

## Relationship between microbiological, physical, and chemical attributes of different soil types under *Pinus taeda* plantations in southern Brazil

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Over the last decades, *Pinus taeda* L. plantations in southern Brazil showed a great increase in average production. However, the gains in productivity obtained by genetic selection and breeding have nowadays stabilized. Research on edaphic factors and silvicultural practices is currently performed with the aim of both increasing the productivity of *P. taeda* plantations and maintaining the soil quality. To this end, soil microbiological attributes are considered better indicators of soil quality as they are more sensitive than chemical and physical ones. In this study, we aimed to evaluate the relationship between microbial activity and the physical and chemical parameters of different soil types under young *Pinus taeda* plantations at five different sites in southern Brazil. Soil samples were collected at depths of 0-5 and 5-10 cm. The soil microbiological attributes evaluated were: potentially mineralizable nitrogen (PMN), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial basal respiration (MBR), and metabolic quotient ( $qCO_2$ ). We also evaluated some physical and chemical soil parameters. Sites with the highest values of C, clay, and nutrients in the soil, showed higher values for the soil microbiological attributes, compared to the other study sites. The previous management with minimal tillage in some sites seems to positively affect soil quality. The MCB and MBR showed better sensitivity in indicating differences between sites and showed a good relationship with clay content, C/N ratio, K, and pH. These results suggest that site-specific characteristics such as soil type or forest management influence soil microbiological attributes in *Pinus taeda* plantations during initial growth in southern Brazil.

**Keywords:** Soil Microbial Activity, Microbial Biomass Carbon, Microbial Basal Respiration, Forest Management

### Introduction

*Pinus taeda* L. is one of the main forest species cultivated in Brazil, and plantations of the genus *Pinus* currently occupy 1.7 million hectares, mostly located in the states

of Paraná (43%) and Santa Catarina (24%) (IBA 2021, Kondo et al. 2021). Recently, the application of silvicultural practices combined with genetic improvement resulted in *Pinus taeda* plantations with high productivity in southern Brazil (Moro et al. 2014). It is recognized that edaphoclimatic factors are crucial in limiting the growth of *Pinus* plantations (Vose & Allen 1988, Dobner & Campoe 2019). However, the relationship between microbiological, physical and chemical attributes in the soil is not well clarified, therefore it has still to be established which attributes better reflect the soil quality affecting the growth of *P. taeda* stands in southern Brazil (Rodriguez et al. 2018, Santos & Reichert 2022).

In *Pinus* plantations, the microbial activity of the soil depends, among other factors, on the soil type, the environmental conditions, and the type of silvicultural management (Albaugh et al. 2010, 2017). Soil pedogenesis is responsible for many edaphic parameters in the soil, along with soil and silvicultural management (Fox et al. 2007). In the long term, these factors are decisive for the increased productivity and sustainability of forest plantations (Zucon et al. 2020).

After a forest exploitation cycle, tree spacing techniques, use of litterfall from

the previous cycle, and addition of fertilizers can contribute to maintaining the supply of nutrients to the soil and guarantee the productivity of the forest site in the long term (Ward et al. 2015). Some researchers have evaluated responses to management practices using improved genetic material, mechanical site preparation, control of competing vegetation, and fertilization in *Pinus* plantations, and found positive results in the relationship between productivity and soil chemical attributes, significantly improving the growth of species when best tillage practices were recommended (Albaugh et al. 2010, Batista et al. 2015). Other studies have reported that successive cycles of *Pinus* plantations without nutrient replacement tend to cause a nutritional deficit for the trees, resulting in low productivity in forest plantations (Gatiboni et al. 2017, Gatiboni et al. 2020). Therefore, assessing soil microbiological attributes and their relationships with chemical and physical soil characteristics, combined with site-specific silvicultural management practices in *Pinus taeda*, provides a better understanding of soil nutrient dynamics (Sixel et al. 2015, Tulio et al. 2022). Several studies showed that nitrogen mineralization and microbial biomass carbon are potential indicators of soil quality in forest

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**Tab. 1** - Location and soil taxonomy of experimental sites of *Pinus taeda* of the initial growth in southern Brazil. (\*): Soil classification according to the USDA Soil Survey (Soil Survey Staff 1999.)

Areas	Municipality/State	Latitude	Longitude	Altitude (m a.s.l.)	Soil (*)
Site 1	Telêmaco Borba/Paraná	24° 22' 57" S	50° 56' 89" W	835	Oxisols
Site 2	Lages/Santa Catarina	27° 79' 28" S	50° 50' 02" W	916	Ultisols
Site 3	Caçador/Santa Catarina	26° 74' 86" S	51° 07' 18" W	1030	Oxisols
Site 4	Vargem Bonita/Santa Catarina	26° 55' 03" S	51° 47' 36" W	1088	Oxisols
Site 5	Campo Belo do Sul/Santa Catarina	28° 00' 29" S	50° 51' 20" W	956	Inceptisols

systems (López-Poma et al. 2020, Lu et al. 2020), as microorganisms are responsible for mineralizing elements in the soil and contribute to the availability of nutrients for plants (Romeo et al. 2020).

Silvicultural management in forest plantations can increase tree growth in *P. taeda* plantations, especially in the long term, through the contribution of forest residues from previous harvest crops (Adam et al. 2021).

We hypothesized that the microbial activity in the soil of *P. taeda* plantations is correlated to some physical and chemical attributes resulting from the soil pedogenesis as well as from the soil and silvicultural management. Thus, our objective was to evaluate the relationship of soil microbiological attributes with chemical and physical parameters, as well as with the initial growth of the *Pinus taeda* in southern Brazil.

