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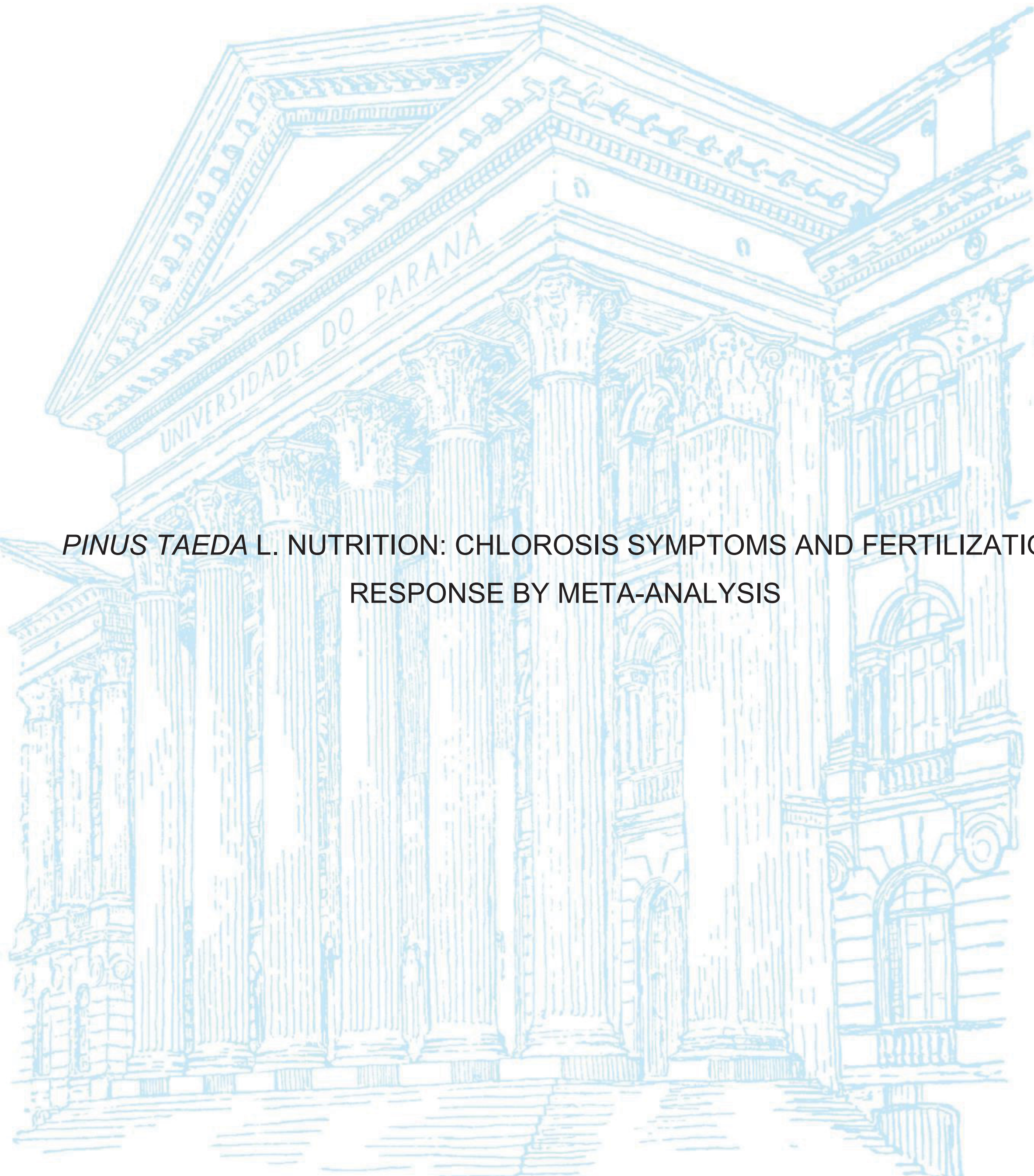

PINUS TAEDA L. NUTRITION: CHLOROSIS SYMPTOMS AND FERTILIZATION RESPONSE BY META-ANALYSIS

CURITIBA

2023

VALDÉCIO DOS SANTOS RODRIGUES

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Tese apresentada ao curso de Pós-Graduação em Ciência do Solo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciência do Solo.

Orientador: Prof. Dr. Antônio Carlos Vargas Motta

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIA DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **VALDÉCIO DOS SANTOS RODRIGUES** intitulada: ***PINUS TAEDA* L. NUTRITION: CHLOROSIS SYMPTOMS AND FERTILIZATION RESPONSE BY META-ANALYSIS** , sob orientação do Prof. Dr. ANTONIO CARLOS VARGAS MOTTA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 15 de Fevereiro de 2023.

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A mim por nunca desistir, buscando
forças para ser resiliente, e continuar
mesmo diante das adversidades.

DEDICO.

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RESUMO

Florestas de pinus (*Pinus taeda* L.) exibindo clorose foliar e morte de árvores após eventos climáticos abruptos têm sido relatadas por empresas florestais no Sul do Brasil nos últimos anos e não se sabe se existe efeito direto com a fertilidade do solo e nutrição das árvores. Para tanto, a tese foi estruturada em 2 capítulos com os seguintes objetivos gerais: i) investigar as propriedades do solo e a nutrição mineral das plantas que possam estar relacionadas a esse distúrbio; ii) realização de uma meta-análise das Américas com base em 44 publicações (1970-2022) de fertilização do *P*. *taeda* em condições de campo. Para avaliar a causa da clorose, cinco pares (áreas controle e cloróticas) de sítios florestais de idade semelhante foram selecionados para análises de amostras de solo, raízes, serapilheira, acículas e discos de madeira. Os solos avaliados são oriundos de fontes ígneas (quatro sítios) e sedimentares (um sítio). Em solos de origem ígnea, sintomas cloróticos foram associados a maior fertilidade do solo e tecidos vegetais (acícula, madeira e casca) com maiores concentrações de nutrientes em relação às áreas controle, diferentemente dos solos de origem sedimentar, onde as áreas cloróticas apresentaram baixa fertilidade do solo, menores concentrações de nutrientes nos tecidos, principalmente magnésio (Mg). Ao avaliar o efeito da fertilização através da metanálise, observou-se que em geral, a fertilização aumentou a produção da madeira com destaque para a adubação com resíduos orgânicos em relação à adubação mineral. Maiores respostas à fertilização foram observadas em solos arenosos, sendo que a aplicação de fertilizantes no plantio (< 1 ano) ou em árvores estabelecidas (2-8 anos) resultou em respostas semelhantes na produção de madeira com maiores incrementos ocorrendo em solos arenosos. Com base em nossa revisão, inferimos que a ‘clorose de *P*. *taeda* ' observada por empresas florestais brasileiras no Sul do Brasil é muito semelhante ao ‘declínio do *P*. *taeda*’ que tem ocorrido em outros países. Assim, concluímos que este é o primeiro relato de declínio de *P*. *taeda* no Brasil. Em relação à meta-análise, o presente estudo revelou respostas do *P*. *taeda* a estratégias de aplicação contrastantes, que podem ajudar a identificar práticas eficientes de manejo da fertilidade do solo para esta espécie de árvore comercialmente significativa.

Palavras-chave: *Pinus taeda* L. Clorose de acículas. Morte de árvores. Solo florestal. Fertilização do pinus.

ABSTRACT

Pine (*Pinus taeda* L.) forests exhibiting leaf chlorosis and tree death after abrupt weather events have been reported by forestry companies in Southern Brazil in recent years and it is not known whether there is a direct effect on soil fertility and tree nutrition. Therefore, the thesis was structured in 2 chapters with the following general objectives: i) to investigate soil properties and mineral nutrition of plants that may be related to this disturbance; ii) carrying out a meta-analysis of the Americas based on 44 publications (1970-2022) on *P. taeda* fertilization under field conditions. To assess the cause of chlorosis, five pairs (control and chlorotic areas) of forest sites of similar age were selected for analysis of samples of soil, roots, litter, needles and wood disks. The evaluated soils come from igneous sources (four sites) and sedimentary sources (one site). In soils of igneous origin, chlorotic symptoms were associated with higher fertility of soil and plant tissues (needle, wood and bark) with higher concentrations of nutrients compared to control areas, unlike soils of sedimentary origin, where chlorotic areas had low fertility soil, lower concentrations of nutrients in tissues, mainly magnesium (Mg). When evaluating the effect of fertilization through meta-analysis, it was observed that, in general, fertilization increased wood production, with emphasis on fertilization with organic residues in relation to mineral fertilization. Greater responses to fertilization were observed in sandy soils, with fertilizer application at planting (< 1 year) or to established trees (2-8 years) resulting in similar responses in wood production with greater increases occurring in sandy soils. Based on our review, we infer that the '*P. taeda* chlorosis' observed by Brazilian forest companies in Southern Brazil is very similar to the '*P. taeda* decline' that has occurred in other countries. Thus, we conclude that this is the first report of *P. taeda* decline in Brazil. Regarding the meta-analysis, the present study revealed *P. taeda* responses to contrasting application strategies, which may help to identify efficient soil fertility management practices for this commercially significant tree species.

Keywords: *Pinus taeda* L.. Needle chlorosis. Tree death. Forest soil. Pine fertilization.

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SUMMARY

1 GENERAL INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is the primary forest species planted in southern Brazil, with an estimated area of 1.6 million hectares (VASQUES *et al.*, 2007; WREGE *et al.*, 2014; IBÁ 2019). This species is fast growing and highly productive (average of 31 m^3 ha^{-1} $year^{-1}$) under regional edaphoclimatic conditions characterized by cold weather (low temperatures and high occurrence of frost), acidic soils, and sloping relief (CARDOSO *et al.*, 2013; KOHLER *et al.*, 2015; GOMES *et al.*, 2016; IBÁ 2019).

Large-scale pine plantations in Brazil began in the 1960s and is currently in the third rotation at several locations (VASQUES *et al.*, 2007; BATISTA *et al.*, 2015). Most planted areas had previously been exploited by the agricultural sector or occupied by fields of native grasses and secondary forests under soil conditions that were generally of low natural fertility (PIOVESAN *et al.*, 2012; BATISTA; *et al.*, 2015; GOMES *et al.*, 2016). Since fertilization and soil acidity correction were not conducted in the vast majority of these pine production areas, the low fertility soils have been further depleted over time (GATIBONI *et al.*, 2020). On the other hand, other areas of high natural fertility in the states of Santa Catarina and Rio Grande do Sul that have eruptive rocks as source material, and were also used for planting pine trees, mainly in conditions of high slope, shallow soils or with occurrence of stonyness.

Pinus taeda originated in the southeastern United States of America (USA) and has been experiencing a disorder known as decline for at least 40 years. *Pinus taeda* decline is a complex tree disorder characterized by symptoms that include crown defoliation, needle chlorosis, reduced growth, and some cases of premature tree mortality (ECKHARDT *et al.*, 2007). Eckhardt *et al.* (2007) observed that symptomatic sites of *P. taeda* decline had smaller amounts of fine roots. Biotic and abiotic factors and their interactions are suggested as probable causes for the onset of Pinus decline (ECKHARDT; MENARD 2008; ECKHARDT *et al.*, 2010), i.e., collectively, pine forest decline is poorly understood yet. Gea-Izquierdo *et al.* (2014) found a relationship between water stress and *P. sylvestris* decline, while Eckhardt *et al.* (2008) observed a direct relationship between land slope and occurrence of *P. taeda* decline.

Effects of soil fertility and pine nutrition have also been examined. In pine decline areas, Brunson (2013) reported higher concentrations of soil organic carbon, macronutrients [calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P)] and micronutrients [manganese (Mn), zinc (Zn), and molybdenum (Mo)] in at least one evaluated soil depth (0–10, 10–20, and 20–30 cm). This same study also found higher macronutrient [nitrogen (N), sulfur (S), Mg, and Ca], micronutrient [boron (B), Zn, Mn, iron (Fe), and copper (Cu)], and aluminum (Al) levels in first and second flush pine needles. Coyle *et al.* (2020) indicated that soil factors such as erosion, granulometry, organic matter level, acidity, and drainage could be related to P. *taeda* decline in the southern USA.

The plateau region of Santa Catarina is characterized by a subtropical climate, with abundant precipitation, which leads to high base leaching, resulting in soils with low pH, high Al and Mn content, which can cause toxicity to plants and negatively affect growth and the proper development of planted species (ALMEIDA *et al.*, 2019). Although *P*. *taeda* develops well in these soils, some studies have shown symptoms of nutritional deficiency and response of the species to fertilization (MORO *et al.*, 2014; BATISTA *et al.*, 2015). However, there are few works relating the decline of *P*. *taeda* with plant nutrition, more precisely deficiency and/or nutritional imbalance.

Snowfall in the winter of 2013 (uncommon scenario for *P. taeda* forests in the region), followed by a long drought in 2014, influenced in the appearance of chlorosis, crown loss, and tree death at some sites in southern Brazil and was considered one of the most intense in recent years (FINKE *et al.*, 2020; MOLLMANN JUNIOR *et al.*, 2021).

Chaves *et al.* (2005) suggested a severe deficiency of Ca and Mg as a possible cause of chlorosis and death of *P. caribaea* trees in tropical areas of Brazil. Magnesium deficiency has also been reported in *P. taeda* (MOTTA *et al.*, 2014; RODRIGUES *et al.*, 2021). Nutritional imbalance between Mn and Fe (especially with high values of Mn) has also been reported in *P. taeda* plantations in southern Brazil (REISSMANN; WISNIEWSKI, 2000).

In Brazilian planted pine environments, soil nutrient depletion suggests a need for implementing fertilizer and lime applications (SIXEL *et al.*, 2015; GATIBONI *et al.*, 2020).

Nutrient application should be directed towards elements limiting (macro and micronutrient) pine growth, which can vary widely depending on soil parent material and can be strongly influenced by soil attributes such as texture and native fertility. Fertilization can further the greater growth of trees with a large root system and greater nutrient uptake capacity.

An integrated evaluation of factors influencing fertilization efficiency is a complex and onerous task when conducted using conventional experimentation. However, several investigations have taken an integrative approach of evaluating plant responses to different management practices through meta-analysis of data from the published literature base (BARBOSA *et al.*, 2022a, 2022b; MARIOTTI *et al.*, 2022). For example, a meta-analysis study evaluating the effects of liming and wood ash on forest ecosystems showed that wood ash provided a greater increase in growth and wood production compared to liming alone (REID; WATMOUGH, 2014). Fertilizing forests can help to get more answers about pine decline in areas with poorer soils.

The general objective of this work was to investigate whether soil properties and nutrient concentrations are involved in the occurrence of pine decline in the state of Santa Catarina- Brazil and, in parallel, a meta-analysis was conducted to evaluate the efficiency of fertilization on production and nutritional status of *P*. *taeda* under growing conditions in America.

2 CHAPTER I: FIRST NUTRITIONAL DIAGNOSIS OF *PINUS TAEDA* L. WITH CHLOROSIS IN THE SOUTHERN REGION OF BRAZIL

2.1 ABSTRACT

Lobolly pine (*Pinus taeda* L.) forests exhibiting foliar chlorosis and tree death following abrupt climatic events have been reported by forestry companies in southern Brazil in recent years. The objective of this work was to investigate soil properties and plant mineral nutrition that might be related to this disorder. Five pairs (control and chlorotic areas) of forest sites of similar age were selected for analyses of soil, root, litter, needle, and timber disc samples. The evaluated soils were derived from igneous (four sites) and sedimentary (one site) parent source material. On igneous derived soils, chlorotic symptoms were associated with higher soil fertility and plant tissues (needle, wood and bark) with higher nutrient concentrations as compared to control areas. Plant tissue concentrations of manganese (Mn) and calcium (Ca) were higher for chlorotic areas, and needle chlorosis was characterized by basal paling of needles. The lack of topsoil (A horizon) was also a relevant characteristic of chlorotic areas. On the sedimentary derived soil, chlorotic areas had low soil fertility, lower tissue nutrient concentrations, particularly magnesium (Mg), and needle tip chlorosis. There was no difference in litter biomass between chlorotic and control areas. Findings suggest two distinct conditions promoting pine chlorosis in southern Brazil: i) high fertility soils may be nutritionally imbalanced due to high levels of Mn and/or Ca; ii) low fertility soils highlighted by large Mg deficiency.

Keywords: *Pinus taeda* L. Needle chlorosis. Tree death. Forest soil. Forest nutrition

2.2 INTRODUCTION

Large-scale pine plantations in Brazil began in the 1960s and is currently in the third rotation at several locations (VASQUES *et al.*, 2007; BATISTA *et al.*, 2015). Loblolly pine is fast growing and highly productive (average of 31 m^3 ha^{-1} $year^{-1}$) under regional edaphoclimatic conditions characterized by cold weather (low temperatures and high occurrence of frost), acidic soils, and sloping relief (CARDOSO *et al.*, 2013; KOHLER *et al.*, 2015; GOMES *et al.*, 2016; IBÁ 2019).

Most planted areas had previously been exploited by the agricultural sector or occupied by fields of native grasses and secondary forests under soil conditions that were generally of low natural fertility (PIOVESAN *et al.*, 2012; BATISTA *et al.*, 2015; GOMES *et al.*, 2016).

Loblolly pine originated in the southeastern United States of America (USA) and has experienced a disorder known as '*Pinus taeda* decline' for at least 40 years. This decline is a complex tree disorder characterized by symptoms that include crown defoliation, needle chlorosis, reduced growth, and some cases of premature tree mortality (ECKHARDT *et al.*, 2007). Eckhardt *et al.* (2007) observed that symptomatic sites of *P*. *taeda* decline had smaller amounts of fine roots. Biotic and abiotic factors and their interactions are suggested as likely causes for the onset of the decline (ECKHARDT; MENARD 2008; ECKHARDT *et al.*, 2010), i.e., the phenomenon is yet poorly understood. Gea-Izquierdo *et al.* (2014) found a relationship between water stress and *P. sylvestris* decline, while Eckhardt *et al.* (2008) observed a direct relationship between land slope and occurrence of *P*. *taeda* decline.

Chaves *et al.* (2005) suggested a severe deficiency of calcium (Ca) and magnesium (Mg) as a possible cause of chlorosis and death of *P. caribaea* trees in tropical areas of Brazil, while Mg deficiency has also been reported for *P. taeda* (MOTTA *et al.*, 2014; ADAM *et al.*, 2021). Nutritional imbalance between manganese (Mn) and iron (Fe) (especially with high values of Mn) has also been reported for *P*. *taeda* plantations in southern Brazil (REISSMANN; WISNIEWSKI, 2000). Snowfall in the winter of 2013 (uncommon scenario for *P*. *taeda* forests in the studied region) followed by a long-term drought in 2014, resulted in the appearance of needle chlorosis, crown loss, and tree death in some sites in southern Brazil; this damage is considered one of the most intense in recent years (FINKE *et al.*, 2020; MOLLMANN JUNIOR *et al.*, 2021).

We hypothesized that the '*P. taeda* chlorosis' observed by Brazilian forestry companies is related to low soil fertility due to natural conditions combined with nutrient exhaustion. The objective of this work was to investigate soil properties and tree mineral nutrition that might be related to *P*. *taeda* chlorosis and death in plateaus of three watersheds in southern Brazil.

2.3 MATERIAL AND METHODS

2.3.1 STUDY SITES

Loblolly pine areas with (chlorotic) and without (control) symptoms of chlorosis previously identified by forestry companies were assessed for homogeneity prior to selection of study sites. Experimental plots representing three regional watersheds were located in plateaus of the state of Santa Catarina, Brazil (FIGURE 1). The regional climate, according to the Köppen climate classification (ALVARES *et al.*, 2013), is mesothermal humid subtropical (Cfb), with well-distributed rainfall (1,494 mm), frequent frosts, and average summer temperature around 17.1 °C. Within each watershed, paired areas (chlorotic and control) were selected; within each pair, seedling origin, tree age, and forest management were the same. There were four sites that had igneous derived soils and one site with sedimentary derived soil, i.e. a total of five pairs of experimental areas (TABLE 1; FIGURE 1).

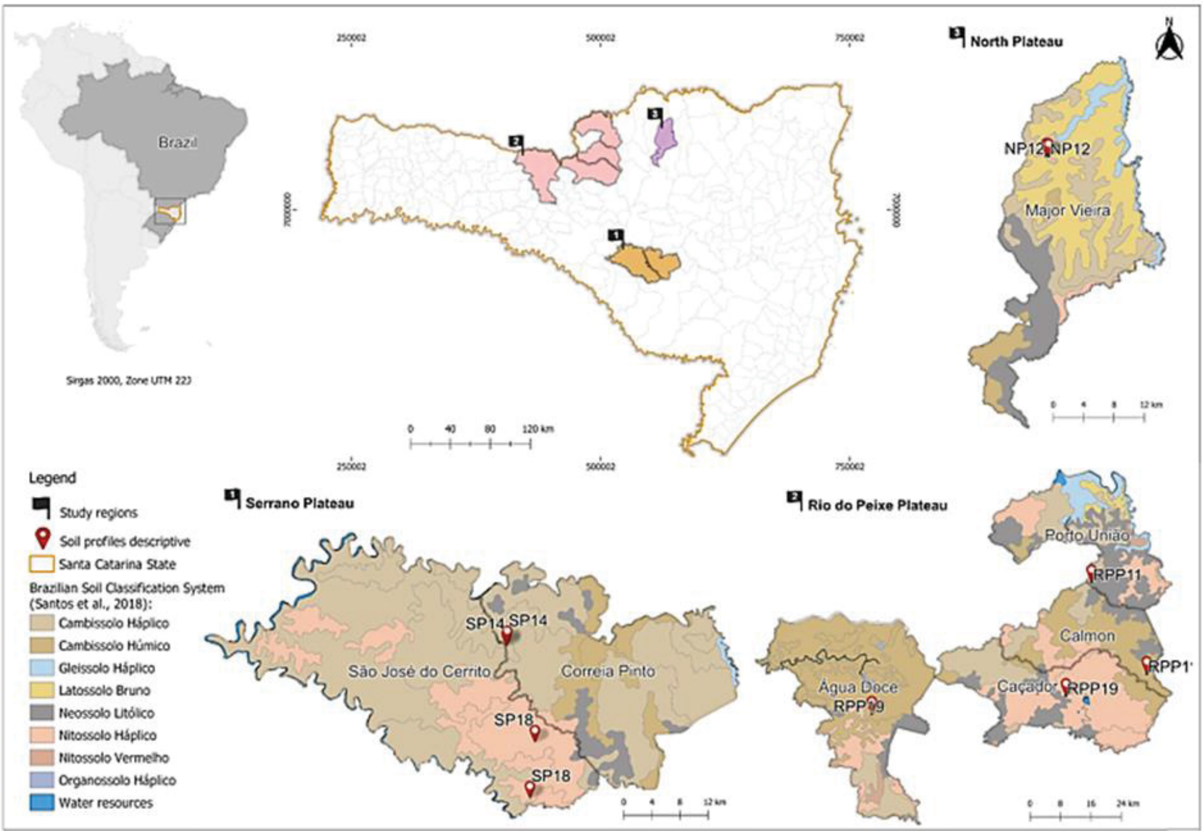
TABLE 1- DESCRIPTION (REGION, LOCATION, PINE AGE, ALTITUDE AND SOIL PARENT MATERIAL) OF PAIRED STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL.

Site	Region	Age (years)	Code	Municipality	Area	Altitude	Parent	DHB (Cm)
1	Rio do Peixe Plateau	19	RPP19	Caçador	Chlorotic	1069	Igneous	27,63
				Água Verde	Control	1308	Igneous	21,55
2	Rio do Peixe Plateau	11	RPP11	Calmon	Chlorotic	1140	Igneous	15,63
				Caçador	Control	1168	Igneous	14,18
3	Serrano Plateau	14	SP14	Correia Pinto	Chlorotic	981	Igneous	23,38
				Correia Pinto	Control	976	Igneous	25,38
4	Serrano Plateau	18	SP18	São José do	Chlorotic	893	Igneous	27,5
				São José do	Control	1002	Igneous	33,08
5	North Plateau	12	NP12	Major Vieira	Chlorotic	740	Sediment	18,45
				Major Vieira	Control	805	Sediment	20,38

SOURCE: The author (2021).

LEGEND: RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years. DBH: Diameter at breast height.

FIGURE 1- LOCATION OF SITES USED FOR SOIL SAMPLING IN THE STATE OF SANTA CATARINA, BRAZIL. RPP19 – RIO DO PEIXE PLATEAU, AGE 19; RPP11 – RIO DO PEIXE PLATEAU, AGE 11; SP14 – SERRANO PLATEAU, AGE 14; SP18 – SERRANO PLATEAU, AGE 18; AND NP12 – NORTH PLATEAU, AGE 12 YEARS



SOURCE: The author (2022).

2.3.2 SOIL AND PLANT SAMPLINGS

Soil and plant tissues were sampled at each experimental area in two stages. The first sampling was performed to characterize the soil profile horizons and for classification of soil taxonomy. Each soil profile location was georeferenced during this sampling. Throughout the second stage, samples (soil, root, and litter) were randomly collected in each area. Litter biomass was sampled randomized below the canopy of four trees using a 20 × 20 cm template, totaling four samples per area (FIGURE 2). Once litter was removed, soil samples were collected from the 0–10 cm soil layer for evaluating fine root biomass (diameter ≤ 2 mm). Soil samples (for fertility assessment) were collected in the same previous local (where litter was sampled) at two soil depths (0–10 and 10–20 cm), totaling four samples per area at each depth.

