches (DEL RE, 2015). Since studies are not identical in their methodologies or/and sampling, these differences will likely to introduce variability among the true effect-sizes and should be modelled in accordance with a random-effect procedure (VIECHTBAUER, 2010).

The effect-sizes from a series of studies are calculated for pairwise observations, that is, the difference between the “treatment” and the “control” groups is calculated for each environment (study), and these effect-sizes are considered in meta-analysis reviews. According to Konstantopoulos (2006), “effect-sizes should put the results of all studies ‘on a common scale’ so that they can be readily interpreted, compared, and combined”. The parameter structure of these models is identical to those of general linear mixed model and incorporates a component of between-study variation into the uncertainty of the effect-size parameters and their estimates (KONSTANTOPOULOS, 2006).

During the last years, the number of meta-analysis studies with topics related to environment or agricultural research has been increasing, showing a trend in using this kind of literature review to effectively summarize agricultural research data and answer certain focused questions (KOUTSOS; MENEXES; DORDAS, 2019). This trend reflects the need for a more transparent, organized, objective and effective method of literature review for estimating more precisely the effect of treatments adjusting them for the experimental heterogeneity (LOVATTO et al., 2007), which may lead to a less biased evidence-base conclusions. Meta-analyses studies can serve as catalyst to separate relevant factors from less relevant ones (DEL RE, 2015) and to guide studies of a given area in a universe of possibilities

3 CHAPTER I

A meta-analysis of physical and chemical changes in the rhizosphere and bulk soil under woodlands

3.1 Abstract

Monocultures of tree species for economic purposes have been increasingly replacing natural environments as the demand for renewable energy sources increases. The choice of species has been more a matter of financial return than sustainability, often at expenses of natural forests, with common practices, such as the lack of nutrient compensation in forested sites, promoting increasing rates of mineral weathering with impacts the soil fertility. To summarize the impact of the forestry activities on edaphic factors, especially in the portion of soil where processes are more intense, we present a meta-analysis of the rhizosphere effects of tree (coniferous and broadleaved) and establishment form (monoculture and natural regeneration) on soil physical and chemical properties. The soil attributes pH, phosphorus, potassium, calcium, aluminum, effective cation exchange capacity, total organic carbon and particle-size distribution were gathered from thirty-two manuscripts (170 studies from 8 countries) published in peer-reviewed journals indexed to Science Direct Core Collection from 2000 to July 2020. The higher acidity in the rhizosphere of coniferous and the greater phosphorus, potassium and aluminum mobilization and higher cation exchange capacity in relation to bulk soil indicates a greater rhizosphere effect of the conifers than broadleaved trees. The higher total organic carbon, phosphorus, potassium, aluminum and cation exchange capacity in naturally regenerated stands indicate higher nutrient cycling and soil fertility levels than in monoculture since greater diversity of species is expected under canopy. The lower fertility levels in monocultures may be attributed to the high nutrient demand by plants that may lead to great mineral weathering rates, as evidenced by the soil claying process under this management system. Overall, we concluded that the influence of conifers and monocultures on soil properties is greater than broadleaved trees and grown in mixed plantations. Furthermore, despite the gap of research in the Southern hemisphere, which is the new agricultural frontier, our conclusions will support decision makers on reassessing the formation of the forest base, mostly in temperate zones, to achieve more effective and sustainable forest management practices.

Keywords: 1. Rhizosphere. 2. . Conifer. 3. Broadleaf. 4. Monoculture. 5. Regeneration.

3.2 Introduction

The increasing demand for renewable energy from woody biomass and the environmental pressure for decreasing net CO_2 emissions have been leading to the development of forest policies that imply in more intensive use of forests worldwide (LAURI et al., 2014), increasing weathering rates and the risks of depleting the soil base cations that support forest growth (AKSELSSON et al., 2019). Rhizosphere, a vital region of approximately ~2 mm from the root surface that is strongly influenced by plant-soil-microorganisms interaction, was firstly defined by Hiltner in 1904 (HINSINGER, 1998; HINSINGER; PLASSARD; JAILLARD, 2006). The rhizosphere plays a key role on sustaining biomass productivity as this small portion of soil affects the nutrient mobility and has direct effects on plant nutrition and crop production (DOTANIYA; MEENA, 2014). An example of the importance of these issues on sustainable forest productivity is the interdisciplinary project Quantifying Weathering Rates for Sustainable Forestry (QWARTS), which involved research groups from 2012 to 2019 in Sweden. It showed that whole-tree harvesting of a Norway spruce forest would markedly deplete exchangeable cation pools (ROSENSTOCK et al., 2019) and it is clearly not sustainable without nutrient compensation (AKSELSSON et al., 2019).

Soil mineral weathering involves chemical and physical processes that are modified by plants and organisms (MCGAHAN; SOUTHARD; ZASOSKI, 2014; UROZ et al., 2016; RONDINA et al., 2019). Temporal and spatial scales are important issues on this process (CORNU et al., 2012), and studies on rhizosphere of trees are promising to predict the long-term supply of weatherable nutrient to sustain forest growth since forest activity requires longer timescales (UHLIG; AMELUNG; BLANCKENBURG, 2020). The number of studies that have been conducted on physical, chemical and biological properties of rhizosphere is appreciable. Also, it has been widely accepted that these properties differ significantly from those of the surrounding bulk soil (HINSINGER, 1998; DIEFFENBACH; MATZNER, 2000; CHEN et al., 2006; CALVARUSO; N’DIRA; TURPAULT, 2011; COLLIGNON et al., 2011; TOBERMAN; CHEN; XU, 2011; BORTOLUZZI et al., 2019; HUMMES et al., 2019; LIU et al., 2019). Although rhizosphere occupies generally between 1% and 5%

of the soil portion, the alteration rates perform up to twice faster than in the bulk compartment (KUZYAKOV; BLAGODATSKAYA, 2015). However, changes in the rhizosphere conditions relative to bulk soil vary accordingly to plant species even when they are growing in the same location (WANG; GÖTTLEIN; BARTONEK, 2001).

The soil nutrients withdrawn upon by leaching and plant uptake are refilled by atmospheric deposition, litter decomposition and mineral weathering (ROSENSTOCK et al., 2019). However, the mineral weathering is the major supply to plant nutrition, and nutrient acquisition is related to intrinsic characteristics of plant species as well as environmental conditions (PAWLIK; PHILLIPS; ŠAMONIL, 2016; UROZ et al., 2016; RONDINA et al., 2019).

Acquisition of nutrients by plants requires a mining strategy. An efficient fine-root system is produced and an elevated colonization of root-microorganisms needs to be maintained in order to increase soil exploitation potential, especially when plants are growing in environments with high light incidence and low input of organic material into the soil (RONDINA et al., 2019), as happens during the establishment of monoculture of trees. In contrast, an established tree population is a shaded environment, with adult trees that present low metabolic rates and slower growth; thus, their strategy for nutrient acquisition is the permanent exudation of acid organic compounds (RONDINA et al., 2019), with variations due to weather conditions (HARTLEY et al., 1999) and between species due to growth and distribution of roots, plant physiology, rate of metabolism and associated microorganisms (CHEN et al., 2006). The strategies of plants are accompanied by pH decrease caused by H^+ released for balance charge, leading to weathering through dissolution of minerals in the rhizosphere indicated by an increase of cation exchange capacity and soluble Al^{3+} (WANG; GÖTTLEIN; BARTONEK, 2001), with transformation of illite into vermiculite due to loss of K from the interlayers (BORTOLUZZI et al., 2012), and the released Al^{3+} in the soil retained int the interlayers of 2:1 clay minerals (KORCHAGIN et al., 2019).

Rhizosphere is considered a “hotspot” of soil where processes take place in a higher rate than in bulk soil despite its small spatial size (KUZYAKOV; BLAGODATSKAYA, 2015). It plays an important role on biogeochemical cycles (TOBERMAN; CHEN; XU, 2011; HE et al., 2020), as the processes strongly influence the soil stock of carbon and nutrient budget (TURPAULT; GOBRAN; BONNAUD, 2007) and can affect pedogenesis (CALVARUSO et al., 2009), no matter the period of contact between the mineral and the active portion of roots (MCGAHAN; SOUTHARD; ZASOSKI, 2014). The greater changes that proceed in the rhizosphere generally cause pH reduction in alkali soils, and pH increase in acid soils, depending on the type of plants grown and the soil mineralogy (MCGAHAN; SOUTHARD; ZASOSKI, 2014), affecting management practices independently of crop or wood production.

The soil parameters are affected in different ways by the vegetation type and the establishment forms and management (MALYSZ; OVERBECK, 2018). Coniferous and broadleaved trees modify the rhizosphere relative to bulk soil differently, as far as mixed-forests relative to monoculture stands (CALVARUSO; N’DIRA; TURPAULT, 2011; GUAN; WANG; ZHANG, 2016). Peng et al. (2020) found higher forest floor C stock in coniferous forest than in broadleaved forests, but according to Hou et al. (2020), the rate of soil organic carbon (SOC) is greater in broadleaved afforestation than in coniferous. According to Bu et al. (2020), the nutrient availability in rhizosphere and bulk soil of mixed coniferous (*Cunninghamia lanceolata*) and broadleaves (*Michelia macclurei* or *Schima superba*) species is increased compared to pure coniferous plantations. Malysz and Overbeck (2018) found differences in the soil conditions due to monoculture and regeneration trees patterns. However, the potential of trees species and the vegetation establishment form to influence the physical-chemical properties in this “hotspot” is not synthetized in a systematic review in a perspective of low-input and sustainable forest growth. Hence, a meta-analysis might be an important tool in helping to clarify the rhizosphere effects in these scenarios.

Meta-analysis is an efficient statistical approach for conducting systematic literature reviews that combines the results from two or more individual quantitative studies (KOUTSOS; MENEXES; DORDAS, 2019), estimates with more precision the effect of treatments adjusting for experimental homogeneity (LOVATTO et al., 2007), and provides objective, transparent and replicable summaries of certain subjects covered in scientific researches (DEL RE, 2015). General reviews on the rhizosphere have been recently published (DOTANIYA; MEENA, 2014; SOKOLOVA, 2015; DESSAUX; GRANDCLÉMENT; FAURE, 2016; BROECKLING et al., 2018), as well as systematic reviews (meta-analyses) considering carbon dynamics in forests (HOU et al., 2020; PENG et al., 2020). However, a synthesis of individual studies across a variety of conditions and sites that allows statistical testing on the rhizosphere effect of tree species and stands establishment form on soil physical-chemical properties is lacking in literature.

Hinsinger et al. (2009) defined the “rhizosphere effect” as the modification of chemical, physical and biological properties around living roots that results from the activity of roots and associated microorganisms. In order to address the rhizosphere effect of trees, a meta-analysis was carried out to examine the soil attributes under different species and establishment forms over the last 20 years. Specifically, we addressed the following research questions: (*i*) Is it possible to infer about soil weathering due to species of trees and establishment form accessing soil data of original researches conducted worldwide? (*ii*) Do different groups of tree species (coniferous and broadleaved) tend to produce distinctive levels of rhizosphere effect? (*iii*) Do rhizosphere effects change due to stands establishment form (monoculture and mixed plantations)? It is expected that this meta-analysis will show the gaps in rhizosphere knowledge to assist in defining the guidelines for the present doctoral research.

3.3 Material and Methods

3.3.1 Literature review

Literature searches in published peer-reviewed journals were conducted from July 11 to 12, 2020, in the Web of *Science Core Collection*. We considered for analysis the studies published from 2000 to 2020 in which soil parameters of the rhizosphere and the bulk soil of forest species grown in field conditions were assessed. We used the specific combination of words “rhizosphere AND bulk AND forest” for the search. The criteria adopted for selecting the manuscripts were: (*i*) tree species (coniferous and broadleaved), (*ii*) studies performed under field conditions, (*iii*) trees grown spontaneously from self-seedling on the regenerated site or close to it (regeneration) or planted seedlings raised in forest nurseries (monoculture), (*iv*) soils without chemical contamination and that did not receive chemical fertilizers, and (*v*) studies whose data presented dispersion measures or, if absent, imputable according to Furukawa et al. (2006).

Our search retrieved 240 publications, among which 90 were downloaded for detailed examination and only 32 fulfilled the established criteria for the meta-analysis (Table 1). A total of 170 studies from eight countries (Figure 1) were used in this study, including 66 on coniferous and 104 on broadleaved trees, 95 planted trees (monoculture) and 75 naturally regenerated in mixed plantations (regeneration) (Table 2). We gathered data on sample size, mean and standard deviation/standard error of ten soil properties for the control (bulk soil) and the treatment groups (rhizosphere): (*i*) pH, (*ii*) available phosphorus (P_{av}), (*iii*) exchangeable potassium (K^+), (*iv*) exchangeable calcium (Ca^{2+}), (*v*) aluminum (Al^{3+}), (*vi*) total organic carbon (TOC), (*vii*) effective cation exchange capacity (*e*CEC), and particle-size distribution – (*viii*) sand, (*ix*) silt and (*x*) clay. These data were extracted from tables in original publications, or digitized from figures using the Web Plot Digitizer software (ROHATGI, 2019). When dispersion measures were not reported, which was observed in Calvaruso; N’Dira; Turpault (2011) for pH and eCEC, and Cloutier-Hurteau et al. (2010) for Al^{3+}, the standard deviation (SD) was imputed according to Furukawa et al. (2006). If dataset presented dispersion measures equal to zero, we changed to 0.0001 to avoid calculation errors by the *RStudio* software (R CORE TEAM, 2019). Prior to statistical analysis, the measurement units were standardized. Furthermore, two moderator variables were considered: (*i*) tree species, which were grouped into coniferous and broadleaved; and (*ii*) establishment forms, which were grouped into monoculture and regeneration in mixed plantations.

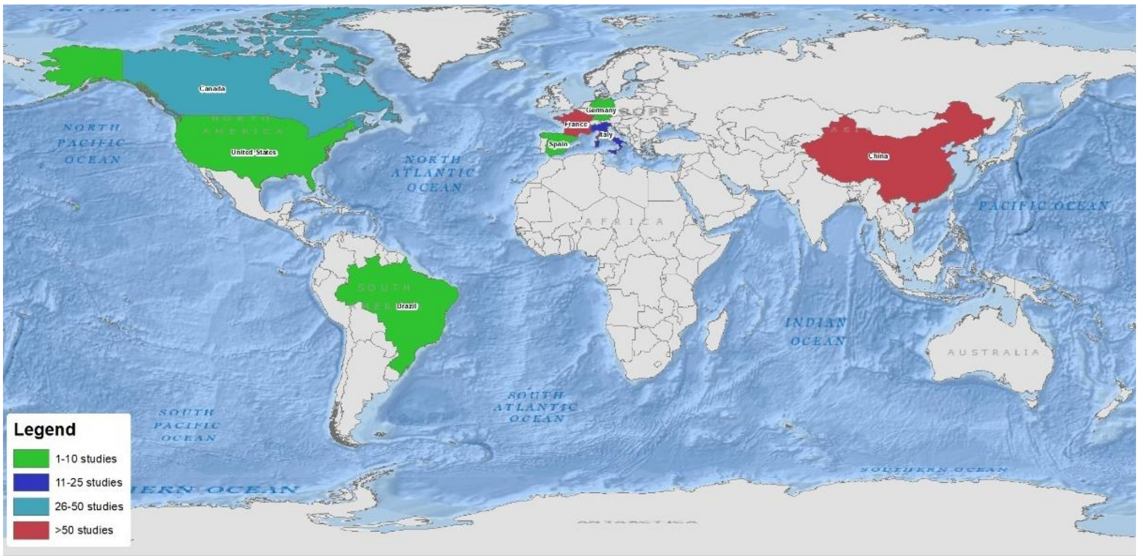
Table 1 - Original publications used in the meta-analysis

Reference	Journal	Number of studies
(Agnelli et al. 2016)	Plant Soil (2016) 400: 297–314	7
(Álvarez et al. 2010)	J Soils Sediments (2010) 10:1236–1245	1
(Álvarez et al. 2011)	J Soils Sediments (2011) 11:221–230	1
(Angst et al. 2016)	Geoderma (2016) 264: 179–187	1
(Bu et al. 2020)	Forests (2020) 11: 461-477	4
(Calvaruso et al. 2011)	Plant Soil (2011) 342:469–480	9
(Chen 2003)	Forest Ecology and Management (2003) 178: 301–310	1
(Chen et al. 2016)	J. Plant Nutr. Soil Sci. (2016) 179: 67–77	4
(Chen et al. 2018)	Soil Biology and Biochemistry (2018) 126: 237–246	12
(Chiellini et al. 2019)	Applied Soil Ecology (2019) 138: 69–79	8
(Cloutier-Hurteau et al. 2007)	Environ. Sci. Technol. (2007) 41: 8104–8110	6
(Cloutier-Hurteau et al. 2010)	J. Environ. Monit. (2010) 12: 1274–1286	2
(Collignon et al. 2012)	Plant Soil (2012) 357:259–274	12
(Collignon et al. 2011)	Plant Soil (2011) 349:355–366	24
(Courchesne et al. 2006)	Environmental Toxicology and Chemistry (2006) 25: 635–642	9
(Dai et al. 2018)	Can. J. For. Res. (2018) 48: 1398–1405	3
(De Feudis et al. 2017)	Geoderma (2017) 302: 6–13	2
(Fang et al. 2017)	PLoS ONE (2017) 12: e0186905	4
(Guan et al. 2016)	Journal of Tropical Forest Science (2016) 28: 159–166	3
(He et al. 2020)	Geoderma (2020) 374: 114424	20
(Hu et al. 2019)	Environ Monit Assess (2019) 191:99-114	1
(Korchagin et al. 2019)	Catena (2019) 175: 132–143	1
(Liu et al. 2018)	Applied Soil Ecology (2018) 132: 91–98	2
(Liu et al. 2019)	Plant Soil (2019) 436: 365–380	2
(Phillips and Yanai 2004)	Water, Air, and Soil Pollution (2004) 159: 339–356	2
(Séguin et al. 2004)	Plant and Soil (2004) 260: 1–17	9
(Turpault et al. 2007)	Geoderma (2007) 137: 490–496	6
(Wang et al. 2016)	J Soils Sediments (2016) 16: 1858–1870	2
(Yin et al. 2014)	Soil Biology & Biochemistry (2014) 78: 213-221	4
(Zhang et al. 2019)	Journal of Soils and Sediments (2019) 19: 2913–2926	4
(Zhao et al. 2015)	J Arid Land (2015) 7: 475–480	2
(Zheng et al. 2016)	Biogeochemistry (2016) 131: 65–76	2

Table 2 – Number of studies by country, tree species and establishment forms

		Number of studies			
Country		**Coniferous**	**Broadleaved**	**Monoculture**	**Regeneration**
Brazil	1	-	1	1	-
Canada	26	4	22	3	23
China/Tibet	46/20	21/14	25/6	32/-	14/20
France	51	27	24	48	3
Germany	1	-	1	1	-
Italy	17	-	17	10	7
Spain	2	-	2	-	2
USA	6	-	6	-	6
TOTAL	**170**	**66**	**104**	**95**	**75**

Figure 1 - Worldwide distribution of the study sites included in this meta-analysis



Sources: Esri, GEBCO, NOAA, National Geographic, Garmin, HERE, Geonames.org, and other contributors; Esri, Garmin, GEBCO, NOAA NGDC, and other contributors

3.3.2 Statistical analysis

The effect size, which is a quantitative index that reflects the magnitude of the association among variables of interest in each study (KONSTANTOPOULOS, 2006), was calculated. Assuming that the methods of the studies and the characteristics of the samples are different and may introduce variability among the true effects, a random-effects model was used as suggested by Viechtbauer (2010). A mixed-effect model analysis was considered based on the assumption of random variation in effect size between studies. Thus, it may account for at least part of heterogeneity in the true effects (VIECHTBAUER, 2010).

To estimate the rhizosphere effect on soil attributes, the effect size for each data point was calculated as the natural log of the response ratio (*ln RR*) as:

$$\ln(R\,R) = \ln\,(X_t/X_c\,)$$

where X_t is the rhizosphere (treatment) mean and X_c is the bulk soil (control) mean. The log transformation was used to balance positive and negative effects and to maintain symmetry in the analysis specially when data presented discrepancies. The RR variance (v) associated with each effect size was calculate by the following equation:

$$v = \frac{SD_t^2}{n_t X_t^2} + \frac{SD_C^2}{n_c\,X_C^2}$$

where SD_t and SD_c are the standard deviations for the treatment and the control groups, respectively; n_t and n_c are the sample sizes for the treatment and the control groups, respectively. Ln (RR) = 0 indicates no rhizosphere effect, ln (RR) > 0 represents positive rhizosphere effect, and ln (RR) < 0 represents negative rhizosphere effect.

The values of the soil attribute estimate, as represented by the effect sizes, were transformed in percentage for better understanding (Appendix I). Negative percentages indicate a decrease in soil attribute numerical value caused by the treatment (rhizosphere) when compared to the control (bulk soil), whereas positive values indicate an increase in rhizosphere attribute levels. If a 95% confidence interval did not overlap with zero, then a significant rhizosphere effect was considered. All analyses were conducted using *metafor* package version 2.4 (VIECHTBAUER, 2010) in RStudio (R CORE TEAM, 2019), and SigmaPlot Version 12.0 was used to create the forest plots.

Correlation analysis (reported using Pearson correlation coefficients, *r*) was carried out to identify the relationships between pH, TOC and *e*CEC in the rhizosphere (treatment) and bulk soil (control) with the climatic factors mean annual precipitation (MAP) and mean annual temperature (MAT). These three soil attributes were chosen due to the greater number of studies (n>40) relatively well distributed among the moderator variables, what was not observed for other attributes.

3.3.3 Heterogeneity and moderator variables

We conducted an overall random effect size meta-analysis, where averages of the effect sizes of all studies were estimated to check whether they were homogeneous or not (Appendix I). Heterogeneity was quantified using I^2, an index that describes the percentage of the total variability over studies and allows to compare meta-analysis of different types and sizes of studies with different outcome data and choices of effect measure (HIGGINS et al., 2003). Percentages of approximately 25, 50 and 75% indicate low, moderate and high heterogeneity, respectively (HIGGINS et al., 2003). When test for heterogeneity was significant, we performed a sensitivity analysis.

Subgroup meta-analysis was performed in order to incorporate moderator variables that could account for at least part of the heterogeneity among effect sizes. Our moderator variables of interest were trees species, i.e. “coniferous” and “broadleaved”, stand establishment forms, i.e. “monoculture” and natural “regeneration” in mixed plantations. However, conclusions were not made on the subgroups that presented only one study in order to avoid misleading statements.

3.3.4 Publication bias and sensitivity analysis

In order to check if the literature review was subject to publication bias, that is significant treatment differences are more likely to be published than non-significant findings, potential publication bias was assessed statistically by the “funnel” function in the *metafor* package, and graphically represented with funnel plots of effect sizes versus their standard errors (Appendices II to IV). In the absence of publication bias, it is assumed that studies with higher precision will be plot near the average, and studies with lower precision will be spread evenly on both sides of the average, creating a funnel shape distribution. Deviation from this shape may indicate publication bias. We also performed the trim and fill analysis, which estimates the number of potentially missing studies from a meta-analysis due to the suppression of the most extreme studies on one side of the funnel plot. It demonstrates how the overall summary effect size would shift if apparent bias would be removed.

Sensitive analysis was performed by assessing both variance and contribution of each study for the overall summary effect size. Studies that presented great variance and low contribution when compared to others in the dataset were removed one at a time and the meta-analysis was performed again. This shows how much heterogeneity and summary effect size change in the absence of the removed studies.

3.4 Results

3.4.1 3.4.1 Particle-size distribution, Al^{3+} and pH

The results of the overall meta-analysis for sand and silt did not show difference between rhizosphere and bulk soil, while clay content decreased 55.51% in rhizosphere (treatment) when compared to bulk (control) (Figure 2a). For the overall meta-analysis, great inconsistences in the risk ratio estimates between the studies were observed for all particle-sizes (I^2=98.95% for sand, 99.71% for silt and 99.76% for clay). These great heterogeneities were not explained by the establishment form moderator variable, whereas the I^2 index remained elevated (>98%) and highly significant ($p \leq 0.01$). Particle-size responses due to tree species moderator variable were not able to be evaluated due to missing studies on coniferous trees.

The contribution of the studies to the summary effect size was also assessed. It was observed that 62.98% of the effect size is explained by 3 out of 19 studies for sand, whereas 4 out of 19 studies accounted for 86.44% of the summary effect size on silt. On the other hand, 54.06% of the effect size is explained by 4 out of 19 the studies for clay.

A reduced number of studies that explain most of effect size is an explanation for the high heterogeneity among them.

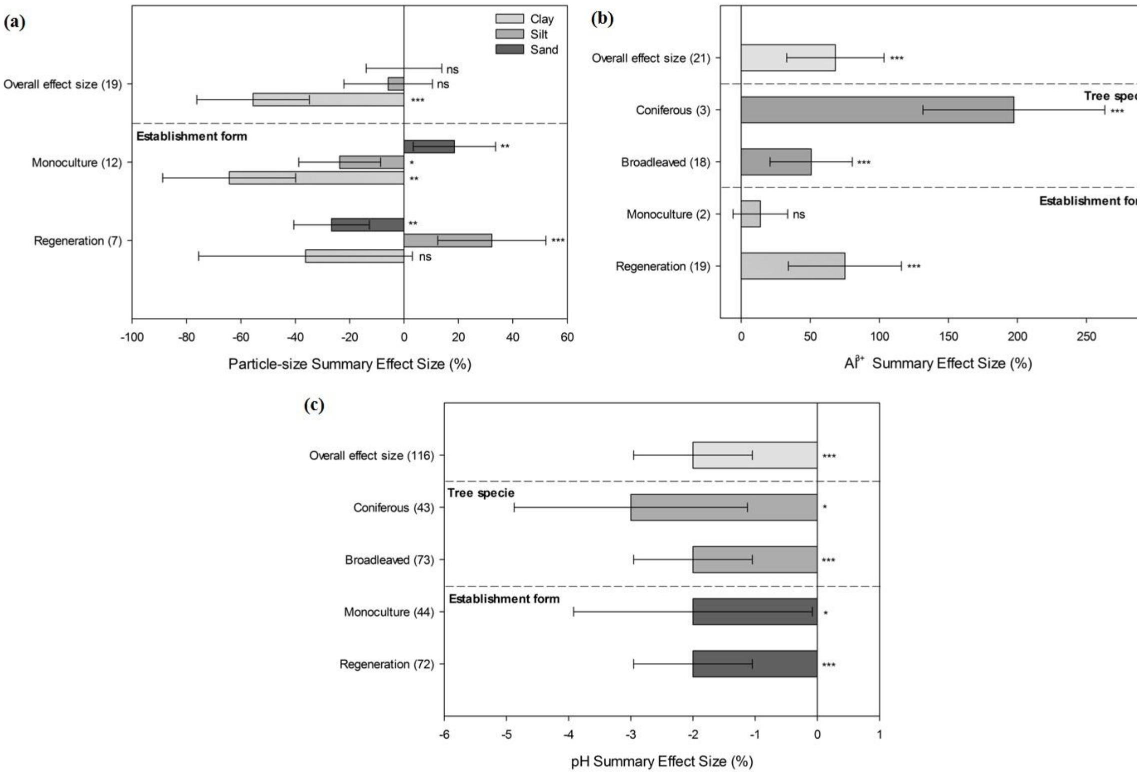
Twenty-two studies were recorded for Al^{3+}, but the sensitivity analysis led us to dispose of one (ÁLVAREZ et al., 2010). Thus, in the overall meta-analysis (n=21), Al^{3+} values increased 68.20% in rhizosphere when compared to bulk (Figure 2b), and in the coniferous rhizosphere Al^{3+} content was 197.43% higher than in the coniferous bulk. Aluminum was 50.68% greater in rhizosphere than in bulk for broadleaved trees. Due to few studies on monoculture (only two), the comparation between Al^{3+} in rhizosphere and bulk soil within establishment form moderator variable could lead to mistakes. The high heterogeneity of the overall meta-analysis (I^2=99.74%; 95% CI: 99.48 to 99.87%; df=20; $p \leq 0.01$) did not change when moderator variables were considered, which means that most of the variability among studies are due to heterogeneity and cannot be fully explained by the moderators. Five out of 22 studies account for 86.30% of the overall summary effect size for Al^{3+}.