## Material and methods

### Study area and experimental design

The experimental sites are part of the Co-operative Program on *Pinus* Research in Brazil, coordinated by the Forestry Science and Research Institute (IPEF). The predominant climate is humid subtropical, with a dry season in winter and classified by Köppen as Cfb (Alvares et al. 2013).

The study was carried out in five experi-

mental plots of *Pinus taeda* planted in 2019 on wavy soft relief, in Santa Catarina and Paraná states, southern Brazil. The sites were selected because of their differences in soil classification (Tab. 1), i.e., differences in soil physical and chemical attributes, but also in the soil use before current *P. taeda* plantation and in soil management. Tree growth was monitored and measurements were performed in 2021. Local climate data were obtained by the annual precipitation and mean annual temperature during the period 2019-2021 (Tab. 2). Climate database were obtained from a high spatial resolution global weather (Fick & Hijmans 2017).

At each study site, 21 replication plots were established in the field with 128 trees (8 rows × 16 trees), including a double border, thus resulting in a set of 32 measured trees per replication plot and 672 trees per site. The mortality index of the sites was around 3%. Starter fertilization was applied 180 days after planting with 20 kg ha<sup>-1</sup> of N, 60 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, and 33 kg ha<sup>-1</sup> of K<sub>2</sub>O in small holes 10 cm distant from the stem of each tree. It was also applied 675 kg ha<sup>-1</sup> of CaO and 150 kg ha<sup>-1</sup> of MgO between rows next to the trees' lines. Complementary fertilization (40 kg ha<sup>-1</sup> of N and 67 kg ha<sup>-1</sup> of K) was applied 360 days after planting on the soil surface under the tree crowns.

### Soil sampling and analysis

Soil samples were collected 360 days af-

ter the last fertilizer application, at depths of 0-5 and 5-10 cm in the rows and between rows. For each site, 21 soil samples were collected at each depth.

Soil samples were air dried, sieved (2.0 mm) and analysed for the following parameters. pH was measured in CaCl<sub>2</sub> 0.01 mol L<sup>-1</sup> solution; exchangeable Al<sup>3+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> were extracted by KCl 1 mol L<sup>-1</sup>; available K<sup>+</sup> and P were extracted by Mehlich I; (H<sup>+</sup> + Al<sup>3+</sup>) were extracted by calcium acetate 0.5 mol L<sup>-1</sup> at pH 7.0; total carbon and nitrogen were obtained by combustion in a Vario EL III<sup>®</sup> analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany).

All the microbiological attributes were determined on the air-dried and sieved soil samples after being moistened to field capacity. Potentially mineralizable nitrogen (PMN) was determined by anaerobic incubation with an adaptation of the Waring & Bremner (1964) method. A first extraction of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N with KCl (2 mol L<sup>-1</sup>) was performed and then the same samples were inserted in centrifuge tubes (50 mL) and filled with 30 mL of a nutritive solution (Na<sub>3</sub>PO<sub>4</sub>-0.005 mol L<sup>-1</sup>, MgSO<sub>4</sub>-0.002 mol L<sup>-1</sup>, CaCl<sub>2</sub>-0.005 mol L<sup>-1</sup>). Samples were then incubated in a BOD chamber in the dark at 30 °C for 15 days until the second KCl extraction. Two more incubation/extraction steps were repeated and in total, we had 4 extractions during 45 days of incubation. Ammonium (NH<sub>4</sub><sup>+</sup>-N) was determined by the phenol method using colorimetric spectrophotometry (APHA 1995). Nitrate (NO<sub>3</sub><sup>-</sup>-N) was determined by ultraviolet spectrophotometry (Heinzmann et al. 1984, Norman & Stucki 1981). The sum of nitrate and ammonium was considered the PMN.

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by irradiation method (Monz et al. 1991). Briefly, one of the two sets of soil samples (20 g) was irradiated, and the other set of soil samples was not irradiated. Subsequently, all samples were extracted with a K<sub>2</sub>SO<sub>4</sub> (0.5 mol L<sup>-1</sup>). The MBC and MBN were determined in a Vario TOC Cube<sup>®</sup> analyzer (Elementar Analysensysteme GmbH, Germany).

Microbial basal respiration (MBR) was performed with 20 g of soil samples moist-

**Tab. 2** - Characteristics of silvicultural management, climate, and average height of trees in the five experimental plots.

Areas	Site 1	Site 2	Site 3	Site 4	Site 5
Previous soil use	<i>Eucalyptus urophylla</i>	<i>Pinus taeda</i>	<i>Pinus taeda</i>	<i>Pinus taeda</i>	<i>Pinus taeda</i>
Age at harvest (years-old)	8	22	38	15	34
Soil management	Removal of residues / Subsoiling 60 cm	Removal of residues / Subsoiling 50 cm	Minimal tillage / Subsoiling 60 cm	Removal of residues / Subsoiling 35 cm	Minimal tillage / Subsoiling 45 cm
Present soil use (since 2019)	<i>Pinus taeda</i>	<i>Pinus taeda</i>	<i>Pinus taeda</i>	<i>Pinus taeda</i>	<i>Pinus taeda</i>
Spacing (m × m)	3.1 × 1.9	2.4 × 2.6	2.5 × 2.5	2.5 × 2.0	3.0 × 2.5
Average temperature (°C)	19.1	16.1	16.4	17.2	15.2
Annual precipitation (mm)	1382	1750	1653	1543	1450
Average height (m)	2.5	3.2	2.5	2.4	2.8

ened to field capacity and placed in hermetically sealed bottles, together with 20 mL of a NaOH solution ( $0.5 \text{ mol L}^{-1}$ ). The bottles were then incubated for 240 hours in the dark in a BOD chamber at  $25^\circ\text{C}$ . After the incubation, 5 mL of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  ( $0.5 \text{ mol L}^{-1}$ ) was added to the NaOH solution to stop the  $\text{CO}_2$  capture, and the NaOH was titrated with HCl ( $0.5 \text{ mol L}^{-1}$ ), and 2 drops of phenolphthalein indicator ( $0.1\%$ ) until the change from red color to lack of color.