FIGURE 2- NEEDLES, LITTER AND SOIL SAMPLES

SOURCE: The author (2019).

Four trees selected randomized near the local of soil sampling in each area were harvested and eight branches from the middle and upper third of the crown were collected. Needle samples from the upper third of the crown were divided into first and second flush. All sampled biomass were stored in plastic bags and transported to the laboratory. Trunk disc samples (5 cm thick) were collected from harvested trees at six positions along the trunk: base, breast height (1.3 m), and 25%, 50%, 75%, and 100% of relative commercial height and four trees were collected from each area (FIGURE 3). Commercial height was considered the total trunk minus portions less than 8 cm diameter.

FIGURE 3- TRUNK DISC SAMPLES

SOURCE: The author (2020).

2.3.3 SOIL CHEMICAL AND PHYSICAL ANALYSES

Soil samples were oven dried at 65 ºC for 48 h, grounded, and passed through a 2 mm sieve. Soil chemical properties evaluated were: potential acidity (H+Al) (0.5 mol L^{-1} calcium acetate extractant, pH 7.0); soil pH in 0.01 mol L^{-1} $CaCl_2$ (soil:solution ratio = 1:2.5) and KCL extractant; exchangeable Ca, Mg and Al (1 mol L^{-1} KCl extractant); available K, P, Fe, Mn, Cu and Zn (Mehlich-I extractant), and soil organic carbon (SOC) according to methods described by Marques and Motta (2003); and total N according to Teixeira *et al.* (2017). Soil granulometry (sand, silt, and clay) was determined by the Bouyoucos densimeter method (GEE; OR, 2002).

2.3.4 PLANT TISSUE ANALYSIS

Needle samples were oven dried at 65 ºC for 72 h and ground in a Wiley mill to pass through a 2 mm sieve (FIGURE 4). Needle digestion was performed by dry combustion process. Produced ashes were diluted with 0.01 mol L^{-1} HCl supplemented with deionized water to determine Ca, Mg, P, K, Al, Mn, Fe, Zn, Cu, and nickel (Ni) concentrations (MARTINS; REISSMAN, 2007) using an inductively coupled plasma optical emission spectrometer (ICP-OES; Varian, 720-ES).

Trunk disc samples were dried at room temperature and separated into bark and wood. Afterwards, bark samples were completely crushed. Wood subsamples were obtained along the surface of the disc using a drill and collecting spall from drilled holes (FIGURE 4). Tissue digestion and analytical processes were identical to those described above for needles; however, analysis of boron (B) (by ICP-OES) was also included.

FIGURE 4- TRUNK DISC ANALYSIS

SOURCE: The author (2020).

2.3.5 STATISTICAL ANALYSIS

Experimental data were submitted to the Shapiro-Wilk's normality test, followed by analysis of variance (ANOVA) based on a completely randomized design with four replications. In our analysis, the 'chlorotic' and 'control' areas were considered factor 1 for comparing the pair of areas by site and age, while factor 2 considered sampled location sites in order to evaluate differences among the five sites (individually within the treatment 'chlorotic' or 'control') for soil chemical properties, nutrient tissue levels, and dry biomass of root, 100 fascicles weight and litter (Tukey at 5% significance). To better visualize clustering of chlorotic areas, principal components analysis (PCA) and discriminant analysis were performed to classify elements of a group. Excel® was used to organize and compose the data, which were then processed by the R Studio software for statistical analysis.

2.4 RESULTS

2.4.1 SOIL HORIZON CHARACTERIZATION

The chlorotic area of the 19-year-old Rio do Peixe Plateau also showed a more basic parent material source, a less developed A horizon, greater relief slope, more stoniness and rockiness when compared to the control area. Both areas had low effective soil depth (TABLE 2).

Pair areas (chlorotic and control) of the 11-year-old Rio do Peixe Plateau (RPP11) showed contrasting characteristics (TABLES 4 and 5). The chlorotic area had more basic parent material source, more undulating relief, more stoniness, and a less developed A horizon compared to the control area.

The pair of areas (chlorotic and control) of the 14-year-old Serrano Plateau (SP14) site showed differences regarding parent material source, type of surficial horizon, granulometry (TABLE 6). Therefore, more basic parent material source, soil with more clay, and eroded horizons were characteristics of areas with chlorosis. The absence of topsoil (A horizon) in the chlorotic SP14 site (due to erosion) indicates the occurrence of stress (TABLE 6).

For the 18-year-old Serrano Plateau (TABLES 8 and 9), differences between chlorotic and control areas were related to parent material source (chlorotic – basic intermediate igneous × control – intermediate igneous), relief (chlorotic – strong undulate × control – wavy to soft wavy), and stoniness (chlorotic – stony to extremely × control – not stony to extremely).

Chlorotic areas derived from igneous rock had a predominance of basic material (basalt), while acid and intermediate igneous rock predominated in control areas, as indicated by total Fe_2O_3 values (APPENDIX 1.2). In general, more undulating relief with greater rockiness and less developed or eroded A horizons were characteristics of chlorotic areas compared to those of the control.

On the other hand, in the 12-year-old North Plateau (TABLES 10 and 11) area derived from sedimentary parent material source (clay-siltite), there were slight differences between chlorotic and control areas in terms of effective soil depth and relief slope.

TABLE 2- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE NITOSSOLO VERMELHO EUTROFÉRRICO CAMBISSÓLICO IN AN AREA WITH CHLOROSIS (19 YEARS), CAÇADOR-SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^{+}	SB	Al^{3}	$H+Al^{3+}$
	Cm	g kg^{-1}					cmolc kg^{-1}					
Ap	0 – 20	50	60	420	470	0,89	8,73	1,67	0,38	10,78	0,15	3,24
Bt1	20 – 50	20	30	220	730	0,3	5,71	1,46	0,28	7,45	0,91	4,27
Bt2	50 - 100+	20	30	190	760	0,25	4,88	1,4	0,31	6,59	1,29	3,38
Horizon	**Layer**	**pH**		**P**	**SOC**	**N total**	**C/N**	**V**	**m**	**CEC_{ef}**	**CEC_{c}**	**Textural class**
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		%		cmol$_c$ kg^{-1}		
Ap	0 – 20	5,5	4,75	0,43	21,35	3,68	5,8	77	1	10,93	20,22	silty clay
Bt1	20 – 50	5	4,19	0,19	10,61	2,18	4,9	64	11	8,36	11,28	Very clayey
Bt2	50 - 100+	5,4	4,34	0,72	5,8	1,34	4,3	66	16	7,88	10,51	Very clayey

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_{c}: cation exchange capacity of clay.

TABLE 3- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE NEOSSOLO REGOLÍTICO HÚMICO LÉPTICO IN AN AREA WITHOUT CHLOROSIS (19 YEARS), ÁGUA VERDE-SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^{+}	SB	Al^{3}	$H+Al^{3+}$
	Cm		g kg^{-1}				cmolc kg^{-1} -					
Ap	0 – 30	50	30	440	480	0,92	0,1	0,13	0,0657	0,3	5,27	16,76
Cr	30 – 65	30	160	520	290	1,79	0,1	0,1	0,0003	0,2	6,76	15,51
R ou RCr	65+	-	-	-	-	-	-	-	-	-	-	-
Horizon	**Layer**	**pH**		**P**	**SOC**	**N total**	**C/N**	**V**	**m**	**CEC_{ef}**	**T**	**Textural class**
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		%		cmol$_c$ kg^{-1}		

Ap	0 – 30	4,2	3,93	1,63	32,48	4,24	7,7	2	95	5,57	20,92	silty clay
Cr	30 – 65	4,4	4,09	1,51	4,23	0,8	5,3	1	97	6,96	52,27	silty clay loam

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; T: cation exchange capacity of clay.

TABLE 4- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE CAMBISSOLO HÁPLICO TB DISTROFÉRRICO NITOSSÓLICO IN AN AREA WITH CHLOROSIS (11 YEARS), CÁLMON-SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^+	SB	Al^3	$H+Al^{3+}$
							---cmolc kg^{-1}---					
	Cm		---g kg^{-1}---							-		
Ap	0 – 20	110	90	300	500	0,6	8,57	1,44	0,25	10,26	0,12	2,93
Bt	20 – 50	70	60	230	640	0,36	7,65	1,55	0,12	9,32	0,11	2,71
Bi	50 - 100+	30	50	270	650	0,42	1,64	0,75	0,1	2,49	2,49	7,05

Horizon	Layer	pH		P	SOC	N total	C/N	V	m	CEC_{ef}	CEC_c	Textural class
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		---%---		$cmol_c$ kg^{-1}		
Ap	0 – 20	5,4	4,8	1,02	21,11	3,22	6,6	78	1	10,38	16,89	clayey
Bt	20 – 50	5,6	4,75	0,22	10,79	2,2	4,9	77	1	9,43	13,94	Very clayey
Bi	50 - 100+	5,1	4,19	1,36	2,73	1,1	2,5	26	50	4,98	13,45	Very clayey

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_c: cation exchange capacity of clay.

TABLE 5- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE CAMBISSOLO HÚMICO DISTRÓFICO LÉPTICO IN AN AREA WITHOUT CHLOROSIS (11 YEARS), CAÇADOR-SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^+	SB	Al^3	$H+Al^{3+}$
	Cm	g kg^{-1}					cmolc kg^{-1}					
Ap	0 – 40	20	40	330	610	0,54	0,1	0,18	0,1	0,38	5,1	16,21
Bi	40 – 75	20	40	190	750	0,25	0,1	0,1	0,03	0,23	4,95	14,03
BC	75 – 85	-	-	-	-	-	-	-	-	-	-	-
CR	85+	-	-	-	-	-	-	-	-	-	-	-
Horizon	**Layer**	**pH**		**P**	**SOC**	**N total**	**C/N**	**V**	**m**	**CEC_{ef}**	**CEC_c**	**Textural class**
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		%		$cmol_c$ kg^{-1}		
Ap	0 – 40	4,3	3,97	1,51	28	3,42	8,2	2	93	5,48	14,62	Very clayey
Bi	40 – 75	4,5	3,96	0,14	5,92	1,34	4,4	2	95	5,18	16,36	Very clayey

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_c: cation exchange capacity of clay.

TABLE 6- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE NITOSSOLO VERMELHO DISTROFÉRRICO CAMBISSÓLICO IN AN AREA WITH CHLOROSIS (14 YEARS), CORREIA PINTO -SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^+	SB	Al^3	$H+Al^{3+}$
							cmolc kg^{-1}					
	Cm	g kg^{-1}								-		
Bt	0 – 50	40	90	200	670	0,3	0,85	0,15	0,08	1,08	4,72	15,68
BC	50 – 80	70	80	260	590	0,44	0,72	0,11	0,05	0,88	3,84	10,99
Cr	80 – 100+	70	70	380	480	0,79	0,77	0,11	0,05	0,93	2,78	7,53
Horizon	**Layer**	**pH**		**P**	**SOC**	**N total**	**C/N**	**V**	**m**	**CEC_{ef}**	**CEC_c**	**Textural class**

	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		---------------%--------------		cmol$_c$ kg^{-1}		
Bt	0 – 50	4,2	3,87	0,94	1,4	2,4	5,9	6	81	5,8	18,65	Very clayey
BC	50 – 80	4,4	3,89	0,52	0,9	1,24	7,1	7	81	4,72	16,14	Clay
Cr	80 – 100+	4,3	4,04	0,96	0,4	0,88	4,5	11	75	3,71	15,81	Clay

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_c: cation exchange capacity of clay.

TABLE 7- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE LATOSSOLO BRUNO DISTRÓFICO HÚMICO IN AN AREA WITHOUT CHLOROSIS (14 YEARS), CORREIA PINTO -SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^{+}	SB	Al^{3}	$H+Al^{3+}$
	Cm	-------------------g kg^{-1}-------------------					---------------cmolc kg^{-1}---------------					
Ap	0 – 30	60	350	120	470	0,26	2,96	0,31	0,06	3,33	1,79	7,12
BA	30 – 50	80	300	140	480	0,29	1,24	0,11	0,04	1,39	2,74	7,96
Bw	50 – 100+	90	280	80	550	0,15	0,43	0,1	0,03	0,56	2,85	6,89
Horizon	**Layer**	**pH**		**P**	**SOC**	**N total**	**C/N**	**V**	**m**	**CEC_{ef}**	**CEC_c**	**Textural class**
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		---------------%--------------		cmol$_c$ kg^{-1}		
Ap	0 – 30	4,8	4,2	1,27	18,1	2,06	8,8	32	35	5,12	14,09	Clay
BA	30 – 50	4,6	4,07	0,55	16,7	1,6	10,4	15	66	4,13	11,96	Clay
Bw	50 – 100+	4,5	4,09	0,34	7,8	1,04	7,5	8	83	3,41	10,05	Clay

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_c: cation exchange capacity of clay.

TABLE 8- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE CAMBISSOLO HÁPLICO TB DISTRÓFICO TÍPICO IN AN AREA WITH CHLOROSIS (18 YEARS), SÃO JOSÉ DE CERRITO -SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^+	SB	Al^3	$H+Al^{3+}$
	Cm	g kg^{-1}					cmolc kg^{-1}					
Ap	0 – 20	40	80	250	630	0,4	2,3	0,79	0,07	3,16	1,77	6,59
Bi	20 – 50	40	70	250	640	0,39	0,45	0,22	0,05	0,72	2,75	8,42
C	50 - 100+	20	60	280	640	0,44	0,1	0,1	0,05	0,25	3,47	8,8
Horizon	**Layer**	**pH**		**P**	**SOC**	**N total**	**C/N**	**V**	**m**	**CECef**	**CEC_c**	**Textural class**
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		%		$cmol_c$ kg^{-1}		
Ap	0 – 20	5,2	4,21	0,46	18,5	2,36	7,8	32	36	4,93	7,16	Very clayey
Bi	20 – 50	4,7	4,11	0,61	12,9	1,56	8,3	8	79	3,47	8,46	Very clayey
C	50 - 100+	4,5	4,11	0,52	6,9	1,06	6,5	3	93	3,72	11,04	Very clayey

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_c: cation exchange capacity of clay.

TABLE 9- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE NEOSSOLO REGOLÍTICO HÚMICO LÉPTICO FRAGMENTÁRIO IN AN AREA WITHOUT CHLOROSIS (18 YEARS), SÃO JOSÉ DE CERRITO -SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^+	SB	Al^3	$H+Al^{3+}$
	Cm	g kg^{-1}					cmolc kg^{-1}					
Ap	0 – 30	50	50	270	630	0,43	1,23	0,27	0,07	1,57	3,95	14,19
RCr	30+	-	-	-	-	-	-	-	-	-	-	-
Horizon	**Layer**	**pH**		**P**	**CO**	**N total**	**C/N**	**V**	**m**	**CECef**	**CEC_c**	**Textural class**
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		%		$cmol_c$ kg^{-1}		
Ap	0 – 30	4,9	3,97	1,51	20,9	2,76	7,6	10	71	5,52	15,6	very Clayey

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; T: cation exchange capacity of clay.

TABLE 10- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE NEOSSOLO REGOLÍTICO TB ALUMÍNICO TÍPICO IN AN AREA WITH CHLOROSIS (12 YEARS), MAJOR VIEIRA -SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^+	SB	Al^3	$H+Al^{3+}$
	Cm		g kg^{-1}				cmolc kg^{-1}					
Ap	0 – 50	10	40	310	640	0,48	0,1	0,1	0,07	0,27	9,46	26,14
C	50 – 75	30	40	320	610	0,52	0,1	0,1	0,07	0,27	6,81	20,02
Cr	75 – 100+	40	40	620	300	2,07	0,1	0,1	0,06	0,26	4,92	9,51

Horizon	Layer	pH		P	CO	N total	C/N	V	m	CECef	CEC_c	Textural class
	Cm	H_2O	KCl	mg dm^{-3}	g kg^{-1}	g kg^{-1}		%		$cmol_c$ kg^{-1}		
Ap	0 – 50	4,2	3,69	1,18	16,94	2,4	7,1	1	97	9,73	33,64	Very clayey
C	50 – 75	4,3	3,82	0,46	6,5	0,34	19,1	1	96	7,08	30,35	Very clayey
Cr	75 – 100+	4,7	4,14	1,3	3,02	0,68	4,4	3	95	5,18	31,2	silty clay loam

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CEC_{ef}: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_c: cation exchange capacity of clay.

TABLE 11- CHEMICAL ATTRIBUTES AND PARTICLE SIZE OF THE CAMBISSOLO HÁPLICO TB ALUMÍNICO TÍPICO IN AN AREA WITHOUT CHLOROSIS (12 YEARS), MAJOR VIEIRA -SC

Horizon	Layer	Coarse sand	Fine sand	Silt	Clay	Silt/clay	Ca^{2+}	Mg^{2+}	K^{+}	SB	Al^{3}	$H+Al^{3+}$
	Cm	----------g kg^{-1}----------					----------cmolc kg^{-1}----------					
Ap	0 – 20	10	70	370	550	0,67	0,1	0,1	0,08	0,28	8,59	22,12
BA	20 – 50	10	100	290	600	0,48	0,1	0,1	0,07	0,27	8,45	16,95
Bi	50 – 80	10	90	260	640	0,41	0,1	0,1	0,05	0,25	6,74	18,73
BC	80 – 100+	10	90	280	620	0,45	0,1	0,1	0,06	0,26	5,45	14,35

Horizon	Layer	pH		P	CO	N total	C/N	V	m	CECef	CEC_c	Textural class
								----------%----------				
	Cm	H_2O	KCl	mg dm³	g kg^{-1}	g kg^{-1}		-		cmol$_c$ kg^{-1}		
Ap	0 – 20	3,8	3,67	1,7	18,97	2,8	6,8	1	97	8,87	32,19	Clay
BA	20 – 50	4,1	3,78	0,76	16,07	2,36	6,8	2	97	8,72	21,47	Clay
Bi	50 – 80	4	3,9	0,37	12,47	1,72	7,3	1	96	6,99	24,05	Very clayey
BC	80 – 100+	4,2	3,98	0,31	7,77	1,46	5,3	2	95	5,71	20,06	Very clayey

SOURCE: The author (2023).

LEGEND: - : Not analyzed; SB: Sum of bases; pH: pH in water (soil: solution ratio = 1:2.5); CECe: Effective cation exchange capacity at current soil pH; SOC: Soil organic carbon; N: Soil total nitrogen; V: Base saturation; m: Al saturation; CEC_c: cation exchange capacity of clay.

2.4.2 SOIL CHEMICAL PROPERTIES

Soils were acidic regardless of region or depth with very low pH (~3.0) found mainly in the North Plateau site (NP12) (TABLE 12). Three out of four sites with igneous derived soils had higher pH (0–10 and 10–20 cm soil layers) in chlorotic areas, but no soil pH difference was observed in the sedimentary site. The control areas with lower pH in igneous derived soils had higher Al^{3+} values and Al^{3+} saturation, and lower exchangeable Ca^{2+} and Mg^{2+} (0–10 and 10–20 cm soil layers) as compared to chlorotic areas, with the exception of SP14 for Ca^{2+} and Al^{3+}. The differences between chlorotic and control areas were very expressive since chlorotic areas had very high levels of Ca^{2+} (> 6 $cmol_c$ dm^{-3}) and Mg^{2+} (> 2 $cmol_c$ dm^{-3}), while control areas had low Ca^{2+} (0.5–1.0 $cmol_c$ dm^{-3}) and Mg^{2+} (0.2–0.4 $cmol_c$ dm^{-3}) to very low Ca^{2+} (< 0.5 $cmol_c$ dm^{-3}) and Mg^{2+} (< 0.2 $cmol_c$ dm^{-3}). Thus, chlorosis symptoms were not related to Ca^{2+} or Mg^{2+} deficiency. For K^+, differences between chlorotic and control areas were only observed for the RPP19 site, where K^+ concentrations were higher in the chlorotic area.

TABLE 12- SOIL CHEMICAL PROPERTIES OF TWO SOIL LAYERS (0-10 AND 10-20) SAMPLED IN PAIRED STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL

Attribute	Layer cm	Area	Site RPP19	RPP11	SP14	SP18	NP12
pH	0-10	Chlorotic	4.42Aa	4.29Aa	3.46Ba	4.15Aa	3.22Ba
		Control	3.46ABb	3.50ABb	3.58Aa	3.57Ab	3.18Ba
	10-20	Chlorotic	4.36Aa	4.29Aa	3.49Ba	3.86ABa	3.02Ca
		Control	3.36Ab	3.36Ab	3.56Aa	3.37Ab	2.95Ba
Ca^{2+} ($cmol_c$ dm^{-3})	0-10	Chlorotic	7.23Aa	6.37Aa	3.36Aa	5.35Aa	0.42Bb
		Control	0.51Db	1.17BCb	3.33Aa	1.85ABb	0.62CDa
	10-20	Chlorotic	5.59Aa	3.94Aa	2.63Aa	2.35Aa	0.65Ba
		Control	0.41Cb	0.63Bb	1.71Aa	0.86ABb	0.66Ba
Mg^{2+} ($cmol_c$ dm^{-3})	0-10	Chlorotic	2.04Aa	2.31Aa	0.77Ba	1.78Aa	0.32Ca
		Control	0.26Bb	0.73Ab	0.61Aa	0.71Ab	0.41ABa
	10-20	Chlorotic	1.51Aa	1.75Aa	0.60Aa	1.26Aa	0.215Ba
		Control	0.25Ab	0.36Ab	0.31Aa	0.29Ab	0.245Aa
P ($mg\ dm^{-3}$)	0-10	Chlorotic	$6{,}04^{ns}$	$4{,}27^{ns}$	$4{,}22^{ns}$	$4{,}78^{ns}$	$4{,}75^{ns}$
		Control	$5{,}16^{ns}$	$4{,}82^{ns}$	$3{,}65^{ns}$	$6{,}07^{ns}$	$3{,}61^{ns}$
	10-20	Chlorotic	$4{,}09^{ns}$	$4{,}14^{ns}$	$3{,}76^{ns}$	$3{,}34^{ns}$	$5{,}11^{ns}$
		Control	$4{,}18^{ns}$	$4{,}42^{ns}$	$3{,}85^{ns}$	$3{,}87^{ns}$	$4{,}07^{ns}$
K^+ ($cmol_c$ dm^{-3})	0-10	Chlorotic	0.58Aa	0.38Ba	0.26BCa	0.23BCa	0.12Ca
		Control	0.20Ab	0.30Aa	0.15Ab	0.23Aa	0.17Aa
	10-20	Chlorotic	0.22^{ns}	0.17^{ns}	0.11^{ns}	0.10^{ns}	0.07^{ns}
		Control	0.11^{ns}	0.15^{ns}	0.07^{ns}	0.07^{ns}	0.08^{ns}
Al^{3+} ($cmol_c$ dm^{-3})	0-10	Chlorotic	0.75Cb	0.39Cb	2.39Ba	0.65Cb	6.90Aa
		Control	5.31ABa	4.55BCa	1.38Da	3.39Ca	6.62Aa
	10-20	Chlorotic	0.55Cb	0.58Cb	2.60Ba	0.97BCb	6.83Aa

		Control	4.93Ba	4.96Ba	1.68Ca	3.67Ba	7.08Aa
Fe^{2+} (mg dm^{-3})	0-10	Chlorotic	59.35Cb	45.28Cb	147.66Ba	59.22Cb	305.27Ab
		Control	173.31Ba	123.88Ba	158.68Ba	127.99Ba	667.84Aa
	10-20	Chlorotic	39.76Bb	29.98Bb	58.79Ba	37.37Bb	395.96Aa
		Control	102.59Ba	101.66Ba	76.83Ba	73.72Ba	430.43Aa
Mn^{2+} (mg dm^{-3})	0-10	Chlorotic	329.69Aa	239.29Aa	219.20Aa	174.62Aa	11.55Bb
		Control	23.99Cb	15.88Cb	83.93ABb	112.23Aa	29.28BCa
	10-20	Chlorotic	294.59Aa	116.33Ba	128.58ABa	109.04ABa	5.82Ca
		Control	8.55ABb	8.19Bb	19.05ABb	33.69Ab	6.27Ba
Zn^{2+} (mg dm^{-3})	0-10	Chlorotic	8.96Aa	6.21ABa	3.65BCa	5.36ABCa	1.61Ca
		Control	3.15Ab	1.79Ab	2.06Aa	2.88Aa	4.27Aa
	10-20	Chlorotic	6.16Aa	2.41ABCa	1.83BCa	4.74ABa	1.29Ca
		Control	1.13Ab	1.35Aa	1.57Aa	1.49Ab	1.48Aa
Cu^{2+} (mg dm^{-3})	0-10	Chlorotic	7.03Ba	7.70Ba	8.22Ba	14.25Aa	1.79Cb
		Control	1.59Db	3.35BCb	4.51ABb	6.55Ab	2.88Ca
	10-20	Chlorotic	7.61Ba	8.59Ba	7.03Ba	15.29Aa	2.06Ca
		Control	1.25Cb	3.19BCb	4.52Bb	8.74Ab	3.07BCa
SOC (g dm^{-3})	0-10	Chlorotic	40.61ABb	34.85ABb	32.16Ba	46.14ABa	53.93Aa
		Control	61.58Aa	65.17Aa	34.23Ba	51.12ABa	51.83ABa
	10-20	Chlorotic	34.34ABb	20.88Bb	28.52ABa	32.39ABa	36.59Aa
		Control	61.53Aa	56.47Aa	26.27Ba	33.59Ba	26.21Bb

SOURCE: The author (2022).