In the overall meta-analysis, it was observed that rhizosphere pH decreased 1.98% relative to bulk soil (Figure 2c). The studies on pH (n=116) presented high heterogeneity among them (I^2=99.93%; 95% CI: 99.91 to 99.96%; df=115; $p \leq 0.01$) which could not be explained by both tree species and establishment form moderator variables, since the I^2 index remained higher than 97%. However, when comparing rhizosphere pH with bulk pH inside both moderator groups, coniferous presented the greatest pH reduction (2.96%) in relation to broadleaved (1.98%), while the pH reduction for monoculture and regeneration rhizospheres was the same (1.98%). As this soil attribute has many studies that account for the overall effect size, the contribution of each one is well distributed across the dataset.

Particle-size distribution (sand, silt and clay), Al^{3+} and pH presented publication bias since they are not evenly distributed in both sides of the average and many studies are plotted marginally or out of the funnel shape (Appendix II). In order to balance the meta-analysis, the trim and fill method suggested that for sand, three studies may be added on the left side of the average, adjusting the I^2 to 99.03% and the effect size to -

7.54%. Five studies may be added on the right side of the average for Al^{3+}, adjusting the I^2 to 99.82% and the effect size to 98.40%.

Figure 2 - Rhizosphere summary effect size on particle-size distribution (**a**), Al^{3+} (**b**) and pH (**c**)



Bars represent the response ratio (%) ± 95% confidence interval. Numbers in parentheses indicate the number of studies (n). Significance at * $p \leq 0.1$; ** $p \leq 0.05$; *** $p \leq 0.01$; “ns” not significant

3.4.2 Available P, exchangeable K, exchangeable Ca and effective CEC

The concentration of available phosphorus (P_{av}) was higher in rhizosphere than in bulk soil (18.53%, n=32) (Figure 3a). Although P_{av} in coniferous soils presented higher concentration than in broadleaves, the increase of P_{av} in the rhizosphere did not differ from bulk soil in both coniferous and broadleaved sites. Taking into account the establishment form moderator variable, P_{av} was higher in regeneration rhizosphere (32.31%) than in regeneration bulk, but in the monoculture rhizosphere, the increase in P_{av} (16.18%) did not differ statistically from the monoculture bulk.

The overall meta-analysis for exchangeable potassium (K^+) showed positive rhizosphere effect, with an increase of 69.89% (*n*=18) relative to bulk (Figure 3b). The coniferous rhizosphere increase was higher (80.40%) than the broadleaved rhizosphere increase (69.90%) when compared to the respective surrounding bulk soil. The regeneration rhizosphere effect was even greater (203.44%) than the monoculture rhizosphere (24.61%), even though the increase of K^+ in the monoculture rhizosphere has not been enough to differ from the regeneration bulk soil concentration.

Exchangeable calcium (Ca^{2+}) in the rhizosphere was not significantly different from bulk soil in the overall meta-analysis (Figure 3c). The large confidence interval (between -30.23% and 29.69%) indicates great data dispersion for this soil attribute, which reduces the estimate precision. Non-significant reduction on Ca^{2+} was also observed within the coniferous and regeneration rhizosphere relative to bulk soil, and non-significant increase was found for broadleaved rhizosphere compared to bulk. For monoculture, the significant reduction (15.63%) was not reliable because only one study was recorded.

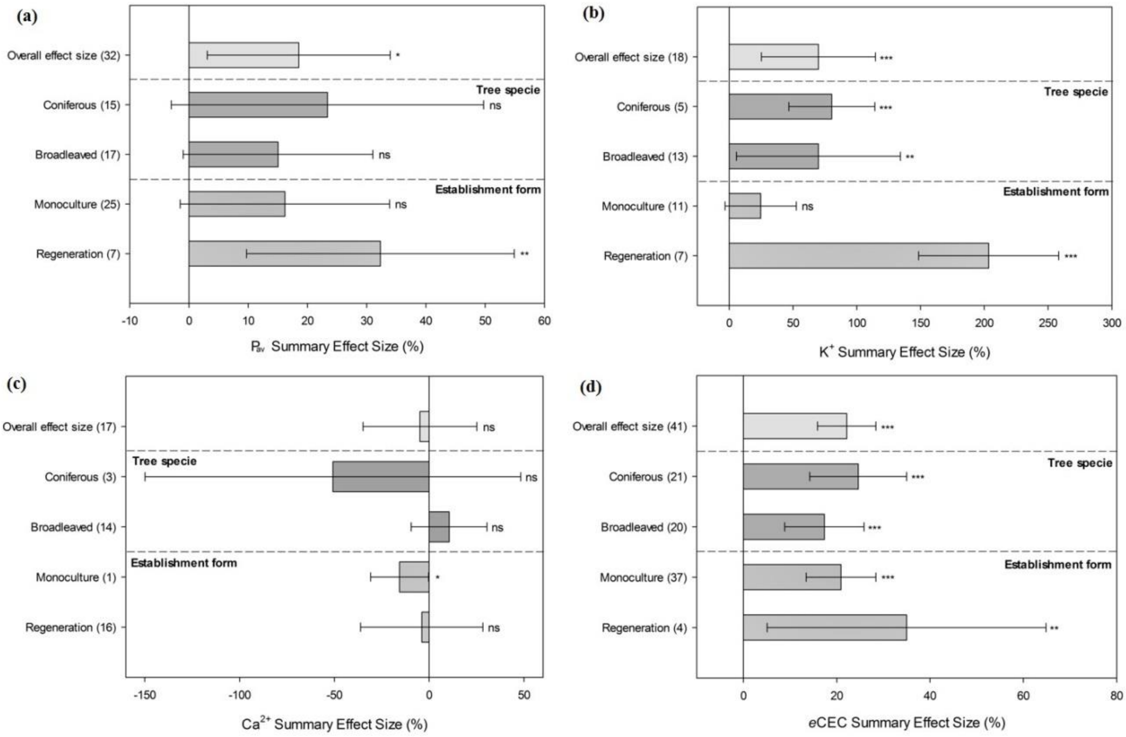
Effective cation exchange capacity (*e*CEC) increased 22.14% (*n*=41) in rhizosphere compared to bulk soil (Figure 3d) considering all recorded studies. Coniferous had a higher *e*CEC (24.61%) in relation to their respective bulk soil than broadleaved (17.35%). Although the regeneration rhizosphere presented greater *e*CEC (34.90%) compared to monoculture bulk, any conclusion on this respect would be premature due to the small number of registers (*n*=4) from only three studies/regions.

High heterogeneity was identified in the overall meta-analysis of all four attributes, as demonstrated by a high I^2 index (93.54% for Pav; 98.92% for K^+; 99.55% for Ca^{2+}, 85.65% for *e*CEC), which may indicate inconsistency across studies. The attempt to explain part of heterogeneity through moderator variables was frustrated for all attributes. However, within P_{av} and K^+ regeneration, the lower heterogeneity is due to all or almost all studies have come from the same publications and conditions. For *e*CEC, the heterogeneity of the regeneration subgroup was reduced to zero probably due to the small number of studies (*n*=4). The same was observed within Ca^{2+} monoculture subgroup (*n*=1).

The publication bias analysis through funnel plots visual interpretation showed that P_{av}, K^+ and Ca^{2+} studies presented publication bias since they are not evenly distributed in both side of the average and more than 40% are plotted marginally or out of the funnel shape. Despite the low number of studies plotted out of the funnel shape for *e*CEC (22%), we also assumed as presence of publication bias (Appendix III). Therefore, the trim and fill method suggested five studies to be added for Ca^{2+}, three for K^+ and seventeen for *e*CEC. For Ca^{2+}, the studies were plotted on the right side of the average, and for K^+ and *e*CEC on the left side of the average. These extra studies adjusted the I^2 and reduced the rhizosphere effect size for these three attributes: for Ca^{2+}, I^2=99.57% and negative effect size -22.79%; for K^+, I^2=98.86% and positive effect size 53.97%; and *e*CTC, I^2=91.24% and positive effect size 9.63%.

The contribution of the studies for P_{av} shows that 5 out of 32 studies accounted for more than 50% of the overall effect size. Three out of 18 studies/registers of K^+ explained 77.02% of the overall effect size, 4 out of 17 studies of Ca^{2+} explained 61.82% of the overall effect size, and for *e*CEC, only one study out of 41 accounted for 76.92% of the overall effect size.

Figure 3 - Rhizosphere summary effect sizes on available P (P_{av}) (**a**), exchangeable K (K^+) (**b**), exchangeable Ca^{2+} (**c**) and effective cation exchange capacity (*e*CEC) (**d**)



Bars represent the response ratio (%) ± 95% confidence interval. Numbers in parentheses indicate the number of studies (*n*). Significance at * $p \leq 0.1$; ** $p \leq 0.05$; *** $p \leq 0.01$; "ns" not significant

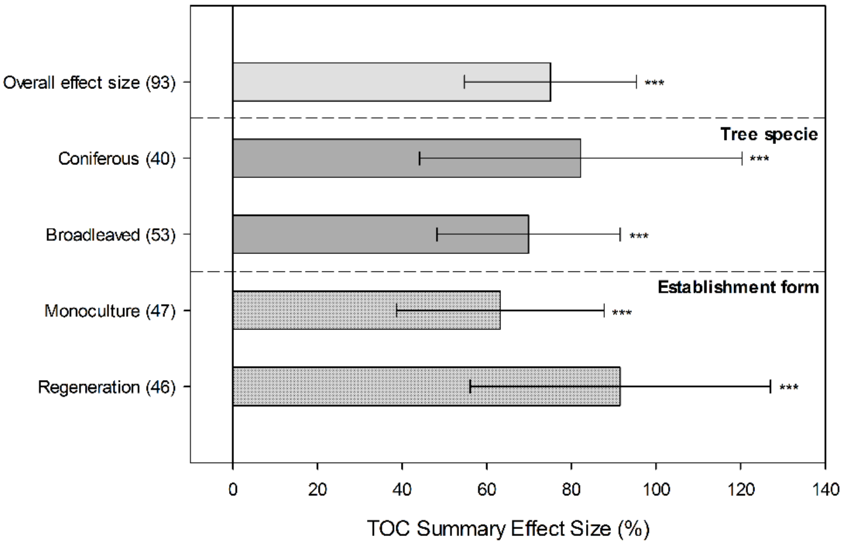
3.4.3 Total organic carbon

Total organic carbon (TOC) was the second attribute most retrieved in our literature review (*n*=96). After examining the overall meta-analysis results for sensitivity, tree studies of Dai et al. (2018) were removed from the TOC dataset due to their high variability and low contribution to the overall summary effect size (0.00049%, 0.00029% and 0.00042%). Thus, considering the overall meta-analysis, TOC significantly increased in the rhizosphere by an average of 75.07% (*n*=93) in relation to bulk soil (Figure 4). The contribution of each study for the overall effect size was not computed since the effect size is uniformly distributed across the dataset.

Analyzing the effect of tree species on TOC, it was observed that in the coniferous rhizosphere, TOC increased 82.21% compared to coniferous bulk soil, which is a higher rate than broadleaved trees rhizosphere (69.89%) when compared to the bulk soil. Furthermore, the rhizosphere effect of the regeneration was greater (91.55%) than monoculture (63.23%).

Heterogeneity across studies was high and statistically significant for TOC (I^2=98.19%), and the moderator variables were not able to explain it since the I^2 index remained high, i.e. there is inconsistency in the risk ratio estimate across studies. Publication bias was observed for TOC (Appendix IV), and trim and fill analysis did not plot any extra studies.

Figure 4 - Rhizosphere summary effect size on total organic carbon (TOC)



Bars represent the response ratio (%) ± 95% confidence interval. Numbers in parentheses indicate the number of studies (*n*). Significance at * $p \leq 0.1$; ** $p \leq 0.05$; *** $p \leq 0.01$; “ns” not significant.

3.4.4 Correlation analysis

pH is significantly correlated with mean annual precipitation (MAP) in both the rhizosphere and the bulk soil (Table 3), but it was not significantly correlated with mean annual temperature (MAT). However, TOC in both rhizosphere and bulk soil was significantly correlated with both MAP and MAT, while *e*CEC does not correlate significantly with neither MAP nor MAT.

Table 3 - Pearson correlation coefficients (*r*) between pH, total organic carbon (TOC), effective cation exchange capacity (*e*CEC) and the climatic factors mean annual precipitation (MAP) and mean annual temperature (MAT)

			MAP	**MAT**	***n***
Total	pH	Rhizosphere	-0.47 **	0.02 ^{ns}	116
		Bulk Soil	-0.36 **	0.02 ^{ns}	
	TOC	Rhizosphere	0.62 **	-0.51 **	93
		Bulk Soil	0.36 **	-0.38 **	
	*e*CEC	Rhizosphere	-0.23 ^{ns}	-0.16 ^{ns}	41
		Bulk Soil	-0.03 ^{ns}	-0.26 ^{ns}	

** Significant at $p \leq 0.05$; "ns" not significant. n = number of studies.

3.5 Discussion

3.5.1 The rhizosphere effect of trees: changes in physical and chemical properties

The particle-size distribution for the overall meta-analysis presented higher and significant reduction on rhizosphere clay content relative to bulk soil, suggesting the effect of plants on weathering of minerals (CAMA; GANOR, 2015; SOKOLOVA, 2015; KORCHAGIN et al., 2019) through the claying process. pH reduction followed by an increase in soluble Al^{3+} and *e*CEC in the rhizosphere compared to the surrounding bulk soil indicates the soil weathering through dissolution mechanisms in the rhizosphere zone (WANG; GÖTTLEIN; BARTONEK, 2001). The higher concentration of K^+ in the rhizosphere compared to bulk suggest the dissolution mechanisms followed by mineral transformation, as there is probably loss of K^+ from illite interlayers, leading to its transformation into vermiculite (BORTOLUZZI et al., 2012).

The high acidity due to an increase in proton activity (H^+) in the rhizosphere is attributed mostly to low-molecular-weight organic acids (LMWOAs) released by microbiota, primarily ectomycorrhizal fungi (SOKOLOVA, 2011), as a mechanism of plant nutrition through the balance charge (WANG; GÖTTLEIN; BARTONEK, 2001). This is also in accordance with the higher significant correlation between pH and MAP in the rhizosphere than in bulk soil (Table 3), and with the higher reduction of clay content followed by an increase in soil acidification and increased exchangeable Al^{3+} in this “hotspot” (WANG; GÖTTLEIN; BARTONEK, 2001). These suggest the action of two mechanisms: (*i*) clay dissolution rate pH dependent on rhizosphere mineral weathering and (*ii*) stoichiometry dissolution, which is pH dependent (CAMA; GANOR, 2015; KORCHAGIN et al., 2019). In response to soil acidification, Al^{3+} is released in the soil to form complex structures as 2:1 mixed-layered minerals with Al-interlayers (VIENNET et al., 2016; KORCHAGIN et al., 2019), and a deeper transformation of micas and illite into expandable mineral is a tendency revealed by Sokolova et al. (2019). Furthermore, the mobilized Al^{3+} may precipitates as poorly crystalline inorganic forms or, preferable, form Al-organic complexes highly stable, preventing plants from its toxic effects (ÁLVAREZ et al., 2010).

Plants colonize and adapt to different environments. To enable this adaptation and favor their growth, they induce modifications in the rhizosphere zone, which are related to the continuous supply of roots exudates, border cells and products from the metabolism of microorganisms (SOKOLOVA, 2015). Among the strategies of plants adaptation is the chemotaxis followed by the association with microorganisms and the increase of organic acids exudation, thus increasing the rate and degree of dissolution of carbonate, silicate and phosphate minerals (LAZO; DYER; ALORRO, 2017; SOKOLOVA et al., 2019), and the mobilization of nutrients (SOKOLOVA, 2015). Mycorrhizal association, for instance, is a strategy of plants roots and fungal hyphae to increase the volume of soil exploitation and mobilization of available P through organic acids release (CLARKSON; HANSON, 1980). The soil acidification affects the pools of base cations and have implications on mineral weathering and forest sustainability (ROSENSTOCK et al., 2019). For example, the Ericaceae strategy to grown in very acid soil is to modify the rhizosphere pH from close to neutral to acid levels with respect to the bulk soil, which may be related to the ability of Ca^{2+} accumulation in the root zone (ÁLVAREZ et al., 2011). In this sense, despite not significant, the reduced levels of Ca^{2+} in the rhizosphere of trees relative to bulk soil may be associated with coniferous and broadleaved adaptation to different environments, suggesting that the low Ca^{2+} availability in soil solution may be due to root charge sites occupied by this element.

Both K^+ and available P increased in rhizosphere compared to bulk soil (69.89 and 18.53%, respectively). Although the dissolution mechanism due to pH may be involved in the availability of these nutrients, the sources may vary. The main mineralogical changes in soils of humid regions is the transformation of micas and illite into chloritized structures and labile minerals (SOKOLOVA, 2011). During the transformation of illite into vermiculite, K is released from the interlayer to the soil, as Bortoluzzi et al. (2012) observed in grape production. This alteration in soil clay mineralogy may occur in very small temporal and spatial scales (TURPAULT; RIGHI; UTÉRANO, 2008). Furthermore, according to Vinichuk et al., (2010), fungal nutritional demands is supposed to regulate P uptake. Apatite is the primary source of soil mineral P due to its relatively high dissolution rate specially in acid medium. However, the organic matter mineralization is an important source of both K (TURPAULT; RIGHI; UTÉRANO, 2008) and P (CHEN, 2003). On sites P deficient, phosphatase activity and the production of organic acids may be enhanced especially in the rhizosphere (CHEN, 2003; FUJII; AOKI; KITAYAMA, 2012; HOFMANN; HEUCK; SPOHN, 2016).

The potential of exchangeable nutrients was improved in the rhizosphere when compared to bulk soil, which is indicated in this meta-analysis by an increase in *e*CEC levels (22.14% higher in rhizosphere relative to bulk). This result confirms that the interaction between roots, soil and microorganism enriches the rhizosphere pool exchangeable sites and ions compared to bulk (HUMMES et al., 2019), independently of the tree species (CALVARUSO; N'DIRA; TURPAULT, 2011). Our results corroborate with Sokolova (2011), who demonstrated the importance of the rhizosphere and, especially, the ectomycorrhizosphere, for the higher CEC due to a more acid reaction compared to the soil beyond the rhizosphere, and with Collignon et al. (2011), which stated that beyond the benefits of the higher biological activity in rhizosphere compared to bulk is an efficient nutrient recycling. However, some species in some sites may have the rhizosphere effect on microbial activity and availability of nutrients reduced by increment in soil fertility (PHILLIPS; FAHEY, 2008).

As related by several studies cited here, higher concentration of TOC was found in the rhizosphere than in bulk soil, and both compartments presented significant correlation of TOC with MAP and MAT, which confirm the influence of these climatic factors on soil C stocks and fluxes. Warming and increased precipitation stimulates the soil respiration and ecosystem photosynthesis, reflecting in increased aboveground biomass and productivity (SULLIVAN et al., 2008; LUO et al., 2009; WU et al., 2011), with larger responses in woody ecosystems (RUSTAD et al., 2001) as consequence of higher microbial activity (SARDANS et al., 2008), enhanced C inputs and net C uptake (LUO et al., 2009; WU et al., 2011), and soil nutrient mineralization (HARTLEY et al., 1999). However, *e*CEC cannot be directly correlated to MAP and MAT since it depends on several cations and each one is related to different soil properties. Furthermore, the ecosystems are less responsive to the interaction of warming and precipitation than expected from single-factors effects (LUO et al., 2008; WU et al., 2011)

3.5.2 The impact of coniferous and broadleaved trees in the rhizosphere effect

Rhizosphere affects the soil solution composition and its effect depends on the specific functioning of different plant species, the related microbial community, and soil mineralogy (MCGAHAN; SOUTHARD; ZASOSKI, 2014; SOKOLOVA et al., 2019). Adjacent soils with the same land-use history, under an uniform tree coverage, may present differentiation of the soil microbial community, especially when exotic species are compared to native ones (KOURTEV; EHRENFELD; HAGGBLOM, 2002), but also when coniferous rhizosphere is compared with broadleaved (SOKOLOVA et al., 2019). But for all tree species, numerous studies demonstrated that Ca, Mn, K, Fe, Mn, Al saturations and proton activity, N, C and base saturation are generally increased in the rhizosphere compared to bulk soil (SÉGUIN; GAGNON; COURCHESNE, 2004; TURPAULT et al., 2005; CALVARUSO; N'DIRA; TURPAULT, 2011; COLLIGNON; RANGER; TURPAULT, 2012).

This meta-analysis shows that the coniferous rhizosphere decreased the pH in a higher percentage (2.96%) than the broadleaved rhizosphere (1.98%). On the other hand, Al^{3+} increased in a greater proportion in the coniferous rhizosphere (197.43%) than in the broadleaved rhizosphere (50.68%). These results suggest that the risk of Al toxicity is higher under coniferous than under broadleaved trees and are in agreement with the higher pH decrease observed under conifers by other studies (FIRN; ERSKINE; LAMB, 2007; COLLIGNON; RANGER; TURPAULT, 2012). In acid soils, the rhizosphere plays an ecological role in Al detoxification by both nutrient accumulation and Al complexation with organic compounds (COLLIGNON; BOUDOT; TURPAULT, 2012). Similar changes in soil acidity due to forest species were observed by Sokolova et al. (2019), who described lower pH values in the rhizosphere and enclosing soil of the conifer Norway spruce (*Picea abies*) than in the rhizosphere and enclosing soil under the broadleaved Norway maple (*Acer platanoides*). Also, in the clay fraction, smaller quantities of mica, kaolinite and illite, which are more labile minerals associated with higher nutrient availability, was found under spruce than under maple. These authors attribute the differences partially due to the woody species and associated microbial community, and partially due to the clay fraction composition.

Along with the higher increase of H^+ activity and Al^{3+} availability in the coniferous than in the broadleaved rhizosphere is the higher P_{av}, K^+, TOC and *e*CEC. All these soil parameters indicate that the impact of conifers on soil is greater than broadleaves (FIRN; ERSKINE; LAMB, 2007; COLLIGNON; RANGER; TURPAULT, 2012; SOKOLOVA et al., 2019). However, as far as the studies are concentrated in temperate zones, further researches in the tropics are needed to find the impact of tree species worldwide.

3.5.3 Changes in rhizosphere properties due to regeneration and monoculture establishment forms

The rhizosphere of naturally regenerated forest soils, where tree species grown spontaneously in a mixed-forest stand, is richer in TOC, K^+, available P_{av} and *e*CEC than the rhizosphere of monocultures when compared to the respective bulk compartment, despite pH decreased at the same intensity (1.98%). These results confirm the positive relationship between soil nutrient availability and the diversity of trees species. Since naturally regenerated woody environments favor the recolonization of species from native flora, the greater diversity of organic materials as leaf litter and roots promote the nutrients availability more similar to natural secondary forest and the maintenance of the original edaphic conditions (FIRN; ERSKINE; LAMB, 2007), with increasing in soil fertility (MALYSZ; OVERBECK, 2018). Our results corroborate with Fan et al. (2019), which found higher content of organic matter and K^+ in secondary forest soils compared to planted forests under the same geological conditions, and with Bu et al. (2020), who stated that soil nutrient availability is promoted by replacing monoculture of Chinese fir for a mixed-species forest.

Forested sites that produce woody biomass are subject to long-term soil acidification due to hydrolysis and cations exportation by the harvest (MCGIVNEY et al., 2019), which impacts the soil chemistry and weathering (ZAHARESCU et al., 2020). The soil weathering spatial scale in forested sites depends on the root density and the microbial community composition, which is greater in old forests relative to regenerating forests (ZAHARESCU et al., 2020). Moreover, planted forests present lower root:shoot ratio relative to natural forests even at the same water availability (WANG; FANG; ZHU, 2008), which explains part of the establishment form effect on rhizosphere properties.

Despite the higher Al^{3+} concentration found in the regeneration forest rhizosphere than in bulk soil when compared to monoculture, the small number of studies on monoculture (only two) did not permit us to accurately infer about this soil attribute on establishment form. The same happens with Ca^{2+}, which presented only one study for monoculture. Thus, inferences considering these elements would lead to misleading conclusions.

The natural succession with tree species depends on their regenerative potential, the distance from seed source and the soil conditions (LEE; HAU; CORLETT, 2005). At stand level, it was facilitated by the small size of the plantations/stands and by the low density of trees (MALYSZ; OVERBECK, 2018). However, the level of germination and recolonization strongly depends on the characteristics of the plantation species (FIRN; ERSKINE; LAMB, 2007) and the combination of nutrients availability (FOLLMER et al., 2021). In order to achieve a low-input and sustainable forest production, a better understanding of the tree species on nutrient availability in soils is crucial. Although monocultures of trees are a major basis for biomass supply, management strategies that aim the transformation of the existing natural woody plant regeneration under plantations into commercial forests is desirable since original soil fertility tend to be preserved (RAMULA et al., 2008; MALYSZ; OVERBECK, 2018). Agronomic and/or breeding approaches that contemplate the manipulation of the rhizosphere environment may enhance the nutrient use efficiency (DOTANIYA; MEENA, 2014).