The Metabolic Quotient ( $q\text{CO}_2$ ) was calculated by the ratio between MBR and MBC (Anderson & Domsch 1993). The result was expressed in  $\text{mg CO}_2\text{-C g of MBC h}^{-1} 10^{-3}$ .

### Statistical analysis

Analysis of variance and Tukey's test ( $P < 0.05$ ) for average comparisons were performed using the statistical package "ExpDes" in R version 1.2.2 (R Development Core Team 2019). Pearson correlations and linear analyses were used to assess the relationships between microbiological attributes and other soil attributes. Principal components analysis (PCA) was done using the program Minitab v.19 (Mckenzie & Goldman 1999). All the diagrams were plotted with SigmaPlot® version 12.0 (Systat Software Inc., Palo Alto, CA, USA).

## Results

### Soil physical and chemical attributes

Small variations were observed in the physical and chemical attributes of the soil between sites (Tab. 3). Site 1 showed the lowest values of clay, carbon, and macronutrients in the surface depth. Sites 3, 4,

**Tab. 3** - Soil chemical and physical attributes in the 0-5 and 5-10 cm soil depth of the sites of *Pinus taeda* of the initial growth in southern Brazil. pH ( $\text{CaCl}_2$   $0.01 \text{ mol L}^{-1}$ );  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  (extracted with  $\text{KCl}$   $1 \text{ mol L}^{-1}$ ); total carbon (C) and nitrogen total (N) (determined by total combustion method); K+ and P (Mehlich<sup>-1</sup> extraction); Base saturation (BS);  $\text{Al}^{3+}$  saturation (m); cation exchange capacity (CEC pH 7.0).

Parameter	Units	Sites									
		Site 1		Site 2		Site 3		Site 4		Site 5	
Depth	cm	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10	0-5	5-10
Clay	$\text{g kg}^{-1}$	300	313	625	575	750	713	714	711	619	623
pH	$\text{CaCl}_2$	3.9	4	4.1	4.1	3.9	3.9	4.4	4.4	4.4	4.4
C	$\text{g kg}^{-1}$	21.8	19.8	32	27.5	53	44.5	42.9	39.9	48.3	41.4
N	$\text{g kg}^{-1}$	1.2	1.2	1.9	1.7	2.5	2.1	2.4	2.3	2.7	2.4
P	$\text{mg dm}^{-3}$	2.8	6	3.9	3.3	5.9	4.8	3.4	3	6.2	4.7
K	$\text{cmolc dm}^{-3}$	0.06	0.05	0.13	0.08	0.15	0.13	0.14	0.12	0.17	0.1
Ca	$\text{cmolc dm}^{-3}$	1.5	1.5	2.7	2.3	5.6	5.1	1.3	1.3	2.4	2
Mg	$\text{cmolc dm}^{-3}$	0.62	0.51	1.12	0.99	0.99	0.88	0.42	0.33	0.68	0.47
Al	$\text{cmolc dm}^{-3}$	1.7	1.6	1.7	2	3.5	3.5	3.7	3.8	3.5	3.8
CEC	$\text{cmolc dm}^{-3}$	11.1	11.1	14.4	12.8	20.8	20.3	17.1	16.7	17.3	17.3
BS	%	19.2	18.8	27.1	24.4	32.3	30.4	11	10.5	18.5	15
m	%	44.7	43.6	30.5	38.4	34.1	36.1	66.4	66.2	52.3	59.4

and 5 had the highest values of clay and nutrients.

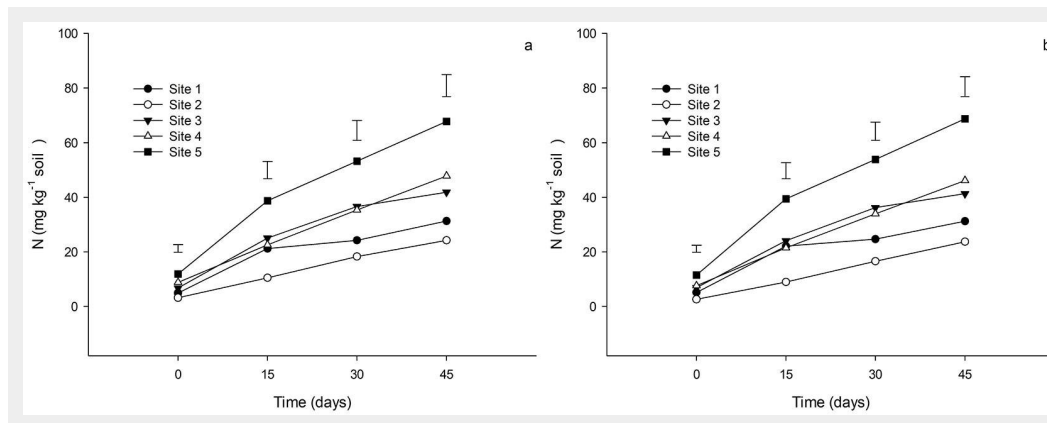
### Soil microbiological attributes

The accumulated potentially mineralizable nitrogen (PMN) along the 45 days of incubation showed a crescent linear behavior which did not reach the stabilization. No identifiable differences were observed between the two soil depths analyzed. Sites 1 and 2 showed the lowest PMN values (less than  $32 \text{ mg N kg}^{-1}$  soil), whereas