LEGEND: The symbol ‘ns’ means ‘not significant’. Means followed by the same uppercase letters in each row (comparing sites) and lowercase letters in the columns (comparing areas, individually for each nutrient and soil layer) do not differ significantly by the Tukey test (p <5%). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years. pH: soil pH in 0.01 mol L^{-1} $CaCl_2$ (soil:solution ratio = 1:2.5); exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} (1 mol L^{-1} KCl extractant); available K^+, Fe, Mn, Zn and Cu (Mehlich-I extractant); SOC: soil organic carbon.

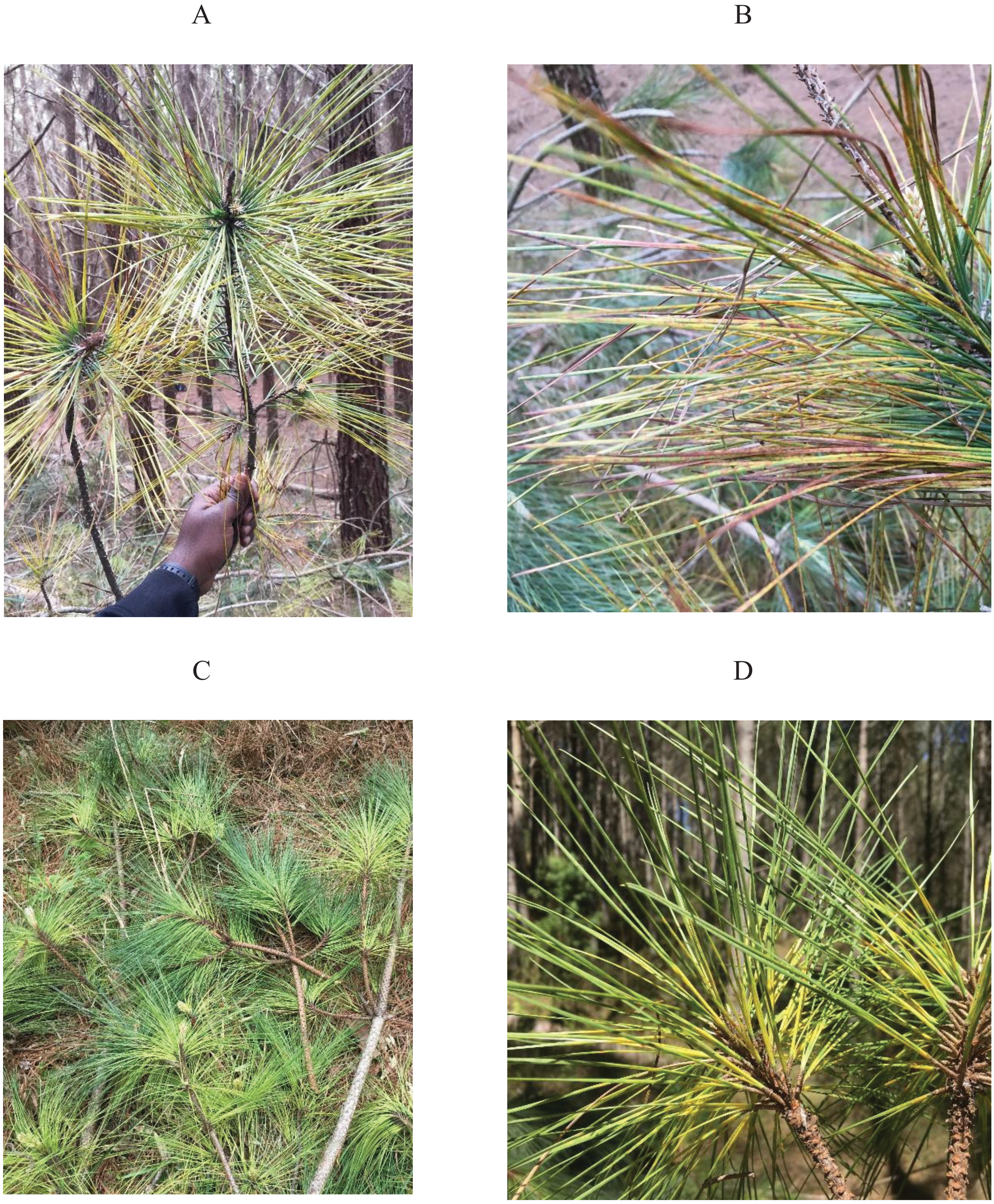
Unlike igneous derived soils, soils originating from sedimentary material in the North Plateau site (NP12) had higher Al^{3+} concentrations (Al^{3+} saturation >80%), and lower values of Ca^{2+}, Mg^{2+}, and K^+ (TABLE 12). No soil differences were observed between chlorotic and control areas except for Ca^{2+} in the 0–10 cm layer. The highest Ca^{2+} concentrations were observed in the control area.

Soils derived from igneous rocks in chlorotic areas had higher values of Mn and Zn in at least one evaluated soil layer, and higher Cu in both soil layers (TABLE 12). The opposite was observed for Fe, since three out of four igneous derived soils had higher Fe concentrations in control areas. Soil of chlorotic area originating from sedimentary rock (NP12), had higher SOC and lower Ca^{2+}, Fe^{2+}, Mn^{2+}, and Cu^{2+} concentrations in the 0–10 cm soil layer (TABLE 12). There were similarities in soil micronutrient levels in the Rio do Peixe Plateau and Serrano Plateau regions, regardless of site and tree age (TABLE 12).

2.4.3 CHLOROSIS SYMPTOMS AND ELEMENT CONCENTRATIONS IN NEEDLES, WOOD AND BARK

Distinct tree chlorosis symptoms were associated with igneous and sedimentary derived soils. Pine needles collected from the sedimentary derived soil site (NP12) displayed normal green color from the base to middle of the needle length but with yellow chlorotic tips (FIGURE 5- A and B). Needles from igneous derived soils had a pale yellowish color extending from the base to mid-length (FIGURE 5- C and D). Loss of crowns had been primarily observed at igneous sites, which was followed by tree mortality.

FIGURE 5- PINE BRANCHES WITH SYMPTOMS OF CHLOROSIS IN TREES GROWN IN SOIL FORMED UNDER SEDIMENTARY ROCK IN THE NORTHERN PLATEAU (A AND B - 12 YEARS) AND BASALTIC ROCK IN THE SERRANO PLATEAU (C AND D - 18 YEARS), IN THE STATE OF SANTA CATARINA - BRAZIL.



SOURCE: The author (2019).

Mineral nutrient concentrations in needles presented different patterns in chlorotic and control areas according to studied sites (TABLES 13 and 14). The RPP19, RPP11, SP14 and SP18 sites derived from igneous parent material source presented magnitude of values that were different than NP12. In general, needle concentrations of Ca and Mg from tissue collected from the lower third (first flush) and upper third (first and second flush) of the crown followed variations in soil Ca^{2+} and Mg^{2+} availability for sites located in the Rio do Peixe Plateau region. Higher concentrations of Ca and Mg were observed in needles of the first and second flush at sites that were 11 and 19 years old (i.e. RPP11 and RPP19) with greater concentrations in chlorotic needles compared to those of the control. Furthermore, Ca concentrations were also higher in needles collected from the lower third of the crown. Surprisingly, lower Ca concentration values were noted in the first needle flush in chlorotic trees, which was contrary to soil Ca^{2+} concentration obtained at 18-years-old sites (SP18).

TABLE 13- CONCENTRATIONS OF Ca, Mg, Al, Fe, Mn, AND Fe/Mn RATIO FOR NEEDLES SAMPLED FROM THE 2ND FLUSH OF THE MIDDLE THIRD OF THE TREE CANOPY AT PAIRED STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL

Nutrient	Area	Site				
		RPP19	RPP11	SP14	SP18	NP12
Ca ($g\ kg^{-1}$)	Chlorotic	4.82Aa	4.69Aa	4.26Aa	4.42Aa	0.52Bb
	Control	1.39ABb	2.93Aa	2.23ABb	2.76Aa	1.08Ba
Mg ($g\ kg^{-1}$)	Chlorotic	1.45Aa	1.64Aa	1.25Aa	1.41Aa	0.32Ba
	Control	0.59Ab	1.05Aa	0.85Aa	0.91Aa	0.46Aa
Al ($g\ kg^{-1}$)	Chlorotic	0.32Ab	0.35Aa	0.42Aa	0.51Aa	0.37Aa
	Control	0.59Aa	0.48ABa	0.38Ba	0.53ABa	0.41ABa
Fe ($mg\ kg^{-1}$)	Chlorotic	91.28ABa	127.64Aa	91.08ABa	72.72BCb	61.32Ca
	Control	96.34ABa	95.31Aa	66.25Cb	102.87Aa	66.34BCa
Mn ($mg\ kg^{-1}$)	Chlorotic	492.52BCa	411.42Ca	1179.55Aa	829.19Ba	149.82Cb
	Control	377.52Ba	460.10ABa	267.06Bb	611.48ABa	732.81Aa
Fe/Mn	Chlorotic	0.22Aa	0.31Aa	0.08Bb	0.09Bb	0.45Aa
	Control	0.28Aa	0.29Aa	0.31Aa	0.17ABa	0.09Bb

SOURCE: The author (2023).

LEGEND: Means followed by the same uppercase letters in each row (comparing sites) and lowercase letters in the columns (comparing areas, individually for each nutrient) do not differ significantly by the Tukey test ($p < 5\%$). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years.

TABLE 14- CONCENTRATIONS OF Ca, Mg, Fe, Mn, Zn, Cu, AND Fe/Mn, K/Ca, K/Mg RATIO FOR NEEDLES OF THE 1st AND 2nd FLUSHES SAMPLED FROM THE UPPER THIRD OF THE TREE CANOPY AT PAIRED STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL

Nutrient	Flush	Area	Site				
			RPP19	RPP11	SP14	SP18	NP12
Ca ($g\ kg^{-1}$)	1º	Chlorotic	3.61Aa	3.54Aa	3.09Aa	1.06Bb	0.49Bb
		Control	0.98Bb	2.12Ab	2.07Aa	1.79ABa	1.44ABa
	2º	Chlorotic	3.70Aa	3.61Aa	3.07Aa	2.53Aa	0.47Ba
		Control	1.15Ab	2.29Ab	2.29Aa	2.48Aa	1.34Aa
Mg ($g\ kg^{-1}$)	1º	Chlorotic	1.16Aa	1.43Aa	0.95Aa	0.49Ba	0.37Bb
		Control	0.50Ab	0.70Ab	0.63Aa	0.68Aa	0.68Aa
	2º	Chlorotic	1.28Aa	1.57Aa	1.04Aa	1.18Aa	0.35Bb
		Control	0.59Ab	0.74Ab	0.79Aa	0.86Aa	0.58Aa
Fe ($mg\ kg^{-1}$)	1º	Chlorotic	111.93Ab	108.85Aa	108.29Aa	73.74Aa	100.49Aa
		Control	207.93Aa	86.26Ba	100.02Ba	129.84ABa	89.38Ba
	2º	Chlorotic	109.96Ab	87.85ABa	84.17ABa	61.26Bb	96.37Aa
		Control	176.51Aa	77.47Ca	80.08Ca	134.92ABa	82.79BCa
Mn ($mg\ kg^{-1}$)	1º	Chlorotic	332.03Ba	291.96Ba	723.53Aa	369.34Ba	153.65Bb
		Control	243.21Ba	282.38Ba	223.63Bb	269.98Ba	575.16Ba
	2º	Chlorotic	505.21ABa	407.19ABa	714.87Aa	507.99ABa	248.18Bb
		Control	363.04ABa	388.97Ba	275.44Bb	415.88ABa	761.39Aa
Zn ($mg\ kg^{-1}$)	1º	Chlorotic	37.79ABa	51.70Aa	30.28ABa	21.08Ba	21.38Bb
		Control	18.27Bb	30.28ABb	24.29ABa	21.33Ba	42.36Aa
	2º	Chlorotic	50.09Aa	61.82Aa	36.65Aa	34.81Aa	20.68Bb
		Control	21.16Ab	34.33Ab	26.89Aa	25.99Aa	32.96Aa
Cu ($mg\ kg^{-1}$)	1º	Chlorotic	3.69Aa	2.77Aa	3.10Aa	3.03Aa	2.69Ab
		Control	2.58Ab	2.96Aa	2.64Aa	3.06Aa	3.60Aa
	2º	Chlorotic	4.03 ns	3.16 ns	3.11 ns	3.36 ns	3.08 ns
		Control	2.86 ns	3.02 ns	3.07 ns	3.34 ns	3.28 ns
Fe/Mn	1º	Chlorotic	0.51ABa	0.37ABCa	0.15Cb	0.22BCb	0.72Aa
		Control	0.89Aa	0.39ABa	0.45Aa	0.49Aa	0.18Bb
	2º	Chlorotic	0.30ABa	0.22ABa	0.12Ba	0.12Ba	0.47Aa
		Control	0.50Aa	0.27ABa	0.30ABa	0.32ABa	0.12Bb
K/Ca	1º	Chlorotic	1.11Bb	1.32Ba	1.19Ba	5.22Aa	8.45Aa
		Control	3.73Aa	2.03ABa	1.62Ba	2.30ABb	3.18ABb
	2º	Chlorotic	1.09Bb	1.22Ba	1.22Ba	1.49Ba	12.78Aa
		Control	3.66Aa	2.07Aa	1.61Aa	1.71Aa	3.18Ab
K/Mg	1º	Chlorotic	3.24Bb	3.7Bb	3.82Ba	9.97Aa	11.69Aa
		Control	7.4Aa	6.66Aa	5.28Aa	6.13Aa	6.82Ab
	2º	Chlorotic	3.05Bb	3.57Bb	3.54Ba	3.10Ba	14.69Aa
		Control	7.41Aa	6.97Aa	4.55Aa	4.89Aa	6.62Ab

SOURCE: The author (2023).

LEGEND: The symbol ‘ns’ means ‘not significant’. Means followed by the same uppercase letters in the rows (comparing sites) and lowercase letters in the columns (comparing areas, individually for each nutrient and flush) do not differ significantly by the Tukey test (p <5%). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years.

Lower Fe concentration and a low Fe/Mn ratio were observed in the second flush in the upper and lower third of the crown in chlorotic trees (TABLES 13 and 14). For 14-year-old trees (SP14), the micronutrient Mn was much higher in needles exhibiting chlorosis compared to those without chlorosis, which was proportional to respective soil Mn concentrations.

In contrast to sites located on igneous parent source material, trees grown on sedimentary derived soil (NP12) had the lowest needle concentrations of Ca, Mg, Mn, Zn, and Cu, and the highest Fe/Mn ratio (TABLE 14), which was proportional to respective soil nutrient concentrations in areas with chlorotic trees (TABLE 12).

Wood Ca concentration generally followed results observed in needle tissue but at lower values (TABLE 15). The highest wood Ca concentration was observed in samples (taken at heights equal to or greater than 25% of the trunk) from chlorotic trees in the Rio do Peixe Plateau region, as compared to the control. In comparison, there was no wood Ca concentration differences between chlorotic and control areas noted in the Plateau Serrano region (SP14 and SP18) (TABLE 15). The opposite was observed for the North Plateau (NP12), where chlorotic samples had lower Ca at sampling heights of 25 and 50% of trunk height (with trends at 75% and 100%). Wood of chlorotic trees from sedimentary parent source material areas (North Plateau region) had lower Ca values at the 1.3 m height compared to that of igneous derived soils.

Numerically, higher Ca concentration values in bark tissue of chlorotic trees (compared to those without chlorosis) occurred along the tree trunk at RPP19 and SP14 but were only significantly different at 100% of height and 50% and 75% of height, respectively (TABLE 16). Lower bark Ca concentration in the North Plateau (compared to Rio do Peixe and Plateau Serrano) was observed mainly for trees with chlorotic symptoms where samples were collected above 1.3 m (TABLE 16). In other words, Ca concentrations in needles, wood, and bark indicated that tree chlorosis symptoms in the Rio do Peixe and Plateau Serrano regions (especially Rio do Peixe) were not associated with Ca concentration.

The concentration of Mg in wood showed larger variations across the three regions compared to Ca concentration (TABLE 15). Values of Mg concentration were two to three times lower in trunks of trees with chlorosis compared to those without chlorosis. Unexpectedly, trunk of trees with chlorosis in the North Plateau had higher Mg concentration than needles. But similar to Ca, significant differences in Mg concentration between trees with and without chlorosis appeared at ~50% of trunk height.

Differences in bark Mg concentration was restricted to the 19-year-old Rio do Peixe Plateau (RPP19), with higher values in chlorotic trees compared to those without chlorosis (TABLE 16). Similar to needle observations, bark Mg concentration in North Plateau trees was much lower than that observed in the other two regions, and lower in chlorotic trees. Therefore, low Mg concentrations in needles, wood and bark of trees from North Plateau region point to a significant deficiency of Mg in that environment. Thus, chlorotic symptoms under these conditions may be associated with a nutritional imbalance of Mg.

In the 14-year-old Serrano Plateau (SP14), Mn concentration in needles, wood, and bark were much higher in chlorotic trees than the control (TABLES 13, 14, 15 and 16). In addition, respective soils in these areas showed clear indications of erosion. The Mn concentration in wood also indicates a distinction between trees grown in the North Plateau compared to that of the two other regions that had very high Mn concentration values in chlorotic and control trees. However, unlike that observed in needle tissue in the North Plateau (where higher Mn concentrations were found in trees without chlorosis), wood Mn concentration showed no significant difference between chlorotic and control areas.

Although there was no clear trend, most sites generally had higher wood Fe concentration in chlorotic areas (TABLE 15). For the assessed areas, the majority of sampled points along trunks showed no differences in bark Fe concentration (TABLE 16). However, at the RPP19 and SP18 sites, bark Fe concentrations were higher in non-chlorotic trees at heights corresponding to 75% of trunk total height (TABLE 16).

Concentrations of K, Al, Cu, Zn, and B in wood and bark sampled at breast height diameter are shown in TABLE 17. Comparing sites, the more important findings were the lower concentrations of K (bark/chlorotic area), Al (bark/control), Cu (wood and bark/control), Zn (wood/control), and B (wood/chlorotic and control) at the NP12 site, and higher Al (bark/chlorotic) and lower Cu (wood/chlorotic) and Zn (wood/chlorotic) at SP18. In turn, comparing areas (chlorotic × control), concentration of elements did not show any pattern among the sites. For instance, chlorotic areas had lower wood concentrations of K, Cu, and B at RPP11, Zn at SP14 and SP18, and Cu at SP18; while in control areas, lower concentrations were observed for Zn at RPP11 and NP12, and B at SP14 and NP12. On the other hand, chlorotic areas had lower bark concentrations of Cu at NP12, and K at RPP1; while only the RPP19 control areas presented lower bark concentrations of K and B, and higher Al.

TABLE 15- MEAN CONCENTRATIONS OF WOOD SAMPLES AT SIX TRUNK HEIGHTS (BASE, 1.3 M, 25%, 50% 75% AND 100%) AT PAIR STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18, NP12) IN SANTA CATARINA STATE, BRAZIL

Trunk height	Area	Ca (g kg^{-1})					Mg (g kg^{-1})				
		RPP19	RPP11	SP14	SP18	NP12	RPP19	RPP11	SP14	SP18	NP12
0	Chlorotic	0.39^{ns}	0.49^{ns}	0.46^{ns}	0.38^{ns}	0.29^{ns}	0.33^{ns}	0.29^{ns}	0.30^{ns}	0.31^{ns}	0.42^{ns}
	Control	0.28^{ns}	0.42^{ns}	0.47^{ns}	0.37^{ns}	0.32^{ns}	0.10^{ns}	0.13^{ns}	0.13^{ns}	0.11^{ns}	0.11^{ns}
1.3 m	Chlorotic	0.42^{ns}	0.53^{ns}	0.43^{ns}	0.32ns	0.25^{ns}	0.33ABa	0.32ABa	0.24Ba	0.29ABa	0.47Aa
	Control	0.30^{ns}	0.47^{ns}	0.39^{ns}	0.36^{ns}	0.31^{ns}	0.10Bb	0.17Ab	0.11Bb	0.12ABb	0.10Bb
25	Chlorotic	0.48ABa	0.52Aa	0.45ABa	0.35BCa	0.26Cb	0.24^{ns}	0.30^{ns}	0.26^{ns}	0.29^{ns}	0.46^{ns}
	Control	0.29Ab	0.39Ab	0.41Aa	0.42Aa	0.39Aa	0.09^{ns}	0.13^{ns}	0.11^{ns}	0.12^{ns}	0.14^{ns}
50	Chlorotic	0.52Aa	0.61Aa	0.59Aa	0.54Aa	0.29Bb	0.37ABa	0.31Ba	0.25Ba	0.32Ba	0.56Aa
	Control	0.39Ab	0.45Ab	0.53Aa	0.50Aa	0.39Aa	0.15Ab	0.16Ab	0.17Ab	0.16Ab	0.14Ab
75	Chlorotic	0.48BCa	0.60Aa	0.56ABa	0.42Ca	0.29Da	0.34Ba	0.31Ba	0.26Ba	0.34Ba	0.53Aa
	Control	0.34Bb	0.44ABb	0.55Aa	0.42Ba	0.37Ba	0.13Ab	0.15Ab	0.16Ab	0.13Ab	0.13Ab
100	Chlorotic	0.45ABa	0.57Aa	0.47ABa	0.41BCa	0.28Ca	0.35Ba	0.31Ba	0.26Ba	0.31Ba	0.49Aa
	Control	0.31Bb	0.44ABb	0.46Aa	0.44ABa	0.35ABa	0.11Ab	0.14Ab	0.13Ab	0.14Ab	0.12Ab

Trunk height	Area	Fe (mg kg^{-1})					Mn (mg kg^{-1})				
		RPP19	RPP11	SP14	SP18	NP12	RPP19	RPP11	SP14	SP18	NP12
0	Chlorotic	11.90^{ns}	2.55^{ns}	9.90^{ns}	16.06^{ns}	10.81^{ns}	45.2^{ns}	53.8^{ns}	89.2^{ns}	57.4^{ns}	142.3^{ns}
	Control	1.56^{ns}	3.24^{ns}	4.09^{ns}	15.81^{ns}	16.00^{ns}	43.3^{ns}	49.9^{ns}	53.2^{ns}	46.0^{ns}	140.8^{ns}
1.3 m	Chlorotic	8.73^{ns}	21.48^{ns}	28.59^{ns}	23.82^{ns}	33.33^{ns}	40.8Bb	39.8Ba	83.9ABa	56.2ABa	150.9Aa
	Control	13.66^{ns}	12.27^{ns}	32.18^{ns}	37.46^{ns}	11.47^{ns}	69.0ABa	45.2BCa	29.7Cb	47.9BCa	144.1Aa
25	Chlorotic	10.66Aa	2.50Aa	4.84Aa	25.51Aa	33.42Aa	43.3Bb	48.8ABa	98.7Aa	63.2ABa	160.4Aa
	Control	0.91Bb	8.80Aa	3.77ABa	12.90Aa	22.65Aa	77.7ABa	48.0Ba	38.1Bb	64.9Ba	194.9Aa
50	Chlorotic	10.48^{ns}	17.50^{ns}	3.87^{ns}	25.11^{ns}	15.37^{ns}	47.9Bb	53.5Ba	124.7Aa	85.7ABa	173.1Aa
	Control	6.21^{ns}	7.15^{ns}	1.43^{ns}	17.45^{ns}	11.80^{ns}	91.8ABa	59.8Ba	57.8Bb	75.7Ba	204.5Aa
75	Chlorotic	15.13^{ns}	21.39^{ns}	4.97^{ns}	37.30^{ns}	10.07^{ns}	44.8Ba	54.3ABa	115.0Aa	66.6ABa	153.0Aa
	Control	2.51^{ns}	9.74^{ns}	3.01^{ns}	19.13^{ns}	11.59^{ns}	77.9ABb	59.1Ba	57.2Bb	67.4Ba	183.2Aa
100	Chlorotic	13.84^{ns}	7.63^{ns}	7.15^{ns}	39.99^{ns}	7.80^{ns}	44.7Ba	49.6ABa	96.7ABa	64.5ABa	131.2Aa
	Control	4.04^{ns}	1.99^{ns}	12.10^{ns}	26.34^{ns}	11.79^{ns}	66.3ABa	59.5Ba	48.2Bb	60.9Ba	165.5Aa

SOURCE: The author (2021).