3.6 Conclusion

Our meta-analysis clearly illustrates the positive rhizosphere effect on nutrient mobilization compared with the surrounding bulk soil, mostly in temperate zone where studies are concentrated. The more acid medium in the vicinity of roots compared to the bulk soil promotes a higher soil weathering followed by mineral dissolution in this compartment, as signalized by clay content decrease and the increase in effective cation exchange capacity and several nutrients essential to plant nutrition. The coniferous rhizosphere effect is greater than broadleaved rhizosphere effect. Contrasting trees naturally regenerated in mixed plantations with monocultures, the soil fertility is higher in the first one which favor the trees establishment and development. The knowledge of rhizosphere effect on soil-plant interaction is critically required for a better understanding of the dynamics of forest systems for biomass production in a low-input perspective, especially in tropical zones where researches on this topic are scarce. A gap of rhizosphere studies in tropical and subtropical regions, which are considered “the new agricultural frontier”, was detected. Tree species selection and current forest management practices must be revisited, as far as replacing monoculture plantations for naturally regenerated trees under canopy and/or mixed-species stands may be consider as an option for multiple purposes forest toward sustainability.

4 CHAPTER II

Higher mineral weathering of a Ferralsol evidenced in the rhizosphere of conifer plantations grown in a subtropical climate

4.1 Abstract

Rhizosphere soil is more sensitive and susceptible to biochemical and physicochemical changes than the surrounding bulk soil. The magnitude and speed of changes in rhizosphere depend on environmental conditions, plant species, their root system architecture and associate microorganism, and soil mineral composition. Thus, the purpose of the present study was to examine the geochemical changes and mineralogical evolution of two soil compartments (Rhizosphere, RZ; and Bulk Soil, BS) in three sites (Natural Araucaria Forest – NAF (control); Reforested Araucaria Forest – RAF; and Reforested Slash Pine Forest – RPF). Soil samples were collected under the canopy of *Araucaria angustifolia* (Bert.) O. Ktze. in both a native forest and a monoculture, and under the fast-growing exotic *Pinus elliottii* (Engelm.) plantation. We evaluated (i) if there was a significant rhizosphere effect in the two monoculture plantations compared to the natural reference site, (ii) if the intensity of the rhizosphere effect was associated to the conifer species, and (iii) if there was evidence of alteration of clay minerals assemblage due to conifers growth compared to MOF. No differences on chemical attributes were found between RAF-RZ and NAF-RZ; however, total organic matter and available phosphorus in RPF-RZ differed from NAF-RZ. Although no differences were observed in silt and clay contents between RZ and BS for all sites, RPF promoted silt decrease followed by clay increase in both compartments, while in RAF the opposite was observed. The diversity of clay mineral assemblage the soil samples was not broad, but variation in the relative proportions of clay minerals was observed, comprising kaolinite, illite and hydroxy-interlayered minerals (HIM), with kaolinite-enrichment in NAF-RZ, RAF-RZ and RPF, and absence of illite in slash pine soil. The proportion of crystalline iron oxides was higher in RPF-RZ suggesting the influence of TOC impact on iron oxides dissolution. This study evidences acceleration of soil weathering by monoculture of conifers, with higher rates in slash pine than araucaria, increasing the risk of soil quality loss when compared with the native Araucaria soil.

Keywords: 1. *Araucaria angustifolia*. 2. *Pinus elliottii*. 3. X-ray diffraction. 4. Ferralsols.

4.2 Introduction

Anthropic activities modify soil properties, among them the clay minerals assemblage and their functions (soil organic matter, water retention, nutrient supply, physical stability), on a time scale ranging from few months to hundreds of years, depending on the environment where the processes occur in soil (CORNU et al., 2012). In the rhizosphere, these changes can be very fast (HOUBEN; SONNET, 2012; HINSINGER; JAILLARD, 1993), affecting the soil over pedogenic time (CALVARUSO et al., 2009).

Firstly defined by Hiltner, in 1904, as the volume of soil surrounding the roots of living plant that is influenced by their activity (HINSINGER, 1998; HINSINGER; PLASSARD; JAILLARD, 2006), the rhizosphere is not a region of definable shape and size due to the complexity and diversity of plants root systems. It consists in a gradient of physical, chemical and biological properties that change along the roots (MCNEAR JR., 2013) and where processes take place in a higher rate than in bulk soil (KUZYAKOV; BLAGODATSKAYA, 2015). The rhizosphere plays an important role on biogeochemical cycles (TOBERMAN; CHEN; XU, 2011; HE et al., 2020), as the processes strongly influence the soil stock of carbon and nutrient budget (TURPAULT; GOBRAN; BONNAUD, 2007) and can affect pedogenesis (CALVARUSO et al., 2009), no matter the period of contact between the mineral and the active portion of roots (MCGAHAN; SOUTHARD; ZASOSKI, 2014).

The impact of root-soil contact time on mineral weathering and nutrient release in pots was investigated by many authors. Naderizadeh, Khademi and Arocena (2010) studied the transformation of phlogopite and muscovite in the rhizosphere of alfalfa (*Medicago sativa* L.) under influence of added organic matter (OM). They observed an increase of acidity, K release and mineral transformation due to OM addition with dependence on the type of mineral. Houben and Sonnet (2012) observed the weathering of smithsonite, a Zn mineral, as consequence of dissolved organic carbon produced by the rhizosphere activity of ryegrass (*Lolium multiflorum* Lam). The effect of a monocot (fescue, *Vulpia myuros*) and a dicot (tomato, *Solanum lycopersicum*) on mineral stability and soil solution composition was investigated by Mcgahan, Southard and Zasoski (2014). They demonstrated a greater influence of the dicot then the monocot rhizosphere, with rhizosphere solution extracts farther from equilibrium than bulk soil solution extracts. These studies were conducted on annual plants and in laboratory, where the conditions are very different from those existing in natural ecosystems since the environment is controlled, resources are limited, and the surface of contact between roots and minerals is larger, which maximizes the impact on mineral weathering.

Several i*n situ* studies on rhizosphere of trees to understand the ecosystem dynamics in temperate climate regions have been conducted, while in subtropical and tropical regions they are scarce, especially for conifers. Turpault, Gobran and Bonnaud. (2007) assessed the differences and the evolution of chemical properties for bulk soil, rhizosphere and rhizosphere interface in a *Pseudotsuga menziesii* (Mirb.) Franco ecosystem and found significant increase in the measured variables (organic matter, cation exchange capacity and exchanged base cations) in the same order (bulk < rhizosphere < rhizosphere interface) in months with higher temperature and biological activity. Calvaruso, N'dira and Turpault (2011) compared physicochemical properties of rhizosphere and bulk soil of five tree species in Breuil-Chenue forest. The soil phosphorus (P) supply due to rhizosphere organic P of two tree species (*Populus simonii* and *Pinus sylvestris*) in semiarid forests of Northeast China was investigated by Zhao et al. (2015), who detected variations in rhizosphere recalcitrant organic P due to tree species. Temporal variations in phosphatase activity and P fractions in bulk soil and rhizosphere of *Larix olgensis* plantations with different developmental stages (16-, 23-, 34- and 49-y-old stands) were examined by Chen, Zhang and Duan (2016). The study indicated that the species development affects P bioavailability through changes in P fractions dynamic and phosphatase activities. Studying the mixture effect of Chinese fir (*Cunninghamia lanceolate*) with broadleaved species, Bu et al. (2020) showed more rhizosphere P availability in mix plantations of the species.

The greater changes that proceed in the rhizosphere are generally related to the strategies of nutrient acquisition by plants, among them the permanent exudation of acid organic compounds (RONDINA et al., 2019) accompanied by pH decrease promoted by

H^+ released for balance charge (HINSINGER et al., 2001; CALVARUSO et al., 2009). Also, most of weathering processes occur due to reaction of minerals in aqueous solution (CHURCHMAN; LOWE, 2012), where the dissolved CO_2 originated from biota respiration and atmospheric inputs reacts with water, forms carbonic acid (H_2CO_3) and releases H^+ into soil solution (CHURCHMAN; LOWE, 2012). However, these processes depend on the environmental conditions, type of plants and associated microorganisms, and on the soil mineralogy (MCGAHAN; SOUTHARD; ZASOSKI, 2014; UROZ et al., 2016; RONDINA et al., 2019), and lead to weathering through dissolution of minerals that is intensified in the rhizosphere. Cation uptake by plants in exchange for H^+ also results in an increase of Al solubility and control the soil pH (LI; JOHNSON, 2016). The mineral dissolution is identified by increases of Al^{3+} released into soil solution (WANG; GÖTTLEIN; BARTONEK, 2001; VIENNET et al., 2016; KORCHAGIN et al., 2019), with transformation of illite into vermiculite due to loss of K^+ from the interlayers (BORTOLUZZI et al., 2012), retention of the Al^{3+} in the interlayers of 2:1 clay minerals (KORCHAGIN et al., 2019), and transformation of hydroxy interlayered 2:1 clay minerals into smectite and vermiculite by depletion of Al-interlayers due to its complexation by low molecular weight organic acids in soil solution (BRAHY; TITEUX; DELVAUX, 2000; INDA et al., 2010; FINK et al., 2014)

Soils in the highlands of Southern Brazil, which are predominantly developed under basaltic and rhyo-dacitic rocks, among them the highly weathered Ferralsols, support the Mixed Ombrophylous Forest, a formation of the Atlantic Forest biome, also known as Araucaria Forest. It consists in an ecosystem of social, cultural and economic relevance that shelters the long-lived pioneer natural conifer *Araucaria angustifolia* (Bertoloni) Otto Kuntze (1898). At the beginning of the 20th century, the native forest biome was destroyed and the landscape converted into agro-ecossystems, among them the plantation fast-growing and low-density exotic wood species such as *Pinus* spp. and *Eucalyptus* spp. that started in the 1970s, has caused the reduction of the araucaria population in over 80% (CNCFlora, 2012). Despite the broad diversity of local flora and fauna supported by the Araucaria Forest (FONSECA et al., 2009; CARVALHO; MOREIRA; CARDOSO, 2012), the fragmentation and habitat reduction have led to loss of biodiversity (HARTLEY, 2002; FONSECA et al., 2009; SILVA; SCHMITT, 2015) and to disruption on soil ecosystem (ISLAM; WEIL, 2000), leading the araucaria to figure on The IUCN Red List of Threatened Species^{TM} as ‘critically endangered’ (THOMAS, 2013) and drawing attention to the need of assessing the impacts on soil (ISLAM; WEIL, 2000).

In a forest ecosystem, the deeper subsoil is an important source of plant nutrition (DAWSON; HAHM; CRUTCHFIELD-PETERS, 2020) since deep fine roots uptake nutrients and water from up to twelve meters deep (GERMON et al., 2020). In this ecosystem, the roots generally represent 13% to 28% of the plant biomass (CAIRNS et al., 1997), and in planted forests, the root:shoot ratio is lower than in natural forests, even at the same water availability (WANG; FANG; ZHU, 2008). The conifer *Pinus elliottii* var. *elliottii* (slash pine) grown from seeds has well-defined tap root with lateral roots developed at 0 to 15 m deep, with fifty percent of the total root surface area concentrated within the first 30 cm of soil, and the remaining fifty percent equally distributed between 30 and 120 cm in soil profile (SCHULTZ, 1972). Furthermore, roots smaller than 2 mm in diameter, which are the most active ones, represent half of the total root surface in this conifer. The conifer *Araucaria angustifolia* (areaucaria) has also a well-developed tap root system (KORNDÖRFER; MÓSENA; DILLENBURG, 2008), but less laterally spread. The root system distribution define how soil resources are explored to support the forest.

The alteration of chemical, physical and biological properties around living roots resulting from their activity and associated microorganisms was defined by Hinsinger et al. (2009) as the “rhizosphere effect”. Although the impacts of the rhizosphere of conifers on soil properties are well documented in the literature (CHEN, 2003; SÉGUIN; GAGNON; COURCHESNE, 2004; COURCHESNE; KRUYTS; LEGRAND, 2006; TURPAULT; GOBRAN; BONNAUD, 2007; CALVARUSO; N’DIRA; TURPAULT, 2011; COLLIGNON et al., 2011; COLLIGNON; BOUDOT; TURPAULT, 2012; ZHAO et al., 2015; GUAN; WANG; ZHANG, 2016; FANG et al., 2017; CHEN et al., 2018; DAI et al., 2018; BU et al., 2020; HE et al., 2020), studies that examine the rhizosphere effect in humid subtropical regions, especially in araucaria plantations, on soil mineralogical changes were not found. Our hypothesis was that, even in highly weathered soils occurring in subtropical regions, exotic fast-growing woody species produce greater rhizosphere effect than native slow-growing species. For this, we evaluated (*i*) if there was a significant rhizosphere effect in the two monoculture plantations compared to the natural reference site, (*ii*) if the intensity of the rhizosphere effect was associated to the conifer species, and (*iii*) if there was evidence of alteration of clay minerals assemblage due to conifers growth. Our approach was the evaluation of physical, chemical and mineralogical properties of the rhizosphere and bulk soil of araucaria, an endangered native long-lived pioneer conifer, and of slash pine, an exotic fast-growing and low-density conifer, grown in a basalt-derived soil within the Mixed Ombrophilous Forest domain.

4.3 Material and methods

4.3.1 Study area

The study area is located at Passo Fundo National Forest (FLONA-PF), Southern Brazil (52°11’12” W, 28°16’47” S) (Figure 1). A secondary native Araucaria Forest (NAF) fragment over 100 years old, a 64-years-old reforested *Araucaria angustifolia* (RAF), and a 50-years old reforested *Pinus elliottii* var. *elliottii* (RPF), were selected. In order to avoid variations of climate, topography and soil type, the samples are located within a radius of 100 meters from the stands’ intersection. The altitude is approximately 700 m above mean sea level. The climate is subtropical humid, classified as Cfa according to Köppen, with average annual temperature of 17°C, average annual rainfall of 1,850 mm, and relative humidity of 72% (ICMBio, 2011). The soil is a Ferralsol (IUSS WORKING GROUP WRB, 2015), also known as Latossolo Vermelho Escuro (Brazilian Classification System), with dominance of kaolinite and oxides, formed predominantly under volcanic basaltic flood. The upper stratum of the natural forest fragments is dominated by araucaria and are in good conservation state in terms of species composition and richness (MALYSZ, 2010). In the lower stratum, or regeneration, individuals of the Myrtaceae family are predominant, followed by Fabaceae, Lauraceae and Euphorbiaceae. Previously to monocultures, intensive agriculture used to be practiced with annual crops and cattle grassing, but details of the land-use history are unknown.

At FLONA-PF, the stands are small (10 – 60 ha), which provides a close contact with adjacent habitats and seed banks. The rotation period of conifer monocultures managed for sustainable multiple use is much longer (> 40 years) than the traditionally adopted by economically-driven regional timber companies (10–18 years). Formerly, the implantation of forest stands was not low impact since both araucaria and slash pine plantations followed soil preparation through conventional tillage with burning. Cultural traits, such as weeding, mowing and crowning, were performed during the next four years. Liming and fertilization practices were not found in the records.

The RAF was established in 1958 in 25.1 ha, with initial density of 5,000 trees ha^{-1}. Selective timber harvests were conducted in 1972/73, 1979 and 1992, and the latest inventory of the timber stock (2011) showed 411.8 m^{3} ha^{-1} and 317 trees ha^{-1}. The estimated mean diameter at breast height (*dbh*) was 36.96 cm, and 19.59 m of total high (*ht*). The RPF was established in 1972 in 17.28 ha, with 2,500 trees ha^{-1}. Nine selective timber harvesting were performed from 1976 until 2000, and according to the latest inventory (2011), the remaining wood stock was 748.1 m3 ha^{-1} with 483 trees ha^{-1}. The *dbh* was 34.73 cm and ht 31.87 m (ICMBio, 2011).

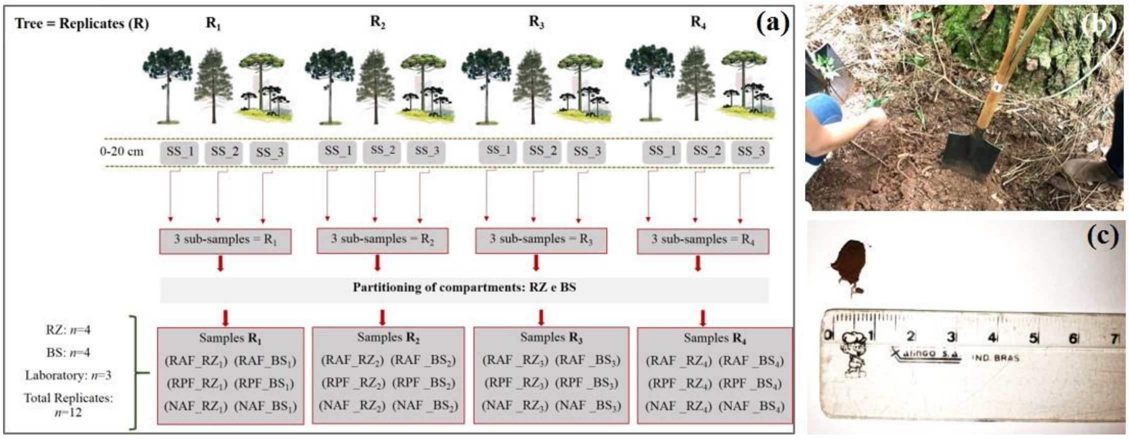
Figure 1 - Study site location

4.3.2 Soil sampling and preparation

In August and September, 2019, four trees with the average population diameter were randomly selected (~37 cm *dbh* for RAF; ~35 cm *dbh* for RPF. The selected native araucaria specimens in the NAF were the remnants in the secondary forest fragment. Three soil subsamples were collected per tree, homogenized to make one composite sample, for a total of 12 samples (Figure 2a). The samples were collected with a cutting blade, under the canopy of trees after litter removal, 10 to 30 cm far from the trunks of trees, at 0-20 cm deep (Figure 2b). The blocks of soil containing roots were stored in plastic bags at 4°C for up to 48 hours, and separated in two aliquots according to Turpault; Gobran and Bonnaud (2007): rhizosphere (RZ) (Figure 2c) and bulk soil (BS), giving 24 experimental units per site.

Soil aggregates with diameter between 1-2cm were separated for determination of bulk density. The disturbed soil samples were air-dried and sieved (<2 mm) to obtain air-dried fine earth (ADFE) which were used for physical analysis. A portion of ADFE samples was manually grounded in an agate grail, passed through a 0.2 mm sieve and reserved for chemical and X-ray fluorescence (FRX) analysis.

Figure 2 - Sampling design (**a**); field soil sampling (**b**) rhizosphere compartment surrounding fine roots (**c**)



4.3.3 Chemical and physical analysis

Soil pH was measured in H_2O (1:2.5 (*w/v*) soil/solution ratio) (TEIXEIRA et al., 2017). Potential acidity (H^+ + Al^{3+}) was determined using the Santa Maria buffer solution (TSM) as an alternative to SMP buffer (TOLEDO, 2011). Total organic carbon (TOC) was determined by wet combustion (YEOMANS; BREMNER, 1988). Available phosphorus (P) and exchangeable potassium (K^+) were extracted by Melich-1 acid solution (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}) and measured using a UV-Vis spectrophotometer at 660 nm and a flame photometer, respectively. The exchangeable cations (calcium: Ca^{2+}; magnesium: Mg^{2+}; and aluminum: Al^{3+}) were extracted in KCl 1 mol L^{-1} and determined by inductively coupled plasma – optical emission spectrometry (ICP-OES). The total cation-exchange capacity (CEC: $K^+ + Mg^{2+} + Ca^{2+} + [H^+ + Al^{3+}]$) and base saturation (%) = ($[K^+ + Mg^{2+} + Ca^{2+}] * 100$)/CEC) were estimated.

Twenty grams of soil were treated with hydrogen peroxide (5% H_2O_2 *v/v*) and temperature (40 °C) to oxidize the organic matter. Particle-size distribution in soil free of organic matter was performed by the pipette method (GEE; BAUDER, 1986). After dispersion with solution of sodium hexametaphosphate, the sand fraction (0.05-2.0 mm) was obtained by sieving, and silt (0.002-0.05 mm) and clay (<0.002 mm) by sedimentation in water. The aliquots of suspended clay were reserved for further analysis. Bulk density (BD) was estimated using the clod method by immerging in water clods coated with paraffin (a water-repellent substance), and the soil particle density (SPD) followed the volumetric flask method (TEIXEIRA et al., 2017). Both BD and SPD make use of the Archimedes' principle. Total soil porosity (TSP) was estimated by the relation between BD and SPD (TEIXEIRA et al., 2017). The samples were assessed in triplicate given a total of 72 samples (n=12).

The abundance of oxides secondary solid phases was estimated in the bulk and rhizosphere clay-sized fraction. An aliquot of suspended clay was lyophilized and reserved for determination of pedogenic and poorly crystalline iron (Fe) oxides and poorly crystalline aluminum (Al) forms by selective chemical dissolutions. Sodium dithionite-citrate-bicarbonate (DCB), identified by subscript "d", was used to extract the combined amount of pedogenic iron oxides (Fe_d), poorly + crystalline forms (MEHRA; JACKSON, 1960). Extraction using acid ammonium oxalate (AAO) at pH 3, identified by subscript "o", was performed to determine low crystalline iron (Fe_o) and aluminum (Al_o) oxides (MCKEAGUE; DAY, 1966). All dissolved Fe and Al forms were determined by inductively coupled plasma – optical emission spectrometry (ICP-OES). Fe_o/Fe_d was calculated to estimate the proportion of poorly-crystallized iron oxides forms related to total iron oxides forms.

4.3.4 Total elemental composition analysis

The total elemental analysis of rhizosphere and bulk soil were performed by energy-dispersive X-ray fluorescence (EDXRF) spectrometry using an Epsilon 3 EDXRF (PANalytical) system. Grounded ADFE (< 0.2 mm; n = 8) was pressed with boric acid (1:7 ratio) into tablets at 25-ton cm-^{2} pressure. Elemental screening with OMNIAN application was performed in 900 seconds per sample using standard reference material for calibration. The total chemical composition was determined and expressed in oxides, and the target elements Al, Si, Fe, Mn, P and K were converted into g kg^{-1}.

4.3.5 Soil clay mineral characterization

The mineralogical composition of the clay-sized fraction (<2 µm) free of organic matter was determined in oriented preparations. Aliquots were Ca-saturated ($CaCl_2$ 0.5 mol L^{-1}) or K-saturated (KCl 1 mol L^{-1}) and then washed to eliminate the excess of salts (BORTOLUZZI; POLETO, 2013). Oriented preparations were obtained by depositing the saturated clay suspensions onto glass slides which were dried at room temperature (air-dried: AD). Ca-saturated slides were also saturated with ethylene glycol (Ca-EG) vapor (inside desiccator) for 24h. K-saturated slides were heated at 150°C (K-150), 350°C (K-350) and 550°C (K-550). XRD patterns of oriented preparations were obtained on a Bruker D8 Advance diffractometer (Cu Kα radiation, $\lambda = 1.5418$ Å), generated at 50 kV and 60 mA.) from 3 to 35^{o} 2θ with steps of 0.02^{o} 2θ and dwelling time of 0.5 s.

The analysis of the clay mineral composition range from 4^{o} 2θ to 14^{o} 2θ, since the higher positions (> 14^{o} 2θ) express the oxy-hydroxy minerals behavior and the broad peaks produced by them reassemble those of the clay minerals, which make it difficult to detect exact peak positions (MOORE; REYNOLDS, 1997) and to obtain an accurate identification.

Air-dried Ca-saturated XRD patterns were decomposed into elementary peaks according to the method proposed by LANSON (1997) using the software DecompXR over 4 to 14^{o} 2θ range. According to the author, the decomposition procedure, also known as modeling, is useful to identify the phases present in a sample and to describe trends. The parameters of the elementary curves (peak position and intensity) were used to identify the clay mineral species according to Brindley and Brown (1980) recommendations.

4.3.6 Statistical analysis

The data were examined for homogeneity of variances by Levene's test, and for normality by Lilliefors' (Kolmogorov-Smirnov) test. If necessary, data were transformed using the Box-Cox function. Outliers were verified through box-plot and eliminated when present in the dataset. The data was subjected to a nested Analysis of Variance (ANOVA) and to Tukey's test ($p \leq 0.05$) using a Linear Mixed Model (LMM) for comparison between sites. Principal Component Analysis (PCA), a multivariate statistic used to explain most of the variance in the dataset while reducing the number of variables to a few uncorrelated components, was applied to the whole set of data. The variables that presented little contribution in explaining the data variability were removed from dataset, and PCA was re-run for grouping according to soil chemical and physical attributes of the rhizosphere. All statistical analyses were performed using RStudio software (R CORE TEAM, 2019) and the graphs were created in both RStudio and SigmaPlot Version 12.0.

4.4 Results

4.4.1 Soil chemical and physical characteristics

The major chemical and physical properties of both rhizosphere and bulk soil in the three sites are presented in table 1. RAF presented the greatest rhizosphere effect, followed by NAF and RPF. Among the assessed soil attributes, 19 out of 27 differed significantly between RAF-RZ and RAF-BS, 8 out of 27 between NAF-RZ and NAF-BS, and 6 out of 27 between RPF-RZ and RPF-BS.

The soil is strongly acidic with pH (H_2O) ranging from 4.0 to 4.3, which is attributed to the very advanced stage of weathering and to the large amount of organic matter (3.5 – 4.5 g org. C kg^{-1}). The exchangeable cations Ca^{2+}, Mg^{2+} and K^+ are very low, while Al^{3+} is high due to the acidic pH, which is typical of high weathered soils. Total CEC ranged from 90.0 to 93.1 mmolc kg^{-1} for slash pine, and from 98.0 to 127.5

mmolc kg^{-1} for araucaria, with the highest values found in the rhizosphere. CEC is related to the organic carbon content and the presence of 2:1 clay-minerals.

When comparing the chemical attributes in the rhizosphere of reforested araucaria (RAF-RZ) and native araucaria (NAF-RZ, control), no differences were detected (Table 1). However, the attributes TOC and available P in RPF-RZ differed from NAF. The RPF-RZ presented lower TOC (3.62 g kg^{-1}) than NAF-RZ (4.48 g kg^{-1}), but it was not different from RAF-RZ (4.14 g kg^{-1}). Available P was extremely low in all sites and compartments (1.52 to 3.97 mg kg^{-1}) (Table 1), whereas total P (P_t) ranged from 462 to 674 mg kg^{-1} (Table 2). Available P and P_t were lower in RPF-RZ (1.52 mg kg^{-1} and 462 mg kg^{-1}) and differed from both NAF-RZ (3.97 mg kg^{-1} and 674 mg kg^{-1}) and RAF-RZ (3.72 mg kg^{-1} and 556 mg kg^{-1}). Total CEC did not differ between RAF-RZ and RPF-RZ; however, CEC of RPF-RZ was lower (93.1 $mmol_c$ kg^{-1}) and differed from RAF-RZ (127.5 $mmol_c$ kg^{-1}). pH, Al^{3+}, $H^+ + Al^{3+}$, Ca^{2+}, Mg^{2+} availability and cation saturation values did not present differences when RAF-RZ and RPF-RZ were compared to NAF-RZ, and when RAF-RZ is compared to RPF-RZ.