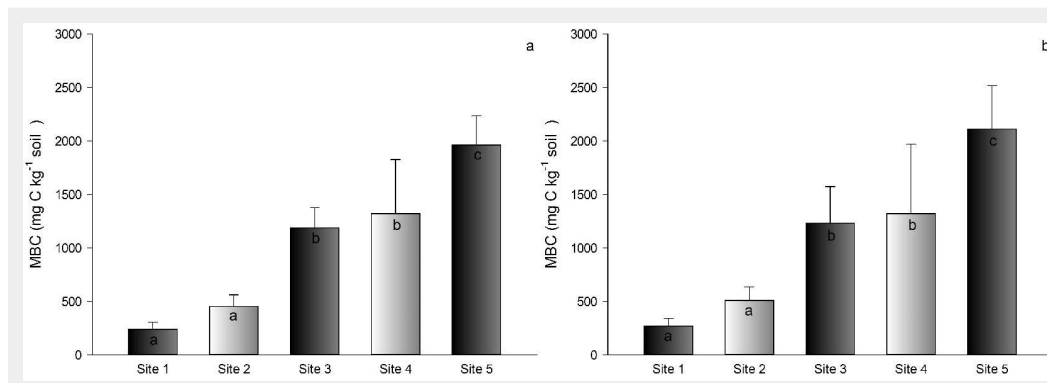
sites 3, 4, and 5 showed the highest PMN values (between  $42$  and  $68 \text{ mg N kg}^{-1}$  soil – Fig. 1a, Fig. 1b).

As for MBC, the results were significantly different between the 5 sites (Fig. 2a, Fig. 2b), with site 5 showing the highest values ( $\sim 2000 \text{ mg C kg}^{-1}$  soil), followed by sites 3 and 4 ( $\sim 1200 \text{ mg C kg}^{-1}$  soil), and the lowest values of MBC for sites 1 and 2 ( $\sim 300 \text{ mg C kg}^{-1}$  soil).

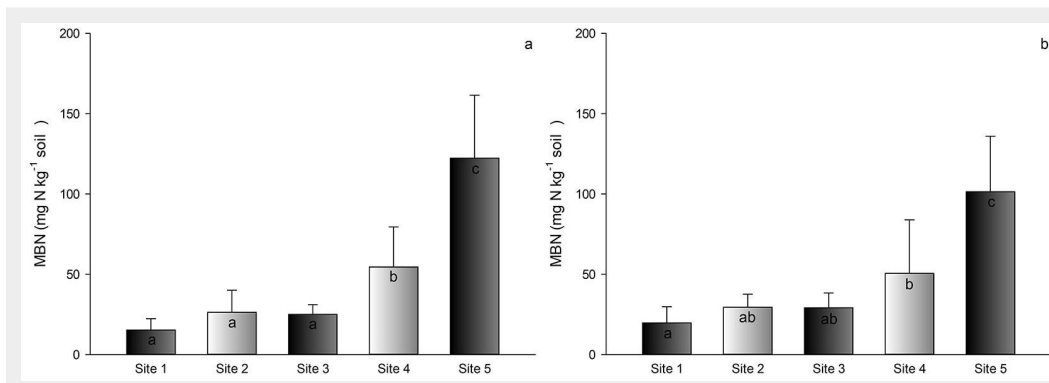
Values of MBN in sites 4 and 5 were higher than those recorded in sites 3, 2,



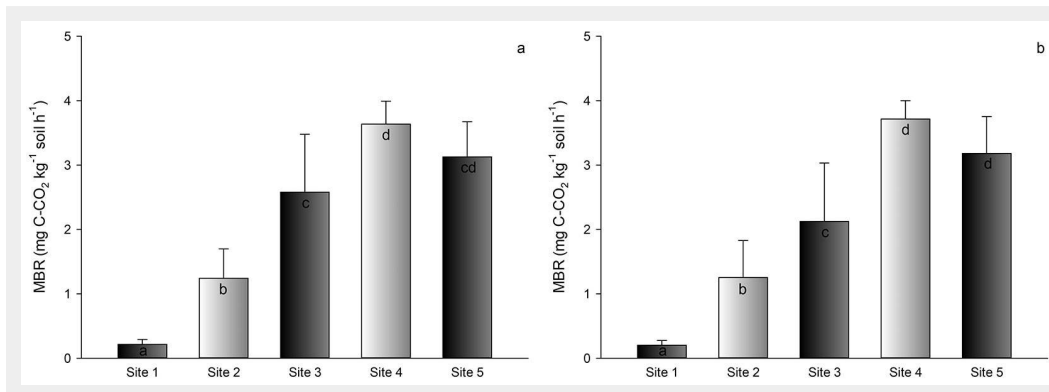
**Fig. 1** - Average of accumulated potentially mineralizable nitrogen (N), as nitrate ( $\text{NO}_3\text{-N}$ ), and ammonium ( $\text{NH}_4\text{-N}$ ) during the 45 days of incubation, in the soil of *Pinus taeda* plantations at different sites in southern Brazil. Depths of 0-5 cm (a) and 5-10 cm (b). The vertical bar represents the least significant difference, according to Tukey's test ( $p < 0.05$ ).