LEGEND: 1.3 m = breast height diameter. Means followed by the same uppercase letters in the rows (individually for each nutrient) and lowercase letters in the columns (individually for each trunk height and nutrient) do not differ significantly by the Tukey test (p <5%). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 11; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years.

TABLE 16- MEAN CONCENTRATIONS OF BARK NUTRIENTS AT SIX TRUNK HEIGHTS (BASE, 1.3 M, 25%, 50% 75% AND 100%) AT PAIR STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18, NP12) IN SANTA CATARINA STATE, BRAZIL

Trunk height	Area	Ca ($g\ kg^{-1}$)					Mg ($g\ kg^{-1}$)				
		RPP19	RPP11	SP14	SP18	NP12	RPP19	RPP11	SP14	SP18	NP12
0	Chlorotic	1.51^{ns}	1.64^{ns}	1.88^{ns}	1.20^{ns}	0.25^{ns}	0.36^{ns}	0.29^{ns}	0.19^{ns}	0.45^{ns}	0.06^{ns}
	Control	0.68^{ns}	1.61^{ns}	1.00^{ns}	1.76^{ns}	0.32^{ns}	0.13^{ns}	0.31^{ns}	0.19^{ns}	0.33^{ns}	0.07^{ns}
1.3 m	Chlorotic	1.39^{ns}	1.46^{ns}	1.75^{ns}	1.69^{ns}	0.22^{ns}	0.43Aa	0.30Aa	0.28Aa	0.33Aa	0.05Bb
	Control	0.82^{ns}	1.36^{ns}	1.04^{ns}	1.38^{ns}	0.34^{ns}	0.16Ba	0.36Aa	0.34Aa	0.40Aa	0.07Ca
25	Chlorotic	1.48ABa	1.95Aa	1.96Aa	0.95BCa	0.37Ca	0.84Aa	0.76Aa	0.74Aa	0.76Aa	0.23Ba
	Control	0.69BCa	1.65Aa	1.22ABCa	1.41ABa	0.55Ca	0.42BCb	0.78ABa	0.89Aa	0.77ABa	0.35Ca
50	Chlorotic	1.14BCa	2.09ABa	2.24Aa	1.06Ca	0.41Ca	0.59^{ns}	0.71^{ns}	0.73^{ns}	0.81^{ns}	0.16^{ns}
	Control	0.76ABa	1.59Aa	1.09ABb	1.63Aa	0.51Ba	0.37^{ns}	0.71^{ns}	0.62^{ns}	0.58^{ns}	0.20^{ns}
75	Chlorotic	1.12Ba	1.39ABa	1.89Aa	1.06Ba	0.18Ca	0.49^{ns}	0.46^{ns}	0.55^{ns}	0.59^{ns}	0.08^{ns}
	Control	0.61BCa	1.38Aa	1.05ABCb	1.32ABa	0.54Ca	0.22^{ns}	0.57^{ns}	0.60^{ns}	0.71^{ns}	0.22^{ns}
100	Chlorotic	1.04Aa	1.47Aa	1.58Aa	1.19Aa	0.18Bb	0.42Aa	0.35Aa	0.39Aa	0.41Aa	0.06Bb
	Control	0.61Bb	1.28Aa	1.25Aa	1.56Aa	0.40Ba	0.21Bb	0.41ABa	0.40Aa	0.49Aa	0.11Ca

Trunk height	Area	Fe ($mg\ kg^{-1}$)					Mn ($mg\ kg^{-1}$)				
		RPP19	RPP11	SP14	SP18	NP12	RPP19	RPP11	SP14	SP18	NP12
0	Chlorotic	24.83Ba	45.87Aa	27.14Ba	27.28Ba	29.72Ba	34.5Ba	38.3ABa	83.7Aa	65.8ABa	39.1ABb
	Control	19.74ABa	18.94ABb	12.13Bb	30.66Aa	26.14ABa	48.5Aa	36.4Aa	25.7Ab	54.4Aa	58.9Aa
1.3 m	Chlorotic	24.55^{ns}	44.86^{ns}	33.87^{ns}	51.26^{ns}	24.56^{ns}	42.2Cb	40.7Ca	110.8Aa	74.1Ba	39.7Ca
	Control	36.13^{ns}	30.59^{ns}	31.24^{ns}	51.01^{ns}	27.64^{ns}	80.7Aa	56.8Ba	39.9Bb	62.4ABa	54.5Ba
25	Chlorotic	40.71^{ns}	49.62^{ns}	36.95^{ns}	39.07^{ns}	38.92^{ns}	92.5Ba	81.4Ba	246.7Aa	94.6Ba	107.6Ba
	Control	36.81^{ns}	18.62^{ns}	29.94^{ns}	51.69^{ns}	31.35^{ns}	75.9Aa	107.6Aa	90.0Ab	107.2Aa	112.1Aa
50	Chlorotic	29.83^{ns}	33.63^{ns}	30.16^{ns}	72.24^{ns}	37.47^{ns}	55.1Ba	68.1Ba	236.6Aa	90.5Ba	64.9Ba
	Control	29.52^{ns}	21.32^{ns}	23.72^{ns}	70.41^{ns}	44.24^{ns}	96.7Aa	96.9Aa	62.7Ab	90.5Aa	95.9Aa
75	Chlorotic	23.97Ab	35.66Aa	27.11Aa	31.49Ab	36.19Aa	51.0Bb	47.5Bb	185.5Aa	75.0Ba	48.6Bb
	Control	43.06ABa	22.27Ba	23.19Ba	59.42Aa	35.97ABa	80.1Aa	79.6Aa	61.2Ab	94.9Aa	90.1Aa
100	Chlorotic	45.13^{ns}	44.30^{ns}	33.08^{ns}	36.16^{ns}	31.64^{ns}	49.3Cb	42.4Cb	140.2Aa	74.5Ba	42.8Cb
	Control	40.01^{ns}	30.37^{ns}	30.25^{ns}	51.40^{ns}	38.19^{ns}	82.3Aa	66.3ABa	50.7Bb	73.3ABa	68.6ABa

SOURCE: The author (2021).

LEGEND: 1.3 m = breast height diameter. Means followed by the same uppercase letters in the rows (individually for each nutrient) and lowercase letters in the columns (individually for each trunk height and nutrient) do not differ significantly by the Tukey test ($p <5\%$). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years.

TABLE 17- CONCENTRATIONS OF NUTRIENTS IN THE WOOD AND TRUNK BARK SAMPLES OF TREES COLLECTED AT BREAST HEIGHT DIAMETER (DBH) AT PAIR STUDY AREAS (CHLOROTIC AND CONTROL) IN FIVE SITES (RPP19, RPP11, SP14, SP18, NP12) IN SANTA CATARINA STATE, BRAZIL

Nutrient	Partition	Area	Sites				
			RPP19	RPP11	SP14	SP18	NP12
K ($g\ kg^{-1}$)	Wood	Chlorotic	0.40Aa	0.43Ab	0.38Aa	0.29Aa	0.45Aa
		Control	0.43Ba	0.89Aa	0.32Ba	0.39Ba	0.31Ba
	Bark	Chlorotic	1.02Aa	0.58Ab	0.49ABa	0.69Aa	0.26Ba
		Control	0.51Bb	1.21Aa	0.52Ba	0.67ABa	0.19Ca
Al ($g\ kg^{-1}$)	Wood	Chlorotic	-	-	-	-	-
		Control	-	-	-	-	-
	Bark	Chlorotic	0.39Cb	0.54BCa	0.76ABa	0.92Aa	0.49Ca
		Control	0.94Aa	0.71ABa	0.71ABa	0.78Aa	0.49Ba
Cu ($mg\ kg^{-1}$)	Wood	Chlorotic	6.20Aa	6.25Ab	6.79Aa	4.38Bb	5.67Aa
		Control	6.45ABa	8.52Aa	6.47ABa	6.12ABa	5.48Ba
	Bark	Chlorotic	3.90Aa	3.65Aa	3.37Aa	3.82Aa	3.44Ab
		Control	3.59Ba	3.73Ba	3.84ABa	5.48ABa	6.51Aa
Zn ($mg\ kg^{-1}$)	Wood	Chlorotic	8.17ABa	19.90Aa	7.22Bb	6.02Cb	22.54Aa
		Control	7.56Ca	11.37Bb	19.59Aa	8.699Ca	6.41Db
	Bark	Chlorotic	-	-	-	-	-
		Control	-	-	-	-	-
B ($mg\ kg^{-1}$)	Wood	Chlorotic	1.86Aa	1.91Ab	2.04Aa	1.46Ba	1.14Ca
		Control	1.88Ba	2.91Aa	1.61Bb	1.56Ba	1.04Cb
	Bark	Chlorotic	2.09Aa	1.25BCa	1.19BCa	1.31Ba	0.75Ca
		Control	1.04Ab	1.33Aa	1.19Aa	1.41Aa	0.95Aa

SOURCE: The author (2021).

LEGEND: Means followed by the same uppercase letters in the rows (site) and lowercase letters in the columns in each partition (with and without symptoms) do not differ significantly by the Tukey test ($p < 5\%$). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years

2.4.4 BIOMASS OF 100 FASCICLES, FINE ROOTS, AND LITTER

Mass of 100 fascicles showed no difference in paired areas (chlorotic and control) within study sites and had an overall average of 17 g (APPENDIX 3). Similarly, fine root mass was non-significant, and values were relatively lower at SP14 site (< 65 kg ha^{-1}) (APPENDIX 3).

Generally, litter biomass was low (TABLE 18). At SP18 and NP12 sites, litter amounts were greater in chlorotic areas, while in SP14 litter biomass was smaller in the chlorotic area. Chlorotic and control areas had similar litter amounts at RPP19 and RPP11 sites.

TABLE 18- LITTER BIOMASS SAMPLED IN PAIRED STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RP11, SP14, SP18 AND NP12) IN SANTA CATARINA STATE, BRAZIL

Site	Litter biomass (kg ha^{-1})	
	Chlorotic area	Control area
RPP19	18.249ABb	29.869Aa
RPP11	15.879Aba	18.152Ba
SP14	9.895Bb	17.886Ba
SP18	21.759Aa	17.943Ba
NP12	26.676Aa	16.641Bb

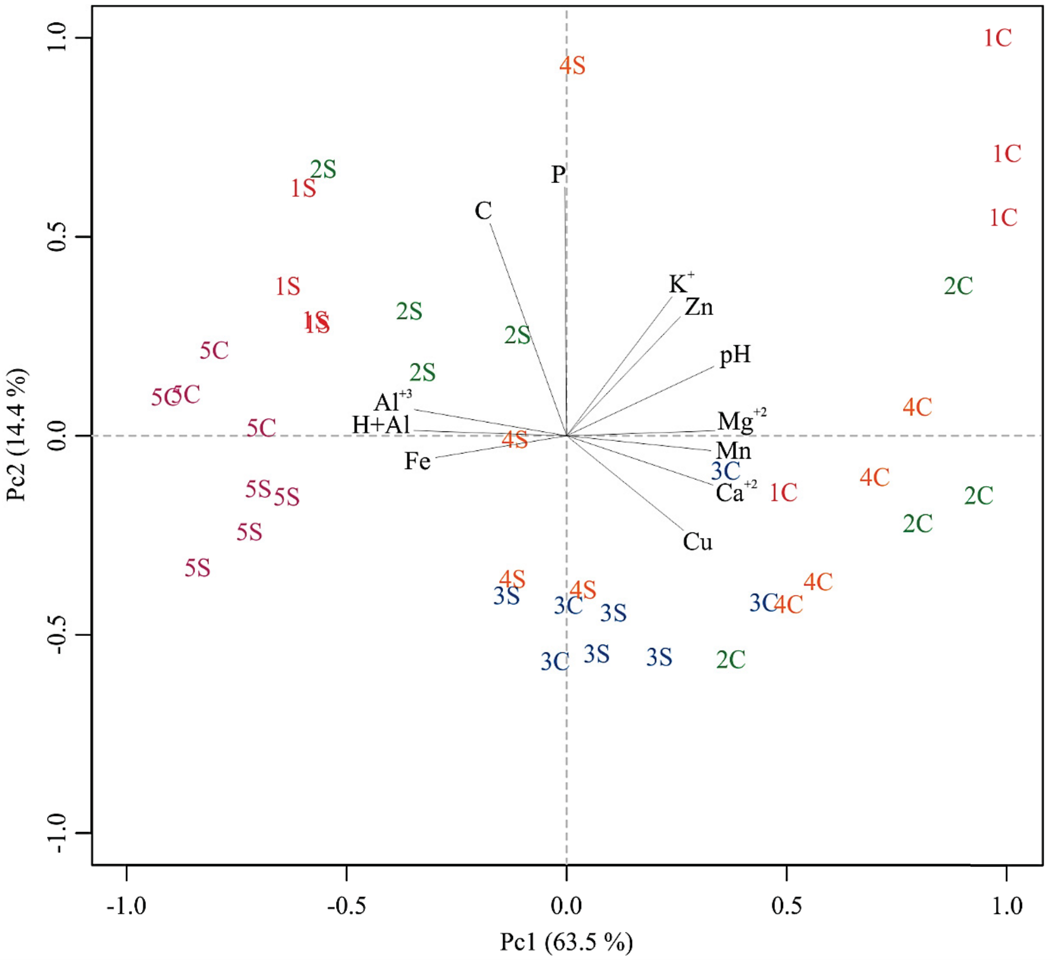
SOURCE: The author (2020).

LEGEND: Means followed by the same uppercase letters in the columns (comparing sites) and lowercase letters in each row (comparing areas) do not differ significantly by the Tukey test (p <5%). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years.

2.4.5 RELATIONSHIP BETWEEN SOIL CHEMICAL PROPERTIES, ELEMENT CONCENTRATION IN NEEDLES, AND LOCATION

Principal components analysis for soil chemical properties (FIGURE 6 and APPENDIX 4) indicated that the NP12 site had different soil characteristics compared to other sites. This site had higher levels of Al^{3+}, H+Al, and $Fe^{2+.}$ However, since chlorotic and control areas shared very similar features, these soil chemical properties were not useful for characterizing chlorotic areas.

FIGURE 6- PRINCIPAL COMPONENTS ANALYSIS FOR SOIL CHEMICAL PROPERTIES [pH; EXCHANGEABLE Ca^{2+}, Mg^{2+} AND Al^{3+}; AVAILABLE K^{+}, P, Fe^{2+}, Mn^{2+}, Cu^{2+} AND Zn^{2+}; H+Al; SOIL ORGANIC CARBON (C)] AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL

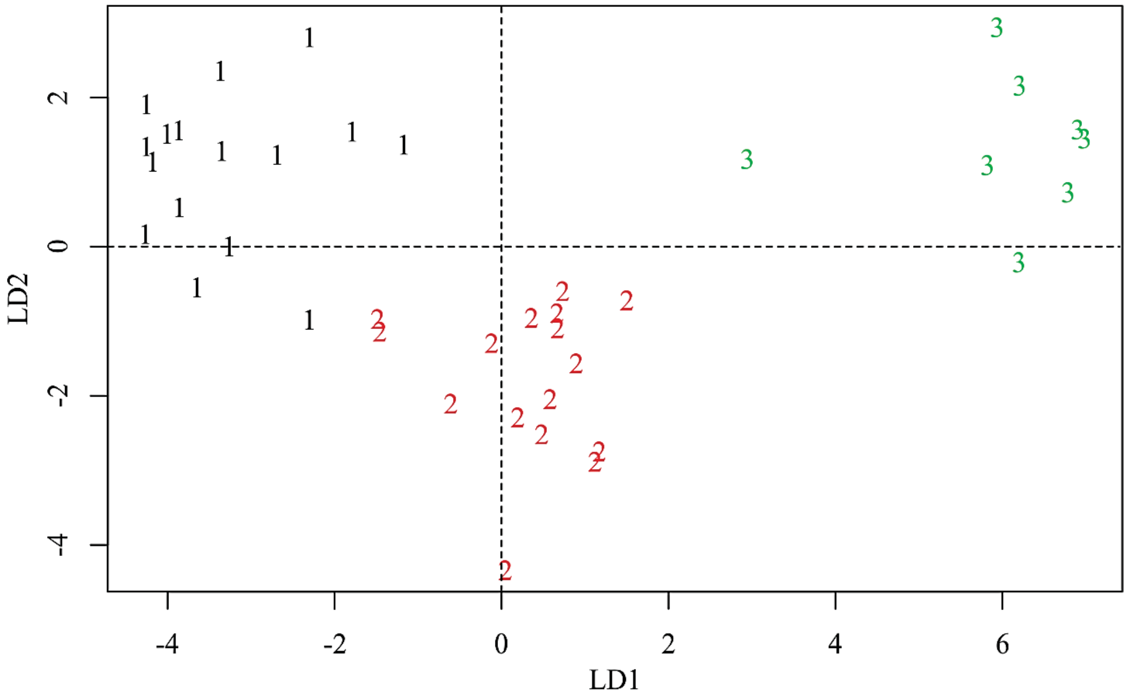


SOURCE: The author (2020).

LEGEND: Pair areas: C = chlorotic. S = supposed control area. Numbering: 1 = RPP19 – Rio do Peixe Plateau, age 19; 2 = RPP11 – Rio do Peixe Plateau, age 11; 3 = SP14 – Serrano Plateau, age 14; 4 = SP18 – Serrano Plateau, age 18; and 5 = NP12 – North Plateau, age 12 years.

Discriminant analysis was effective in clustering sites into three distinct groups (FIGURE 7). The first group was composed by RPP19, RPP11, SP14, and SP18 with chlorosis symptoms. The second group included the same sites but related to control areas. Finally, a third group was represented by NP12 (chlorotic and control areas). When using these groups in a prediction model with a regression tree, it was observed that the main difference between NP12 and other sites was associated with higher soil available Fe, while chlorotic and control areas were separated by soil available Mn (FIGURE 8). Prediction for chlorotic and control areas was based on a threshold value of 92.73 mg kg^{-1} Mn; values above this were chlorotic areas, while value below this were control areas. In the regression tree model, only one sample from chlorotic areas was confounded with samples from control areas.

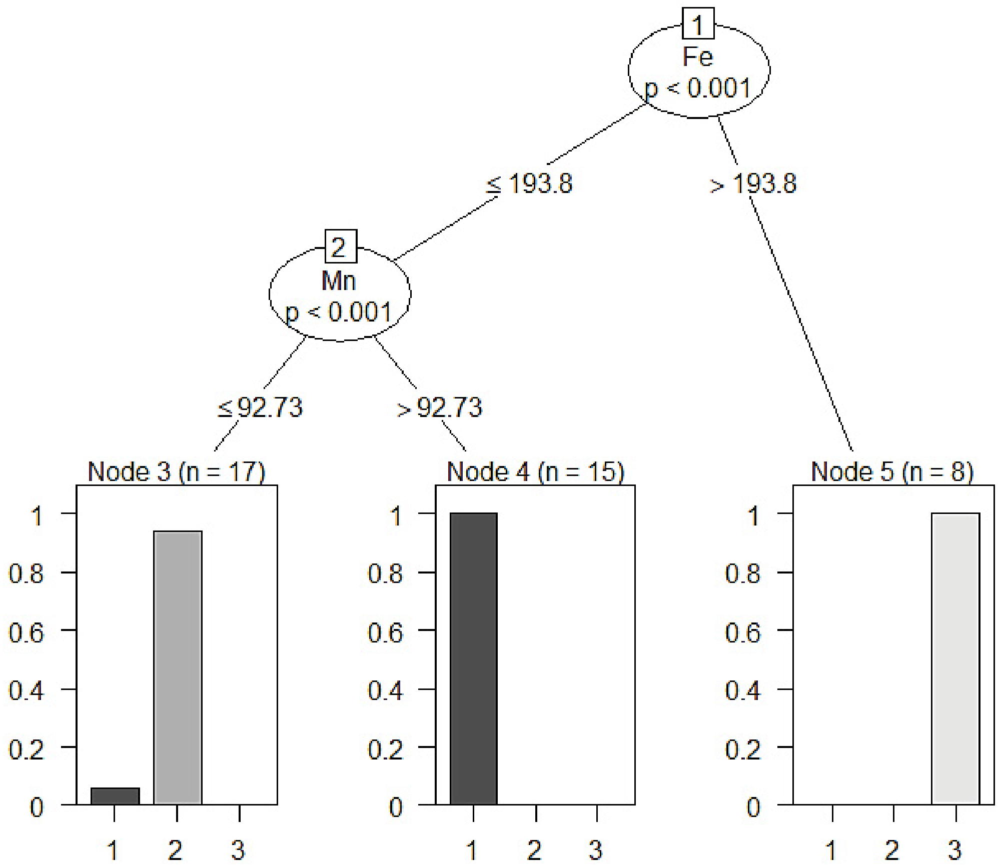
FIGURE 7- DISCRIMINANT ANALYSIS OF SOIL CHEMICAL PROPERTIES (pH; EXCHANGEABLE Ca^{2+}, Mg^{2+} AND Al^{3+} AVAILABLE K^{+}, P, Fe^{2+}, Mn^{2+}, Cu^{2+} AND Zn^{2+}; H+Al; SOIL ORGANIC CARBON) AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL



SOURCE: The author (2020).

LEGEND: Numbering: 1 = chlorotic areas [from sites 1 (RPP19), 2 (RPP11), 3 (SP14) and 4 (SP18)]; 2 = control areas [from sites 1, 2, 3 and 4]; and 3 = chlorotic and control areas from site 5 (NP12). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years.

FIGURE 8- REGRESSION TREE FOR SOIL CHEMICAL PROPERTIES USED IN PRINCIPAL COMPONENTS ANALYSIS (FIGURE 6) AND DISCRIMINANT ANALYSIS (FIGURE 7) PREDICTING GROUPS GENERATED IN THE DISCRIMINANT ANALYSIS AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL

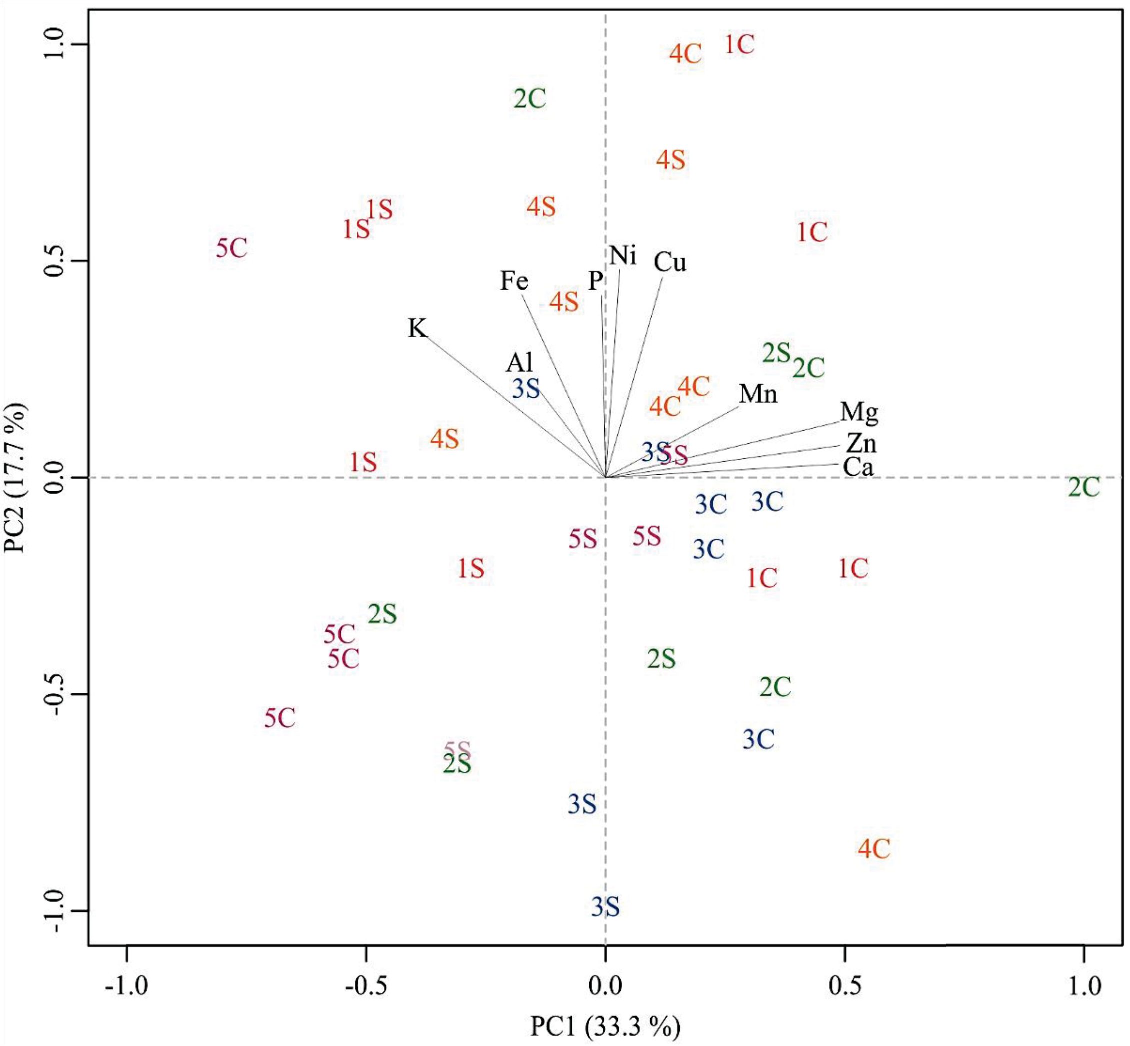


SOURCE: The author (2023).