The proportion of clay and silt in the rhizosphere did not differ from the proportions in bulk soil for NAF-RZ and RAF-RZ or RPF-RZ (Figure 3). However, in RAF and RPF it was detected a decrease of clay and silt content, respectively, related to NAF-RZ. BD, SPD and TSP did not show difference when comparing NAF-RZ with RAF-RZ and RPF-RZ (Table 1). Nevetheless, differences were found only for SPD in RAF, which was lower in RZ (2.45 g cm^{-1}) compared to BS (2.63 g cm^{-1}), and for BD and TSP in RPF. BD was lower in RZ (1.29 g cm^{-1}) compared to BS (1.38 g cm^{-1}), and TSP was higher in RZ (0.48 m^3 m^{-3}) related to BS (0.45 m^3 m^{-3}).

4.4.2 Iron and aluminum forms in the clay fraction and total elemental content in soil

The amount of iron extracted by dithionite-citrate-bicarbonate (Fe_d) and of iron and aluminum extracted by acid ammonium oxalate (Fe_o and Al_o) are presented in table 2. We did not find rhizosphere effect in NAF nor in RAF. However, in RAF the rhizosphere presented higher Fe_o and Al_o contents than in BS probably due to the restrictive impact of organic matter on iron oxide crystallinity. Fe_o/Fe_d ratio, which gives the proportion of poorly-crystallized iron oxides within pedogenic iron, was less than 0.10, suggesting the prevalence of crystalline forms (hematite, goethite and maghemite). These results are in accordance with total Fe and Al (Fe_t and Al_t) measured by XRF (Table 2), whose values did not differ between RZ and BS for NAF and RPF, only for RAF, which presented higher amount in RZ than in BS. A depletion of total silicon (Si_t) content was observed in RAF-RZ compared to RAF-BS, but without difference between the compartments for NAF and RPF.

To evaluate the species effect on soil, RAF-RZ and RPF-RZ were compared to the reference site (NAF-RZ). Poorly-crystallized Al-oxides (Al_o) did not differ among RAF-RZ and RPF-RZ and the reference site NAF-RZ. Pedogenic iron (Fe_d) was lower in RAF-RZ, while low crystalline iron (Fe_o) was lower in RPF-RZ, causing lower Fe_o/Fe_d ratio in RPF-RZ compared to rhizosphere of the reference NAF-RZ. Sit was higher in RAF-RZ, oppositely to Al_t and Fe_t, whose levels in RAF-RZ were lower than in NAF-RZ and RPF-RZ. Si_t and Al_t in RPF-RZ did not differ from the control, only Fe_t was higher in RPF-RZ than in the control site.

Phosphorus (P) and potassium (K), two major elements for plant nutrition, presented depleted total content in RPF-RZ compared to RAF-RZ (Table 2). Total P (P_t) content was 462, 556 and to 674 mg kg^{-1} in RPF-RZ, RAF-RZ and NAF-RZ, respectively. Total K (K_t) was also lower in RPF-RZ (2.039 mg kg^{-1}), differing from both NAF-RZ and RAF-RZ (2.247 and 2.428 mg kg^{-1}, respectively).

Table 1 - Chemical and physical soil properties for rhizosphere (RZ) and bulk soil (BS) under native araucaria forest (NAF), reforested *Araucaria angustifolia* (RAF), and reforested *Pinus elliottii* (RPF). Values are means ± confidence interval in parentheses (*n* = 12)

Soil properties	No obs.	NAF				RAF				RPF			
		RZ		BS		RZ		BS		RZ		BS	
Chemical													
pH (H_2O)	66	**4.09 (± 0.13)**	aA	4.14 (± 0.13)	a	**4.03 (± 0.15)**	bA	4.10 (± 0.15)	a	**4.26 (± 0.13)**	aA	4.27 (± 0.13)	a
Al^{3+} (mmol$_c$ kg^{-1})	72	**13.9 (± 8.46)**	aA	13.1 (± 8.48)	a	**15.0 (± 8.46)**	aA	14.3 (± 8.48)	a	**19.9 (± 8.49)**	aA	21.1 (± 8.49)	a
H^++Al^{3+} (mmol$_c$ kg^{-1})	69	**111.2 (± 22.75)**	aA	88.8 (± 22.45)	b	**111.8 (± 23.05)**	aA	89.1 (± 22.80)	b	**81.6 (± 22.60)**	aA	81.0 (± 22.85)	a
TOC (g kg^{-1})	72	**4.48 (± 0.34)**	aA	3.91 (± 0.34)	b	**4.14 (± 0.34)**	aAB	3.49 (± 0.34)	b	**3.62 (± 0.34)**	aB	3.65 (± 0.34)	a
K^+ (mmol$_c$ kg^{-1})	72	**3.37 (± 0.82)**	aA	2.46 (± 0.83)	b	**2.88 (± 0.82)**	aA	1.33 (± 0.82)	b	**2.29 (± 0.82)**	aA	1.34 (± 0.82)	b
Ca^{2+} (mmol$_c$ kg^{-1})	70	**1.85 (± 0.84)**	aA	1.77 (± 0.84)	a	**1.75 (± 0.84)**	aA	1.54 (± 0.84)	b	**1.78 (± 0.84)**	aA	1.6 (± 0.84)	a
Mg^{2+} (mmol$_c$ kg^{-1})	70	**5.97 (± 3.92)**	aA	4.99 (± 3.92)	a	**8.12 (± 3.92)**	aA	6.33 (± 3.93)	b	**7.46 (± 3.92)**	aA	6.06 (± 3.92)	b
Available P (mg kg^{-1})	72	**3.97 (± 0.67)**	aA	3.47 (± 0.66)	a	**3.72 (± 0.67)**	aA	2.09 (± 0.66)	b	**1.52 (± 0.66)**	aB	1.53 (± 0.66)	a
CEC (mmol$_c$ kg^{-1})	70	**122.4 (± 19.45)**	aAB	98.0 (± 19.15)	b	**127.5 (± 19.45)**	aA	99.2 (± 19.50)	b	**93.1 (± 19.10)**	aB	90.0 (± 19.15)	a
Base Saturation (%)	71	**9.15 (± 7.53)**	aA	9.59 (± 7.51)	a	**11.70 (± 7.52)**	aA	10.74 (± 7.54)	b	**12.76 (± 7.53)**	aA	10.30 (± 7.51)	b
Physical													
Sand (%)	72	**9.79 (± 0.82)**	aB	9.55 (± 0.83)	b	**12.43 (± 0.79)**	aA	12.35 (± 0.84)	a	**10.22 (± 0.80)**	bB	10.50 (± 0.81)	a
Silt (%)	71	**31.1 (± 1.25)**	aA	30.9 (± 1.2)	a	**31.8 (± 1.2)**	aA	32.6 (± 1.2)	a	**28.1 (± 1.25)**	aB	27.8 (± 1.2)	a
Clay (%)	71	**59.1 (± 1.6)**	aA	59.5 (± 1.55)	a	**55.8 (± 1.6)**	aB	55.0 (± 1.55)	a	**61.7 (± 1.6)**	aA	61.7 (± 1.6)	a
BD (g cm^{-3})	71	**1.37 (± 0.06)**	aA	1.35 (± 0.06)	a	**1.34 (± 0.06)**	aA	1.4 (± 0.06)	a	**1.29 (± 0.06)**	bA	1.38 (± 0.06)	a
SPD (g cm^{-1})	70	**2.48 (± 0.07)**	aA	2.51 (± 0.07)	a	**2.45 (± 0.07)**	bA	2.63 (± 0.07)	a	**2.49 (± 0.07)**	aA	2.50 (± 0.07)	a
TSP (m^3 m^{-3})	71	**0.45 (± 0.03)**	aA	0.46 (± 0.03)	a	**0.45 (± 0.03)**	aA	0.47 (± 0.03)	a	**0.48 (± 0.03)**	aA	0.45 (± 0.03)	b

Means sharing the same letter are not significantly different (Tukey, $p \leq 0.05$). Lowercase letters compare compartments within the site; uppercase letters compare rhizosphere compartment between the three sites. Bold values represent rhizosphere means. Underlined values present significant difference between the three sites. H^++Al^{3+} – potential acidity; TOC – total organic carbon; CEC – total cation exchange capacity; BD – soil bulk density; SPD – soil particle density; TSP – total soil porosity.

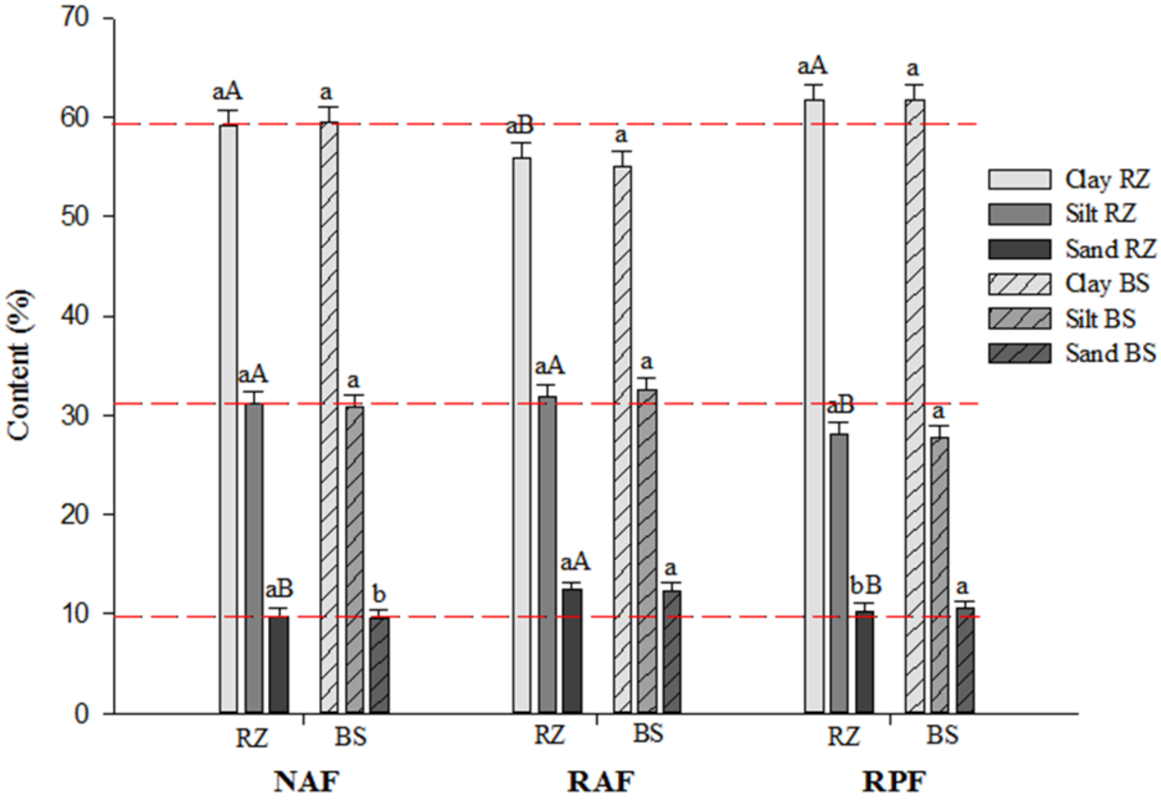
Table 2 – Total soil fraction elements for rhizosphere (RZ) and bulk soil (BS) under native araucaria forest (NAF), reforestation with *Araucaria angustifolia* (RAF), and reforestation with *Pinus elliottii* (RPF) sites (*n* = 8). Iron (Fe) and aluminum (Al) in clay faction for rhizosphere (RZ) and bulk soil (BS) under native araucaria forest (NAF), reforestation with *Araucaria angustifolia* (RAF), and reforestation with *Pinus elliottii* (RPF) sites (*n* = 12). Values are means ± confidence interval in parentheses.

Soil fraction	No obs.	NAF		RAF		RPF	
		RZ	BS	RZ	BS	RZ	BS
Total elemental composition (XRF)							
Al (g kg^{-1})	48	**120 (± 4.50)** aA	120 (± 4.50) a	**111 (± 5.00)** aB	110 (± 4.50) b	**127 (± 4.50)** aA	128 (± 4.50) a
Si (g kg^{-1})	48	**205 (± 3.50)** aB	205 (± 3.50) a	**213 (± 4.00)** bA	215 (± 4.00) a	**198 (± 3.50)** aB	198 (± 4.00) a
Fe (g kg^{-1})	48	**85.7 (± 2.05)** aB	85.2 (± 2.05) a	**80.7 (± 2.05)** aC	78.9 (± 2.05) b	**89.9 (± 2.05)** aA	90.1 (± 2.05) a
Mn (g kg^{-1})	44	**1.19 (± 0.23)** aA	1.10 (± 0.24) b	**1.27 (± 0.24)** aA	1.26 (± 0.23) a	**0.92 (± 0.24)** aA	0.95 (± 0.23) a
P (mg kg^{-1})	48	**674 (± 40)** aA	594 (± 39.50) b	**556 (± 40)** aB	500 (± 40) b	**462 (± 40)** aC	477 (± 40) a
K (mg kg^{-1})	48	**2.247 (± 107)** aA	2.120 (± 107) b	**2.428 (± 107)** aA	2.266 (± 107) b	**2.039 (± 107)** aB	1.983 (± 107) b

Clay fraction	No obs.	NAF		RAF		RPF	
		RZ	BS	RZ	BS	RZ	BS
Ions extraction (g kg^{-1})							
Fe_d	71	**74.9 (± 3.1)** aA	72.7 (± 3.1) a	**68.8 (± 3.1)** aB	69.0 (± 3.1) a	**71.9 (± 3.2)** aAB	71.2 (± 3.05) a
Fe_o	71	**2.91 (± 0.22)** aA	2.68 (± 0.22) a	**2.95 (± 0.22)** aA	2.62 (± 0.22) b	**2.03 (± 0.22)** aB	2.05 (± 0.22) a
Al_o	71	**2.95 (± 0.37)** aA	2.91 (± 0.36) a	**3.33 (± 0.36)** aA	2.77 (± 0.37) b	**2.71 (± 0.36)** aA	2.66 (± 0.37) a
Ion Ratio							
Fe_o/Fe_d	71	**0.04 (± 0.003)** aA	0.04 (± 0.003) a	**0.043 (± 0.003)** aA	0.038 (± 0.004) b	**0.03 (± 0.003)** aB	0.03 (± 0.003) a

Means sharing the same letter are not significantly different (Tukey, $p \leq 0.05$). Lowercase letters compare compartments within the site; uppercase letters compare rhizosphere compartment between the three sites. Bold values represent rhizosphere means. Underlined values present significant difference between the three sites. Fe_d – sodium ditionite-citrate-bicarbonate extraction; Fe_o, Al_o – acid ammonium oxalate extraction.

Figure 3 – Particle-size distribution for rhizosphere (RZ) and bulk soil (BS) under native araucaria forest (NAF), reforestation with *Araucaria angustifolia* (RAF), and reforestation with *Pinus elliottii* (RPF) (n = 12)



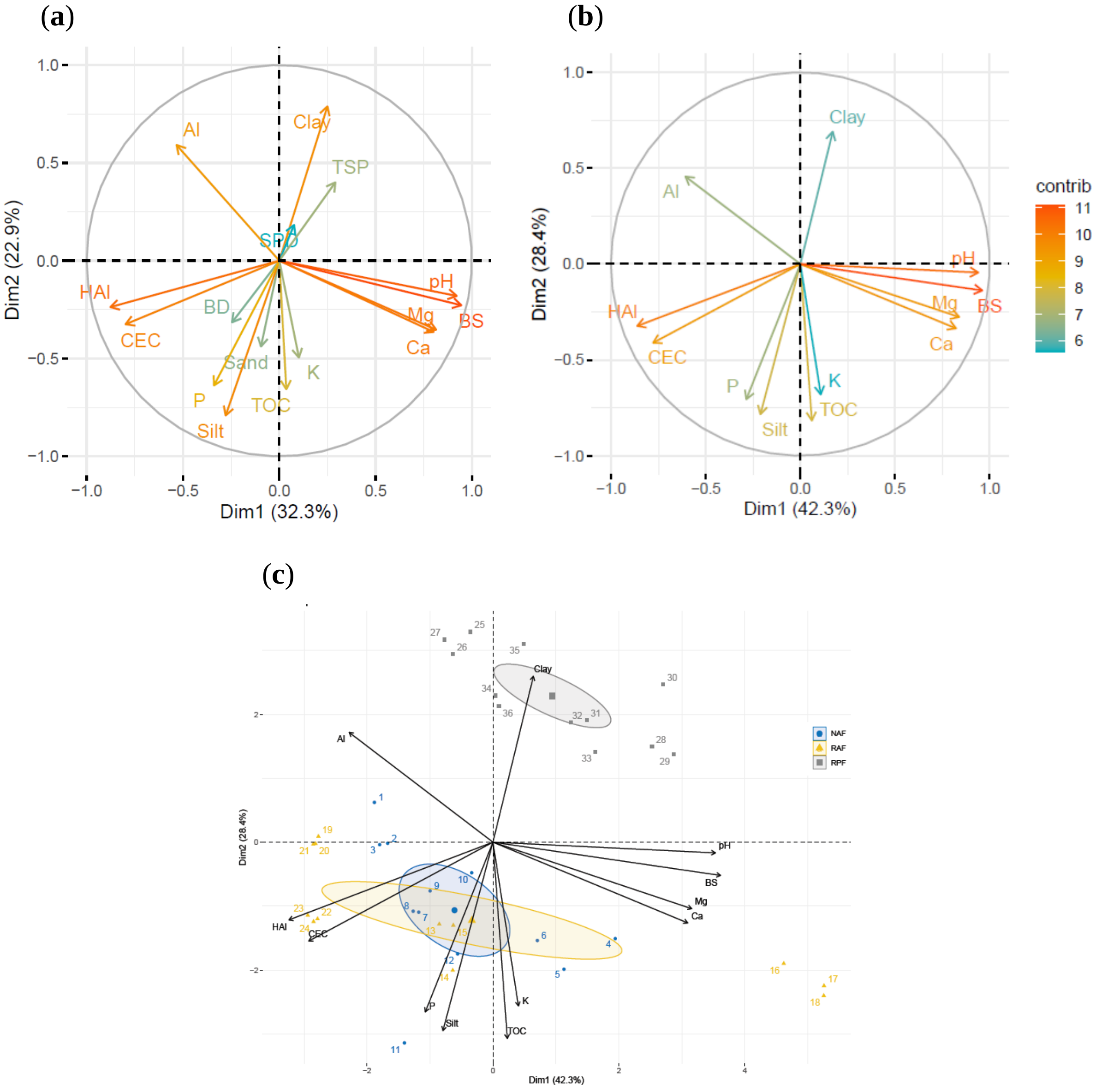
Lowercase letters compare the compartments within the site; uppercase letters compare rhizosphere compartment between the three sites

4.4.3 Principal component analysis

The relationship between soil chemical and physical properties assessed using principal component analysis (PCA) showed that PC1 and PC2 with all sixteen observed variables explained 55.2% of the total variation (Figure 4a). However, 4 out of 16 were excluded (Sand, BD, TSP and SPD), and the new PCA showed that PC1 and PC2 explained 70.7% of the total variation in the dataset (Figure 4b). The contribution of the variables to PC1 and PC2 follow the color gradient (blue to orange) and the increasing length of the vectors.

PCA revealed that the cluster RAF overlapped the cluster NAF, while slash pine cluster did not (Figure 3c), meaning that changes in chemical and physical soil properties were promoted by the exotic species. The soil properties that are most related to NAF and RAF clusters are silt, TOC, K^+ and available P, while the variable negatively correlated to them, the clay content, is more associated with the RPF cluster.

Figure 4 – Principal component analysis (PCA) ordination biplot for rhizosphere chemical and physical properties in the three sites, with all observed (16) variables (**a**) and after the exclusion of four variables (**b**); clusters of native Araucaria Forest (NAF), reforested Araucaria forest (RAF), and reforested Slash Pine forest (RPF) (**c**)



4.4.4 Mineralogy of the clay fraction

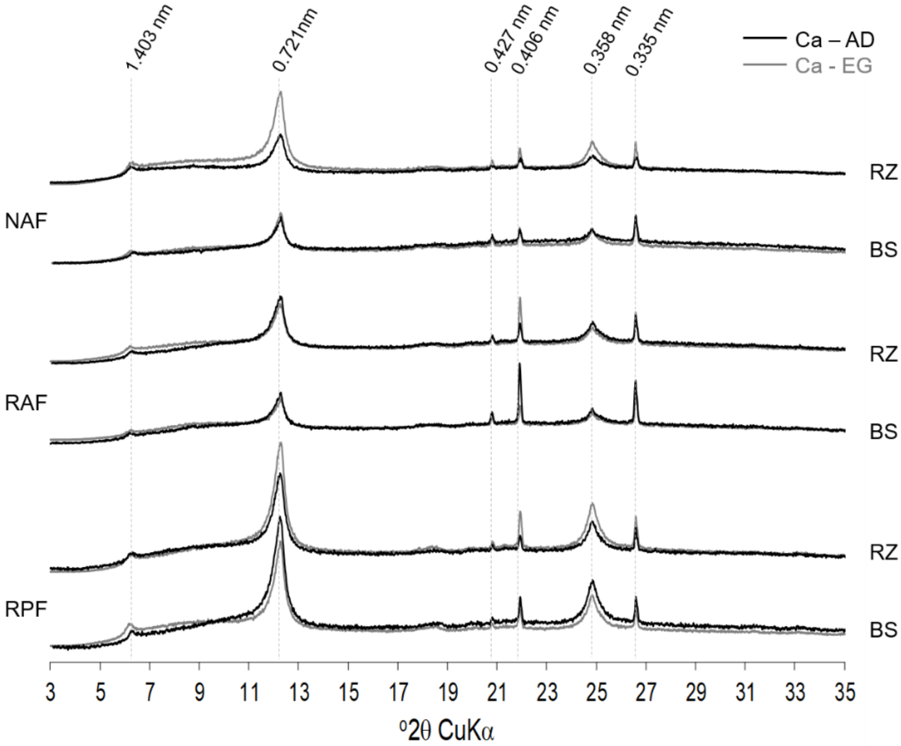
XRD patterns of the oriented Ca-saturated samples (Figure 5) exhibited peaks of quartz (*d* ~ 0.426 and 0.358 nm), feldspars (*d* = 0.406 nm), kaolinite (one peak (*001*) at *d* = 0.721 nm and one peak (*002*) at d = 0.358 nm) and hydroxy-interlayered minerals (*d* = 1.403 nm). The collapse of the 0.721 nm-peak after heating at 550 °C (K-550) (Figure 6) that did not shift following EG solvation (Figure 5) confirms the presence of kaolinite in all sites and compartments (MOORE; REYNOLDS, 1997). The peak *d* = 0.721 nm under hydrated and non-hydrated conditions presupposes the presence of meta-hallosyte (BRINDLEY; BROWN, 1980).

XRD patterns of oriented K-saturated samples (Figure 6a, 6b, 6c) showed peaks that indicate the presence of kaolinite (*d* ~ 0.715 nm and ~ 0.356 nm), goethite (*d* = 0.42 and 0.27 nm), hematite (*d* = 0.27 nm), quartz (*d* ~ 0.426 and ~0.334 nm) and feldspars (*d* = 0.406 nm).

The behavior of the 1.40-nm peak in both Ca- and K-saturated samples, chemically and thermally treated, indicates the presence and/or absence of 2:1 clay-minerals (MOORE; REYNOLDS, 1997). Peaks at *d* ~ 1.40 nm that did not shift after EG solvation excluded the presence of smectite (*d* = 1.51-1.52 nm) (Figure 5). Hydroxy-interlayered minerals/vermiculite are suggested after the combination of the treatments with EG solvation and K-350: no expandability in EG patterns, and a partial collapse of *d* ~ 1.40 nm toward ~ 1.00 nm after K-350 (INOUE et al., 1989, MOORE; REYNOLDS, 1997). The partial displacement of the vermiculite peak at *d* = 1.0 after K-350 is due to the hydroxy-Al polymers bonds in the siloxane surface. The partial collapse of the 1.0 nm-peak at K-550 confirms the presence of Al polymers in the interlayers (BORTOLUZZI et al., 2008; INDA et al., 2010; BORTOLUZZI et al., 2012). The absence of chlorite is also confirmed by the peak at 1.40-1.44 nm that is not affected by ionic saturation and heating treatments (BARNHISEL; BERTSCH, 1989).

In the XRD data, no other low-intensity peaks are evident in AD and EG patterns, only at K-550. A precise differentiation between vermiculite and hydroxy-interlayered vermiculite (HIV) or hydroxy-interlayered smectite (HIS) was not possible due to the high noise levels at K-550 patterns; thus, from the experimental data, it is assumed the presence of 2:1 hydroxy-interlayered minerals (HIM).