**Fig. 2** - Average microbial biomass carbon (MBC) in the soil of *Pinus taeda* plantations at different sites in southern Brazil. Depths of 0-5 cm (a) and 5-10 cm (b). Different letters indicate significant ( $p < 0.05$ ) differences among means after Tukey's test. Vertical bars represent the standard deviation.



**Fig. 3** - Average microbial biomass nitrogen (MBN) in the soil of *Pinus taeda* plantations at different sites in southern Brazil. Depths of 0-5 cm (a) and 5-10 cm (b). Different letters indicate significant ( $p < 0.05$ ) differences among means after Tukey's test. Vertical bars represent the standard deviation.



**Fig. 4** - Average microbial basal respiration (MBR) in the soil of *Pinus taeda* plantations at different sites in southern Brazil. Depths of 0-5 cm (a) and 5-10 cm (b). Different letters indicate significant ( $p < 0.05$ ) differences among means after Tukey's test. Vertical bars represent the standard deviation.

and 1. The lowest values varied between 25 and 29 mg N kg⁻¹ soil, and the highest ones were between 51 and 123 mg N kg⁻¹ soil (Fig. 3a, Fig. 3b).

The results for MBR were a little bit different when comparing sites. Site 4 showed the highest value of MBR among all sites, with a value of 3.7 mg CO₂-C kg⁻¹ soil h⁻¹.

Site 5 showed the second highest value (3.2 mg CO₂-C kg⁻¹ soil h⁻¹), followed by sites 3, 2, and 1 (less than 2.0 mg CO₂-C kg⁻¹ soil h⁻¹ - Fig. 4a, Fig. 4b).

The lowest values of  $qCO_2$  were observed for sites 1 and 5, the highest ones for sites 2 and 4, and the intermediary value for site 3 (Tab. 4).

**Tab. 4** - Average of the metabolic quotient ( $qCO_2$ ). Depths of 0-5 cm and 5-10 cm, the ratio between MBR (microbial basal respiration) and MBC (microbial biomass carbon), in the soil of sites of *Pinus taeda* of the initial growth in southern Brazil. Averages followed by the same letter in the line are not significantly different by Tukey's test ( $P < 0.05$ ).

Depth	$qCO_2$ (mg CO₂-C g⁻¹ MBC h⁻¹ 10⁻³)				
	Site 1	Site 2	Site 3	Site 4	Site 5
0-5 cm	1.28 <sup>a</sup>	3.00 <sup>b</sup>	2.32 <sup>ab</sup>	3.24 <sup>b</sup>	1.63 <sup>a</sup>
5-10 cm	0.77 <sup>a</sup>	2.84 <sup>ab</sup>	1.84 <sup>ab</sup>	3.91 <sup>c</sup>	1.59 <sup>a</sup>

**Tab. 5** - Pearson's correlation coefficients of microbiological variables (PMN: accumulated potentially mineralizable nitrogen; MBC: microbial biomass carbon; MBR: microbial basal respiration;  $qCO_2$ : metabolic quotient) with chemical and physical attributes of the soil (pH CaCl₂; C/N: ration between total carbon and total nitrogen; P: phosphorous content; Ca, Mg, K: calcium, magnesium, potassium content, respectively; BS: base saturation; m: Al³⁺ saturation), at depths of 0-5 and 5-10 cm, in *Pinus taeda* plantations at different sites in southern of Brazil. The pairs of variables showing positive and significant correlation coefficients tend to increase together. (\*):  $p < 0.05$ .

Soil Variables	PMN	MBC	MBR	$qCO_2$
Clay	0.371	0.514	0.299	0.826*
pH	0.723*	0.725*	0.817*	0.716*
C/N	0.518	0.601*	0.233	0.633*
P	0.316	0.269	0.235	-0.015
Ca	-0.052	0.092	-0.2	0.073
Mg	-0.472	-0.306	-0.342	-0.333
K	0.487	0.637*	0.474	0.734*
BS	-0.447	-0.316	0.136	-0.38
m	0.635*	0.555	0.166	0.657*