LEGEND: Regression tree for soil chemical properties used in principal components analysis (FIGURE 6) and discriminant analysis (FIGURE 7) predicting groups generated in the discriminant analysis at five sites (RPP19, RPP11, SP14, SP18 and NP12) in the state of Santa Catarina, Brazil. Numbering: 1 = chlorotic areas from the sites 1 (RPP19), 2 (RPP11), 3 (SP14) and 4 (SP18); 2 = control areas from sites 1, 2, 3 and 4; and 3 = chlorotic and control areas from site 5 (NP12). RPP19 – Rio do Peixe Plateau, age 19; RPP11 – Rio do Peixe Plateau, age 11; SP14 – Serrano Plateau, age 14; SP18 – Serrano Plateau, age 18; and NP12 – North Plateau, age 12 years

Principal components analysis of needle nutrient concentration in the second flush showed that the chlorotic area at NP12 had different characteristics, thereby making differentiation possible based mainly on needle Mn concentration (FIGURE 9 and APPENDIX 5). This result also characterizes the chlorotic area at NP12 as having low concentrations of Ca, Mg, Mn, and Zn in relation to the other sites.

FIGURE 9- PRINCIPAL COMPONENTS ANALYSIS FOR CONCENTRATIONS OF P, K, Ca, Mg, Al, Fe, Ni, Cu, Mn, AND Zn IN NEEDLES FROM THE SECOND FLUSH AT FIVE SITES (RPP19, RPP11, SP14, SP18 AND NP12) IN THE STATE OF SANTA CATARINA, BRAZIL



SOURCE: The author (2023).

LEGEND: Pair areas: C = chlorotic area; S = supposed control area. Numbering: 1 = RPP19 – Rio do Peixe Plateau, age 19; 2 = RPP 11 – Rio do Peixe Plateau, age 11; 3 = SP14 – Serrano Plateau, age 14; 4 = SP18 – Serrano Plateau, age 18; and 5 = NP12 – North Plateau, age 12 years.

2.5 DISCUSSION

The distinct conditions for expression of *P. taeda* chlorosis in soils derived from basic igneous or sedimentary parent source materials are individually discussed henceforth. Chlorotic areas in the Rio do Peixe Plateau region (RPP11 and RPP19) had more basic or alkaline parent material sources, less developed A horizon, more rockiness and stoniness, and higher slope relief based on soil surveys. Chlorotic sites had higher soil pH, lower exchangeable Al^{3+}, higher exchangeable bases (Ca^{2+} and Mg^{2+}), higher concentrations of micronutrients (Mn, Zn, and Cu), and lower Fe availability. Leaf tissue had higher concentrations of Ca and Mg; this was also true for wood and partially true for bark. Lower Fe and Al concentrations in needles and Mn in wood and bark were observed at one site. Therefore, soil conditions and slope relief were more limiting to trees in chlorotic areas compared to control sites with more favorable conditions in terms of soil chemical properties and plant nutrition. Therefore, both acidity parameters and availability of macro- and micronutrients indicate a greater degree of soil fertility in areas displaying symptoms of chlorosis.

The Serrano Plateau region exhibited differences among sites. The 14-year-old chlorotic site (SP14) was derived from more basic parent source material and had an eroded A horizon based on soil surveys. Soil chemical analyses showed small differences between sites, which was restricted to Mn and Cu. For trees, high Mn concentrations in needles, wood and bark led to low values of Fe/Mn ratio. In addition, higher Ca concentration was observed in needles and bark, while higher Mg concentration was noted in wood. Thus, the presence of soil erosion and high Mn concentrations in soil and plant tissues (needles, wood and bark) were characteristics of the chlorotic site. In turn, the 18-year-old chlorotic site (SP18) had parent source material that was also more basic and had higher soil pH and concentrations of Ca, Mg, Mn, Zn, and Cu, and lower Fe concentration. The lower values of Fe in needles and bark led to a lower Fe/Mn ratio in these tree tissues.

The combination of higher slope relief with more stoniness and rockiness likely increases plant stress and, consequently, the potential for tree chlorosis symptoms on soils derived from igneous parent source material. The influence of slope has been well recognized as a condition that increases abiotic stress and the occurrence of Pinus decline in the USA (ECKHARDT; MENARD 2008; COYLE et al., 2015). The importance of soil granulometry in relation to chlorosis occurrence was reported by Coyle et al. (2020). They noted that a higher silt fraction was associated with chlorosis. However, the occurrence of clayey to very clayey soils in the chlorotic and control areas in the current study does not confirm this hypothesis. The importance of erosion on the incidence of *P. taeda* decline, as reported by Eckhardt and Menard (2008), has a high likelihood for explaining the occurrence of chlorosis at the 14-year-old Serrano Plateau site (SP14).

Soil organic carbon (an indirect indication of soil N availability) was generally high in all study sites and did not exhibit great difference between chlorotic and control areas. However, some sites showed a slightly higher SOC concentration in control areas and in topsoil horizons. Coyle et al. (2020) found slightly lower SOC (30%) and soil N values (20%) in the 15–30 cm layer in non-chlorotic areas compared to others with decline symptoms. However, higher SOC were observed in *P. resinosa* displaying symptoms of tree decline (KLEPZIG et al., 1991).

Lower soil acidity (higher pH and lower Al^{3+}) and higher soil exchangeable bases (Ca^{2+} and Mg^{2+}) in igneous chlorotic sites (Rio de Peixe and Serrano Plateau) may be related to other factors such as disease and weed incidence. In Germany, Jung et al. (2000) noted that decline symptoms in 35 Quercus stands were related to clayey and less acidic soils that favored pathogen proliferation and subsequent infection of fine roots. Furthermore, Brunson (2013) found higher levels of N and exchangeable bases in areas with greater weed infestation, which in turn may be associated with pine decline.

The lower soil acidity and nutrient availability at chlorotic sites were reflected by higher nutrient concentrations in plant tissues. In needles (upper third of the canopy), nutrient concentrations were above the critical levels of 4.0 g kg^{-1} for K, 1.5 g kg^{-1} for Ca, 0.8 g kg^{-1} for Mg, 40 mg kg^{-1} for Mn, 20 mg kg^{-1} for Fe and Zn, and 3 mg kg^{-1} for Cu (SYPERT, 2006). In a study involving 2,663 plots in the southeastern USA, Albaugh et al. (2010) reported maximum concentrations of 3.1 g kg^{-1} Ca, 1.5 g kg^{-1} Mg, 916 mg kg^{-1} Mn, and 64.9 mg kg^{-1} Zn. Viera and Schumacker (2009) found that needles of P. taeda grown on two soils derived from basalt parent source material in southern Brazil had values of 5.2 and 4.1 g kg^{-1} Ca, 1.5 and 1.1 g kg^{-1} Mg, 1,068 and 731 mg kg^{-1} Mn, and 26.5 and 32.7 mg kg^{-1} Zn. This suggests that igneous derived soils were associated with high concentrations of nutrients.

A relationship between Mn concentration and occurrence of Pseudotsuga menziesii ('Douglas fir') decline has been reported by Kaus and Wild (1998).

Although fallen needles from low growth trees had high Mn concentration, they could not establish a direct relationship with Fe deficiency; thus, the authors stated that more detailed work was required to determine the influence of Mn concentration on other nutrients in plant organs. An inverse relationship between leaf Mn concentration and *P. taeda* growth observed by Pereira et al. (2022) suggested that Mn toxicity may play a role in tree growth. However, Reissmann and Wisniewski (2000) pointed out that higher concentration of Mn (in relation to Fe) can be a sign of nutritional imbalance that can be evaluated by the Fe/Mn ratio.

High soil acidity, greater soil water retention, low soil oxygen content, and presence of ferromagnesian minerals in soil favor high Mn availability (COYLE et al., 2020). In the current study, the main factor for high soil Mn concentration was not soil pH, since chlorotic sites had equal or greater soil pH values; however, clayey granulometry and soil compaction could have resulted in more water retention and lower soil aeration (SILVA et al., 2006), which favors soil Mn availability (SILVA et al., 2018).

The higher slope relief of some sites can also have an effect on Mn availability. Fernando et al., (2015) reported that – in addition to exacerbating Mn toxicity by photodegradation and photo-oxidative stress – sunlight may also be responsible for increasing leaf Mn accumulation. An excess supply of Mn can also impose stress by competing with other ions during plant uptake, thus decreasing the uptake of other nutrients with the same charge (ST CLAIR; LYNCH 2005). Through visual observations, we noted that slopes with greater exposure to solar radiation displayed greater intensity of *P. taeda* chlorosis symptoms.

Chlorotic and control areas at the 12-year-old North Plateau site (NP12) showed very similar pedological characteristics, except for relief depression (visualized in the field) that occurred only in the chlorotic area. In contrast to igneous sites, soil in the chlorotic area at NP12 had lower concentrations of Ca, Fe, Mn, and Cu than the control. Needle tissue showed lower concentrations of Ca, Mg, Mn, Zn, and Cu, while bark had lower concentrations of Ca, Mg and Mn, and higher values of Zn, and wood had lower concentrations of Ca. In addition, concentrations of Ca and Mg in needle tissue from the chlorotic area were below the respective critical levels of 1.5 and 0.8 g kg^{-1} for pine in the USA (SYPERT, 2006) or 0.5 and 0.8 g kg^{-1} for Pinus in Brazil (REISSMANN, 1981).

Symptoms of Mg deficiency have been described as yellowing of needle tips with the base remaining green (ENDE; EVERS 1997; LAING et al., 2000), which corroborates our observations in the North Plateau site (FIGURE 5). The control area at RPP19 site also had Mg concentration in plant tissues below or close to the critical level (agreeing with soil results) but did not display chlorotic symptoms, indicating that other factors such as weather conditions and nutritional imbalance influenced appearance of symptoms. Needle K/Ca and K/Mg ratios observed at the RPP19 site were lower than those of the North Plateau control site. Several reports highlight the importance of these nutrient ratios for Pinus spp (MITCHELL, 2000; SUN; PAYN 1999; XIE et al., 2021). Cakmak and Marschner (1992) reported a correlation between Mg deficiency symptoms and light intensity. This has been noted for needles exposed to greater sunlight incidence (ENDE; EVERS, 1997). Thus, this fact may explain why Mg deficiency tends to appear in the upper mid-crown. Fink (1991) reported yellow needles of mature spruce (*Picea abies* Karst) trees as a deficiency of Mg in acidic soils, which was described as a 'new type of forest decline'. This deficiency resulted in starch accumulation, which suggests less carbohydrate availability for roots, thereby impacting their growth and function.

In general, soil concentrations of Fe lower than 194 mg kg^{-1} and Mn higher than 93 mg kg^{-1} were associated with pine chlorosis. Soils with greater fertility and very high Mn availability may also be indicative of a likely problem with pine decline (ZAITSEV et al., 2020). Contrary to our observations, Coyle et al. (2020) observed symptoms of *P. taeda* decline when Mn was lower at soil layers of 0–15 and 15–30 cm. For the 0–15 cm soil layer, these authors reported Mn concentrations of 60 and 36 mg dm-3 for chlorotic and control areas, respectively.

The mass of 100 fascicles can also be a good indicator of plant nutritional status; however, obtained values were very close to 17 g for both chlorotic and control areas. This indicates that trees were under stress but needle growth was not negatively affected. For *P. taeda* of different ages (12 to 25 years) and different locations in the southeastern USA, Carlson et al. (2014) found a mean mass of 100 fascicles around 15 g (thus, near to our findings) showing that this needle characteristic may be relatively constant within age and position in the tree.

Although we observed no significant changes in fine root biomass regardless of area and symptoms, values were very low compared to observations of other researchers (LOPES et al., 2010; KASEKER et al., 2012; RABEL et al., 2020). In

Pinus plantations located at Cambará do Sul (state of Rio Grande do Sul, southern Brazil), Lopes et al. (2010) found 1.4 Mg ha^{-1} of fine roots in the 0–10 cm soil layer, thus about 22 times higher than that observed at SP14 site in our study. The low fine root biomass obtained in our study may indicate a soil restriction for fine root growth and a predisposition to water and mineral nutrition stress. We also highlight the importance of root colonization by mycorrhizae as a factor to be considered in the interpretation of chlorosis symptoms. The symbiosis between fine roots and mycorrhizae allows for greater exploitation of soil volume that can facilitate the uptake of nutrients, especially those with low soil mobility (LANDEWEERT et al., 2001). In this context, Trautwig et al. (2017) associated mycorrhiza presence with the health of pines in the USA.

Litter biomass was below the range of 40–90 Mg ha^{-1} reported for other low-growth sites in southern Brazil (MOTTA et al., 2014; ZUCON et al., 2020; PEREIRA et al., 2022). In our study, litter biomass between 10 and 30 Mg ha^{-1} were similar to those reported by Schumacher et al. (2008) and Rabel et al. (2020) for basalt and sedimentary derived soils. An inverse relationship between site quality and litter accumulation has been noted by Reissmann and Wisniewski (2000). The lower litter biomass at 19-year-old Rio do Peixe Plateau region (RPP19 and RPP11) and 14-year-old Serrano Plateau chlorotic sites (SP14, SP18) could be related to more open crown canopy that allowed for more light penetration, while the lower amount of litter at the North Plateau site (NP12) could be associated low soil fertility.

2.6 CONCLUSIONS

Although not conclusive, the present study suggests a possible relationship between soil relief slope and an eroded topsoil A horizon, as well as nutritional imbalance and nutrient deficiency in some areas. Whether observed chlorotic symptoms are a cause or an effect of pine decline has also not been well established. In igneous derived sites, chlorotic areas had higher soil fertility and higher nutrient concentrations in tree tissues (needle, wood and bark) than control areas. Concentrations of Mn and Ca were higher for chlorotic areas, and needle chlorosis was characterized by basal paling of needles. Lack of an A horizon was also characteristic of areas exhibiting chlorosis. On sedimentary derived soil, the chlorotic site had low soil fertility, lower tissue nutrient concentrations (particularly

Mg), and needle tip chlorosis. There was no difference in litter biomass between chlorotic and control sites. Findings suggest two distinct conditions that may be causing *Pinus taeda* chlorosis in southern Brazil: low fertility soils resulted in a lack of Mg, while high fertility soils may be nutritionally imbalanced due to high levels of Ca and/or Mn. A predominance of tree chlorosis symptoms on very clayey soils in some areas was observed. In this context, very clayey soils tend to have more problems with compaction, which increases soil bulk density that impairs root growth. Therefore, additional research focused on soil physical properties can help elucidate causes of tree chlorosis symptoms. Further investigation is required to better understand factors influencing *Pinus taeda* chlorosis, such as the association of biotic and environmental stresses.

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3 CHAPTER II: RESPONSE TO FERTILIZATION AND LIMING IN PINUS *TAEDA* L.: A META-ANALYSIS OF THE AMERICAS

3.1 ABSTRACT

Loblolly pine (*Pinus taeda* L.) is one of the most planted forest species in the Americas. Since few studies have comprehensively assessed loblolly pine responses to fertilization, the present study performed a meta-analysis of the Americas based on 44 publications (1970-2022) of loblolly pine fertilization under field conditions. In general, fertilization increased root dry matter (+33%), litter (+21%), plant height (+6%), trunk diameter (+9%), wood productivity (+30%), and needle concentrations of P (+9%), K (+36%), Ca (+17%), Mg (+14%), and S (+12%). Wood production was higher with residue fertilization, primarily with use of composite residues (cellulosic sludge + ash), compared to mineral fertilization. In regards to mineral applications, wood production was higher when multiple nutrients were added from fertilization and liming operations. Early fertilizations (< 1 year) or on established trees (2-8 years), showed similar increases in wood production with higher responses occurring on sandy soils. These factors generally increased needle nutrient concentrations, except for no alteration or slight decreases in N under most conditions. The present study revealed loblolly pine responses to contrasting application strategies, which can help identify efficient fertility management practices for this commercially forest species.

Keywords: Loblolly pine. Planted forest. Waste. Sandy soil. Needle composition

3.2 INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is the most planted conifer in the southeastern United States of America (USA) being native of this region. In Brazil and other South American countries, loblolly is also widely cultivated in support of several timber industries (IBÁ, 2019). In the last few decades, natural regeneration of harvested forests in the southeastern USA has moved toward systematic introduction of improved genetic materials and intensification of weed control, soil preparation, and fertilization (FOX *et al.*, 2007; CARLSON *et al.*, 2014; ZHAO *et al.*, 2019; CARTER *et al.*, 2021). When introduced in South America, similar silvicultural practices have been largely followed, except for fertilizer use, even if plantings occurred on low fertility soils (MOTTA *et al.*, 2014). In Brazilian planted pine environments, soil nutrient depletion suggests a need for implementing fertilizer and lime applications (SIXEL *et al.*, 2015; GATIBONI *et al.*, 2020).

Nutrient application should be directed towards elements limiting pine growth, which can vary widely depending on soil parent material and can be strongly influenced by soil attributes such as texture and native fertility. In the US, nitrogen (N) responses have been reported for soils with organic matter levels of 1.33 and 0.37 %

for six sites under Pleistocene terraces and seventeen sites under other soil formations, respectively (CARLSON *et al.*, 2014). These organic matter levels were much lower than the 5.2, 4.2, 3.3, and 2.4% noted for soils originating from basalt, granite, claystone, and sandstone in pine production areas of southern Brazil (HOOGH, 1981). Such observations may help explain why few studies have evaluated N fertilization in this region. In contrast, pine responses to phosphorus (P) application have frequently been reported for the Americas (FOX *et al.*, 2005; CARLSON, 2014; ALBAUGH *et al.*, 2021; CONSALTER *et al.*, 2021a, b), especially on sandy soils (STAHL, 2018). Unlike P, even soils with low available potassium (K) have shown lower response (CARTER *et al.*, 2021) or no response (ALVES *et al.*, 2013; CONSALTER *et al.*, 2021b). Use of micronutrients has garnered some interest since their application with macronutrients has been shown to increase tree production (CARLSON *et al.*, 2014).

Use of limestone and alkaline organic residues to elevate soil pH and supply of calcium (Ca) and magnesium (Mg) has been evaluated since Mg deficiency (associated with or without Ca deficiencies) has been reported for pines in the USA and Brazil (CHAVES; CORRÊA *et al.*, 2005; SOUTH *et al.*, 2017; ROCHA *et al.*, 2019; ADAM *et al.*, 2021). Alkaline organic residues may also contain several macro- and micronutrients that increase the possibility of pine responses (RODRIGUEZ *et al.*, 2018; SASS *et al.*, 2020; RABEL *et al.*, 2021). In such cases, joint application of more than one nutrient could result in synergistic effects (ESPINOZA *et al.*, 2012; CARLSON *et al.*, 2014; ALBAUGH *et al.*, 2021).

As a function of fertilizer and soil pH corrective applications, changes in loblolly pine leaf nutrient concentrations have exhibited variability between sites. Sypert (2006) evaluated addition of 10 elements (N - nitrogen, P - phosphorus, K - potassium, Ca - calcium, Mg - magnesium, S - sulfur, Mn - manganese, Zn - zinc, B – boron, and Cu - copper) at several locations and reported increased foliar concentrations for 9, 6, 4, and 1 elements for sites in Georgia, Texas, South Carolina, and Alabama, respectively. In this same study, a decrease was registered for 4 elements at South Carolina sites and 1 element at Texas sites, which was possibly related to a dilution effect and/or an interaction. Similarly, Pereira *et al.* (2022) associated residue use with decreased foliar P, Ca, S, Fe, Mn, and B and increased tree growth, which suggests a significant dilution effect.

Given the long growth cycles of loblolly pine, time of application can also play a role in responses to nutrient and soil corrective applications. Such applications can commonly occur at planting or mid-rotation following first or second thinning operations. Application at planting can correct for deficient elements and favor initial growth (MORO *et al.*, 2014; MOTTA *et al.*, 2014). However, applications at stand initiation can lead to nutrient losses due to reduced ability of young plants with slow growth (i.e., less developed and inefficient root system) to intercept and uptake highly soil mobile nutrients, such as N and K. Mid-rotation fertilization can favor greater growth of trees with wide root systems and uptake capacity. The formation and accumulation of litter following nutrient and corrective applications can allow for nutrient retention within this horizon, thereby contributing as source of Cu and Zn, and soil acidity corrective (ADAM et al., 2021).

An integrated evaluation of factors influencing fertilization efficiency is a complex and onerous task when conducted using conventional experimentation. However, several investigations have taken an integrative approach of evaluating plant responses to different management practices through meta-analysis of data from the published literature base (BARBOSA *et al.*, 2022a, 2022b; MARIOTTI *et al.*, 2022). For example, a meta-analysis study evaluating the effects of liming and wood ash on forest ecosystems showed that wood ash provided a greater increase in growth and wood production compared to liming alone (REID; WATMOUGH, 2014).

In the present study, a meta-analysis was conducted to evaluate the efficiency of fertilization on the production and nutritional status of loblolly pine under various growth conditions in the Americas. Our hypothesis was that fertilization and liming, whether with mineral or residue applications, favors the growth and nutrition of loblolly pine, especially when multiple nutrients were provided together.

3.2 MATERIAL AND METHODS

3.2.1 LITERATURE SEARCH AND DATA COMPILATION

A search for publications that evaluated the response of loblolly pine to fertilization was performed on Google Scholar between October 2021 and June 2022. The following combinations of terms were used in this search: “*Pinus taeda*”, “fertilization”, “America” and “Brazil”, or “USA”. We reviewed each publication to determine whether these studies met the following criteria: (1) study conducted under field conditions in the Americas; (2) contained treatments without fertilization (control treatment) and treatments with fertilization (experimental treatment); and (3) results could be directly extracted from the text, tables, and/or figures.

After careful evaluation, 44 publications representing field trials conducted at 65 sites (Argentina n = 5; USA n = 20; Brazil n = 40), were selected for this meta-analysis (MOSCHLER *et al.*, 1970; VAN LEAR , 1980; TORBERT JR. ; BURGER, 1984; ALBAUGH *et al.*, 1998; FERNANDEZ *et al.*, 1999; MAIER; KRESS, 2000; PIATEK; ALLEN, 2000; LEE; JOSE, 2003; ALBAUGH *et al.*, 2004; IBAÑEZ *et al.*, 2004; RODRIGUES, 2004; SAYER *et al.*, 2004; FOX *et al.*, 2005; PAIM, 2007; ALBAUGH *et al.*, 2008; COYLE *et al.*, 2008; SAMUELSEN *et al.*, 2008; SAMUELSEN *et al.*, 2009; HASHIMOTO *et al.*, 2011; MARTINS, 2011; PÉRTILE, 2011; SCHNEIDER, 2011; ALBAUGH *et al.*, 2012; SCOTT; BLISS, 2012; CAMPOE *et al.*, 2013; FAUSTINO *et al.*, 2013; ALBAUGH *et al.*, 2014; MORO *et al.*, 2014; BATISTA *et al.*, 2015; MAGGARD *et al.*, 2016; ALBAUGH *et al.*, 2017; MAGGARD *et al.*, 2017; PIVA; DLUGOSZ, 2018; RODRIGUEZ *et al.*, 2018; STAHL, 2018; TRAZZI *et al.*, 2019; VANCE, 2019; SCHULTE *et al.*, 2020; SASS *et al.*, 2020; ZUCON *et al.*, 2020; ADAM *et al.*, 2021; CONSALTER *et al.*, 2021a; RABEL *et al.*, 2021; and PEREIRA *et al.*, 2022).

General information on these research factors (location, soil texture, fertilization type, tree age at fertilization, and nutrients applied), means (X), standard deviations (SD), and number of replications (n) were extracted to compose the values for assessed tree attributes (production of wood, litter, and roots; tree height and trunk diameter (DBH); and needle concentrations of N, P, K, Ca, Mg, and S). In studies that reported only the coefficient of variation (CV%), equation (1) was used to obtain SD values:

$$SD = \frac{CV\%}{100}X \quad (1)$$

For studies that did not report data variability information, the mean SD was calculated for the control and experimental treatments using all data of each study. All data were extracted and compiled into a spreadsheet.

3.2.2 DATA CATEGORIZATION

To evaluate the response of loblolly pine to fertilization, three groups of control factors were considered: fertilization factor; plant factor; and soil factor. Only control factors with more than ten paired comparisons were considered. For the fertilization factor, fertilizer type (mineral or residue), number of nutrients applied (one, two, three or more than three), type of nutrients applied (macronutrients; macro + micronutrients), and mineral fertilization (with or without lime application) were considered. Due to the low number of paired comparisons, it is worth noting that it was not possible to analyze liming in isolation, but only in association with fertilizer applications. Plant factors were categorized based on tree age at the time of nutrient application (< 1 yr, 2 - 8 yrs, and 9 - 16 yrs). Soil factors were based on clay content (< 15%, 15 - 30%, and 31 - 65%).