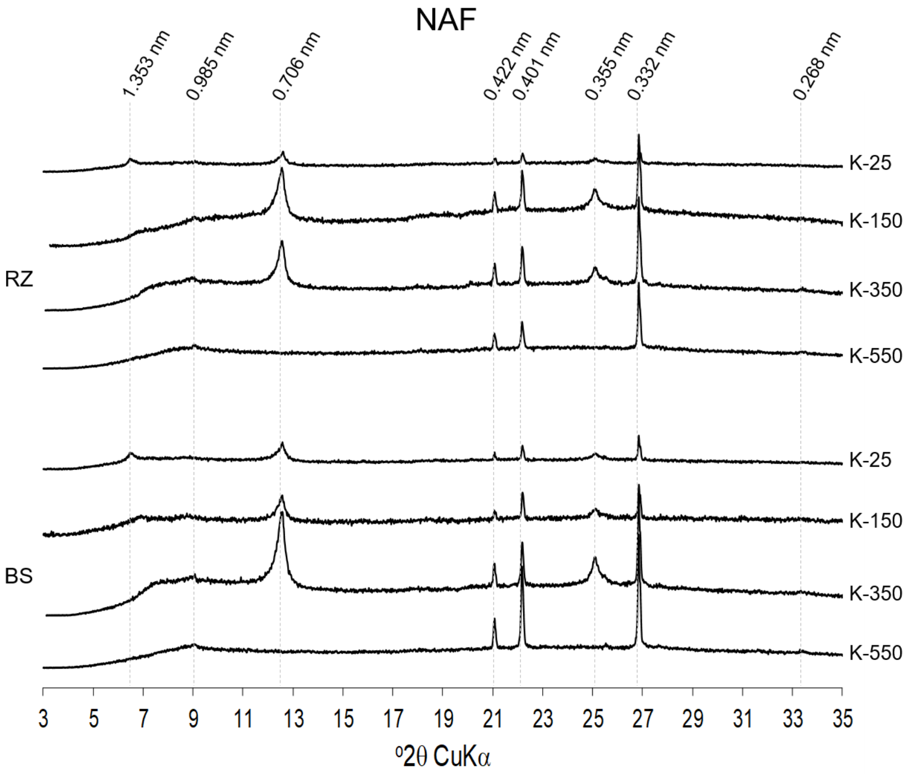
Figure 5 – X-ray diffraction (XRD) pattens of the clay fraction (< 2 µm) Ca-saturated samples under native Araucaria forest (NAF), reforested Araucaria forest (RAF) and reforested Slash Pine forest RPF. RZ: rhizosphere; BS: bulk soil. Oriented slides were scanned for air-dried (Ca-AD) and ethylene glycol solvation (Ca-EG)



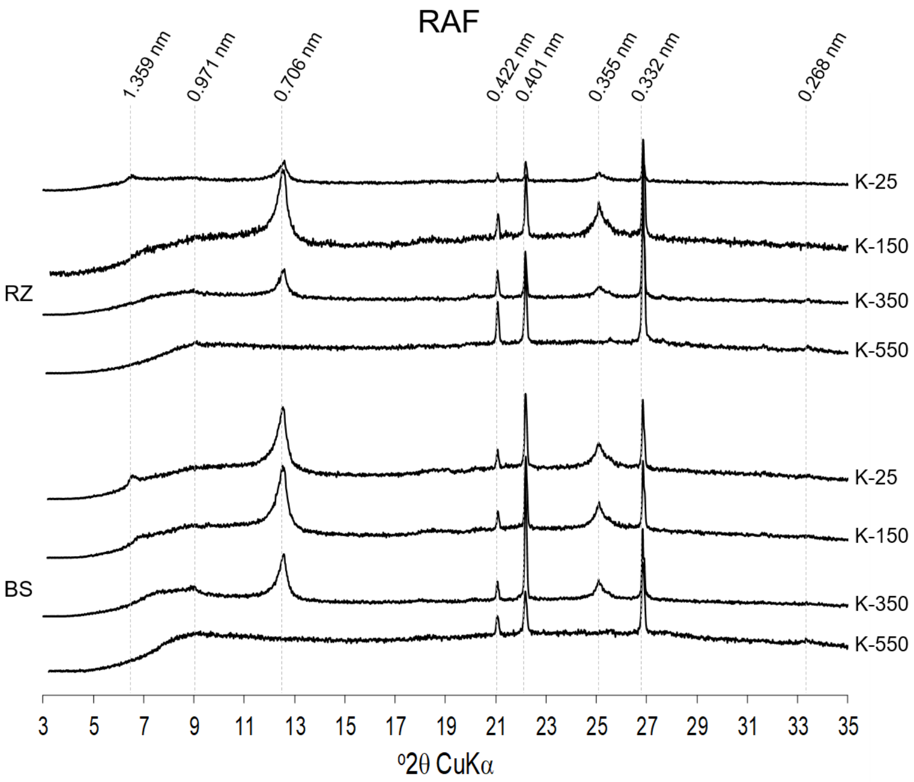
Solid black lines represent air-dried treatment (Ca–AD), and solid gray lines, ethylene glycol treatment (Ca–EG)

Figure 6 – X-ray diffraction (XRD) pattens of the clay fraction (< 2 µm) K-saturated samples under native Araucaria forest (NAF) (**a**), reforested Araucaria forest (RAF) (**b**) and reforested Slash Pine forest RPF (**c**). RZ: rhizosphere; BS: bulk soil. Oriented slides were scanned for air-dried (K-25) and heated at 150 °C (K-150), 350 °C (K-350) and 550 °C (K-550)

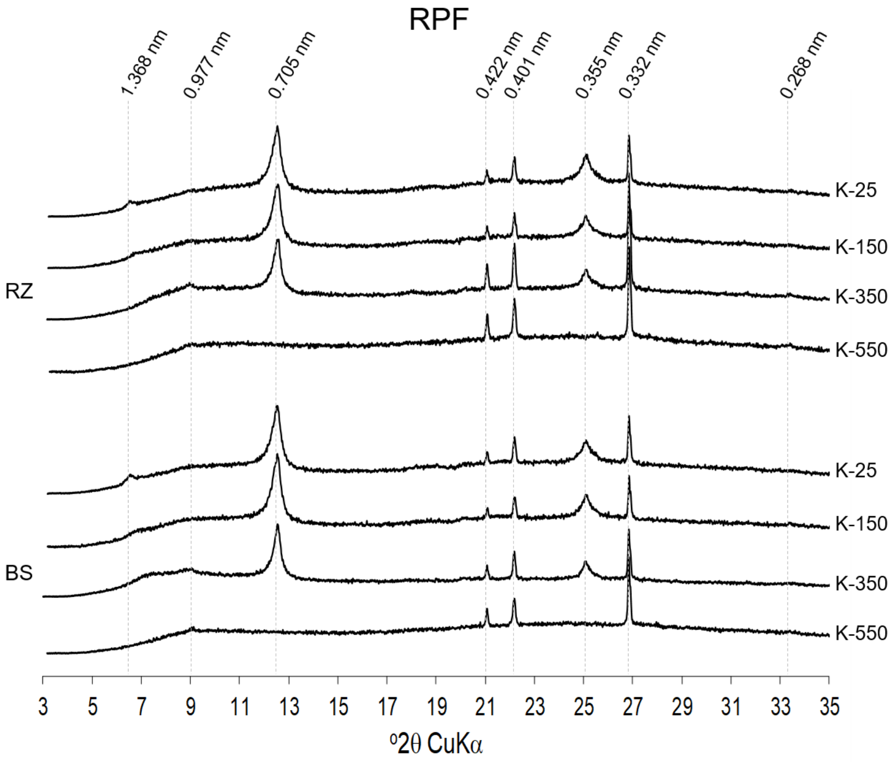
(**a**)



(b)


RAF
1.359 nm
0.971 nm
0.706 nm
0.422 nm
0.401 nm
0.355 nm
0.332 nm
0.268 nm
RZ
K-25
K-150
K-350
K-550
BS
K-25
K-150
K-350
K-550
3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
°2θ CuKα

(c)

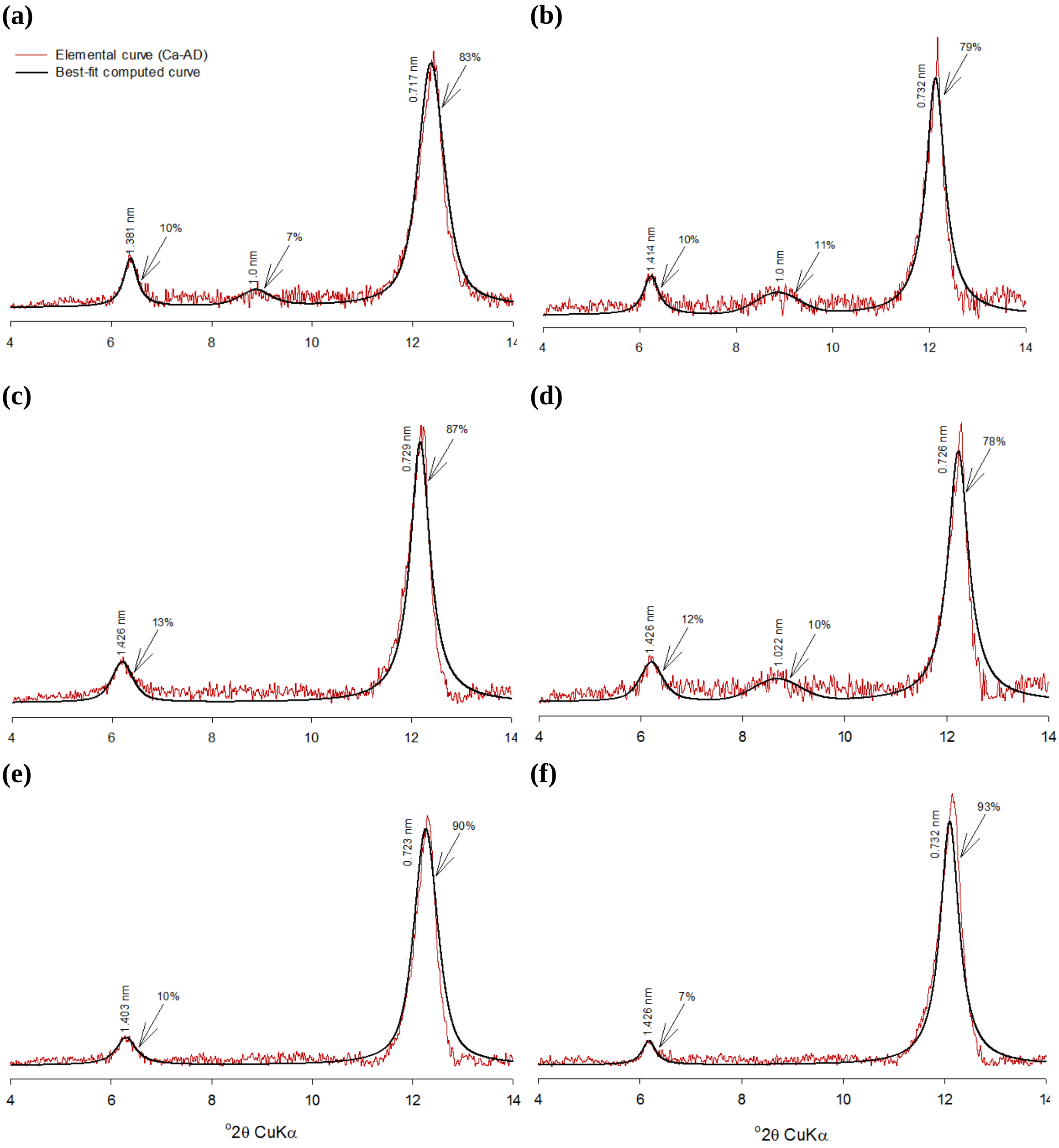

RPF
1.368 nm
0.977 nm
0.705 nm
0.422 nm
0.401 nm
0.355 nm
0.332 nm
0.268 nm
RZ
K-25
K-150
K-350
K-550
BS
K-25
K-150
K-350
K-550
3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
°2θ CuKα

4.4.5 XRD modeling of the clay fraction

Considering that the mineral particle size and the degree of crystallinity are similar (MOORE; REYNOLDS, 1997), the differences in the peaks on XRD patterns are explained by the relative quantity of the clay minerals in the samples. By modelling the XRD patterns and their peaks, it was possible to detect the relative contribution of the clay mineral in soil samples. However, XRD patterns of K-saturated samples both air-dried and thermally treated were not modeled due to the high noise levels, which could lead to errors.

Modeling the XRD Ca-saturated pattens revealed that kaolinite and HIM are the common minerals in the clay fraction in all samples, in different proportions and with kaolinite dominance. Furthermore, the intensity of the kaolinite peak (d ~ 0.7 nm) in the air-dried Ca- and K-saturated samples was higher in the RPF than in NAF and RAF (Figures 5 and 6 a, b, c), which indicate grater relative contribution of the kaolinite peak in slash pine soil. The total surface areas of the kaolinite peak in NAF and RAF were higher in RZ compared to BS (Figure 7a-7d), while in RPF the opposite was observed (Figure 7e-7f). Illite (d = 1.0 nm) was detected only in NAF-RZ, NAF-BS and RAF-BS, with lower relative proportion in NAF-RZ. The relative contribution of HIM peaks was lower in RPF than in RAF. The surface area of all peaks in the air-dried Ca-saturated samples are presented in figures 7a to 7f.

Figure 7 – X-ray diffraction (XRD patterns modeling for the clay fraction performed on air-dried Ca-saturated (Ca-AD). Rhizosphere of native Araucaria forest (**a**); bulk soil of native Araucaria forest (**b**); rhizosphere of reforested Araucaria forest (**c**); bulk soil of reforested Araucaria forest (**d**); rhizosphere of reforested Slash Pine forest (**e**); bulk soil of reforested Slash Pine forest (**f**)



4.5 Discussion

Ferralsols are deep, intensively weathered, yellow to red tropical soils, with a residual concentration of resistant primary mineral such as quartz, and a clay assemblage dominated by the low-active clay kaolinite, and a high content of sesquioxides (IUSS WORKING GROUP WRB, 2015; BORTOLUZZI et al., 2015). These soils are well drained, with good permeability and stable microstructure. Their poor chemical fertility makes them rapidly depleted of plant nutrients if the process of nutrient cycling is interrupted, as occurs with harvesting (VANMAEKELBERGH, 2009). The studied Ferralsol presented high clay and organic matter (humic A horizon), with quantities altered by use. Crystalline iron form was present in higher amount than the poorly-crystallized form, confirming the intensive weathering of this class of soils.

Analyzing the soil most active surface layer under RAF and RPF, evidences of increased mineral weathering found in the rhizosphere confirm the results previously obtained by many studies (HINSINGER; JAILLARD, 1993; COURCHESNE; GOBRAN, 1997; CALVARUSO et al., 2009; TURPAULT; NYS; CALVARUSO, 2009; HOUBEN; SONNET, 2012; KORCHAGIN et al., 2019). Changes were detected due to monoculture of trees, especially in soil organic carbon, available phosphorus, cation exchange capacity (CEC) and soil granulometry. Here, positive effect was observed in NAF-RZ and RAF-RZ concerning total organic carbon (TOC) and CEC, while in RPF no differences were observed between RZ and BS. It was evidenced that growing araucaria and slash pine in monoculture for over 50 years, without fertilizer replacement and managed for sustainable multiple use, resulted in changes on soil granulometry, total elemental composition, oxides, chemistry and proportion of clay minerals. Below, the main evidences and consequences of these changes are discussed.

4.5.1 Soil chemical and physical properties affected by monoculture of conifers

The present study evidenced a greater rhizosphere effect promoted by the native species, especially when araucaria is cultivated in monoculture. This finding may be related to the root system architecture of the conifer. The rhizosphere is related to the activity of living roots and associated microorganisms, and as soon as they die, the portion of soil associated with them is no longer rhizosphere, but bulk soil (HINSINGER; PLASSARD; JAILLARD, 2006). As the slash pine has an extensive lateral root system with a moderate tap root (CAREY, 1992), the transformation from rhizosphere to bulk soil, and vice versa, is more dynamic than in araucaria plantations since it presents a well-developed tap root system (REITZ, 1988) less laterally spread. In general, it can be stated that the slash pine has more impact on its surrounding soil than the araucaria.

The RAF-RZ presented soil acidification (pH_{H2O} = 4.03), which was not observed in slash pine soil, contesting the findings that highly productive woody species are associated with lower pH and exhaustion of nutrient (FIRN; ERSKINE; LAMB, 2007; KORCHAGIN et al., 2019). Lower pH was followed by increase of TOC, potential acidity, available P and available K^+, Ca^{2+}, Mg^{2+}. Consequently, an increase in CEC and base saturation was observed in RZ related to BS. Several studies demonstrate that, whatever the tree species, Ca, Mn, K, Fe, Mn, Al and proton activity, N, C and base saturation are generally increased in the rhizosphere compared to bulk soil (SÉGUIN; GAGNON; COURCHESNE, 2004; TURPAULT et al., 2005; CALVARUSO; N'DIRA; TURPAULT, 2011; COLLIGNON; CALVARUSO; TURPAULT, 2011; COLLIGNON; RANGER; TURPAULT, 2012). The acidification process in the rhizosphere caused by H^+ released for balance charge has been previously describe in the literature (APRIL; KELLER, 1990; CAMA; GANOR, 2015; LAZO; DYER; ALORRO, 2017; KORCHAGIN et al., 2019), what occurs due to permanent exudation of organic acids compounds and hydrolysis (SOKOLOVA, 2011; RONDINA et al., 2019).

Higher TOC content was observed in NAF-RZ and RAF-RZ related to BS especially due to fine roots concentration in topsoil, which represents the highest carbon sink of root system (DANJON; STOKES; BAKKER, 2013). However, TOC in RPF-RZ did not differ from RPF-BS, and it was lower than the content found in the reference NAF-RZ, confirming the influence of the root system architecture on soil properties. Also, TOC correlates with K^+, a cation of high nutritional demand subject to a faster turnover and greater retention time in plant biomass (CÉSPEDES-PAYRET et al., 2012). These two soil attributes are expected to be higher in secondary forest soils compared to planted forests under the same geological conditions (FAN et al., 2019). Hence, the high correlation between TOC and K^+ and the association with NAF and RAF clusters (Figure 4 a, b, c) can be explained by the retention of K by the organic matter and K mobilization from mineral to soil solution (NADERIZADEH; KHADEMI; AROCENA, 2010).

Available P was higher in RAF-RZ than in RPF-RZ, following the tendency observed by Michelsen et al. (1996) in native areas. Highly weathered soils in subtropical regions are rich in Al- and Fe-oxides, and the higher P contents are associated with the site occupation by soil organic matter that are located on the surface of Fe- and Al-oxides (INDA et al., 2010; OLIVEIRA et al., 2020).These oxides act as P sink due to the strong adsorption in oxi-hydroxides surfaces (BORTOLUZZI et al., 2015), and also have a greater potential to preserve TOC than soils dominated by primary minerals such as quartz and felspars (YEASMIN et al., 2017), which are quite present in coarse textured soils. On the one hand, the acidic condition of these soils also facilitates specific P adsorption with Fe- and Al-oxides (KIM et al., 2011), and on the other, the root exudation of organic acids are a strategy of P mobilization in the rhizosphere (FUJII; AOKI; KITAYAMA, 2012). As demonstrated by Inda, Fink and Santos (2018), Fe_o/Fe_d ratios lower than 0.10 indicate the prevalence of crystalline forms (hematite, goethite and maghemite) over low crystallinity types (ferrihydrite). Also, the increased TOC suggest the dissolution of crystalline iron oxides (FINK et al., 2014) in rhizospheres of NAF and RAF, with lower poorly crystalline forms in RPF-RZ followed by proportion of lower Fe_o/Fe_d ratio than NAF-RZ and RAF-RZ.

Forest plantations induce alteration of soil constituent proportion, as observed by other studies (AGNELLI et al., 2016; BORTOLUZZI et al., 2019; KORCHAGIN et al., 2019; KORCHAGIN et al., 2020). No differences were observed in silt and clay content between RZ and BS for all studied sites. However, RPF promoted silt decrease followed by clay increase in both compartments, while in RAF the opposite was observed (Figure 3). The claying process observed in the soil cultivated with slash pine suggests that the fast-growing exotic conifer and the root-associated microorganisms had greater effect on the fracturing of mineral grains. As revealed by April and Keller (1990), a greater degree of fragmentation occurs in mineral grains with edges oriented toward root surfaces than in grains with sides faced oppositely to roots. The mechanical forces exerted by roots growth accelerate mineral degradation, exposing the mineral surfaces to root-induced chemical gradients and, consequently, increasing soil weathering rates. Mineral disaggregation is also induced by microorganisms whose growth is stimulated by compounds, especially fixed carbon, released by photosynthetic members of the symbiosis (BANFIELD et al., 1999).

The multivariate principal component analysis (PCA) showed that clay content is the single soil attribute associated with RPF cluster, confirming the impact of the exotic tree in the claying process. Clay is inversely related to available P and silt, which are attributes most related to NAF and RAF, followed by total organic carbon, available K^+, cation exchange capacity and potential acidity (Figure 4c). Although aluminum, calcium, magnesium, base saturation and soil acidity (pH_{H2O}) are not directly related to a particular cluster, what is confirmed by the non-significant difference of these attributes between the rhizosphere in all sites (Table 1), they are also important in explaining the data variability since vectors are long.

4.5.2 Clay mineralogy under conifer soils

The mineral assemblage of the clay fraction presents some differences that may vary due to the intensity of weathering and the soil environment. Highly weathered soils are characterized by the presence of thermodynamically stable primary and secondary minerals (QAFOKU et al., 2004). The samples of the three sites presented very similar clay mineralogy, comprising 1:1-layer silicates of the kaolinite group, 2:1 dioctahedral mica (illite-like minerals), hydroxy-interlayered minerals (HIM) (Figure 7a-7f), quartz, and iron oxides. As expected for highly weathered subtropical soils, such as the studied Ferralsol, it was not found a broad diversity of minerals, but variation in the proportions of the main minerals or variation within the same type of mineral (QAFOKU et al., 2004), with dominance of kaolinite (BORTOLUZZI et al., 2015).

Kaolinite-enrichment was detected in RAF-RZ compared to RAF-BS, oppositely to what was found in RPF site (Figure 7a-7f). Kaolinite is a highly hydrated 1:1 clay mineral, thus presenting less thermal stability than the 2:1 mineral. Its steady-state dissolution is stoichiometric, with regard to Al/Si ratio in both acid and alkaline medium (CAMA; GANOR, 2015), and is considered an intermediate phase in alteration of silicates to oxides and oxy-hydroxides (VELDE; BARRÉ, 2010). The presence of kaolinite in soil indicates reduction of silica content in the clay assemblage, what means increased weathering rates (VELDE; BARRÉ, 2010).

Illite is a mica specimen (detected at d = 1.0 nm), a 2:1 clay mineral whose interlayer is occupied by anhydrous K ions forming a non-expansible layer. The mineralogical composition of the samples, along with the greater amount of available K (Table 1) and total K (Table 2) in the RZ compared to BS in all sites confirms the rhizosphere effect on illite dissolution. The lower total K in RPF-RZ compared to RAF-RZ may be due to the absence of illite in RPF (Figure 7e-7f,). Illite-like minerals are the main reservoir of K in soils of temperate regions (BARRÉ; VELDE; ABBADIE, 2007) and essential for plant nutrition (WILSON, 2004; VELDE; BARRÉ, 2010). The roots act to release K from interlayers, thus inducing the formation of interlayered-Al polymers as observed in other studies (BORTOLUZZI et al., 2008; INDA et al., 2010), leading to illite transformation into vermiculite (HINSINGER; JAILLARD, 1993). Therefore, the decline of illite confirms the greater slash pine demand for K, especially in topsoil.

Whereas an ionic depletion is expected around the roots, especially of K^+ (BREWSTER; TINKER, 1970; HINSINGER; JAILLARD, 1993), it was observed a K-enrichment in the rhizosphere. This probably occurred due to the high biological activities, which favor the nutrient recycling in the root zone and tree nutrition (COLLIGNON; CALVARUSO; TURPAULT, 2011). Polyvalent cations, especially aluminum, within the soil solution alter the potassium uptake by roots (CUMMING; ECKERT; EVANS, 1985). High concentrations of aluminum and other cations may be responsible for a net potassium accumulation in the root zone, resulting in retardation of mica weathering in the araucaria sites (i.e., K-release) and, perhaps, potassium reincorporation into interlayer and reconstitution of some previously degraded micas (SCOTT; SMITH, 1966; APRIL; KELLER, 1990).

The presence of one or another clay mineral is probably related to the transformation of primary minerals (BORTOLUZZI et al., 2012). The relative increase of kaolinite (1:1) with illite decline was observed in the present study and in grassland soil cultivated 25 years with eucalyptus in the temperate region of South America (CÉSPEDES-PAYRET et al., 2012). Cama and Ganor (2015) affirm that the dissolution rate of illite is mainly controlled by the solution pH, that have the adsorption of H^+ by the edges of particles as the first step of the dissolution process. Furthermore, Céspedes-Payret et al. (2012) affirm that the dissolution by organic acids could be a major mechanism of vermiculitization or smectization of illite-like minerals.

Based on the results of K-saturated XRD pattens (Figure 6 a, b, c), the partial collapse of the 2:1 hydroxy-interlayered minerals (~ 1.40 nm peak toward ~ 1.00 nm peak after K-550 treatment) was evidenced, as observed in many other Brazilian weathered soils containing hydroxy-Al interlayered vermiculite (HIV) and hydroxy-Al interlayered smectite (HIS) (BORTOLUZZI et al., 2008; INDA et al., 2010; CANER et al., 2014). HIM presents hydroxy coordinated interlayer ions, which in natural conditions is favored by the fixation of Al (OH) cations. These 2:1 mineral present similar nature and crystallographic expression, but their response vary according to their physic-chemical properties when investigated by X-ray diffraction (VELDE; BARRÉ, 2010). When Al is the interlayer prevalent ion and the layer is incomplete, the minerals are identified as vermiculite (MEUNIER, 2007), and chlorite is identified when the interlayer is more complete and Mg may be present. If K displaces the hydroxy layer ions Al and/or Mg, the minerals are high charge smectites (VELDE; BARRÉ, 2010). However, a reliable identification of HIM was not possible due to the high noise exhibited by the XRD patterns exposed to thermal treatments.

4.5.3 Implications of land-use change for soil properties

The Ferralsol under conifer plantations, in which RZ and BS environments were assessed, presented characteristics that are expected for high weathered soils of the humid subtropical region of Brazil, with high amounts of kaolinite-group minerals that coexist with 2:1 clay minerals (BORTOLUZZI et al., 2008; INDA et al., 2010; BORTOLUZZI et al., 2012; BORTOLUZZI et al., 2013; OLIVEIRA et al., 2020). The greater susceptibility of the 2:1 minerals to land use changes and agricultural practices, such as liming and K fertilization for annual crops, or acidification due to slash pine and eucalyptus monocultures (CANER et al., 2014; KORCHAGIN et al., 2019), lead to a tendency of aluminization to form hydroxy-Al-interlayered minerals (BORTOLUZZI et al., 2008; BORTOLUZZI et al., 2012; KORCHAGIN et al., 2019). These changes of interlayered cations may occur in a short time scale, from decades in soil horizons to months in the rhizosphere (CORNU et al., 2012), and have consequences for soil fertility as it affects the CEC (MEUNIER, 2007; KORCHAGIN et al., 2019).

A shift in soil use from native forest to conifer plantations leads to changes in soil properties, no matter if they are native or exotics. However, exotic species such as slash pine, when cultivated in monoculture, has greater soil alteration potential, decreasing silt fraction and organic matter contents in the rhizosphere. The silt fraction is associated with both medium- and long-term nutrient supply (WILSON, 2004); however, the finest soil fraction, the clay, is more susceptible to changes due to soil use and management (BORTOLUZZI et al., 2012). Furthermore, the higher proportion of well-crystallized iron related to the poorly-crystallized (Fe_o/Fe_d) indicates increased weathering promoted by the fast-growing exotic conifer, as well as the absence of illite along with greater amount of total K and higher kaolinite proportion in soil. Araucaria plantation also promoted changes in rhizosphere properties, but to a lesser extent than slash pine, and are related to total element content, with lower P_t, Al_t and Fe_t, and higher Si_t, which may also indicate some degree of weathering.