**Correlations between soil attributes**

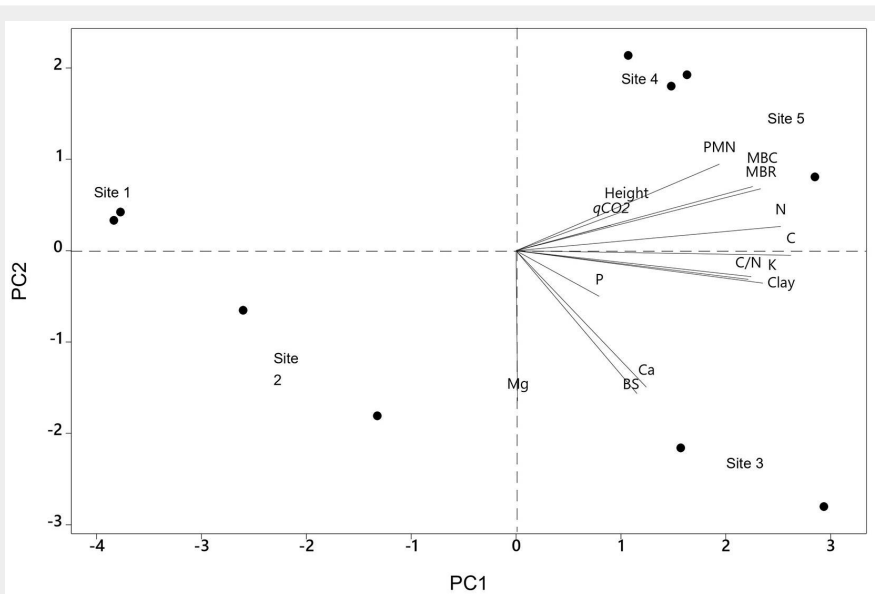
Pearson's correlation between the soil microbiological attributes and the physical and chemical attributes with data from all sites was performed. We observed a tight relationship between some of the microbiological attributes and soil characteristics such as clay, pH, C/N ratio, and potassium (K). However, no significant correlations were found between phosphorus, calcium, and magnesium and basic cations saturation (BS) with the microbiological attributes (Tab. 5).

In the PCA, principal components (PC) 1 and 2 accounted for 70% of the variation in the data. PC1 explains 50% of the total variation in the matrix data, and PC2 explains 20% of the variation. The eigenvector matrix represents the weight of each soil attribute factor, in the formation of the principal components. For PC1 the most associated eigenvectors were C, N, K, C/N ratio, MBC, MBR, and PMN, resulting in a direct association. PC2 was influenced by PMN, MBC, and MBR. (Tab. 6, Fig. 5).

**Discussion**

*Effect of soil types on microbiological attributes*

In general, the soil microbiological attributes were not influenced by the depth



**Fig. 5** - Principal component analysis (PCA) for sites of *Pinus taeda* of the initial growth in southern Brazil.

**Tab. 6** - Results of principal component analysis (PCA) for *Pinus taeda* plantations at different sites in southern Brazil. (PMN): accumulated potentially mineralizable nitrogen; (MBC): microbial biomass carbon; (MBR): microbial basal respiration; ( $qCO_2$ ): metabolic quotient; (P): phosphorus; (K): potassium; (Ca): calcium; (Mg): magnesium; (C): carbon; (N): nitrogen; (C/N): carbon/nitrogen content ratio; (BS): base saturation.

	Principal components	
	PC1	PC2
Eigenvalue	6.9	3.2
Proportion	0.5	0.2
Cumulative	0.5	0.7
Variable	Eigenvectors	
PMN	0.28	0.30
MBC	0.32	0.22
MBR	0.32	0.21
$qCO_2$	0.13	0.09
Height	0.15	0.14
P	0.11	-0.16
K	0.34	-0.11
Ca	0.18	-0.47
Mg	0.00	-0.52
C	0.38	-0.02
N	0.36	0.08
Clay	0.32	-0.09
C/N	0.32	-0.10
BS	0.17	-0.49

of soil sampling (0-5 and 5-10 cm). The differences identified for the microbiological attributes in the soil were related to site-specific conditions.

Despite all the sites having the same species and with similar ages in the present, previous use of the soil was quite different among sites (Tab. 2). During the initial growth, the effects on soil microbiological attributes are not related to the species planted (Samuelson et al. 2004, 2009), but rather to soil conditions resulting from the history of soil use and the management of forest system of the sites (Ward et al. 2015).

For most of the microbiological attributes evaluated, except for the  $qCO_2$ , site 5 showed the highest values and site 1 the lowest ones. At site 1, the current *P. taeda* stand replaced a previous *Eucalyptus urophylla* plantation, and this could partly explain the lowest soil clay content among all sites. In contrast, at all other sites current *Pinus taeda* stands replaced previous plantations of the same species. The quality of the remaining organic matter certainly affected the studied microbial attributes (Harrison-Kirk et al. 2014, Fine et al. 2018). Most of the residues from tree harvest were removed from site 1 before planting *Pinus taeda* and we can hypothesize that this has determined the low values of soil microbiological activity recorded. Also, site 1 presented the lowest levels of C, N, K, Ca, and Mg at both soil depths. Therefore, many soil conditions of site 1 could be related to the lowest values of its microbiological attributes.

Biesek (2012) studied soil microbiological attributes in crop areas, native and planted pasture, and a forest ecosystem, finding that the microbiological characteristics were attributed to the carbon and nitrogen stocks (forest and native pasture) or to the

nutrient availability (crop areas) in the soil. Site 5 showed high values for clay, C, N, P, K, Ca, and Mg, likely related to the maintenance of harvesting residues of previous *P. taeda* plantations during the last 34 years (Gonçalves et al. 2001). The combination of all these factors may explain the highest values for the microbiological attributes at this site.

Site 2 had the second lowest values for the microbiological attributes, as well as C, N, P, K, Ca, and Mg in the soil. In this case, most of the vegetal residues were removed when the former *P. taeda* plantation was harvested. Sites 3 and 4 showed values of clay, C, N, P, K, Ca, and Mg very close to those observed at site 5, but the soil type is an Oxisols, whereas site 5 has an Inceptisols which is less altered when compared to Oxisols. The higher elevation at sites 3 and 4, along with their altered clay content in the soil, may explain the lower values of the microbiological attributes in comparison to site 5.

The levels of C, N, P, K, Ca, and Mg in the soil surface are closely related to the quality of the soil organic matter. Suitable forest management practices, such as soil fertilization and silvicultural techniques, can improve site productivity by incorporating organic matter to the soil, that in turn can improve the soil microbiological quality (López-Poma et al. 2020).