3.2.3 DATA ANALYSIS

Magnitude of the fertilization effect was calculated using the natural logarithm of the response ratio (lnRR; equation 2) as the effect size (HEDGES *et al.*, 1999):

$$\text{lnRR} = \ln\frac{X_e}{X_c} \qquad (2)$$

where Xe and Xc are the mean values for the experimental and control treatments, respectively. Variance (v) was calculated as (equation 3):

$$v = \frac{SD_e^2}{n_e X_e^2} + \frac{SD_c^2}{n_c X_c^2} \qquad (3)$$

where SDe, ne, SDc, and nc represented standard deviations and numbers of replications for experimental and control treatments, respectively. Response ratio variance was required to obtain balanced response ratio values and 95% confidence intervals (CI). Thus, the effect of fertilization was considered significant when the 95% CI of the response ratio did not overlap zero. Mean response ratio and CI values were generated using random-effects method with restricted maximum likelihood estimation.

To facilitate interpretation of variations between experimental and control treatments, the response ratio and CI of the treatments were transformed (equation 4):

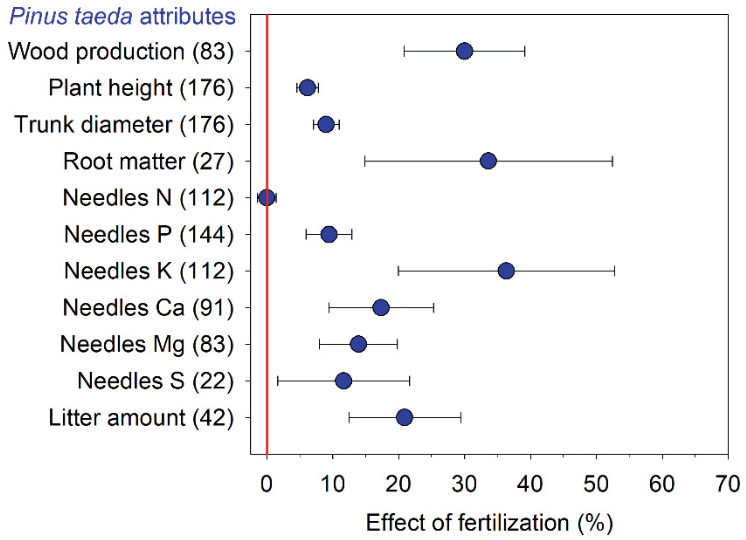
$$\%\text{change} = (e^{\text{lnRR}} - 1) \times 100 \qquad (4)$$

All analysis were performed using OpenMEE software (WALLACE *et al.*, 2017).

3.3 RESULTS

Fertilization provided a significant increase in several analyzed loblolly pine attributes, such as wood production (+30%), root dry matter (+33%), and needle K concentration (+36%) (FIGURE 10). Fertilization increased plant height, trunk diameter, and litter amount by 6%, 9%, and 21%, respectively. Concentrations of other nutrients (i.e., P, Ca, Mg, and S) displayed increases between 9% and 17%, and only N concentrations were not affected.

FIGURE 10- EFFECT OF FERTILIZATION ON LOBLOLLY PINE ATTRIBUTES COMPARED TO THE ABSENCE OF FERTILIZATION.

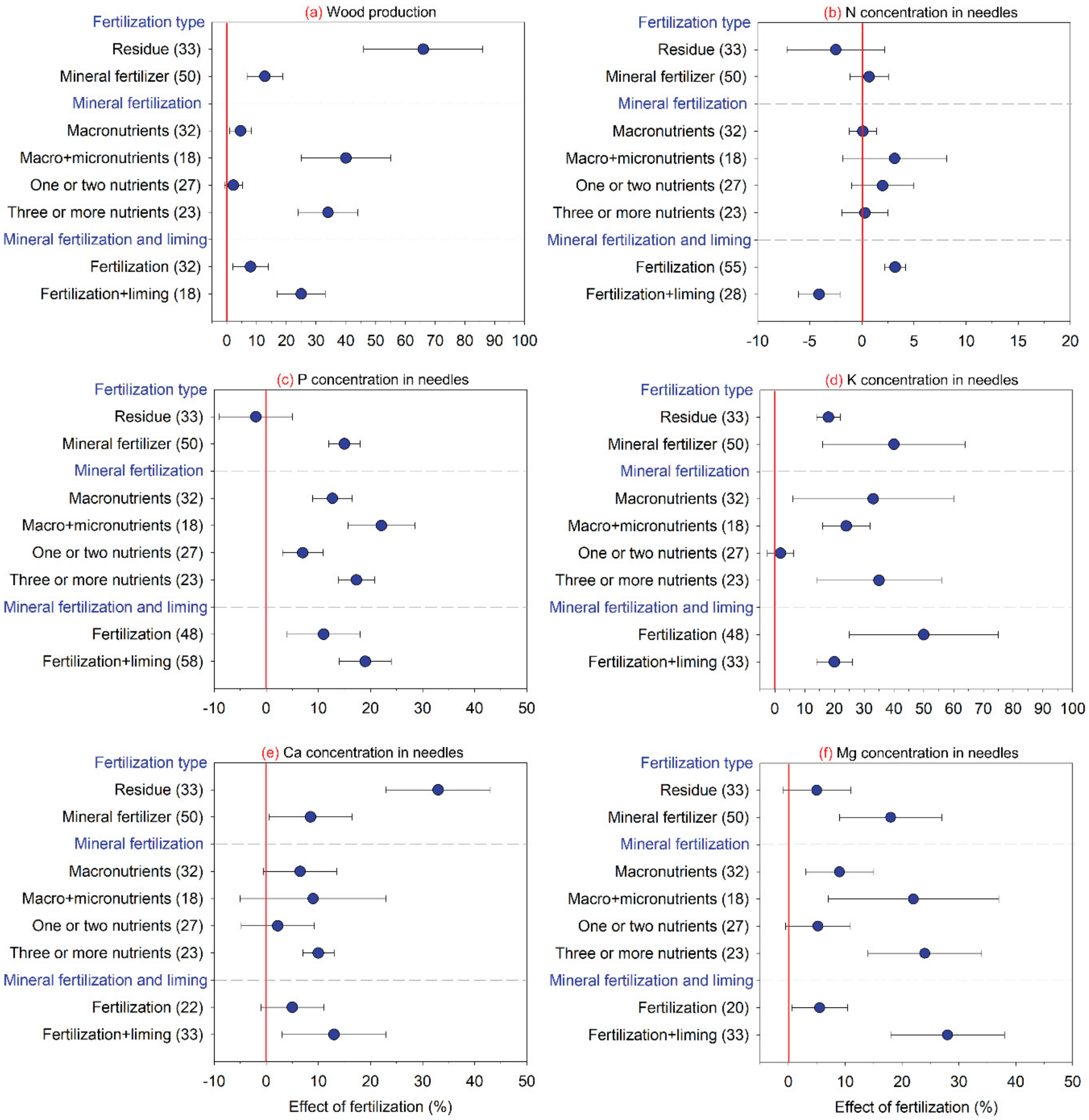


SOURCE: The author (2022).

LEGEND: Effect of fertilization is significant when the 95% CI response ratio value did not overlap zero. Values are means ± 95% of the confidence interval (CI), and the number of comparisons used in the analysis of each attribute is presented in parentheses.

There was a direct relationship between increased loblolly pine wood production and fertilization, especially with residue applications (FIGURE 11). Analysis of residue type indicated that loblolly pine response was greater with applications of composite waste (cellulosic sludge + ash) compared to only applying cellulosic sludge (FIGURE 12). For mineral fertilization, wood production was greater with macro- and micronutrient applications (three or more nutrients applied) and when fertilization was combined with liming (FIGURE 11).

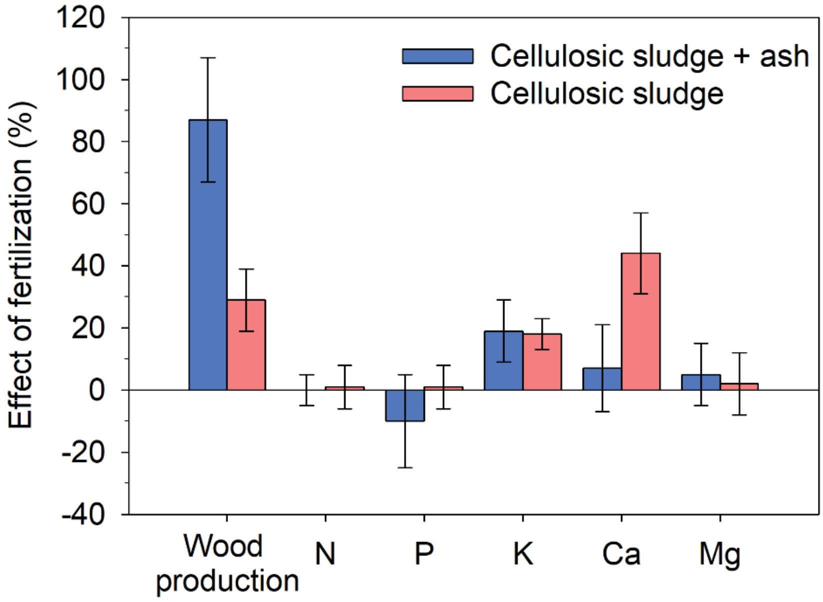
FIGURE 11- EFFECT OF FERTILIZATION ON WOOD PRODUCTION AND NUTRIENT CONCENTRATION (N – NITROGEN; P – PHOSPHORUS; K – POTASSIUM; Ca – CALCIUM; AND Mg – MAGNESIUM) IN LOBLOLLY PINE COMPARED TO THE ABSENCE OF FERTILIZATION



SOURCE: The author (2022).

LEGEND: Values are means ± 95% of the confidence interval (CI), and the number of comparisons used in the analysis of each attribute is presented in parentheses. effect of fertilization is significant when the 95% ci response ratio values did not overlap zero.

FIGURE 12- EFFECT OF FERTILIZATION WITH DIFFERENT RESIDUES ON WOOD PRODUCTION AND NUTRIENT CONCENTRATION (N – NITROGEN; P – PHOSPHORUS; K – POTASSIUM; Ca – CALCIUM; AND Mg – MAGNESIUM) IN LOBLOLLY PINE COMPARED TO THE ABSENCE OF FERTILIZATION



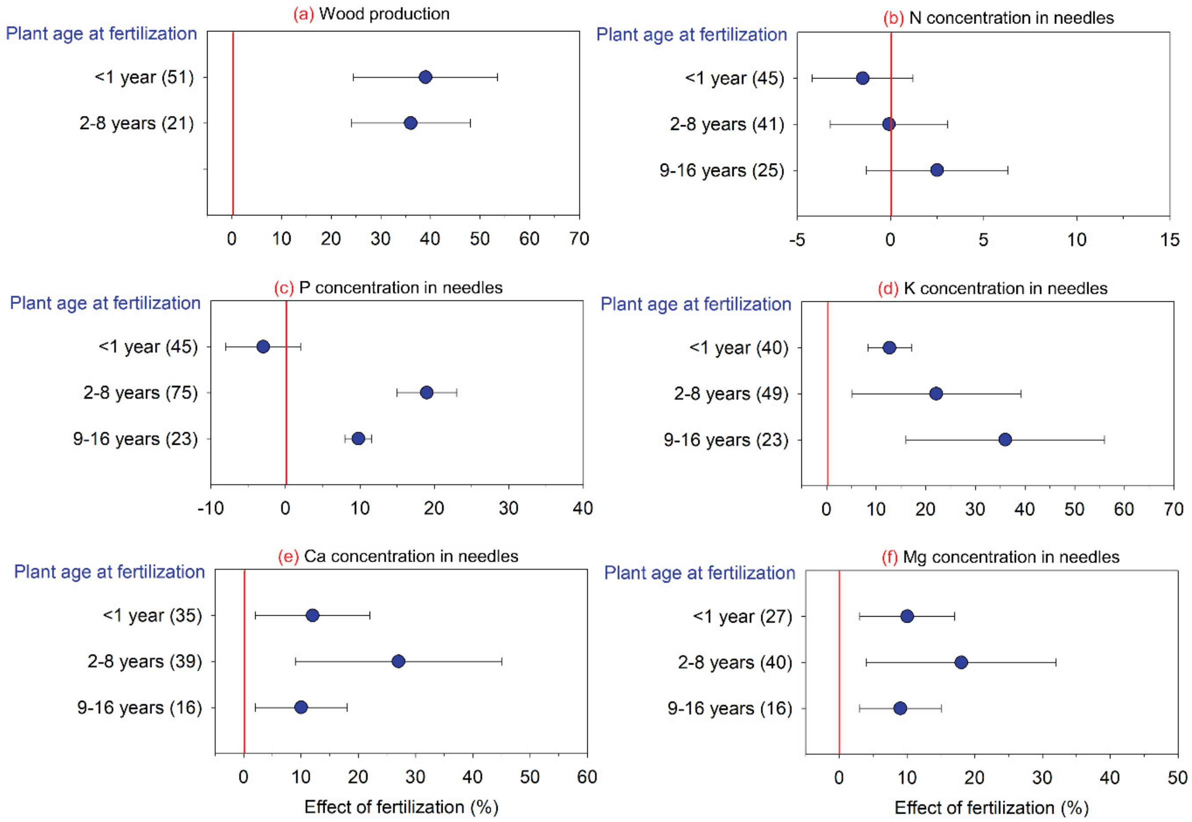
SOURCE: The author (2022).

LEGEND: Values are means ± 95% of the confidence interval (CI). effect of fertilization is significant when the 95% ci response ratio values did not overlap zero.

Regarding needle nutrient composition, mineral fertilization increased concentrations of various nutrients (i.e., P, K, Ca, and Mg), while fertilization with residue only increased K and Ca concentrations (FIGURE 11). Mineral fertilization with three or more nutrients was more efficient in increasing concentrations of P, K, Ca, and Mg in needles. Application of macronutrients alone or in association with micronutrients increased needle P, K, and Mg concentrations. Likewise, fertilization alone or in association with liming increased needle P, K, and Mg concentrations. However, Ca and Mg concentrations were higher as a result of liming with the opposite being observed for N concentrations.

Increased loblolly pine wood production in response to fertilization occurred regardless of plant age at application since similar efficiency patterns where observed for trees less than one year-old up to trees 8 years-old (FIGURE 13). On the other hand, this control factor had contrasting results based on the nutritional composition of loblolly pine needles: no effect for N; increases in P concentrations only when fertilization was performed on trees aged between 2 and 16 yrs; and increases in concentrations of other elements (i.e., K, Ca, and Mg) for all tree ages with fertilization.

FIGURE 13- EFFECT OF FERTILIZATION ON WOOD PRODUCTION AND NUTRIENT CONCENTRATION (N – NITROGEN; P – PHOSPHORUS; K – POTASSIUM; Ca – CALCIUM; AND Mg – MAGNESIUM) IN LOBLOLLY PINE COMPARED TO THE ABSENCE OF FERTILIZATION IN RESPONSE TO PLANT AGE AT FERTILIZATION

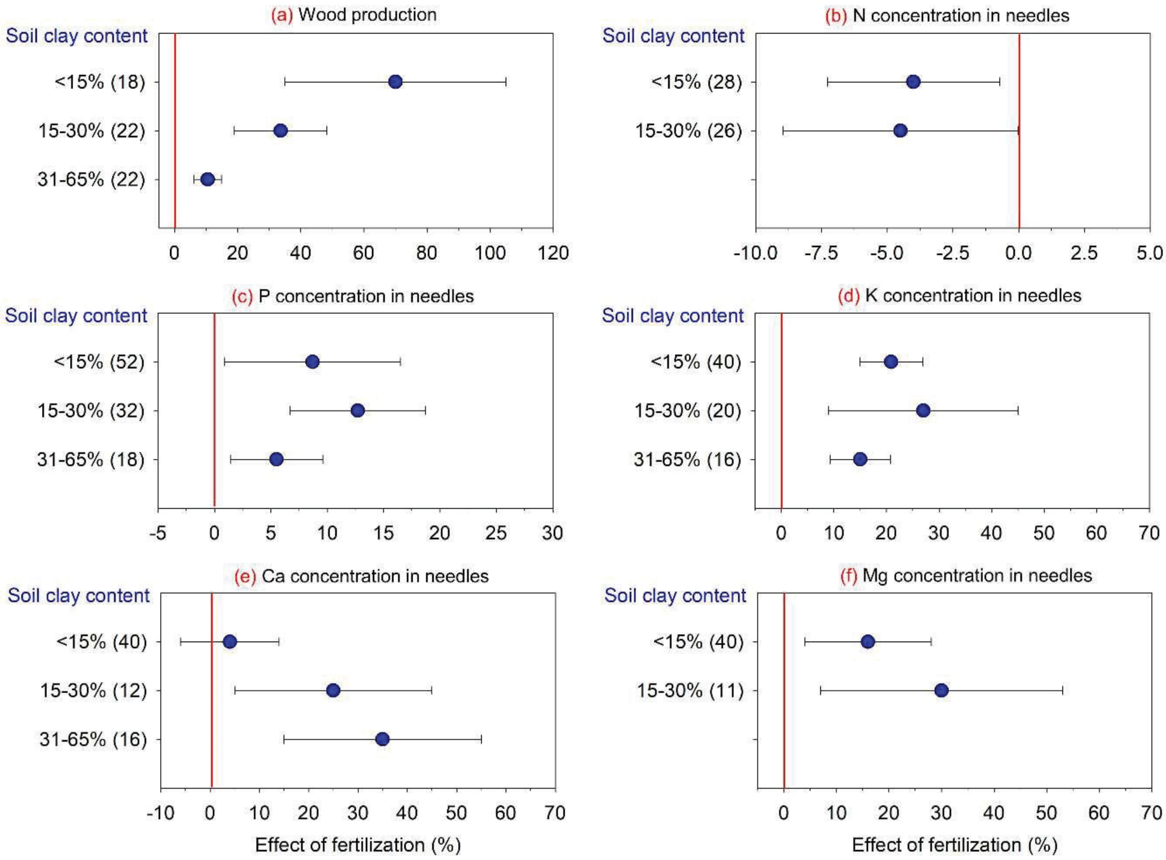


SOURCE: The author (2022).

LEGEND: Values are means ± 95% of the confidence interval (CI), and the number of comparisons used in the analysis of each attribute is presented in parentheses. effect of fertilization is significant when the 95% ci response ratio values did not overlap zero.

Soil granulometry was an important factor in understanding variations in fertilization efficiency on loblolly pine wood production (FIGURE 14). Although fertilization increased wood production on soils with different clay values, the greatest tree response (+70%) occurred for soils with clay contents below 15%. Regarding nutritional composition of needles, fertilization decreased N concentrations and increased P, K, and Mg concentrations regardless of soil clay content. The only exception was Ca concentration in needles, which only increased for soils with clay contents between 15 - 30% and 31- 65%.

FIGURE 14- EFFECT OF FERTILIZATION ON WOOD PRODUCTION AND NUTRIENT CONCENTRATION (N – NITROGEN; P – PHOSPHORUS; K – POTASSIUM; Ca – CALCIUM; AND Mg – MAGNESIUM) IN LOBLOLLY PINE COMPARED TO THE ABSENCE OF FERTILIZATION IN RESPONSE TO SOIL CLAY CONTENT



SOURCE: The author (2022).

LEGEND: Values are means ± 95% of the confidence interval (CI), and the number of comparisons used in the analysis of each attribute is presented in parentheses effect of fertilization is significant when the 95% ci response ratio values did not overlap zero.

3.4 DISCUSSION

3.4.1 GENERAL EFFECT OF FERTILIZATION

Our meta-analysis revealed that fertilization caused a significant increase in wood production (+30%) of loblolly pine, which supports findings of multiple studies conducted in the Americas (ZHAO *et al.*, 2019; CARTER *et al.*, 2021; FOX *et al.*, 2007; CARLSON *et al.*, 2014; MORO *et al.*, 2014; ADAM *et al.*, 2021; PEREIRA *et al.*, 2022). Although less expressive, fertilization was also responsible for increasing tree height and trunk diameter. Similarly, Consalter *et al.* (2021a) reported smaller increases in height and diameter and greater wood volume responses with loblolly pine fertilization.

Fertilization favored enhanced root growth, probably due to improved nutritional status (ALVAREZ-CLARE; MACK, 2015). Increases in pine root systems were reported by Albaugh *et al.* (1998, 2004) and Campoe *et al.* (2013) as an effect of fertilization. In a 16-year-old forest, Albaugh *et al.* (2004) found increases of 100 and 130 % in total aboveground and belowground biomass, respectively; this corroborates findings obtained in the present study. In an 11-year-old forest litter layer, Adam *et al.* (2021) found an increase in fine root growth with fertilizer and limestone use, which was associated with a large decrease in the Ca/Al ratio of roots.

Litter accumulation may be associated with at least three factors: quantity of deposited material, quality of deposited material, and soil fertility at the forest site. Higher litter deposition was due to increased plant growth in response to fertilization (FIGURE 10). However, surface addition of nutrients and soil acidity correctives at mid-rotation showed no change (ADAM *et al.*, 2021; RABEL *et al.*, 2021) or increased (CONSALTER *et al.*, 2021a; PEREIRA *et al.*, 2022) litter deposition. Prescott *et al.* (1992) suggested that higher microbial activity could accelerate litter decomposition, and perhaps this occurred in studies reporting a decrease in litter amounts; furthermore, fertilization may have decreased litter C/N ratio and resulted in greater decomposition (SANCHEZ, 2001). Non-alteration of needle N, a primary nutrient influencing decomposition processes, may explain the maintenance of litter. After evaluating 110 needle samples from *P. taeda* regions in the USA, Albaugh *et al.* (2010) ranked N as the least variable (Ca > K > Mg > P > S > N), suggesting low sensitivity to soil variation and possibly fertilization. Also, symptoms of N deficiency in Brazil are rare and restricted to areas having very shallow soils with growth occurring directly on the C horizon (MOTTA *et al.*, 2014). However, response to N use has been observed under different conditions in the USA (SYPERT, 2006; CARLSON *et al.*, 2014). Since N acts as a great promoter of vegetative growth, it is likely that the needle response to N use is influenced by the dilution effect, which causes an apparent decrease in response. The decrease in N concentration in needle fall and increases in litter fall from P alterations reported by Wienand and Stock (1995) were used to explain decreased litter decomposition and litter accumulation in a P. *elliotti* system.

A significant increase in needle K (+30%) was observed. Such changes may be associated with soil type in pine cultivations; these soils generally have low effective CEC and no K fixation (CARLSON *et al.*, 2014; BATISTA *et al.*, 2015; MOTTA *et al.*, 2014), which decreases K adsorption capacity and allows for ready availability when applied. In relation to the plant, K is a nutrient required in high concentrations and associated with luxury consumption (MARSCHNER, 2011), which together favors greater K concentration increases in loblolly pine needles.

In contrast to K, smaller increases in P concentration may be related to the specific adsorption of this nutrient to soil colloids, especially in very acidic soils (MOTTA *et al.*, 2014; POGGERE *et al.*, 2020). Additionally, increases in P concentration were less frequent and to a lesser extent than K (SYPERT, 2006; CONSALTER *et al.*, 2021b). Small variations in P concentration have been reported in the literature; Sypert (2006) indicated a significant variation in P concentration with an increase from 1.1 to 1.2 g kg^{-1}, confirming small leaf variation. Small variations were also reported by Albaugh *et al.* (2010) with a median P value (g kg^{-1}) of 1.1 and a P range from 1.0 (25% of samples) to 1.1 (75% of samples) for 110 sites in the USA.

Limestone use increased Ca and Mg concentrations; in comparison, these values were lower than K and higher than P. Commonly observed in pine systems, Mg deficiencies could promote tree responses to fertilization and/or liming (ENDE; EVERS, 1997). However, the smaller variation in needle Ca and Mg (compared to K concentration) can be explained by slow mobility from roots to needles since high adsorption within xylem tissue can act as a chromatographic column (HEIJDEN *et al.*, 2015).

3.4.2 EFFECT OF FERTILIZATION STRATEGIES

Greater tree response was observed when more than one nutrient was supplied due to synergistic effects as reported for N and P (Albaugh *et al.*, 2021) or NPK plus micronutrient additions (CARLSON *et al.*, 2014; CARTER *et al.*, 2021). Fertility experiments that add only one element (without addressing other required nutrients) can limit loblolly pine response on soils lacking multiple nutrients. Positive responses to fertilizer and lime use can be associated with the addition of three or more nutrients since lime can be a Ca and Mg source (BATISTA *et al.*, 2015; ADAM *et al.*, 2021; ROCHA *et al.*, 2019). The prominent lack of Mg observed in the USA (ALBAUGH *et al.*, 2004), Brazil (ADAMS *et al.*, 2021; ROCHA *et al.*, 2019), and worldwide (ENDE; EVERS, 1997) forest systems clearly suggests that liming can complement fertilizer applications.