4.6 Conclusion

The chemical, physical and mineralogical conditions in rhizosphere and bulk soil of conifers under a high weathered Ferralsol confirm the higher intensity of the processes occurring in the rhizosphere and has evidenced geochemical changes due to monoculture of ~ 50 years old native and exotic conifers. The fast-growing exotic slash pine promotes silt reduction followed by increase of clay contents and of crystalline iron oxides, along with complete dissolution of illite and increase of kaolinite proportion in clay assemblage. Araucaria plantations also promote changes in rhizosphere properties compared to the rhizosphere of araucaria in its native environment, although in lower proportions than slash pine.

This study evidences the soil geochemical and mineralogical evolution in conifer plantations and the potential capacity of a non-native woody species in altering the soil properties when introduced in an environment for timber production. Our findings suggest an acceleration of weathering rates and the increasing risk of soil quality loss when monocultures are favored over mixed plantations. Thus, a broad discussion based on large approach studies on chemical, physical and mineralogical aspects of soils-plant interactions in different scales merits particular attention due to their importance in ensuring sustainable use of edaphic resources.

5 CHAPTER III

Edaphic changes in sustainable use conifer plantations within the Mixed Ombrophilous Forest domain

5.1 Abstract

The introduction of large-scale plantations brings recognized economic benefits at the expense of natural environments. Understanding how forest plantations affect the natural resources may match conservation with sustainable timber production. This study evaluated the chemical, physical and microbiological soil attributes that are affected when monoculture of the native *Araucaria angustifolia* (araucaria) and the exotic *Pinus elliottii* var. *elliottii* (slash pine) are introduced in a Mixed Ombrophilous Forest (MOF) ecosystem. Soil samples were collected at Passo Fundo National Forest in a secondary Araucaria Forest fragment, and in long-term araucaria and slash pine monocultures managed for sustainable multiple use. Samples were characterized in their physical (sand, silt, clay, bulk density, soil particle density and total soil porosity), chemical (pH_{H2O}, Al^{3+}, H^+ $+Al^{3+}$, total organic carbon, K^+, Ca^{2+}, Mg^{2+}, available P, total cation exchange capacity and base saturation) and microbiological (soil basal respiration, microbial biomass carbon, metabolic and microbial quotients) attributes. Slightly edaphic changes promoted by araucaria and slash pine were detected when the soil attributes were assessed individually. However, the discrimination between the three sites by multivariate analysis highlights different impacts of conifer species on soil, demonstrating the relevance of two factors and the attributes associated to them: “soil acidity factor” and “total organic carbon factor”. Slash pine impacted greatly the soil constituents since a claying process could be observed, suggesting higher weathering rates. The factors soil acidity (pH_{H2O}, $H^+ + Al^{3+}$, Ca^{2+}, Mg^{2+} and base saturation) and soil organic carbon (total organic carbon, K^+, available P, sand and clay), are the best indicators to discriminate soil changes due to vegetal substitution over time. The present study also highlights the importance of the sustainable managed Brazilian National Forests in protecting the natural ecosystems and the biodiversity, thus meeting the Sustainable Development Goals established by the United Nations and the OMS One Health approach.

Keywords: 1. *Araucaria angustifolia*. 2. *Pinus elliottii*. 3. National Forests. 4. Sustainable Management. 5. Soil weathering.

5.2 Introduction

Araucaria angustifolia (Bertoloni) Otto Kuntze is a conifer of the Mixed Ombrophilous Forest (MOF), also called Araucaria Forest, a subtropical component of the Atlantic Forest Biome restricted to the highlands of Southern Brazil, with some extension to Southeastern Brazil and Argentina. It is a long-lived pioneer that occupies the MOF upper canopy and supports a broad diversity of local flora and fauna (WEBB et al., 1984; CARVALHO, 2002; FONSECA et al., 2009; CARVALHO; MOREIRA; CARDOSO, 2012). Originally, the Araucaria Forest covered an estimated surface area of 200,000 km^2 (HUECK, 1972) inhabited by the Amerindians of Guarani group (PETRY, 2003). In the early 20th century, the non-sustainable exploitation and natural forest conversion to other uses caused a reduction of 12.8% of its original extent only in Rio Grande do Sul state (CORDEIRO; HASENACK, 2009). Consequently, there was a fragmentation and habitat reduction with loss of biodiversity (HARTLEY, 2002; FONSECA et al., 2009; SILVA; SCHMITT, 2015), leading araucaria to figure on The IUCN Red List of Threatened SpeciesTM as 'critically endangered' (THOMAS, 2013).

Currently, the harvest of araucaria is restricted by law, and a movement toward the exploitation of exotic, fast-growing and low-density wood species, such as *Pinus* spp. and *Eucalyptus* spp. by timber companies, has been noticed since the 70's as these exotics are more profitable in South America (CUBBAGE et al., 2007). In Brazil, the total area planted with these exotic species reached 7.3 million ha in 2018, of which slash pine occupied 1,6 million ha. Contrastingly, only around 13 thousand ha was planted with araucaria (IBA 2019). The decline of Araucaria Forests resulted in disruption on soil ecosystem and native species, drawing attention to the need of assessing the impacts on soil quality (DORAN; PARKIN, 1994; BREJDA et al., 2000; ISLAM; WEIL, 2000; USHARANI; ROOPASHREE; NAIK, 2019). As an example, the introduction of exotic tree species often introduce non-native microorganisms in association with the plant root system, with potential to impact the indigenous community which, in turn, may affect the restauration of native vegetation (IZUMI et al., 2008), particularly in relation to slash pine, which is considered an invasive species.

To address the conservation of forest biodiversity and forest restoration are among the biggest challenges faced by forest managers globally (LINDENMAYER, 2019).

The reforested araucaria soils are chemically different from the natural araucaria ones (CARVALHO; MOREIRA; CARDOSO, 2012; BERTINI et al., 2015), but it is more similar than slash pine or eucalyptus plantations (FONSECA et al., 2009; FAGOTTI et al., 2012; BINI et al., 2013; PAZ et al., 2015; MALYSZ; OVERBECK, 2018). Highly productive woody species are associated with lower pH and exhaustion of nutrient (FIRN; ERSKINE; LAMB, 2007; KORCHAGIN et al., 2019), while native tree plantations allow the recolonization, thus ensuring the soil heterogeneity and the maintenance of original properties (MALYSZ; OVERBECK, 2018). The Chapter II presented different rhizosphere effects of araucaria and slash pine, but the effect of these species in bulk soil related to a native forest soil is still a gap to be filled. Also, studies on the impact of *Araucaria angustifolia* (araucaria) and *Pinus elliottii* var. *elliottii* (slash pine) plantations, managed for multiple and sustainable use of native and exotic forest species, on a large range of edaphic attributes considering a single and contiguous soil unit, are scarce in the literature.

The present research was carried out at Passo Fundo National Forest (FLONA-PF) to evaluate to the effect of plantations of the native araucaria and the exotic slash pine in the soil matrix when they are introduced in a MOF ecosystem. Although monocultures of non-native trees are commonly considered ‘green deserts’, sustainable forest practices of exotic species are among the specific objectives of the Brazilian National Forests for conservation and sustainable multiple use of natural resources since they support some biodiversity when compared to primary forest (FONSECA et al., 2009; BREMER; FARLEY, 2010; HORÁK et al., 2019). Here, the hypothesis is that neighboring forested soils, within the same topographic and climatic conditions, reforested with araucaria and slash pine, following sustainable use management approaches, would differ from a secondary Araucaria Forest soil. Besides, as araucaria is a native species that physiognomically dominates the local forest, we expect that the soil reforested with araucaria presents higher overall similarity with the preserved native forest soil than the soil planted with slash pine. Statistical approaches are used to identify the soil chemical, physical and microbiological variables that are able to discriminate the three sites and monitor the edaphic changes over time.

5.3 Material and methods

5.3.1 Site description

The study was carried out at Passo Fundo National Forest (FLONA-PF), located on the Campos Gerais plateau in the Northern region of Rio Grande do Sul state, and belong to Mato Castelhano municipality (52° 11’ 12” W, 28° 16’ 47” S), following previous authorization (Appendix V). The National Forest is a Conservation Unit for Sustainable Use created in 1968 to conserve the in-situ biodiversity, including araucaria. It is currently managed by the government environmental agencies *Chico Mendes Institute of Biodiversity Conservation* (ICMBio), and *Brazilian Institute of Environment and Renewable Natural Resources* (IBAMA). FLONA-PF is considered a biodiversity hotspot inserted in a landscape matrix dominated by annual crop fields forming a heterogeneous mosaic with remnants of Araucaria Forest and monocultures of woody species. It protects 38.63% of the largest fragment of existing native forests in the area corresponding to its buffer zone, adding to its own area. It also represents 12% of the area covered by forest remnants in this area. If araucaria plantations at FLONA-PF are considered, this percentage rises to 25% (ICMBio, 2011). The reserve encompasses 1,333.61 ha with elevation between 632.3 and 757.6 m. The climate is humid subtropical Cfa (Köppen), with mean annual temperature of 17°C, mean annual rainfall between 1,800 – 1,900 mm, and 72% of air relative humidity (ICMBio, 2011).

Previous reforestation, intensive agriculture used to be practiced with annual crops and cattle farming, but details of these land-use history are unknown. The remaining fragments of natural forests are over one hundred years old and are in good conservation state in terms of species composition and richness (MALYSZ, 2010), with phytosociological dominance in the horizontal forest structure of the *Araucaria angustifolia* and the co-occurrence of many broadleaf species (ICMBio, 2011). The regenerative component of both the Araucaria Forest and the monoculture plantations (araucaria and slash pine) are dominated by the Myrtaceae family, followed by Fabaceae, Lauraceae and Euphorbiaceae, with almost twice more individuals in the reforested Araucaria forest than in the reforested Slash Pine forest (MALYSZ; OVERBECK, 2018).

At FLONA-PF, the stands are small (10 – 60 ha), which provides a close contact with adjacent habitats and seed banks. The rotation period of conifer monocultures of sustainable multiple use is much longer (> 40 years) than the traditionally adopted by economically-driven regional timber companies (10–18 years). Formerly, the implantation of forest stands was not low impact since both araucaria and slash pine plantations followed soil preparation through conventional tillage with burning. Cultural traits, such as weeding, mowing and crowning, were performed during the next four years. Liming and fertilization practices were not found in the records.

The reforested Araucaria forest (RAF, stand 19) was established in 1958 (25.1 ha), with initial density of 5,000 trees ha^{-1}. Selective timber harvests were conducted in 1972/73, 1979 and 1992, and the last evaluated remaining wood stock (2011) consisted of 411.8 m^3 ha^{-1} and 317 trees ha^{-1}. The estimated mean diameter at breast height (*dbh*) was 36.96 cm and 19.59 m of total high (*ht*). The reforested Slash Pine forest (RPF, stand 24) was established in 1972 (17.28 ha), with 2,500 trees ha^{-1}. Nine selective timber harvesting were performed from 1976 until 2000, and according to the latest inventory (2011), the remaining wood stock was 748.1 m^3 ha^{-1} with 483 trees ha^{-1}. The *dbh* was 34.73 cm and *ht* 31.87 m (ICMBio, 2011).

5.3.2 Soil sampling and preparation

In 2018, three adjacent sites with the same soil type – a loamy, well-drained and deep Ferralsol (Latossolo Vermelho Escuro by the Brazilian Classification System) – were selected for sampling: *i*) a native secondary Araucaria Forest fragment (NAF) as the reference site; and two mature monocultures managed for sustainable multiple use: *ii*) a reforested *Araucaria angustifolia* forest (RAF); and *iii*) a reforested *Pinus elliottii* forest (RPF) as the treatments. However, small nuances in the soil type cannot be disregarded.

Disturbed soil samples were taken using a blade in August and September, 2019, under tree canopy, 10 cm far from the trunks and at 0-20 cm deep, after litter removal. From each site, four spots under the trees canopy were randomly selected. One composite soil sample was collected per spot (three soil subsamples homogenized to make one sample), for a total of 12 samples. The soil samples were air-dried and sieved (<2 mm) to obtain air-dried fine earth (ADFE) used for physical and granulometric characterization. An aliquot of the samples was manually grounded in an agate grail for chemical characterization. All samples were assessed in triplicate given 36 experimental units. For microbiological analysis, the soil samples were collected in March, 2020, following the same criteria; however, they were sieved (<2 mm) to remove roots and litter, and stored for up to 24 hours at 4°C before conducting the analysis in order to minimize microbial death. Also, soil clods were reserved to obtain undisturbed samples for the assessment of bulk density

5.3.3 Physical and chemical analysis

Soil pH was measured in H_2O (1:2.5 (*w*/*v*) soil/solution ratio) (TEIXEIRA et al., 2017). Potential acidity ($H^+ + Al^{3+}$) was determined using the Santa Maria buffered solution (TSM) as an alternative to SMP buffer (TOLEDO, 2011). Total organic carbon (TOC) was determined by wet combustion (YEOMANS; BREMNER, 1988). Available phosphorus (P) and exchangeable potassium (K^+) were extracted by Mehlich^{-1} (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}) and measured using a UV-Vis spectrophotometer at 660 nm and a flame photometer, respectively. Calcium (Ca^{2+}), magnesium (Mg^{2+}) and aluminum (Al^{3+}) were extracted with 1 mol L^{-1} KCl and determined using an inductively couple plasma optical emission spectroscopy (ICP-OES). The total cation exchange capacity (CEC: $K^+ + Ca^{2+} + Mg^{2+} + [H^+ + Al^{3+})$ and base saturation (BS% = $([K^+ + Mg^{2+} + Ca^{2+}] * 100)$/CEC) were estimated.

Particle-size distribution was performed to obtain sand (0.05-2.0 mm), silt (0.002-0.05 mm) and clay (< 0.002 mm) fractions by sieving and sedimentation (GEE; BAUDER, 1986) after organic matter oxidation with hydrogen peroxide (5% H_2O_2 *v*/*v*) and temperature (40 °C). Soil bulk density (BD) was estimated using the clod method by immerging in water clods coated with paraffin (a water-repellent substance). The soil particle density (SPD) followed the volumetric flask method while total soil porosity (TSP) was calculated by the ratio between BD and SPD (TEIXEIRA et al., 2017).

5.3.4 Microbiological analysis

Gravimetric soil moisture was determined at 105°C for 24h to standardize moisture levels whenever necessary and to express laboratory results. To measure the soil basal respiration (SBR), an indicator of the organic carbon microbial mineralization, moister was adjusted to 70% of water holding capacity (WHC) and C-CO_2-C released within 10 days was determined by extraction with NaOH 1 mol L^{-1} and titration with HCl 1 mol L^{-1} (STOTZKY, 1965). For microbial biomass carbon (C_{mic}), which represents a reservoir of C that acts as drain and source, the samples were preincubated at 70% WHC for seven days inside a dark chamber, at 25°C, and estimated according to the fumigation-incubation method described by Reis Junior and Mendes (2007). The metabolic effectiveness of soil microbial communities, expressed by the index qCO_2, was obtained by the ratio between SBR/C_{mic}, which predicts that higher values are associated to higher consumption of readily mineralized carbon and elevation of CO_2 losses. The microbial quotient index (*q*MIC), calculated by the ratio between C_{mic} /TOC, indicates the contribution of C_{mic} to TOC. It is a reliable parameter, superior to its single components, in describing changes in anthropized ecosystems (INSAM; DOMSCH, 1988).

5.3.5 Statistical analysis

All variables were examined for homogeneity of variances with Levene’s test and, for normality, with Lilliefors’ test (Kolmogorov-Smirnov). Dataset was check for outliers through box-plot. When necessary, data were transformed using the Box-Cox function in order to obtain homogeneous variances and data normality. The dataset was subjected to a nested Analysis of Variance (ANOVA) and to Tukey's test ($p \leq 0.05$) using a Linear Mixed Model (LMM) for comparison between sites (n=12). Pearson correlation coefficients (r) were calculated to identify the relationship between soil attributes. Principal Component Analysis (PCA), a multivariate statistic used to explain most of the variance in the dataset, was applied to the whole set of data. Linear Discriminant Analysis (LDA) was performed to check possible misclassification of the forest types and to find the linear combination of original variables that provide the best possible separation between groups. All statistical analyses were performed using the free RStudio version 3.6.1 (R CORE TEAM, 2019), and the graphs were created in both RStudio and Sigmaplot version 13.

5.4 Results

5.4.1 Soil physical, chemical and microbiological properties

The majority of physical, chemical and microbiological soil attributes were not sensitive to conifer species when assessed individually (Table 1). Only available P, soil particle-size distribution and *q*MIC differed among the treatments. Available P was higher in the control (NAF) than in the reforested sites (RAF and RPF). The lowest *q*MIC in NAF indicates less carbon immobilization by the microbial biomass in the native than in the reforested soils. Among the reforested sites, no difference in silt content between RAF and NAF was found, only slash pine presented lower silt. Clay content in RPF was not different from NAF (control), and in both it was higher than in NAF (Figure 1).

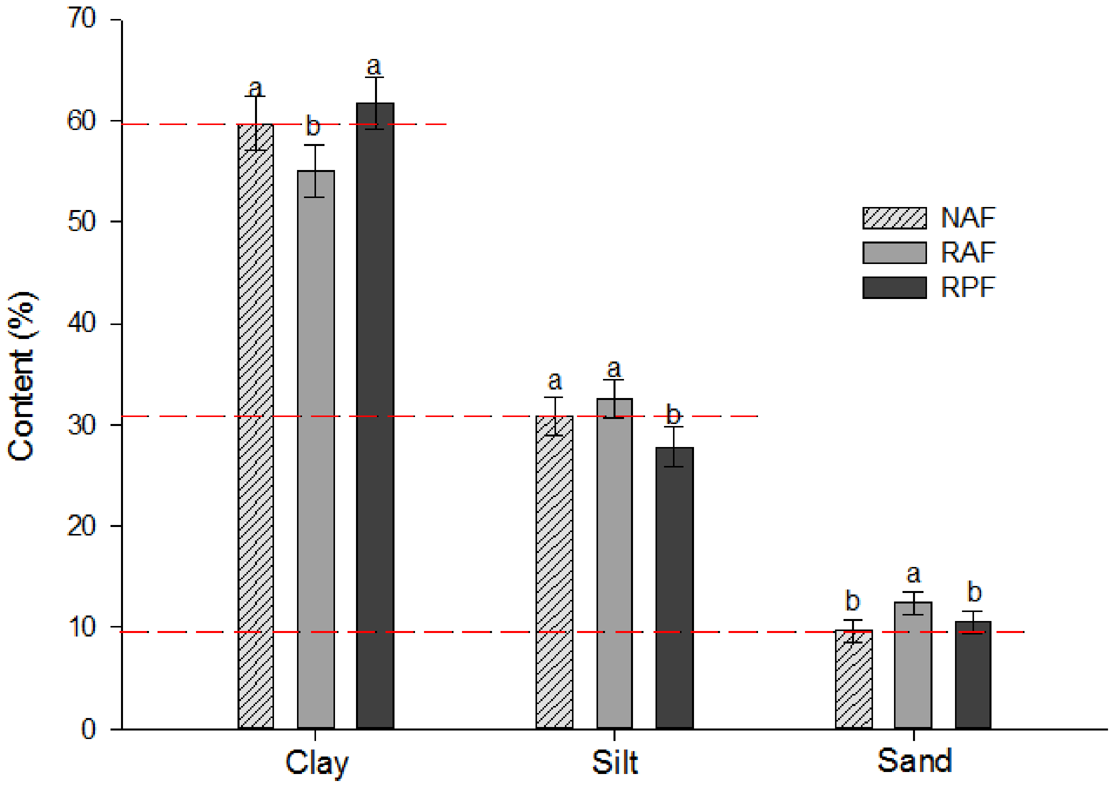
Significant correlation ($p < 0.05$) was observed among 52 out of 190 soil attribute pairs (Appendix VI). The higher frequency of strong correlations ($-0.75 \leq r \geq 0.75$) was observed for base saturation (BS), which was negatively correlated with CEC (r = -0.80) and $H^+ + Al^{3+}$ (r = -0.80), and positively correlated with pH (r = 0.92), Ca^{2+} (r = 0.93) and Mg^{2+} (r = 0.97). $H^+ + Al^{3+}$, Ca^{2+} and Mg^{2+} also presented high positive correlation with pH (*r* = 0.77, 0.82 and 0.89, respectively) and Ca^{2+} with Mg^{2+} (*r* = 0.94). Other high positive correlations were between K^+ and available P (*r* = 0.81), CEC and H^++Al^{3+} (*r* = 0.97), SBR and qCO_2 (*r* = 0.82), and C_{mic} and *q*MIC (*r* = 0.87). However, the strongest negative correlations were between silt and clay (*r* = -0.96) and BD and TSP (*r* = -0.94). BD and TSP correlated only with each other, and the microbiological soil attributes SBR, C_{mic} and qCO_2 were correlated with the fewest number of soil attributes (2, 2 and 3 attributes, respectively).

Table 1 – Soil chemical, physical and microbiological attributes in native Araucaria Forest (NAF), reforested *Araucaria angustifolia* (RAF), and reforested *Pinus elliottii* (RPF)

Soil attributes	**No. obs**	**Forest type**		
		NAF	**RAF**	**RPF**
Chemical				
pH$_{(H2O)}$	33	4.1 (± 0.2) a	4.1 (± 0.2) a	4.3 (± 0.2) a
Al^{3+} (mmol$_c$ dm^{-3})	36	13.1 (± 10.9) a	14.3 (± 10.9) a	21.1 (± 10.9) a
H$^+$ + Al^{3+} (mmol$_c$ dm^{-3})	36	88.8 (± 26.4) a	89.1 (± 26.7) a	81.0 (± 26.8) a
TOC (g kg^{-1})	36	3.9 (± 0.5) a	3.5 (± 0.5) a	3.7 (± 0.5) a
K$^+$ (mmol$_c$ dm^{-3})	36	2.5 (± 1.0) a	1.3 (± 1.0) a	1.3 (± 1.0) a
Ca^{2+} (mmol$_c$ dm^{-3})	35	1.8 (± 1.1) a	1.5 (± 1.1) a	1.6 (± 1.1) a
Mg^{2+} (mmol$_c$ dm^{-3})	35	5.0 (± 5.1) a	6.4 (± 5.1) a	6.1 (± 5.1) a
Available P (mg dm^{-3})	**36**	**3.5 (± 0.9) a**	**2.1 (± 0.9) b**	**1.5 (± 0.9) b**
CEC (mmol$_c$ dm^{-3})	36	98.0 (± 20.8) a	99.2 (± 20.7) a	90.0 (± 20.8) a
Base saturation (%)	35	9.6 (± 10.0) a	10.8 (± 10.0) a	10.3 (± 10.0) a
Physical				
Sand (%)	36	**9.6 (± 1.1) b**	**12.4 (± 1.1) a**	**10.5 (± 1.1) b**
Silt (%)	35	**30.8 (± 1.9) a**	**32.6 (± 1.9) a**	**27.8 (± 1.9) b**
Clay (%)	35	**59.7 (± 2.6) a**	**55.0 (± 2.6) b**	**61.7 (± 2.5) a**
BD (g cm^{-3})	35	1.4 (± 0.1) a	1.4 (± 0.1) a	1.4 (± 0.1) a
SPD (g cm^{-1})	36	2.5 (± 0.1) a	2.6 (± 0.1) a	2.5 (± 0.1) a
TSP (m^3 m^{-3})	35	0.5 (± 0.03) a	0.5 (± 0.03) a	0.5 (± 0.03) a
Microbiological				
SBR (µg CO$_2$ g^{-1} 10 d)	36	347 (±164.2) a	241 (± 164.1) a	353 (± 163.9) a
C$_{mic}$ (µg C g^{-1})	35	3,459 (± 457.5) a	3,809 (± 454.5) a	4,013 (± 454.5) a
*q*CO$_2$	35	0.1 (± 0.05) a	0.1 (± 0.05) a	0.1 (± 0.05) a
*q*MIC	36	**0.8 (± 0.1) b**	**1.0 (± 0.1) a**	**1.1 (± 0.1) a**

Means sharing the same letter are not significantly different (Tukey, $p \leq 0.05$). Bold values represent significant difference among forest types. Numbers in parentheses represent confidence interval (*n*=12). H$^+$+Al^{3+} – potential acidity; TOC – total organic carbon; CEC – total cation exchange capacity; BD – bulk density; SPD – soil particle density; TSP – total soil porosity; SBR – soil basal respiration; Cmic – microbial biomass carbon; *q*CO2 – metabolic quotient; *q*MIC – microbial quotient.

Figure 1 – Particle-size distribution in the soil of native Araucaria Forest (NAF), reforested *Araucaria angustifolia* (RAF), and reforested *Pinus elliottii* (RPF)



Letters compare clay, silt and clay among the sites. Means sharing the same letter are not significantly different (Tukey, $p \leq 0.05$)

5.4.2 Principal Component and Linear Discriminant Analyses

The relationship between soil chemical, physical and microbiological attributes in the forest sites (NAF, RAF and RPF) was assessed using principal component analysis (PCA). PC1 and PC2 with all 20 observed variables explained 49.2% of the total variation (Figure 2a). After evaluating the variables that presented low contribution in explaining data variability and few significant correlations among soil attributes, 5 out of 20 were excluded (BD, TSP, SBR, Cmic and *q*CO2). Thereafter, a linear discriminant analysis (LDA) was performed with the remaining 15 variables in order to check the probability of grouping the soil samples without misclassification.

PCA was re-run with the remaining 15 variables. The principal components (PCs) with eigenvalues >1 were selected to interpret the variability in the observations since they explain better the total variation in the dataset than the soil attributes individually, as far as PC with eigenvalues <1 explain less the total variation than the soil attributes individually. The analysis showed that four PCs presented eigenvalues >1 and explained 86.3% of the total variance, in which 64.7% was explained by PC1 and PC2 (Table 2). The contribution of each soil variables to the PCs is presented in table 2, and the ones whose contribution to a PC was greater are highlighted in bold.

The soil correlated attributes were grouped according to their contribution to the respective PC, and the magnitude of the PCs' eigenvalues determined the order that the components, or factors, were interpreted (Table 2). PC1 explained 37.3% of the total variation in the observations and was named "soil acidity factor" because the soil attributes that most contribute to it are BS, pH, Ca^{2+}, Mg^{2+} and H^{+}+Al^{3+}, which are significantly correlated with each other and strongly correlated ($-0.75 \leq r \geq 0.75$) with soil pH. The second PC (PC2) explained 27.4% of the total variance, with major contribution from sand, clay, TOC, K^{+} and available P. However, despite the lack of correlation between clay and available P, clay was considered within this factor due to its high contribution (14.5%). PC2 was named "soil organic carbon factor" because all these soil attributes are related to soil organic matter quality. PC3 explains about 13.9% of the total variability in the observations, being highly contributed by silt and clay, which are strongly correlated to each other ($r = -0.96$). PC4 described approximately 7.7% of the overall variability, with major influence from the significantly correlated soil attributes CEC and SPD. PC3 and PC4 have minor importance in explaining the total variance in the dataset (only 21.6%) and will not be discussed here.