The C/N ratio is another important factor controlling the rate of mineralization and the microorganism community composition (Kuz'yakov et al. 2019). Based on C/N ratio, the soil mineralization rate can be estimated, as well as the retention of mineral nitrogen in the soil and the variation in organic matter, which contributes to promoting microbial biomass (Veloso et al. 2019). Manirakiza et al. (2019) evaluated the nitrogen mineralization rates in soil with the ap-

plication of mineral and organic fertilizer, finding that fertilization improved the C/N ratio to adequate levels (around 14), thus increasing microbial biomass (Lee & Jose 2003, Chen et al. 2010).

#### Relationships between soil attributes

The soil pH showed a close relationship with the soil microbiological attributes, mainly with MBC, MBR, and PMN, despite the very small range of variation (3.9 to 4.4) among the study sites. The pH of the soil is an important variable in regulating microbial diversity and the mineralization process (Gonçalves et al. 2007), although it seems unlikely that differences in the microbiological attributes among sites could be explained by the small pH variation observed.

MBC and MBR showed the highest correlations with the C/N ratios, and similar results were found in other studies (Miller et al. 2005). Samuelson et al. (2009) showed that MBR can be an efficient indicator of soil microbiota in *P. taeda* plantations, and along with the MBC, they were the most sensitive indicators of the microbiological activity across *P. taeda* sites. In our study, MBR showed a strong relationship with clay content and was the most sensitive microbiological attribute. Low MBR values

can indicate lower substrate availability for soil microorganisms (Veloso et al. 2019, Yao et al. 2020); therefore, a balanced MBR rate in soils could maintain soil quality.

The  $qCO_2$  showed a positive correlation with clay content across the studied sites. Similar  $qCO_2$  values for pine plantations, but of different ages and at different soil depths, were found in other studies (Joergensen & Emmerling 2006). Also, Tulio et al. (2022) did not find any significant relationships between the metabolic quotient and chemical and physical attributes in the soil of *P. taeda* plantations. However, in the soil under *P. sylvestris* stands in northern China, Yao et al. (2020) found a strong correlation between  $qCO_2$  and soil water content, and this allowed to better understand the soil organic matter (SOM) dynamics in relation to soil physical characteristics (Yao et al. 2020).

Sites 1 and 2 showed lower average values of soil microbiological attributes and a weaker relationship with other soil attributes, while sites 3, 4, and 5 showed the highest average values of microbiological attributes and the strongest correlations with soil attributes. Meanwhile, at sites 4 and 5 microbiological activity was mostly associated with other soil attributes, such as PMN, MBC, MBR, carbon, and nitrogen total, whereas at site 3 it was more related to soil bases, K and Ca. Therefore, we speculate that soil microbiological attributes can be influenced by both the SOM and the nutrient content in the soil.

## Conclusion

Our results indicate that physical and chemical attributes intrinsic to the soil types have site-specific effects on soil microbiological attributes. Moreover, previous history of land use at the study sites remarkably affects soil microbiological activity. MBC and MBR were the soil microbiological attributes most sensitive to the differences between sites and showed a close relationship with clay content, pH, C/N ratio, and K in the soil. Considering the sensitivity of microbiological soil attributes to soil management practices, monitoring these attributes is a feasible way to evaluate changes in soil quality.