Compared to mineral fertilization, the greater response to residue applications can be related to multi-element additions and higher rates of nutrients and organic compounds (PEREIRA *et al.*, 2022). This can be illustrated with use of cellulose sludge plus ash (rich in Ca) compared to application of cellulose sludge alone. In pine plantations, respective increases of 127% and 113% from use of such residue mixtures were reported by Rodriguez *et al.* (2018) and Pereira *et al.* (2022), while increase of ~16% were noted with only using cellulose sludge (RABEL *et al.*, 2021). A further consideration regarding organic residue use is the slow release of nutrients such as N, P, and S (ZECH *et al.*, 1997) and how this benefits soil biota, contributes to nutrient cycling, and reduces pathogenic actions (D'HOSE *et al.*, 2018; LUO *et al.*, 2018).

Organic residue use could improve sustainability of forest plantations (RABEL *et al.*, 2021). These low-cost residue inputs represent a viable fertility option since the high cost of mineral fertilizer can be an impediment factor (ALLEN *et al.*, 2005). Albaugh *et al.* (2019) reported that increases in fertilizer consumption from 1969 to 1999 were followed by decreases in 2016, primarily due to higher costs. In intensively managed plantations, fertility practices targeting adequate nutrient levels in soil will allow for better productivity and increased wood production (Wear; Greis 2002; SIXEL *et al.*, 2015; CONSALTER *et al.*, 2021a, b). Although companies in the forestry sector follow strict rules at various stages of cultivation, primarily aimed at certification of wood or derivatives for exports (ARAÚJO *et al.*, 2009), adequate replacement of exported nutrients (from harvests) is often not considered (MOTTA *et al.*, 2014). Thus, to avoid low tree growth and soil degradation from nutrient depletion, fertilization and liming could be important practices ensuring greater sustainability of loblolly pine production systems.

Regarding needle nutrient concentrations, mineral fertilization increased P, K, Ca, and Mg. Once again this reinforces the idea that trees quickly uptake readily available nutrients from mineral fertilizer applications, while residue use primarily increases K and Ca concentrations. When applying residues from the cellulose industry, increases in needle Ca were expected since the material composition contained high amounts of this nutrient (RODRIGUEZ *et al.*, 2018; PEREIRA *et al.*, 2022). The confirmed high mobility of K has been shown to significantly affect needle levels under most fertilization strategies.

Although fertilization can generally lead to improved K concentration, results clearly showed that lime added with fertilizer can lead to decreased K concentration since other nutrients can compete for transporters and active absorption sites of roots. Excess Ca^{2+} and Mg^{2+} ions can lead to lower uptake of the K^{+} ion (CONSALTER *et al.*, 2021b). Batista (2011) observed that Ca and Mg had reduced uptake in response to K fertilization; this antagonistic effect demonstrates the importance of calibrating soil correctives and fertilizer rates used in forest plantations.

Regarding N, there were no major changes in loblolly pine needles. Even with N application, growth stimulation (MARSCHNER, 2011) can lead to a dilution effect with lower levels of N in needles. Nitrogen is the nutrient most commonly limiting to loblolly pine growth, and fertilization can increase availability, which increases leaf area/sunlight capture and growth (FOX *et al.*, 2007), thereby lowering N concentrations.

3.4.3 EFFECT OF FERTILIZATION TIMING

Wood production did not differ between ages of loblolly pine at fertilization, although a consistent response to fertilization has been reported for mid-rotation applications (FOX *et al.*, 2007; CARLSON *et al.*, 2014; CARTER *et al.*, 2014; CARTER *et al.*, 2021). Moro *et al.* (2014) also reported similar findings with fertilization of *Pinus* at different ages (1, 5, and 9 yrs). Although adequate response to fertilization at mid-rotation have been documented, early fertilization could be justified on sites displaying low initial growth.

At all tree ages, fertilization increased needle K, Ca, and Mg concentrations, while P responded only in the age range of 2 – 16 yrs. Fertilization responses may be closely related to regional soil and climatic conditions. The rapid accumulation of nutrients observed by Barros Filho *et al.* (2017) in trees up to 4 years-old is a strong indicator that fertilization should be applied before stands reach this age. However, needle N concentration did not change with age in response to fertilization. Similar results were reported by Moro *et al.* (2014) in pine needles at 18 months after NPK fertilization and by Pereira *et al.* (2022) after application of organic residues.

3.4.4 EFFECT OF SOIL TEXTURE

Higher tree response (+70%) occurred for soils with clay contents below 15% since nutrient deficiency conditions of these soils favored positive responses to fertilization (BATISTA *et al.*, 2015; STAHL *et al.*, 2018; SASS *et al.*, 2020, ADAM *et al.*, 2021; CONSALTER *et al.*, 2021a, b). In southern Brazil, ample nutrient reserves can be associated with very clayey soils derived from basalt parent material, while sandstone derived soils are low in nutrient reserves (HOOGH *et al.*, 1987; MOTTA et al., 2020). In the United States, large areas originating from marine sediments formed low fertility sandy soils that reflect a wide response to fertilization (ALBAUGH *et al.*, 2019). Bognola *et al.* (2010) observed higher average annual growth increases for 13 and 14-year-old loblolly pine suggesting sandy soils with correct fertility management had good potential for further growth increases in these high humidity areas.

Regardless of soil clay content, there was an increase in K, P, and Mg needle concentrations likely due to a general lack of these soil nutrients. A similar response was found with organic residue applied to loblolly pine on soils with 30% clay (RABEL *et al.,* 2021), where K was increased in the first needle flush 10 years after application, suggesting that clay content did not impede K uptake. On the other hand, N levels were reduced for any soil texture. For Ca, increases only occurred in the textural range of 15 to 65%; this is possibly related to more ideal water availability and evapotranspiration since Ca is known to be more absorbed by mass flow (MARSCHNER, 2011).

3.5 CONCLUSION

This meta-analysis indicated that fertilization and liming increased loblolly pine wood production due to increases in plant height and diameter. These production benefits were associated with increased root growth and improved nutritional status of trees. As a result of increased tree growth, there was also an increase in litter deposition that likely impacted forest nutrient cycling.

Although fertilization efficiency in loblolly pine was similar for applications at planting and on established sites of 2 to 8 years (especially for wood production), we verified slight variations in function of other controlling factors. In this regard, the most expressive tree response occurred with residue applications (vs fertilization with mineral sources), with emphasis on residues with more balanced amounts of nutrients. When evaluating only mineral fertilization, multiple nutrients supplied by the joint application of fertilizer and lime was the most beneficial strategy for tree growth, possibly related to addressing lack of Ca and Mg. Regarding soil attributes, loblolly pine on sandy soils (<15% clay) was more responsive to fertilization. In summary, results obtained in this meta-analysis could be a useful guide for fertilization and liming practices in areas destined for reforestation with loblolly pine, aiming to guarantee greater sustainability of these production systems.

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4 GENERAL CONCLUSION

There are two distinct symptoms of chlorosis occurring in southern Brazil, one in areas of low productivity, with symptoms of multi-element deficiencies, mainly Mg, and the other in areas of high productivity with high production potential, where in some of these areas toxicity and /or various interactions between biotic and abiotic factors may be the causal factor.

Although pine fertilization is a practice that is still little used, it can help to improve the productivity of the areas and, consequently, bring better financial returns.

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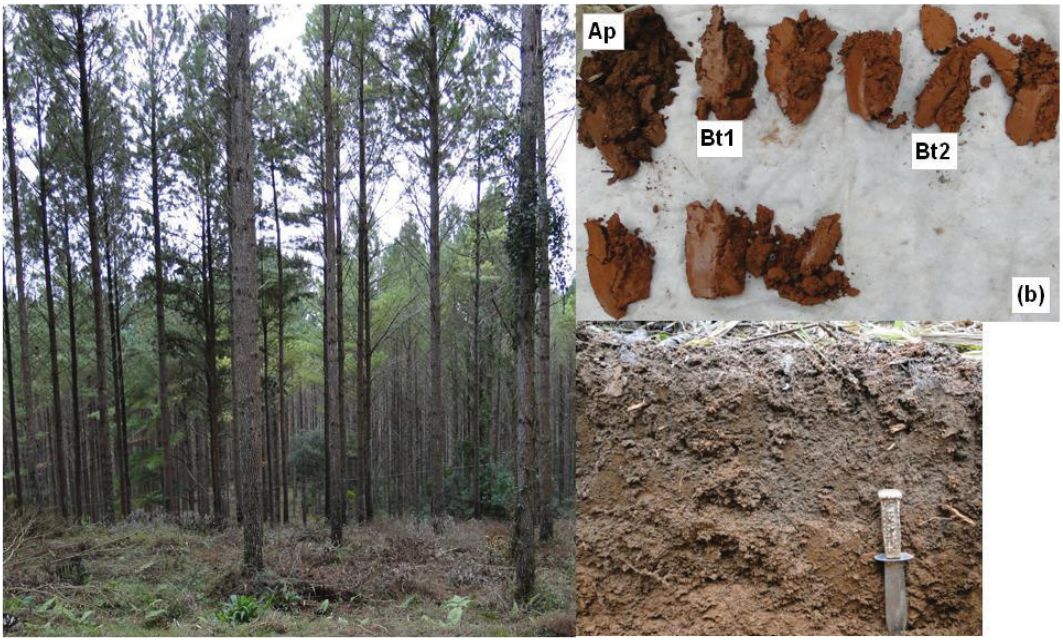
APPENDIX 1 – PROFILE PHOTOS AND SOILS DATA (CHAPTER I).

APPENDIX 1.1- Photos

PROFILE 1: RPP 19 with chlorosis

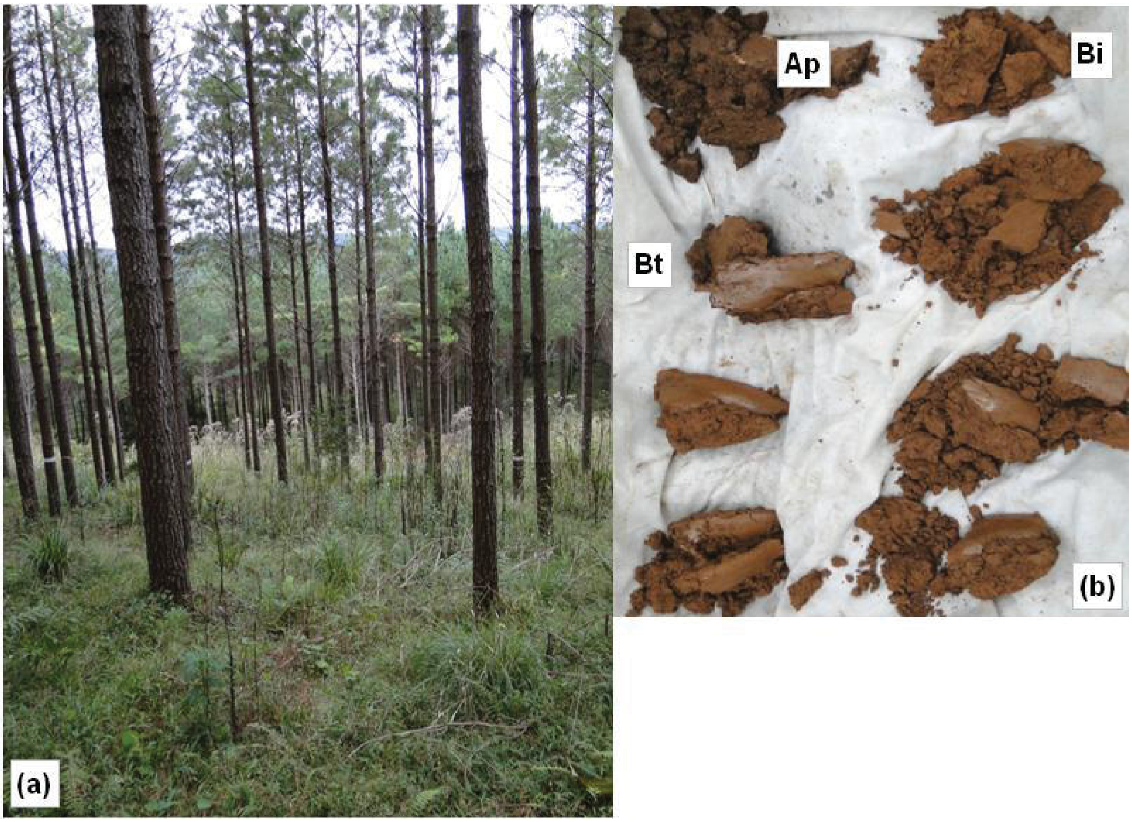


SOURCE: The author and collaborators (2019)

PROFILE 2: RPP 19 without chlorosis

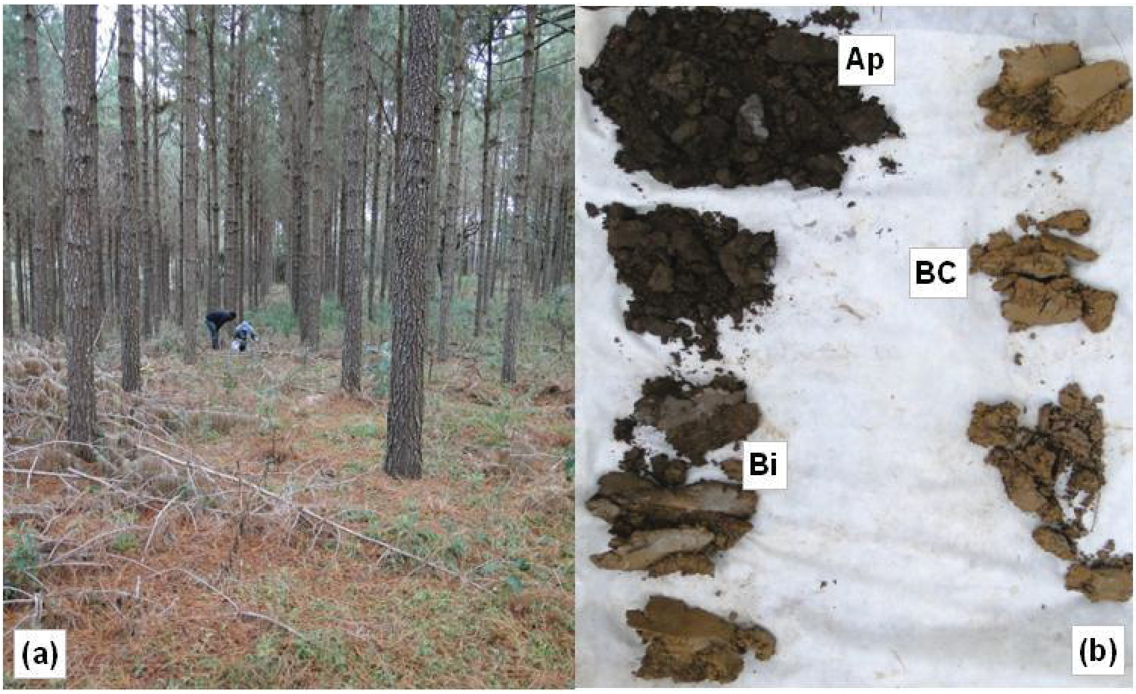
SOURCE: The author and collaborators (2019)

PROFILE 3: RPP 11 with chlorosis



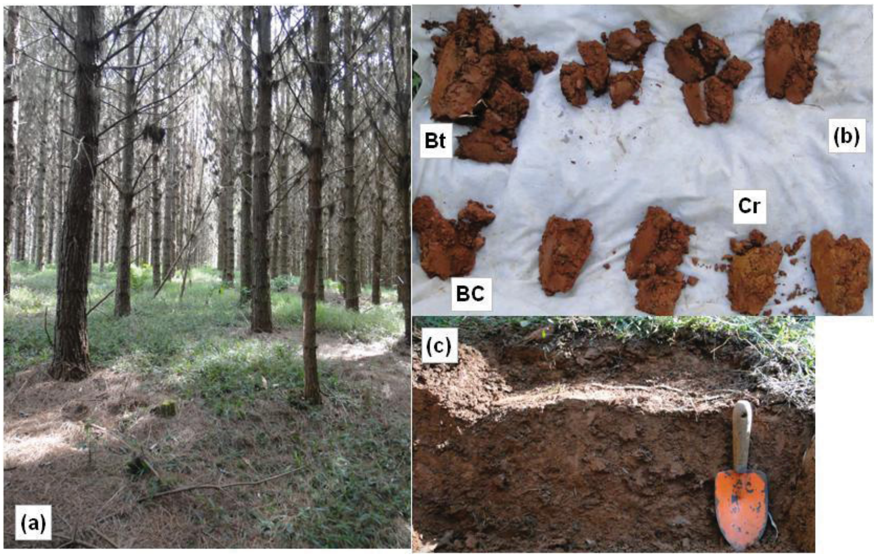
SOURCE: The author and collaborators (2019)

PROFILE 4: RPP 11 without chlorosis



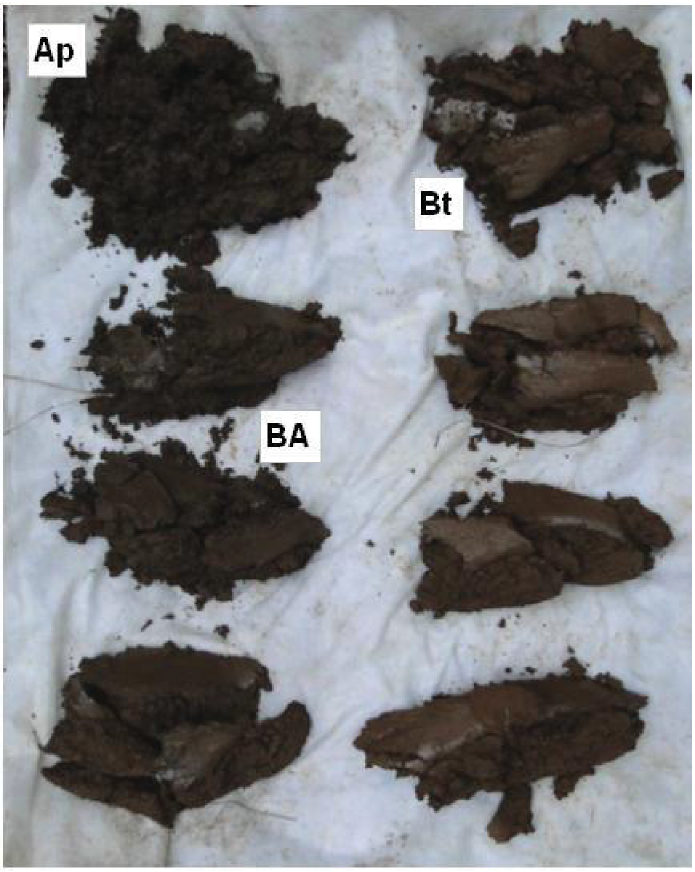
SOURCE: The author and collaborators (2019)

PROFILE 5: SP 14 with chlorosis



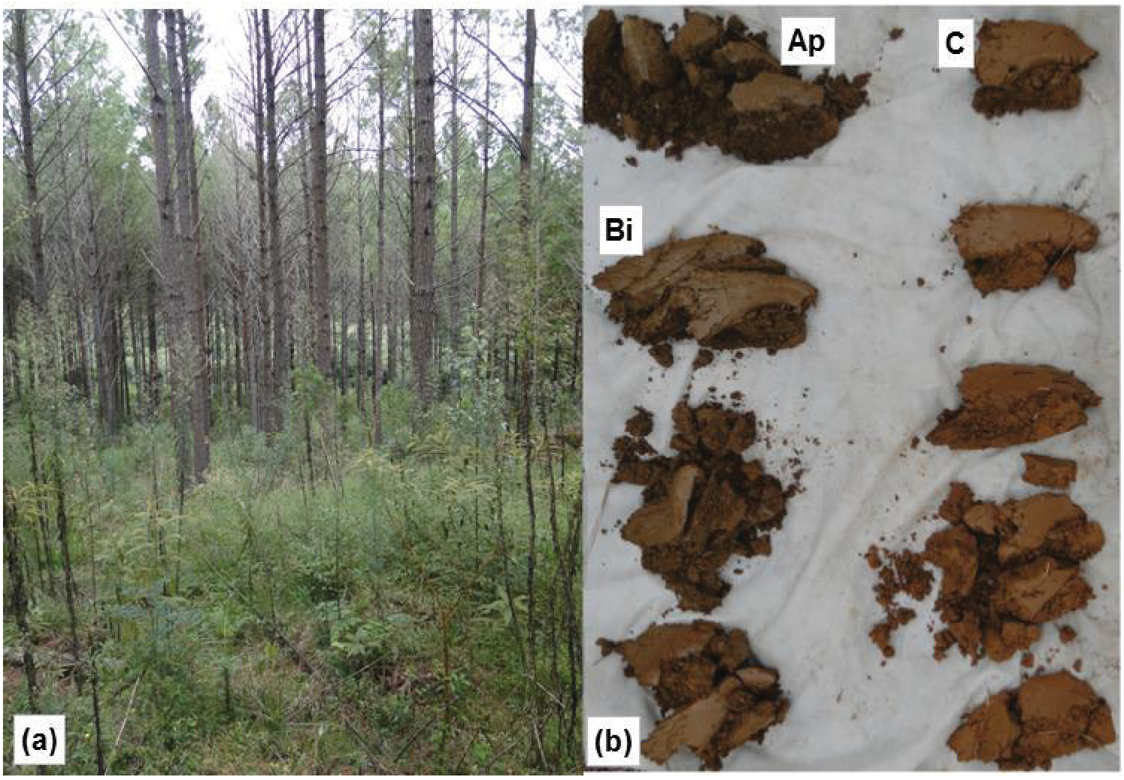
SOURCE: The author and collaborators (2019)

PROFILE 6: SP 14 without chlorosis



SOURCE: The author and collaborators (2019)

PROFILE 7: SP 18 with chlorosis



SOURCE: The author and collaborators (2019)

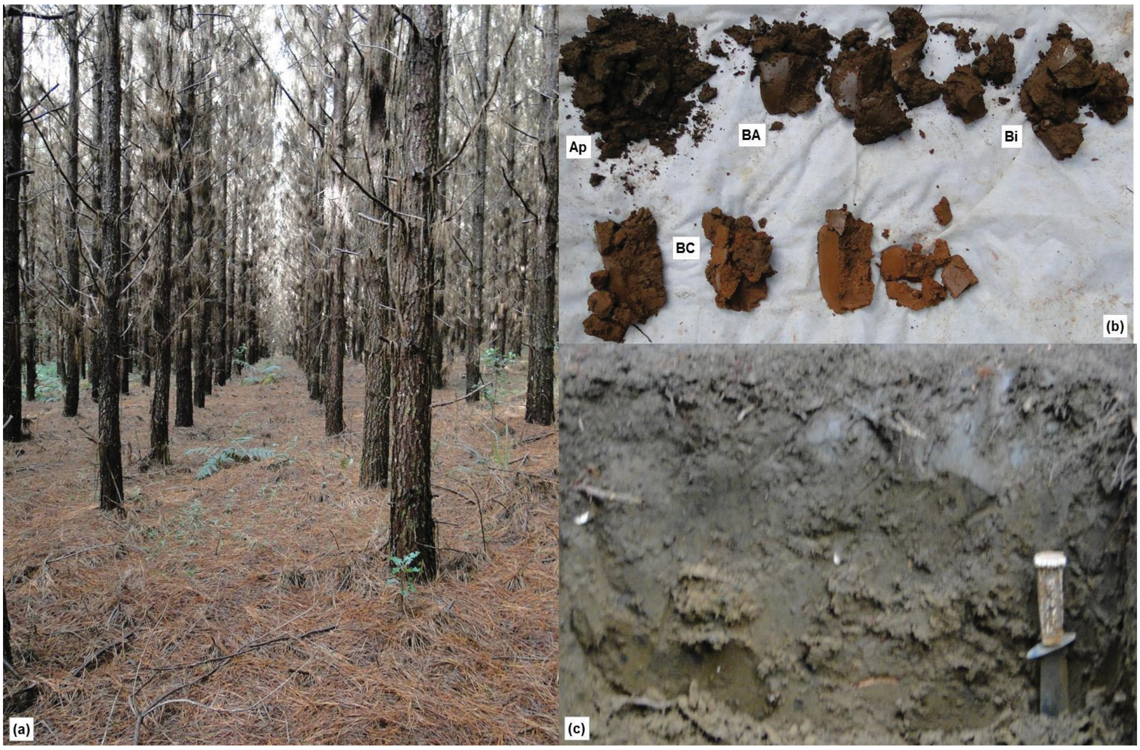
PROFILE 8: SP 18 without chlorosis

SOURCE: The author and collaborators (2019)

PROFILE 9: NP 12 with chlorosis

SOURCE: The author and collaborators (2019)

PROFILE 10: NP 12 without chlorosis



SOURCE: The author and collaborators (2019)