The contribution of the variables to PC1 and PC2 are according to the increasing color gradient and length of the vectors (Figure 2b). TOC, available P and K^{+} are negatively correlated with *q*MIC and SPD; Ca^{2+} is negatively correlated with Al^{3+}; pH, Mg^{2+} and BS are negatively correlated with CEC and H^{+}+Al^{3+}; and clay is negatively correlated to silt and sand (Appendix VI). Silt, *q*MIC and SPD are the variables that present the lowest contribution in explaining the data variability.

PCA revealed differences between the sites based on soil chemical, physical and microbiological properties (Figure 3a). NAF, RAF and RPF were grouped without overlapping, what means that although cultivated in the same soil type, topographic and climate conditions, the conifer species and the understorey composition affected the soil properties differently. The variables more associated with NAF cluster are clay, TOC, K^+ and available P, while the variables negatively correlated to them are more associated with RAF cluster, which are *q*MIC, SPD, silt and sand (Figure 3a).

LDA presented equal prior for all three groups (NAF, RAF and RPF), what means that given the 15 assessed variables, the probability of a soil sample of belonging to the correct site is the same (0.33%), and no misclassification is observed. The percentage separation that is achieved by the first linear discriminant function (LD1), a linear combination of the fifteen variables, was 64.8%, and by the second linear discriminant function (LD2) it was 35.2% (Figure 3b). LD1 is suitable to separate the native sites (NAF and RAF) from the exotic (RPF), while LD2 enable a clear separation of NAF from RAF, and RAF from RPF, but does not permit to separate NAF from RPF since there is some overlapping in the horizontal projection.

Table 2 – Soil attributes contribution for the principal components

	Principal Component (PC)			
	1	**2**	**3**	**4**
Explained Variance (%)	37.3	27.4	13.9	7.7
Eigenvalues	5.6	4.1	2.1	1.2
	Variables contribution on PCs (%)			
pH (H_2O)	**14.5**	1.4	0.5	2.8
Al^{3+}	10.3	0.1	7.7	6.6
$H^+ + Al^{3+}$	**13.7**	0.4	3.5	8.7
TOC	0.4	**15.3**	0.4	2.6
K^+	1.4	**13.4**	5.4	0.2
Ca^{2+}	**14.3**	0.3	0.0	10.2
Mg^{2+}	**14.5**	0.4	0.0	12.7
Available P	0.6	**13.0**	**15.4**	0.8
CEC	9.7	0.6	5.4	**22.0**
BS	**16.7**	0.1	0.0	4.6
Sand	0.4	**20.2**	0.5	1.9
Silt	0.1	8.7	**23.4**	4.8
Clay	0.2	**14.5**	**16.2**	1.6
SPD	2.4	5.7	7.5	**16.8**
*q*MIC	0.7	5.6	**13.9**	3.7

Bold values indicate greater contribution to PCs. H^++Al^{3+} – potential acidity; TOC – total organic carbon; CEC – total cation exchange capacity; BD – soil bulk density; SPD – soil particle density; TSP – total soil porosity; SBR – soil basal respiration; C_{mic} – microbial biomass carbon; *q*CO2 – metabolic quotient; *q*MIC – microbial quotient.

Figure 2 – Principal component analysis (PCA) ordination biplot for chemical, physical and microbiological soil attributes of the three sites, with the twenty observed variables (**a**) and after the exclusion of five variables (**b**)

(**a**) (**b**)

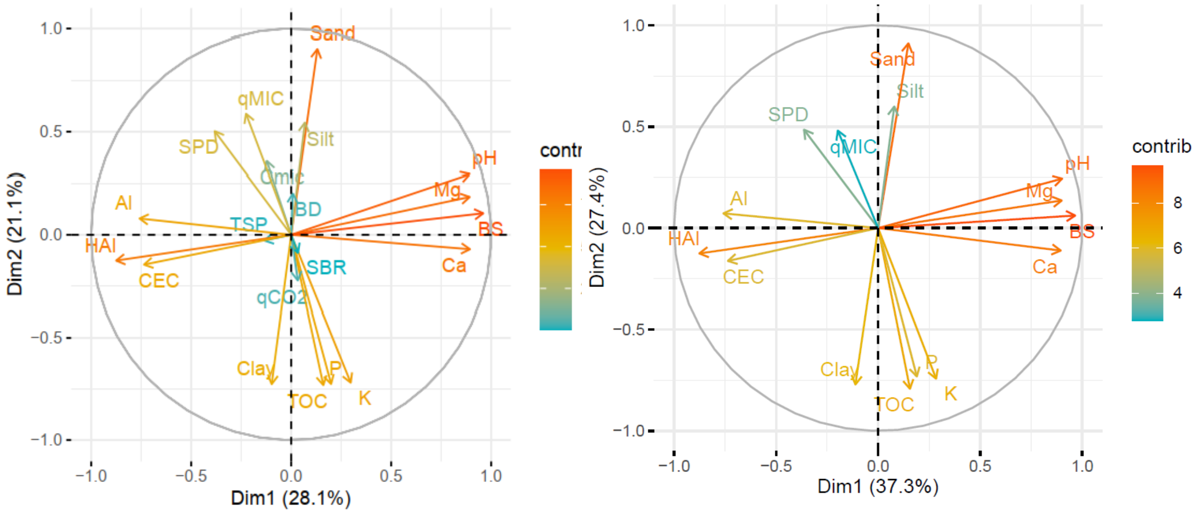
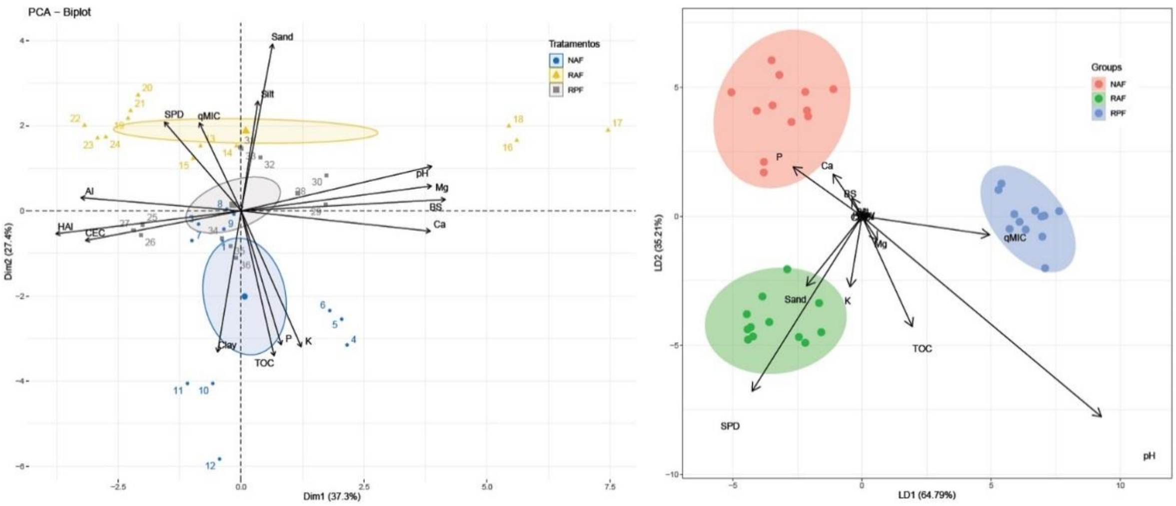


Figure 3 – Clusters of the forest sites native Araucaria Forest (NAF), reforested Araucaria forest (RAF), and reforested Slash Pine forest (RPF) based on PCA (**a**) and LDA (**b**)

(**a**) (**b**)



5.5 Discussion

5.5.1 Implications of species on edaphic changes

In the native Araucaria Forest, the conditions for natural soil functioning are provided, so it can be considered a reference for soil quality. In the reforested sites, most chemical properties were not sensitive to the conifer species (i.e., araucaria and slash pine) (Table 1), except for available P. The available P was higher in the native than in the reforested sites, following the tendency observed by Michelsen et al. (1996) and Malysz et al. (2019). Highly weathered soils, as the Ferralsols in subtropical regions, act as P sink due to the strong adsorption in Al and Fe oxi-hydroxides surfaces (BORTOLUZZI et al., 2015); however, tropical forests immobilize great amounts of P in its biomass (CLEVELÁRIO JUNIOR, 1996) and the litter mineralization is the source of organic P that sustain the forest. In managed environments, as RAF and RPF, the soil disturbance probably promoted an increase in soil organic matter decomposition rates and P immobilization. At FLONA-PF, previous studies show higher K^+ concentrations in the native forest than in araucaria and slash pine plantations (MALYSZ; OVERBECK, 2018; MALYSZ et al., 2019), as well as higher organic matter content (MALYSZ et al., 2019); however, we did not find significant differences among sites for these attributes.

Forest plantations induce alteration of soil constituent proportion, as observed in previous studies (AGNELLI et al., 2016; BORTOLUZZI et al., 2019; KORCHAGIN et al., 2019; KORCHAGIN et al., 2020). Although clay content in the RPF did not differed from the reference NAF site, a reduction on silt content in the RPF site compared to NAF was observed (Table 1, Figure 1). Therefore, as these two attributes are highly correlated (r = -0.96, Appendix VI), it can be suggested that the slash pine had a greater effect on soil weathering expressed by a claying process. As revealed by April and Keller (1990), a greater degree of fragmentation occurs in mineral grains with edges oriented toward root surfaces than in grains with sides faced oppositely to roots. The mechanical forces exerted by roots growth accelerate mineral degradation, exposing the mineral surface to root-induced chemical gradients and, consequently, increasing soil weathering rates. Mineral disaggregation is also induced by microorganisms whose growth is stimulated by compounds, especially fixed carbon, released by photosynthetic members of the symbiosis (BANFIELD et al., 1999)

Regarding the biological response due to conifer plantations, variations in the microbial biomass C (C_{mic}), soil basal respiration (SBS) and metabolic quotient (qCO$_2$) were not observed among the monoculture and the reference site. However, the results do not necessarily mean that there is no influence of the species on soil microbiological activity, but confirm the low-impact management practices and the mature stage of the conifer forests. In fact, the microbial biomass from fungus, bacteria, actinomycetes and algae is the main component of the living soil organic matter, but with shorter cycling time, representing 1-3% of the total organic carbon in tropical soils (SILVA; MENDONÇA, 2007). According to Odum's evolution theory and ecosystem development (ODUM, 1983), as far as the ecosystems move toward an advanced stage, the dependence on new entries decreases and the efficiency of energy and use of nutrients increases. For example, the mycorrhizal networks that connect roots of neighboring trees can positively affect the growth of mature trees (BIRCH et al., 2021) by transferring water, carbon and essential nutrients between trees connected by a net formed by their root systems and associated organism.

Despite variation due to climatic (INSAM, 1990) and edaphic factors, the soil biological activity is a good indicator of ecosystem stability. Basal respiration, a parameter used to evaluate the general activity of microbial biomass, shows that the lowest loss of C as CO_2 through respiration indicates a more efficient microbial biomass (INSAM; DOMSCH, 1988). Metabolic quotient, an index that evaluates the efficiency of the microorganisms' function (INSAM, 1990), shows that the more efficient they are, the greater is the C fraction incorporated into their biomass and the smaller is the loss of C per biomass unit through respiration (BEHERA; SAHANI, 2003).

The relationship between microbial biomass carbon (C_{mic}) and total soil organic carbon (TOC), expressed by qMIC, was the only microbiological variable that differed among the reference NAF (0.8) and the reforested sites (1.0 for RAF, and 1.1 for RPF). As the native forest presents higer diversity of species, with a higher number of individuals in the regenerative stratum (MALYSZ; OVERBECK, 2018), it produces a greater diversity of easier degradation residues, which improve the conditions for the establishment of microbial community (BINI et al., 2013). Also, as NAF is a more balanced ecosystem, besides the lower *q*MIC confirms this, it suggests that organic carbon is better distributed among living and dead fractions of organic matter, and that most of the carbon is found in humic substances, which are more stable. Comparing agricultural and forest areas, the contribution of C_{mic} to the TOC decreases with time more rapidly in forests than in agricultural sites (INSAM; DOMSCH, 1988), what indicates a greater stability of the forest ecosystems.

For evaluating the sustainability of agriculture and land management practices, a big volume of data and the analysis of multiple attributes simultaneously are required since the soil variables individually almost do not bring usable information (DORAN; PARKIN, 1994). In this sense, the significant correlation among several variables and the low level of information that they contain individually are the reasons why multivariate statistical methods were used to investigate the relationships between variables (THIOULOUSE; PRIN; DUPONNOIS, 2012).

PCA showed that the four primary PCs are suitable to interpret the total variability in the observations. PC1, or soil acidity factor, presented greater contribution in explaining the data variability. The soil acidity is related to the capacity of the soil to mobilize and supply nutrients that promote plants growth and the alteration of soil constituents (BORTOLUZZI et al., 2019; HUMMES et al., 2019; KORCHAGIN et al., 2020). Especially in established tree monocultures, a shaded environment with adult trees with low metabolic rates and slower growth, the strategy for nutrient acquisition is the permanent exudation of soil acid organic compounds (RONDINA et al., 2019; SOKOLOVA, 2011). This strategy is accompanied by pH decrease caused by H^+ released for balance charge, leading to weathering through dissolution of minerals (CAMA; GANOR, 2015; LAZO; DYER; ALORRO, 2017; KORCHAGIN et al., 2019).

PC2, the soil organic carbon factor, is dominated by variables significantly correlated to TOC and associated with the NAF cluster. The total amount of organic carbon is originated mainly from residues of animals and plants, from soil microbial carbon and soil humus (MARTENS; REEDY; LEWIS, 2004). In most soils, the fine mineral fraction is determinant on soil organic matter storage and stabilization (WIESMEIER et al., 2019) due to the organic matter ability to form chemical bonds with particles that present large specific superficial area, such as silt and clay (SILVA; MENDONÇA, 2007). Soils enriched with Al and Fe oxides or phyllosilicates (clay minerals), such as the Ferralsol, have a greater potential to preserve TOC than soils dominated by primary minerals like quartz and felspars (YEASMIN et al., 2017). Through the mineralization of organic matter, an important biochemical process, nutrients are released into the soil and supplied to plants (MARINARI et al., 2010), such as the essentials potassium (TURPAULT; RIGHI; UTÉRANO, 2008) and phosphorus (CHEN, 2003).

5.5.2 Conifer plantations as a strategy of ecosystem sustainability

In a scenario of increasing demand for wood, maintaining or increasing the soil fertility is the major objective of a sustainable forest management (RANGER; TURPAULT, 1999). However, the preservation of biological diversity has emerged as an objective in forest management due to its increasing loss (ZAVALA; ORIA, 1995). According to these authors, three goals must be achieved to obtain sustainable forest practices: (*i*) to perpetuate forest cover being economically profitable, (*ii*) to preserve ecosystem structure in terms of biodiversity, and (*iii*) to preserve ecosystem function in nutritional terms. In this sense, low-impact practices such as small stand size, reduced tillage, selection and retention systems, longer rotation cycle length, reduced impact logging, steam removal instead of whole-tree harvesting have to be considered. Although suitability of forest practices is highly site dependent, the tree harvesting method is the most important silvicultural practice to not compromise long-term sustainability since essential nutrients such as N and P are very sensitive to overexploitation and nutrient reserves may be strongly negatively affected by whole-tree removal (BLANCO et al., 2005).

According to the United Nations Organization, sustainably manage forests and halt biodiversity loss are among the 17 Sustainable Development Goals (SDGs) to be reach in order to promote prosperity while protecting the planet. To incentive tree plantations with native species, such as araucaria in South Brazil, instead of exotics, goes along with the goals that are expected (*i*) to end all forms of poverty (SDGs 1, 2 and 3) considering that its seeds are edible and source of income mainly for smallholder farmers; (*ii*) to propose renewable energy (SDG 7) because its wood is highly appreciated by the industry; (*iii*) to tackle climate change (SDG 13) and; (*iv*) to value and protect all forms of life on earth (SDGs 14 and 15) since araucaria plantations allow the recolonization with other native species, thus ensuring the soil heterogeneity and the maintenance of original properties (MALYSZ; OVERBECK, 2018) besides hosting Araucaria Forest biodiversity (FONSECA et al., 2009; MALYSZ; OVERBECK, 2018).

To encourage revegetation of areas, included degraded ones, with native species that present slower growth potential and longer-term financial return, such as araucaria, in spite of exotics with faster economical return, such as slash pine, is a big challenge when only general soil properties are considered since both species are able to maintain them in quite similar conditions compared to Araucaria Forest fragments. However, differences exist as was clearly discriminated by multivariate analyses. The plantations established in the past with araucaria and (mostly) with the non-natives slash pine and eucalyptus, changed the forest composition and the natural ecosystem dynamics. In general, monospecific tree plantations in the Araucaria Forest ecosystem present loss of overall diversity compared to the natural environment (FONSECA et al., 2009; PAZ et al., 2015; PEREIRA et al., 2020). The maintenance of species and functional diversity under native rather than under exotic plantations is the major point in favor of the native (PAZ et al., 2015). Thereafter, the exploitation of native forests and plantations on a sustainable basis may allow for sustainable forest management in the long-term besides offering immediate income opportunities (CUBBAGE et al., 2007).

The guidelines of the Brazilian National Forests are aligned with FAO's principles (FAO, 2014) of sustainable use of forest resources (ICMBio, 2011). Low tree density and frequency of thinning, with reduced impact of logging are among the adopted practices to ensure sustained yields (MALYSZ; OVERBECK, 2018). Pesticide-free production allows achieving the environmental health predicted in the OMS One health approach (FAO, 2008) since we live in a world of microbiomes, and in this sense, sustainable agriculture is a feasible associated practice (BAPTESTE et al., 2021). Land use modifications and deforestation lead to a loss of biodiversity and are expected to increase the pest infestations in urban areas and the risk of many emerging infectious disease (MORRIS et al., 2016). Gradual improvement of the soil quality is obtained over time (HARIPAL; SAHOO, 2013), and long-term rotations are in compliance with FLONA's practices of sustainable multiple use.

5.6 Conclusion

The properties of a weathered Ferralsol of a subtropical humid region, when individually assessed, were slightly affected by monocultural plantations of *Araucaria angustifolia* (native) and *Pinus elliottii* var. *elliottii* (exotic) in a Mixed Ombrophilous Forest ecosystem and managed for a sustainable multiple use. The soil cultivated with slash pine is more impacted than the araucaria soil as a claying process was observed, which suggests increasing weathering rates. Multivariate analysis clearly discriminates among native Araucaria Forest, reforested Araucaria forest and reforested Slash Pine forest soils, evidencing edaphic changes due to conifers species. Principal Components Analysis (PCA) explains most of variability between soil. Soil acidity factor (pH_{H2O}, $H^+ + Al^{3+}$, Ca^{2+}, Mg^{2+} and base saturation) and soil organic carbon factor (total organic carbon, K^+, available P, sand and clay), are the best indicators to discriminate soil changes due to vegetal substitution over time. The present study also highlights the importance of the Brazilian National Forests managed for sustainable multiple use in protectig the natural ecosystems and the biodiversity, and to show that it is possible to increase the areas cultivated with forest monocultures in order to meet the Sustainable Development Goals established by the United Nations and the OMS One Health approach.

6 FINAL CONSIDERATIONS

Soils, whether natural or cultivated, are an open system resulting from the complex interaction between mineral and live worlds (MEUNIER; BORTOLUZZI; MEXIAS, 2016). In the rhizosphere, where processes occur in higher rates compared to the surrounding bulk soil, to assess geochemical and mineralogical evolution is a good approach to detect and monitor the short-term impact of trees on soil. Although studies on soil physic-chemical characteristics under slash pine and araucaria are reported in the literature, implications of monocultures with these conifers on soil geochemical and mineralogical evolution under tropical and subtropical climates are missing, but of great relevance to ensure the sustainability of timber production, especially in areas considered the new agricultural frontier in the southern hemisphere.

In Brazil, the total area planted with exotic woody species such as *Pinus* spp. that started in the 70’s has been increasing, reaching 1,6 million ha in 2018. Contrastingly, only around 13 thousand hectares were planted with the woody *Araucaria angustifolia* (IBA, 2019). This native long-live pioneer species is characterized by its imposing size, high wood value and appreciable edible seeds; however, its population decline has been resulting in loss of diversity due to fragmentation and habitat reduction, causing disruption on soil ecosystem (HARTLEY, 2002; ISLAM; WEIL, 2000; FONSECA et al., 2009; SILVA; SCHMITT, 2015; USHARANI; ROOPASHREE; NAIK, 2019).

In an attempt to preserve the remaining fragments of native forest and specimens, Brazil has established the strictest environmental laws and the largest required reserves of natural forests among South America countries (Argentina, Chile, Uruguay and Brazil) and the South United States (CUBBAGE et al., 2007). Despite the restricted rules on the conservation and use of the Atlantic Forest Biome (Law No. 11,428/06), together with inspection, have reduced deforestation and the araucaria cutting (SCHNEIDER et al., 2018), they also discouraged the planting of this species because of the requirements and applied sanctions, which do not occur when the choice is for planting the exotics *Pinus* spp. and *Eucalyptus* spp.

The *World Commission on Environmental and Development* defines sustainable development as the “development that meets the needs of the present without compromising the ability of future generations to meet their own needs”, where natural systems that support life on Earth such as the atmosphere, the water, the soils and the living beings must not be endangered (BRUNDTLAND, 1987). In this context, to preserve and monitor the soil quality, defined as “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health” (DORAN; PARKIN, 1994), is crucial. It is not a matter of just replacing the entire established forest base with exotic species by native trees, but of make the requirements more flexible and really encourage monocultures with native woody species, such as araucaria, in order to match timber production with conservation.

7 GENERAL CONCLUSIONS

The present study highlights the potential of conifers to change soil physical-chemical and mineralogical characteristics and intensify the soil weathering. In the meta-analysis study, it was found that the ability of conifers and monocultures to alter the soil properties is greater than broadleaved and naturally established trees in mixed forest fragments. The higher fertility of the mixed forest soils compared to monoculture soils is probably related to the greater diversity of species, with different root systems and soil mining skils in addition to higher nutrient cycling. The meta-analysis also presented a gap in the study of conifer soils in tropical and subtropical regions.

The exotic *Pinus elliottii* var *elliottii* presents a greater potential in changing the general characteristics of the rhizosphere compared to the native *Araucaria angustifolia*. The most affected soil attributes detected by univariate statistical analysis were particle-size fractions, microbial quotient, total organic carbon and available phosphorus; however, the multivariate statistics clearly discriminate slash pine from araucaria by the assessed soil properties, which suggest that this is a great tool to detect and monitor soil changes under plantations of different conifers. The mineralogical evolution of the clay fraction in the rhizosphere and bulk soil of both conifers evidence a slight claying process in slash pine soil, followed by complete illite dissolution and increase of kaolinite proportion in clay assemblage, suggesting an acceleration of weathering rates and the increasing risk of soil quality loss when monocultures of this conifer is established in high weathered soils of humid subtropical regions. *Araucaria angustifolia* also promoted changes in soil properties compared to the species grown in its native environment, although in lower proportions than slash pine.

In south Brazil, monoculture with the exotics, fast-growing and low-density woody species *Pinus* spp. and *Eucalyptus* spp. initiated in the early 1970 as an alternative to the increasing demand and decline of the native long-lived pioneer conifer *Araucaria angustifolia* due to its uncontrolled exploitation and slow growth of the plantations. The introduction of exotic species in an ecosystem, as well as the management practices, must be preceded by multidisciplinary studies of environmental impact, at different scales, to understand the potential of environmental changes and to find alternatives to mitigate them. The present study shows that studies on the rhizosphere of trees are a feasible strategy to detect and monitor the short-term impact of long-lived woody species on soil, which can be further investigated especially on the interactions between microorganisms and clay minerals.