APPENDIX 1.2- Sulfuric Attack

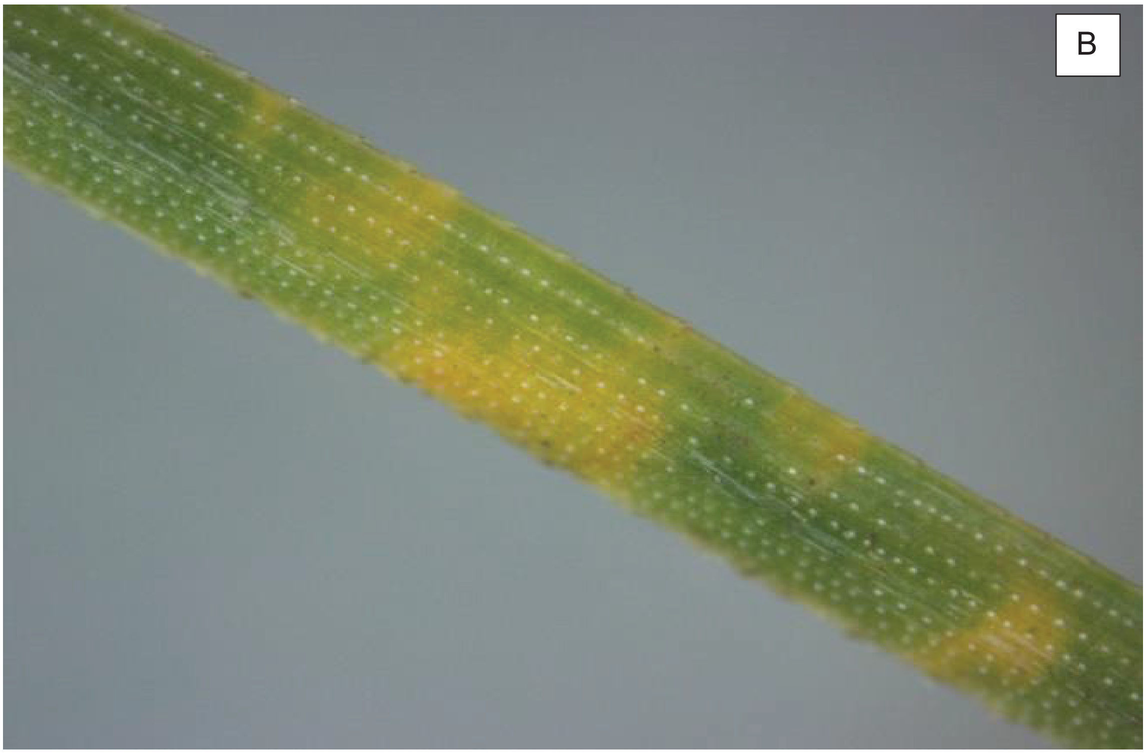
Profile	Horizon	sulfuric attack							
		SiO_2	Al_2O_3	Fe_2O_3	TiO_2	P_2O_5	Ki[3]	Kr[3]	Al_2O_3/Fe_2O_3[3]
RPP19-C	Bt1	26,98	19,86	18,83	1,52	0,28	2,31	1,44	1,66
RPP19-NC	Ap	11,76	16,58	7,31	0,5	0,31	1,21	0,94	3,56
RPP11-C	Bt	23,2	19,22	19,46	1,69	0,25	2,05	1,25	1,55
RPP11-NC	Bi	26,85	21,11	7,09	1,12	0,2	2,16	1,78	4,67
SP14- C	Bt	25,08	21,24	18,15	1,34	0,35	2,01	1,3	1,84
SP14-NC	Bw	16,93	18,14	10,09	1,57	0,23	1,59	1,17	2,82
SP18-C	Bi	21,78	17,17	16,74	1,3	0,6	2,16	1,33	1,61
SP18-NC	Ap	19,75	16,41	13,45	1,26	0,36	2,05	1,34	1,92
NP12-C	Ap	23,35	15,62	5,09	0,44	0,16	2,54	2,1	4,82
NP12-NC	Bi	20,75	16,43	6,77	0,48	0,14	2,15	1,7	3,81

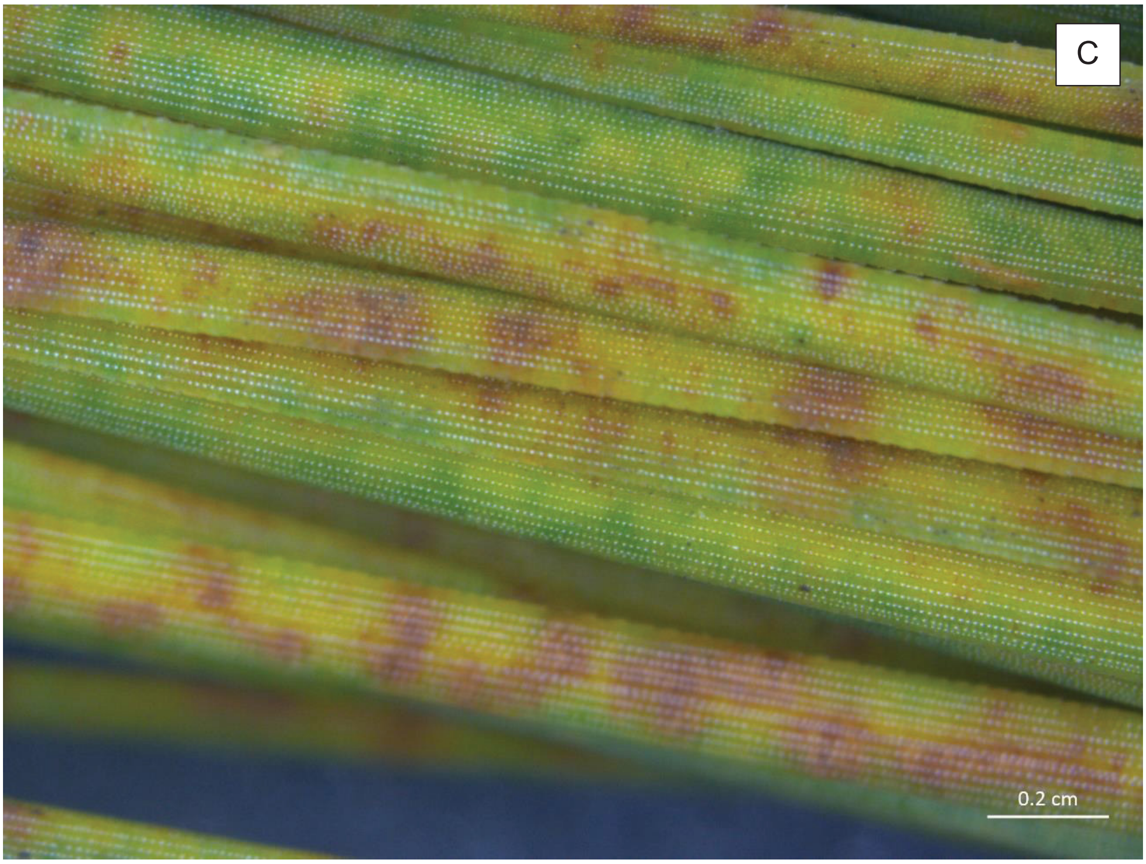
SOURCE: The author and collaborators (2019)

LEGEND: Molecular relationships: Ki – SiO_2/Al_2O_3; Kr - $SiO_2/(Al_2O_3+FE_2O_3)$ and Al_2O_3/Fe_2O_3. C- Chlorosis; NC- Not Chlorosis.

APPENDIX 2 – PHOTOS OF COLLECTED SAMPLES (CHAPTER I).

APPENDIX 2.1- A – BUSH OF NEEDLES WITHOUT CHLOROSES. B AND C - FASCICLE WITH LOW INTENSITY OF CHLOROSES. D – NEEDLES WITH SYMPTOMS OF CHLOROSES.


B


C
0.2 cm

APPENDIX 2.1- SOIL (A), LITTER (B), ROOT (C) AND NEEDLE


C


D

APPENDIX 3 – ROOT BIOMASS (Kg.há$^{-1}$) AND 100 FASCICLES WEIGHT (g) OF CHAPTER I.

	Root biomass	
	With	Without
RPP19	293,75	435,625
RPP11	237,5	227,5
SP14	53,75	77,5
SP18	221,25	214,375
NP	605	757,5
	100 fascicles weight	
RPP19	17,58	16,60
RPP11	16,65	14,88
SP14	16,02	18,17
SP18	18,56	18,82
NP	19,02	19,04

APPENDIX 4 – RESULTS OF PRINCIPAL COMPONENT ANALYSIS OF SOIL CHEMICAL PROPERTIES OF CHAPTER I.

Variable	CP1		CP2	
	Eigenvector	σ^2	Eigenvector	σ^2
H + Al	-0.35	12	0.01	0
Al^{+3}	-0.35	12	0.07	0
Fe	-0.30	9	-0.05	0
OC	-0.17	3	0.53	29
P	0.00	0	0.63	39
K^{+}	0.24	6	0.35	12
Zn	0.26	7	0.30	9
Cu	0.27	7	-0.24	6
Mn	0.33	11	-0.04	0
Ca^{+2}	0.33	11	-0.12	2
pH $CaCl_2$	0.33	11	0.17	3
Mg^{+2}	0.34	11	0.01	0
Eigenvalues	2.8		1.3	
% explained	63.5		14.4	
% accumulated	63.5		77.9	

APPENDIX 5 - RESULTS OF PRINCIPAL COMPONENT ANALYSIS OF NUTRIENT CONCENTRATIONS IN NEEDLES FROM THE SECOND FLUSH OF CHAPTER I.

Variable	CP1 Eigen	CP2 Eigen	CP3 Eigen	CP4 Eigen
Mg	0.49	0.13	0.16	0.12
Zn	0.49	0.07	-0.0 8	0.03
Ca	0.49	0.03	0.14	0.07
Mn	0.28	0.16	0.06	-0.71
Cu	0.12	0.46	-0.45	-0.23
Ni	0.03	0.48	-0.31	0.31
P	-0.01	0.42	0.42	0.47
Al	-0.14	0.21	0.64	-0.31
Fe	-0.17	0.42	0.18	-0.09
K	-0.37	0.32	-0.18	-0.13
Eigenvalu	1.8	1.3	1.2	1.0
%	33.3	17.7	14.5	10
%	33.3	51	65.5	75.5

APPENDIX 6 – CORRELATION BETWEEN NUTRIENT CONCENTRATIONS IN THE SOIL (AVERAGE VALUES OF THE 0-10 AND 10-20 CM LAYER), IN THE NEEDLES (UPPER THIRD-2ND FLUSH) AND IN THE WOOD AT 1.30 M (DBH) IN TREES IN AREAS WITH CHLOROSIS (CHAPTER I).

Nutrients	Wood and needle	Wood and soil	Needle and soil
Ca	0,57*	0,59*	0,54*
Mg	-0,27	-0,17	0,55*
K	0,19	0,10	-0,45*
P	0,42	-0,22	-0,50*
Al	0,31	0,28	0,05
Fe	-0,28	0,05	0,22
Mn	0,48*	-0,44	0,28
Cu	0,15	-0,24	0,22
Zn	0,33	0,03	0,53*
Ni	0,14	-	-

APPENDIX 7 – CONCENTRATIONS OF P, K, Ni, FOR NEEDLES OF THE MIDDLE THIRD, 1ST AND 2ND FLUSHES SAMPLED (CHAPTER I).

	P				
	RPP19	RPP11	SP14	SP18	NP12
	Middle third				
Chlorotic	1,05	1,26	1,13	1,16	1,04
Control	1,27	1,16	1,31	1,06	1,04
	1st				
Chlorotic	0,87	1,27	0,92	1,07	1,08
Control	1,02	1,08	0,93	1,13	1,07
	2nd				
Chlorotic	1,06	1,47	1,12	1,33	1,08
Control	1,19	1,22	1,21	1,29	1,00
	K				
	RPP19	RPP11	SP14	SP18	NP12
	Middle third				
Chlorotic	4,65	5,22	5,28	4,70	4,88
Control	6,02	5,59	5,30	4,51	4,87
	1st				
Chlorotic	3,68	3,69	3,45	3,98	4,09
Control	3,64	4,03	3,34	3,96	4,60
	2nd				
Chlorotic	3,85	3,65	3,64	3,46	4,96
Control	4,21	4,22	3,52	4,05	3,69
	Ni				
	RPP19	RPP11	SP14	SP18	NP12
	Middle third				
Chlorotic	1,89	1,82	2,33	3,78	2,99
Control	1,36	0,94	3,61	1,45	1,36
	1st				
Chlorotic	2,64	2,97	1,98	18,18	6,78
Control	2,14	1,16	1,74	4,08	2,65
	2nd				
Chlorotic	6,94	9,63	2,69	11,83	7,97
Control	5,69	2,64	3,36	6,11	5,30

APPENDIX 8 – MEAN CONCENTRATIONS OF WOOD SAMPLES AT SIX TRUNK HEIGHTS (BASE, 25%, 50% 75% AND 100%) AT PAIR STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18, NP12) IN SANTA CATARINA STATE, BRAZIL (CHAPTER I).

APPENDIX 8.1- Base

		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
	Control	0,10	0,13	0,13	0,11	0,11
K	Chlorotic	0,30	0,31	0,25	0,29	0,37
	Control	0,30	0,39	0,30	0,28	0,39
P	Chlorotic	0,04	0,05	0,03	0,05	0,04
	Control	0,04	0,06	0,04	0,04	0,05
Al	Chlorotic	0,15	0,01	0,03	0,03	0,03
	Control	0,01	0,01	0,02	0,03	0,03
Cu	Chlorotic	6,67	6,24	6,27	4,56	6,38
	Control	4,39	6,94	6,64	4,99	7,06
Zn	Chlorotic	7,22	6,50	7,61	5,66	6,08
	Control	5,06	6,77	5,31	6,02	6,67
B	Chlorotic	1,93	1,81	1,91	1,39	1,12
	Control	1,32	2,02	1,62	1,56	1,03
Ni	Chlorotic	0,20	0,10	0,12	0,18	0,17
	Control	0,17	0,07	0,16	0,56	0,05

APPENDIX 8.2- 25%

		With interaction				
		RRP19	RRP11	SP14	SP18	NP12
Zn	Chlorotic	7,81Aa	6,83Ab	6,91Aa	5,62Aa	5,77Aa
	Control	7,05ABa	8,49Aa	5,24Bb	6,54ABa	6,79ABa
B	Chlorotic	1,82Aa	1,88Ab	1,91Aa	1,43Ba	1,10Ca
	Control	1,73Ba	2,06Aa	1,51Cb	1,47Ca	1,07Da
		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	0,36	0,32	0,23	0,28	0,43
	Control	0,30	0,39	0,21	0,30	0,45
P	Chlorotic	0,03	0,05	0,03	0,07	0,04
	Control	0,03	0,04	0,03	0,05	0,05
Al	Chlorotic	0,02	0,01	0,02	0,05	0,08
	Control	0,02	0,02	0,01	0,03	0,05
Cu	Chlorotic	6,43	6,28	6,32	4,48	5,70

	Control	5,98	8,20	6,05	5,38	6,79
Ni	Chlorotic	0,15	0,27	0,11	0,38	0,02
	Control	0,09	0,13	0,00	0,28	0,00

APPENDIX 8.3- 50%

				With interaction		
		RRP19	RRP11	SP14	SP18	NP12
P	Chlorotic	0,06Ba	0,05Ba	0,04Bb	0,11Aa	0,06Ba
	Control	0,067Aa	0,06Aa	0,07Aa	0,07Ab	0,05Aa
B	Chlorotic	1,93Aa	1,99Ab	2,09Aa	1,59Ba	1,32Ca
	Control	1,90Ba	2,18Aa	1,67BCb	1,56Ca	1,15Db
				Without interaction		
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	0,57	0,44	0,35	0,48	0,59
	Control	0,50	0,58	0,46	0,44	0,57
Al	Chlorotic	0,02	0,03	0,01	0,05	0,08
	Control	0,01	0,04	0,01	0,03	0,03
Cu	Chlorotic	7,05	7,10	6,76	4,89	7,04
	Control	7,90	7,63	6,19	5,36	6,30
Zn	Chlorotic	14,91	10,72	6,19	6,80	6,23
	Control	9,81	7,85	8,57	5,88	7,25
Ni	Chlorotic	0,63	0,63	0,45	1,49	0,14
	Control	0,05	0,19	0,53	0,28	0,06

APPENDIX 8.4- 75%

				With interaction		
		RRP19	RRP11	SP14	SP18	NP12
Al	Chlorotic	0,03ABa	0,02ABa	0,01Ba	0,06Aa	0,02Ba
	Control	0,01Cb	0,01Cb	0,01BCa	0,05Aa	0,02ABa
Zn	Chlorotic	7,63ABa	8,32Aa	8,38ABa	5,97ABa	5,56Ba
	Control	7,42Aa	6,79Aa	5,57Ab	6,69Aa	7,02Aa
B	Chlorotic	1,95Aa	1,98Aa	1,99Aa	1,57Ba	1,18Ca
	Control	1,78Ba	2,12Aa	1,68Bb	1,55Ba	1,16Ca
				Without interaction		
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	0,47	0,46	0,39	0,39	0,57
	Control	0,46	0,48	0,42	0,36	0,46
P	Chlorotic	0,05	0,06	0,05	0,07	0,06
	Control	0,05	0,06	0,05	0,05	0,05
Cu	Chlorotic	6,30	7,22	6,53	4,98	6,18
	Control	6,15	6,87	6,38	5,08	6,60
Ni	Chlorotic	0,36	1,21	0,23	1,44	0,13

	Control	0,22	0,40	0,11	0,18	0,13

APPENDIX 8.5- 100%

		With interaction				
		RRP19	RRP11	SP14	SP18	NP12
P	Chlorotic	0,04BCa	0,07Aa	0,03Ca	0,07Aa	0,06ABa
	Control	0,05Aa	0,06Aa	0,04Aa	0,04Ab	0,05Aa
Zn	Chlorotic	13,16Aa	7,31ABa	6,64ABa	6,06Ba	5,30Bb
	Control	7,34Aa	6,49Aa	6,01Aa	7,34Aa	7,18Aa
		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	0,41	0,41	0,25	0,35	0,51
	Control	0,45	0,45	0,31	0,36	0,44
Al	Chlorotic	0,12	0,01	0,02	0,08	0,02
	Control	0,01	0,01	0,02	0,05	0,03
Cu	Chlorotic	8,85	7,36	6,32	5,22	7,00
	Control	7,06	6,06	6,13	5,95	6,47
B	Chlorotic	2,03	2,00	1,87	1,43	1,16
	Control	1,71	2,07	1,59	1,51	1,09
Ni	Chlorotic	0,30	0,12	0,03	1,10	0,03
	Control	0,17	0,00	0,12	0,16	0,00

APPENDIX 9 – MEAN CONCENTRATIONS OF BARK NUTRIENTS AT SIX TRUNK HEIGHTS (BASE, 25%, 50% 75% AND 100%) AT PAIR STUDY AREAS (CHLOROTIC AND CONTROL) AT FIVE SITES (RPP19, RPP11, SP14, SP18, NP12) IN SANTA CATARINA STATE, BRAZIL.

APPENDIX 9.1- Base

		With interaction				
		RRP19	RRP11	SP14	SP18	NP12
P	Chlorotic	0,1575Ba	0,2375Ba	0,2625Ba	0,3925Aa	0,15Ba
	Control	0,1175ABCa	0,2375Aa	0,0975BCb	0,2175ABb	0,0475Cb
B	Chlorotic	3,3125Aa	1,1725ABa	1,165ABa	1,3325ABa	0,79Ba
	Control	0,845Ab	1,2825Aa	1,0675Aa	1,265Aa	0,9875Aa
		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	1,12	0,71	0,44	0,91	0,36
	Control	0,51	1,40	0,44	0,81	0,18
Al	Chlorotic	0,46	0,48	0,61	0,9075	0,48
	Control	0,74	0,62	0,52	0,61	0,43
Cu	Chlorotic	4,04	3,43	3,59	4,20	3,47

	Control	3,58	3,83	3,27	4,13	6,01
Zn	Chlorotic	42,22	51,39	42,95	91,04	19,06
	Control	38,51	53,46	26,05	40,25	26,15
Ni	Chlorotic	2,11	1,24	1,16	3,18	0,28
	Control	0,50	2,89	0,41	0,61	0,16

APPENDIX 9.2- 25%

		With interaction				
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	2,19Aa	1,48Ab	1,27Ab	2,44Aa	1,76Aa
	Control	2,42ABa	3,25Aa	2,73ABa	2,25ABa	1,56Ba
Cu	Chlorotic	8Aa	6,26ABa	6,08ABa	5,81ABa	3,91Bb
	Control	4,69Ab	5,55Aa	6,31Aa	5,64Aa	6,57Aa
Zn	Chlorotic	94,22Aa	87,99Aa	54,59Aa	67,21Aa	18,32Ba
	Control	46,71Bb	85,68Aa	58,48ABa	47,92ABa	30,39Ba
B	Chlorotic	5,52Aa	2,58ABa	2,54ABa	1,89Ba	1,49Ba
	Control	1,89Ab	2,60Aa	2,68Aa	2,34Aa	1,61Aa
Ni	Chlorotic	5,89Aa	6,97Aa	3,72Aa	5,15Aa	0,66Ba
	Control	1,53ABb	0,44Bb	1,86Ab	1,90Ab	0,61Ba

		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
P	Chlorotic	0,2175	0,36	0,39	0,38	0,17
	Control	0,495	0,63	0,72	0,69	0,3025
Al	Chlorotic	0,46	0,55	0,74	0,89	0,52
	Control	0,73	0,63	0,58	0,68	0,495

APPENDIX 9.3- 50%

		With interaction				
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	1,44Aa	1,39Ab	1,21Aa	1,92Aa	1,06Aa
	Control	1,94ABa	2,82Aa	1,65ABa	1,21Ba	0,89Ba
Al	Chlorotic	0,53Ba	0,57Ba	0,84ABa	1,09Aa	0,51Ba
	Control	0,91Ab	0,69ABa	0,63ABa	0,85ABb	0,48Ba
Cu	Chlorotic	4,93Aa	4,81Aa	4,34ABa	5,28Aa	3,62Ba
	Control	4,03Aa	4,89Aa	4,71Aa	4,35Aa	5,06Aa
Zn	Chlorotic	45,49Aa	30,09Aa	45,9Aa	60,07Aa	11,08Ba
	Control	27,74Aa	31,92Aa	39,85Aa	23,16Ab	19,64Aa
Ni	Chlorotic	3,04Aa	4,14Aa	2,36Aa	5,17Aa	0,46Ba
	Control	0,74ABb	0,35Cb	1,1Aa	0,96ABb	0,39BCa

		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
P	Chlorotic	0,23	0,31	0,30	0,37	0,15
	Control	0,3875	0,50	0,39	0,36	0,17

B	Chlorotic	3,63	2,1225	2,4775	1,99	1,16
	Control	1,72	2,28	2,01	1,78	1,33

APPENDIX 9.4- 75%

		With interaction				
		RRP19	RRP11	SP14	SP18	NP12
Al	Chlorotic	0,50Ba	0,51Ba	0,88ABa	1,01Aa	0,5Ba
	Control	0,94Ab	0,74ABa	0,76ABa	0,89ABa	0,51Ba
Cu	Chlorotic	4,09BCa	4,35Ba	4,18Ba	5,49Aa	3,35Cb
	Control	3,88Ba	4,41ABa	4,58ABa	4,83Ab	5,19Aa
Zn	Chlorotic	28,78Aa	23,33Aa	32,37Aa	37,12Aa	5,89Bb
	Control	15,28Bb	23,27ABa	28,36ABa	35,59Aa	15,813Ba
B	Chlorotic	2,73Aa	1,65Aa	1,86Aa	1,82Aa	0,86Bb
	Control	1,24Bb	1,9ABa	1,89ABa	2,065Aa	1,47ABa
Ni	Chlorotic	2,33a	2,22a	1,36a	2,71a	0,76a
	Control	0,39b	0,29b	0,85a	0,85b	0,26b
		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	1,01	0,94	0,80	1,34	0,49
	Control	0,905	1,99	1,20	1,35	1,0925
P	Chlorotic	0,22	0,29	0,31	0,34	0,14
	Control	0,20	0,36	0,28	0,41	0,18

APPENDIX 9.5- 100%

		With interaction				
		RRP19	RRP11	SP14	SP18	NP12
Al	Chlorotic	0,48Bb	0,55Ba	0,84ABa	1,09Aa	0,49Ba
	Control	1,00Aa	0,75ABa	0,72ABa	0,79ABb	0,52Ba
Zn	Chlorotic	18,28Aa	17,12Aa	21,88Aa	21,35Aa	3,90Bb
	Control	12,64Aa	16,50Aa	14,57Aa	16,44Aa	9,46Aa
B	Chlorotic	2,21Aa	1,35Aa	1,45Aa	1,4Aa	0,72Ba
	Control	1,24Ab	1,68Aa	1,55Aa	1,59Aa	1,04Aa
Ni	Chlorotic	1,51Aa	1,34Aa	0,99Aa	1,68Aa	0,23Ba
	Control	0,54Aa	0,28Ab	0,47Aa	0,72Aa	0,28Aa
		Without interaction				
		RRP19	RRP11	SP14	SP18	NP12
K	Chlorotic	0,92	0,66	0,53	0,85	0,32
	Control	0,765	1,33	0,62	1,00	0,405
P	Chlorotic	0,18	0,32	0,29	0,34	0,14
	Control	0,1625	0,25	0,17	0,29	0,0675
Cu	Chlorotic	3,86	3,72	3,74	3,85	3,40
	Control	3,66	5,28	3,75	4,53	5,29