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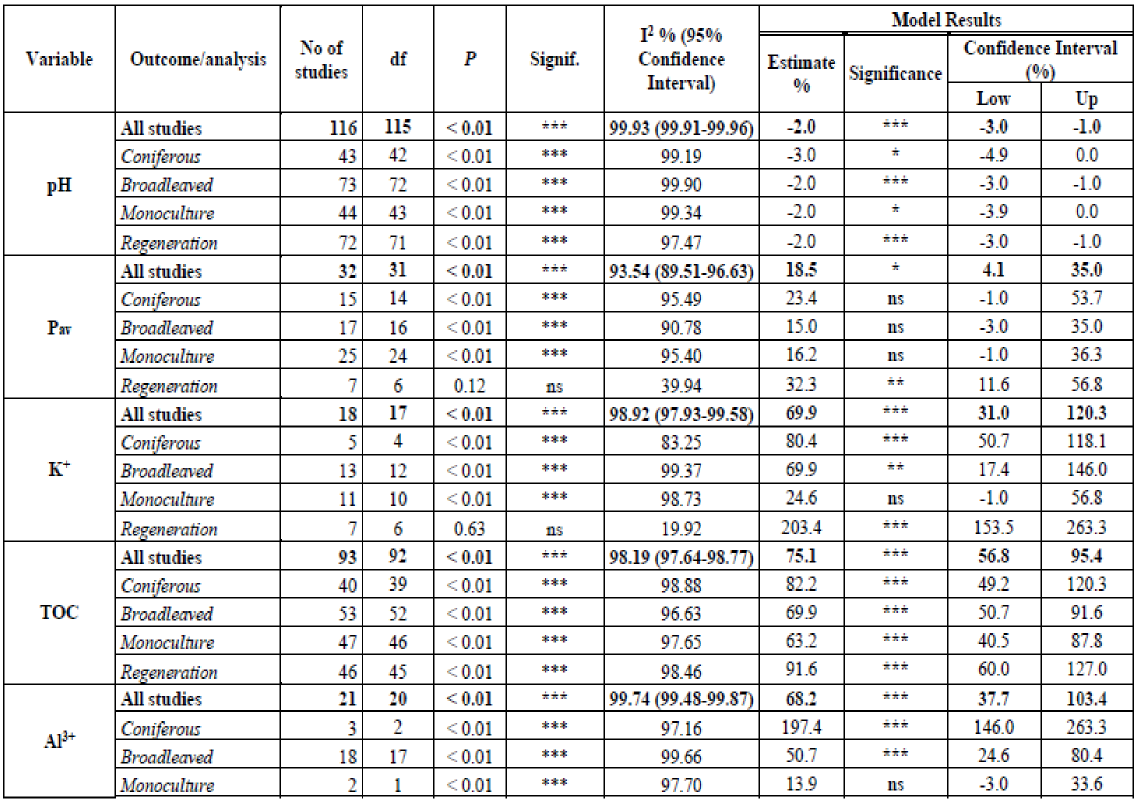
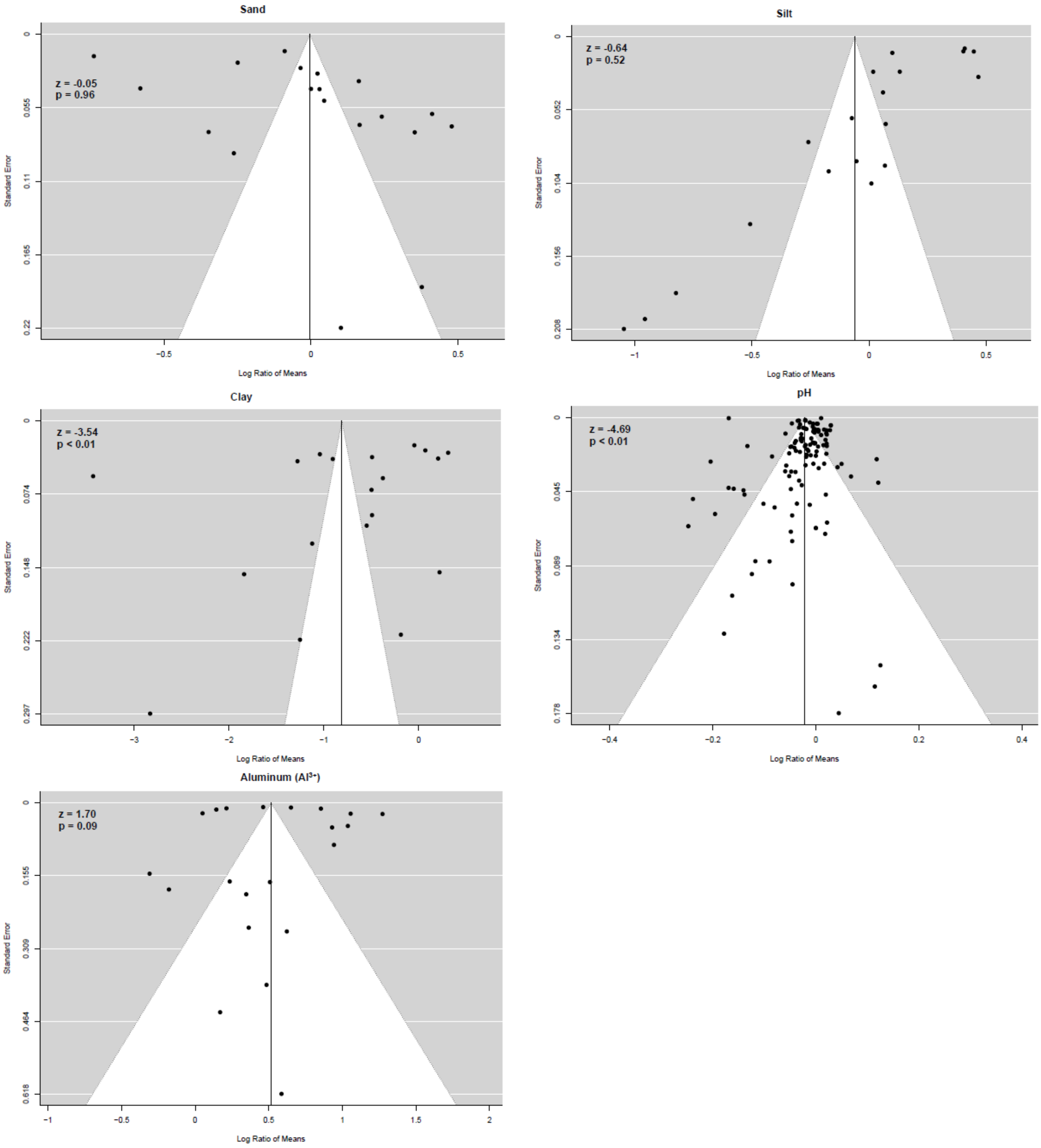
APPENDICES

Appendix I. Supplemental table

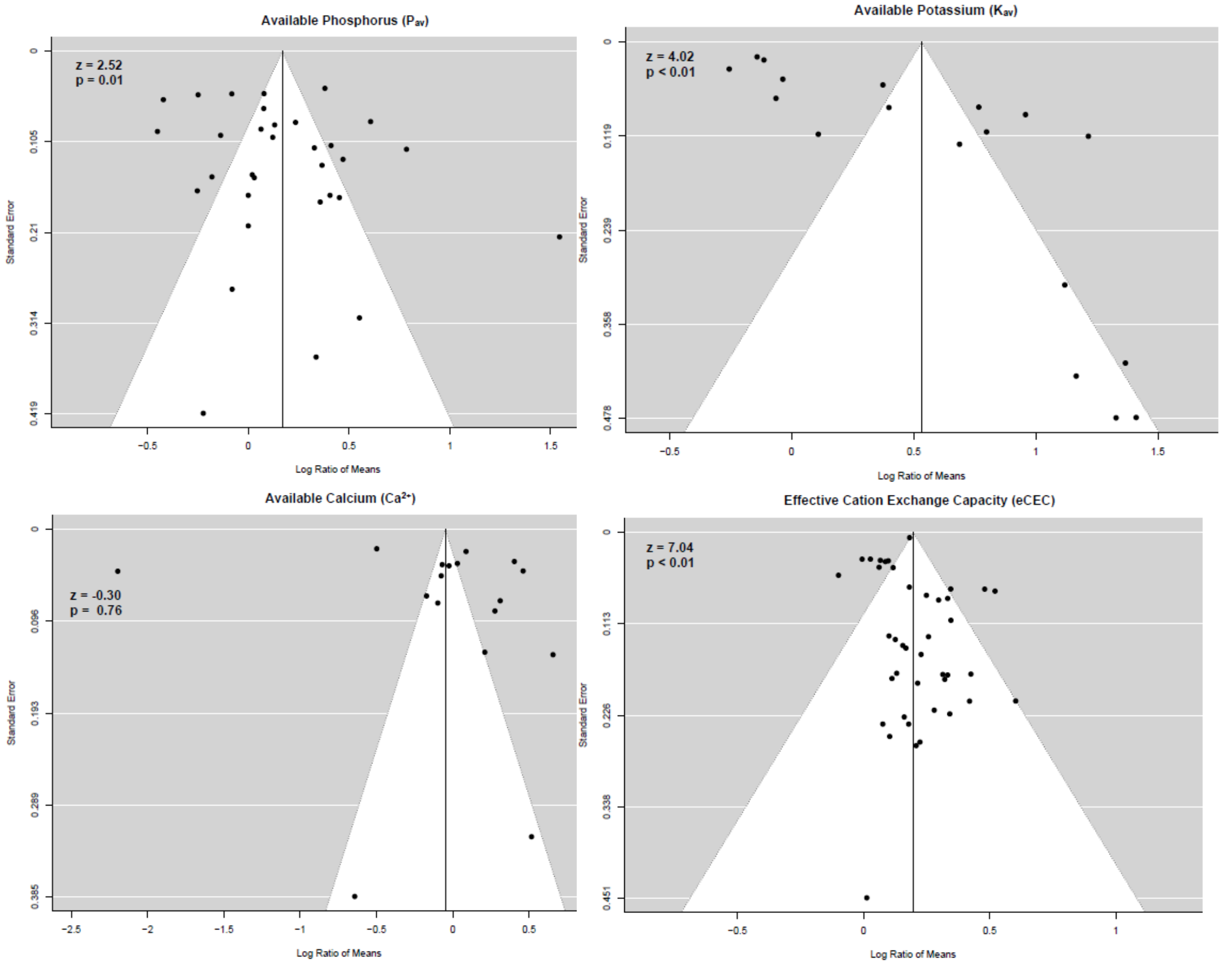


Variable	Outcome/analysis	No of studies	df	*P*	Signif.	I^2 % (95% Confidence Interval)	Model Results			
							Estimate %	Significance	Confidence Interval (%)	
									Low	Up
pH	**All studies**	**116**	**115**	**< 0.01**	***	**99.93 (99.91-99.96)**	**-2.0**	***	**-3.0**	**-1.0**
	Coniferous	43	42	< 0.01	***	99.19	-3.0	*	-4.9	0.0
	Broadleaved	73	72	< 0.01	***	99.90	-2.0	***	-3.0	-1.0
	Monoculture	44	43	< 0.01	***	99.34	-2.0	*	-3.9	0.0
	Regeneration	72	71	< 0.01	***	97.47	-2.0	***	-3.0	-1.0
P_{av}	**All studies**	**32**	**31**	**< 0.01**	***	**93.54 (89.51-96.63)**	**18.5**	*	**4.1**	**35.0**
	Coniferous	15	14	< 0.01	***	95.49	23.4	ns	-1.0	53.7
	Broadleaved	17	16	< 0.01	***	90.78	15.0	ns	-3.0	35.0
	Monoculture	25	24	< 0.01	***	95.40	16.2	ns	-1.0	36.3
	Regeneration	7	6	0.12	ns	39.94	32.3	**	11.6	56.8
K^+	**All studies**	**18**	**17**	**< 0.01**	***	**98.92 (97.93-99.58)**	**69.9**	***	**31.0**	**120.3**
	Coniferous	5	4	< 0.01	***	83.25	80.4	***	50.7	118.1
	Broadleaved	13	12	< 0.01	***	99.37	69.9	**	17.4	146.0
	Monoculture	11	10	< 0.01	***	98.73	24.6	ns	-1.0	56.8
	Regeneration	7	6	0.63	ns	19.92	203.4	***	153.5	263.3
TOC	**All studies**	**93**	**92**	**< 0.01**	***	**98.19 (97.64-98.77)**	**75.1**	***	**56.8**	**95.4**
	Coniferous	40	39	< 0.01	***	98.88	82.2	***	49.2	120.3
	Broadleaved	53	52	< 0.01	***	96.63	69.9	***	50.7	91.6
	Monoculture	47	46	< 0.01	***	97.65	63.2	***	40.5	87.8
	Regeneration	46	45	< 0.01	***	98.46	91.6	***	60.0	127.0
Al^{3+}	**All studies**	**21**	**20**	**< 0.01**	***	**99.74 (99.48-99.87)**	**68.2**	***	**37.7**	**103.4**
	Coniferous	3	2	< 0.01	***	97.16	197.4	***	146.0	263.3
	Broadleaved	18	17	< 0.01	***	99.66	50.7	***	24.6	80.4
	Monoculture	2	1	< 0.01	***	97.70	13.9	ns	-3.0	33.6

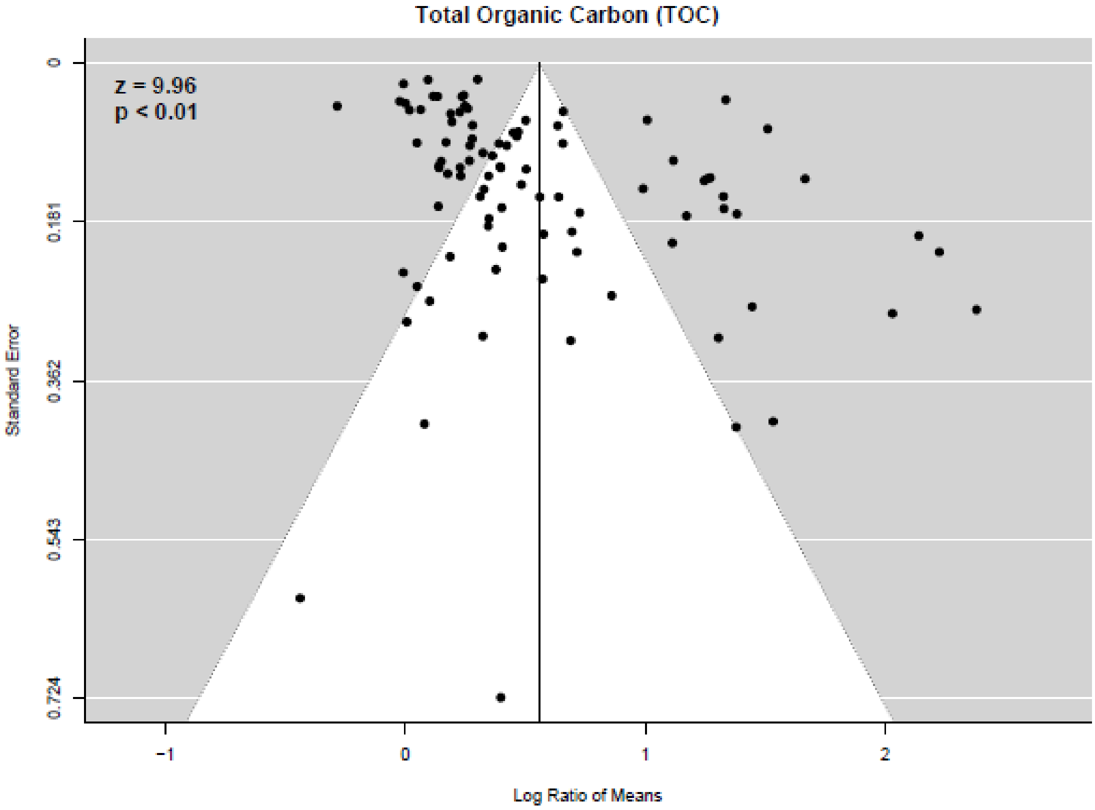
Appendix II. Funnel plots of effect sizes versus standard errors for sand, silt, clay, pH and Al^{3+}



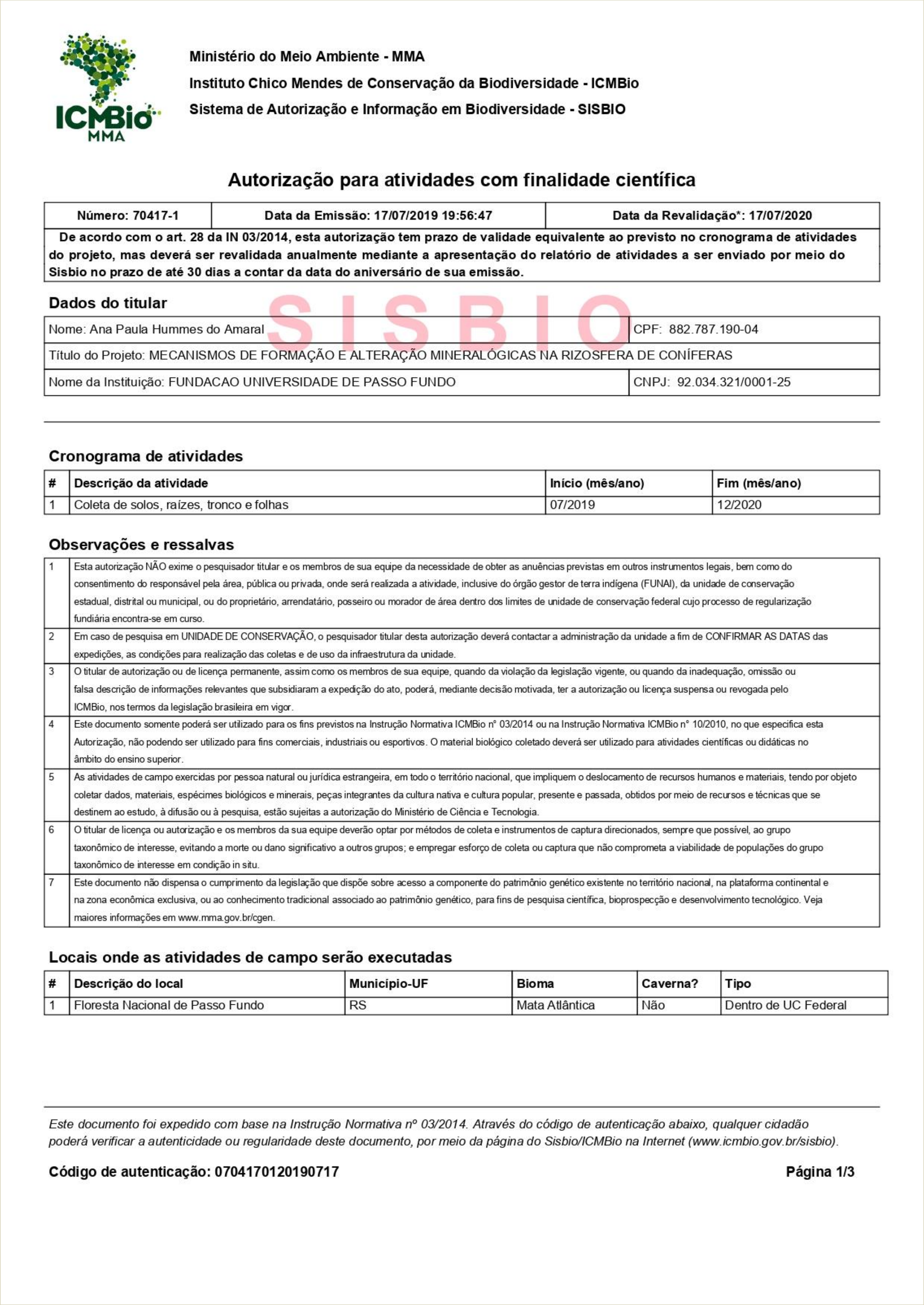
Appendix III. Funnel plots of effect sizes versus standard errors for P_{av}, K_{av}, Ca^{2+} and eCEC



Appendix IV. Funnel plots of effect sizes versus standard errors for TOC



Appendix V – Authorization for research at FLONA-PF (page 1)



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Autorização para atividades com finalidade científica

Número: 70417-1	**Data da Emissão: 17/07/2019 19:56:47**	**Data da Revalidação*: 17/07/2020**

De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: Ana Paula Hummes do Amaral	CPF: 882.787.190-04
Título do Projeto: MECANISMOS DE FORMAÇÃO E ALTERAÇÃO MINERALÓGICAS NA RIZOSFERA DE CONÍFERAS	
Nome da Instituição: FUNDACAO UNIVERSIDADE DE PASSO FUNDO	CNPJ: 92.034.321/0001-25

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Coleta de solos, raízes, tronco e folhas	07/2019	12/2020

Observações e ressalvas

1	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
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Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Floresta Nacional de Passo Fundo	RS	Mata Atlântica	Não	Dentro de UC Federal

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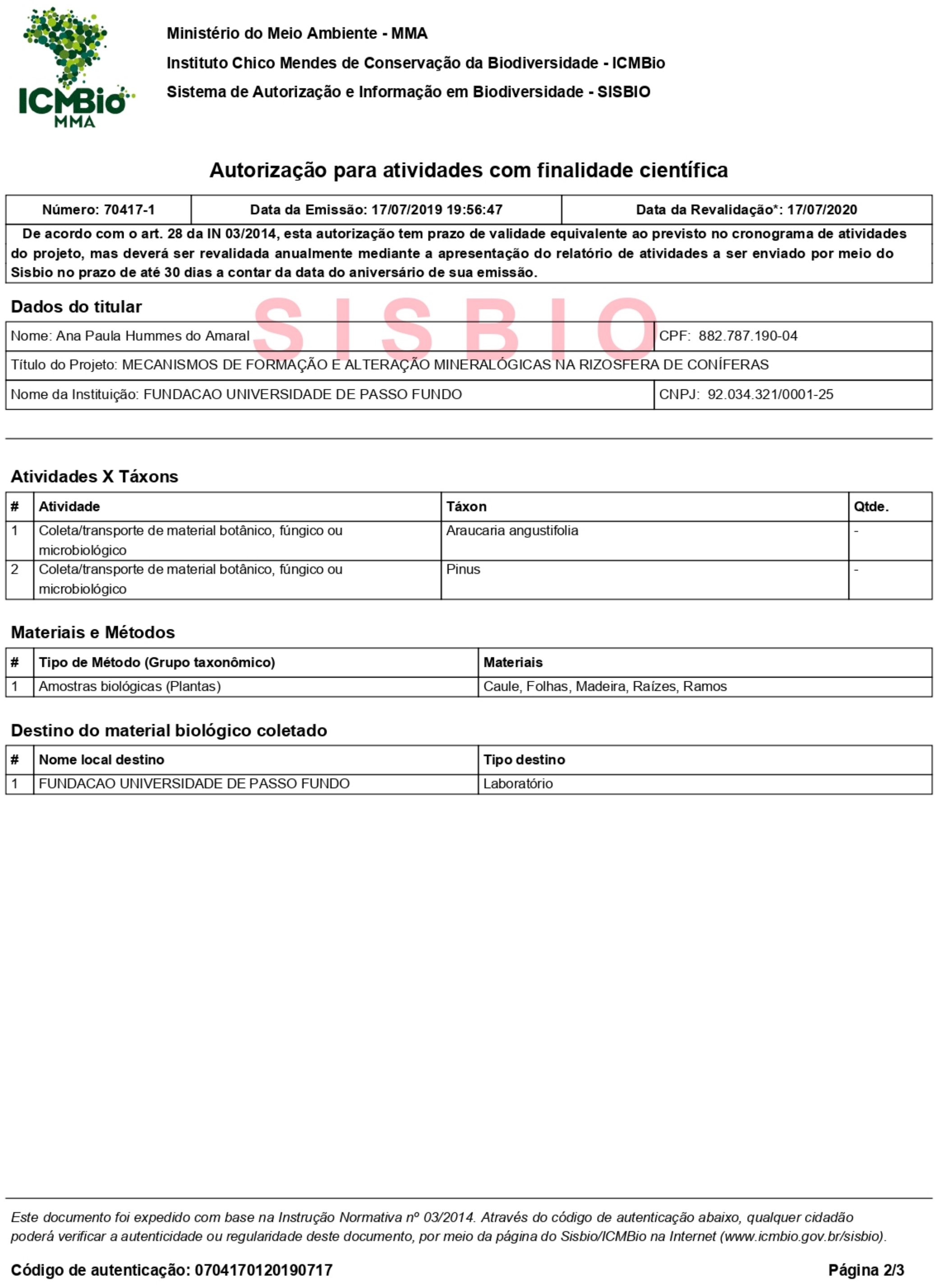
Appendix V – Authorization for research at FLONA-PF (page 2)



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Dados do titular

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Nome da Instituição: FUNDACAO UNIVERSIDADE DE PASSO FUNDO	CNPJ: 92.034.321/0001-25

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de material botânico, fúngico ou microbiológico	Araucaria angustifolia	-
2	Coleta/transporte de material botânico, fúngico ou microbiológico	Pinus	-

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Plantas)	Caule, Folhas, Madeira, Raízes, Ramos

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	FUNDACAO UNIVERSIDADE DE PASSO FUNDO	Laboratório

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Appendix V – Authorization for research at FLONA-PF (page 3)

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Nome da Instituição: FUNDACAO UNIVERSIDADE DE PASSO FUNDO	CNPJ: 92.034.321/0001-25

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	**Qtde.**	**Tipo de Amostra**	**Qtde.**	**Data**

* Identificar o espécime do nível taxonômico possível.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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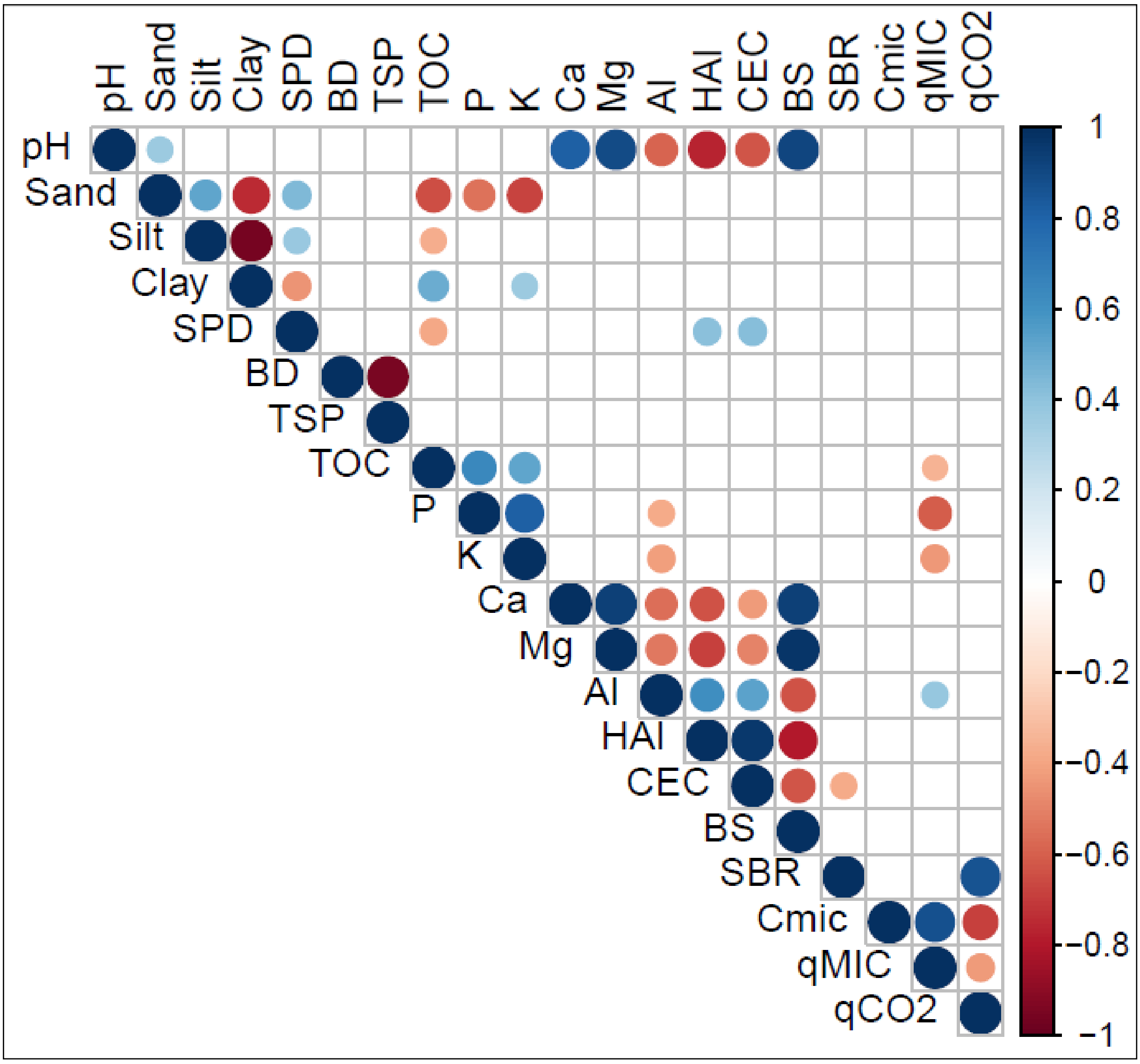
Appendix VI – Pearson correlation, *r* (significant at $p < 0.05$)



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