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## References

- Adam WM, Rodrigues VS, Magri E, Motta ACV, Zambon LM, Lima RLD (2021). Mid-rotation fertilization and liming of *Pinus taeda*: growth, litter, fine root mass, and elemental composition. *iForest* 14: 195-202. - doi: [10.3832/IFOR3626-014](https://doi.org/10.3832/IFOR3626-014)
- Albaugh JM, Blevins L, Allen HL, Albaugh TJ, Fox TR, Stape JL, Rubilar RA (2010). Characterization of foliar macro- and micronutrient concentrations and ratios in loblolly pine plantations in the Southeastern United States. *Southern Journal of Applied Forestry* 34 (2): 53-64. - doi: [10.1016/j.sjaf.2010.03.003](https://doi.org/10.1016/j.sjaf.2010.03.003)
- Albaugh TJ, Fox TR, Rubilar RA, Cook RL, Amateis RL, Burkhart HE (2017). Post-thinning density and fertilization affect *Pinus taeda* stand and individual tree growth. *Forest Ecology and Management* 396: 207-216. - doi: [10.1016/j.foreco.2017.04.030](https://doi.org/10.1016/j.foreco.2017.04.030)
- Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711-728. - doi: [10.1127/0941-2948/2013/0507](https://doi.org/10.1127/0941-2948/2013/0507)
- Anderson T, Domsch K (1993). The metabolic quotient for  $CO_2$  ( $qCO_2$ ) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biology and Biochemistry* 25: 393-395. - doi: [10.1016/0038-0717\(93\)90140](https://doi.org/10.1016/0038-0717(93)90140)
- APHA (1995). Standard methods for the examination of water and wastewater (19<sup>th</sup> edn). American Public Health Association - American Water Works Association - Water Pollution Control Federation, New York, USA, pp. 541.
- Batista AH, Motta ACV, Reissmann CB, Schneider T, Martins IL, Hashimoto M (2015). Liming and fertilisation in *Pinus taeda* plantations with severe nutrient deficiency in savanna soils. *Acta Scientiarum: Agronomy* 37 (1): 117-125. - doi: [10.4025/actasciagron.v37i1.18061](https://doi.org/10.4025/actasciagron.v37i1.18061)
- Biesek MF (2012). Indicadores químicos e microbiológicos da qualidade do solo em agrossistemas e sistemas nativos na região dos campos gerais, Castro-PR [Indicators of chemical and microbiology soil quality and system in agrosystems native in the region of general fields, Castro-PR]. Msc dissertation, Soil Science and Agricultural Engineering Department, Federal University of Paraná, Curitiba, PR, Brazil, pp. 50. [in Portuguese]
- Chen B, Liu S, Ge J, Chu J (2010). Annual and seasonal variations of  $Q_{10}$  soil respiration in the sub-alpine forests of the eastern Qinghai-Tibet plateau, China. *Soil Biology and Biochemistry* 42: 1735-1742. - doi: [10.1016/j.soilbio.2010.06.010](https://doi.org/10.1016/j.soilbio.2010.06.010)
- Dobner M, Campoe OC (2019). Meteorological effects on 30-years-grown *Pinus taeda* under a gradient of crown thinning intensities in southern Brazil. *Forest Ecology Management* 453: 117624. - doi: [10.1016/j.foreco.2019.117624](https://doi.org/10.1016/j.foreco.2019.117624)
- Fick SE, Hijmans RJ (2017). WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37 (12): 4302-4315. - doi: [10.1002/joc.5086](https://doi.org/10.1002/joc.5086)
- Fine AK, Schmidt MP, Martínez CE (2018). Nitrogen-rich compounds constitute an increasing proportion of organic matter with depth in Oi-Oe-Oa horizons of temperate forests. *Geoderma* 323: 1-12. - doi: [10.1016/j.geoderma.2018.02.039](https://doi.org/10.1016/j.geoderma.2018.02.039)
- Fox TR, Allen HL, Albaugh TJ, Rubilar RA, Carlson CA (2007). Tree nutrition and forest fertilization of pine plantations in the southern United States. *Southern Journal of Applied Forestry* 31 (1): 5-11. - doi: [10.1093/sjaf/31.1.5](https://doi.org/10.1093/sjaf/31.1.5)
- Gatiboni LC, Vargas CO, Albuquerque JA, Almeida JA, Stahl J, Chaves DM, Brunetto G, Dall'Orsoletta DJ, Rauber LP (2017). Phosphorus fractions in soil after successive crops of *Pinus taeda* L. without fertilization. *Ciência Rural* 47: 1-8. - doi: [10.1590/0103-8478CR20160595](https://doi.org/10.1590/0103-8478CR20160595)
- Gatiboni LC, Da Silva WC, Mumbach GL, Schmitt DE, Iochims DA, Stahl J, Vargas CO (2020). Use of exchangeable and nonexchangeable forms of calcium, magnesium, and potassium in soils without fertilization after successive cultivations with *Pinus taeda* in southern Brazil. *Journal of Soils and Sediments* 20: 665-674. - doi: [10.1007/s11368-019-02460-x](https://doi.org/10.1007/s11368-019-02460-x)
- Gonçalves JLM, Mendes KCFS, Sasaki CM (2001). Mineralização de nitrogênio em ecossistemas florestais naturais e implantados do estado de São Paulo [Nitrogen mineralization in natural and forest plantation ecosystem of São Paulo state]. *Revista Brasileira de Ciência do Solo* 25: 601-616. [in Portuguese] - doi: [10.1590/S0100-06832001000300009](https://doi.org/10.1590/S0100-06832001000300009)
- Gonçalves JLM, Wichert MCP, Gava JL, Masetto AV, Arthur Junior JC, Serrano MIP, Mello SLM (2007). Soil fertility and growth of *Eucalyptus grandis* in Brazil under different residue management practices. *Journal of Southern Hemisphere Earth Systems Science* 69: 95-102. - doi: [10.2989/SHFJ.2007.69.2.4.289](https://doi.org/10.2989/SHFJ.2007.69.2.4.289)
- Harrison-Kirk T, Beare MH, Meenken ED, Condon LM (2014). Soil organic matter and texture affect responses to dry/wet cycles: changes in soil organic matter fractions and relationships with C and N mineralisation. *Soil Biology and Biochemistry* 74: 50-60. - doi: [10.1016/j.soilbio.2012.10.008](https://doi.org/10.1016/j.soilbio.2012.10.008)
- Heinzmann FX, Miyazava M, Pavan MA (1984). Determinação de nitrato por espectrofotometria de absorção ultravioleta [Nitrate determination by ultraviolet absorption spectrophotometry]. *Revista Brasileira de Ciência do Solo* 8: 159-163. [in Portuguese] - doi: [10.18188/sap.v8i1.3836](https://doi.org/10.18188/sap.v8i1.3836)
- IBA (2021). Relatório anual [Annual Report of the Brazilian Tree Industry]. Indústria Brasileira de Árvores, Brazil, pp. 117. [in Portuguese] [online] URL: <http://iba.org/datafiles/publicacoes/relatorios/relatorio-iba-2020.pdf>
- Joergensen RG, Emmerling C (2006). Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. *Journal of Plant Nutrition and Soil Science* 169: 295-309. - doi: [10.1002/jpln.200521941](https://doi.org/10.1002/jpln.200521941)
- Kondo YR, Kaschuk G, Cruz SP (2021). Inoculation of plant growth-promoting bacteria in *Pinus taeda* seedlings. *Agropecuária Catarinense* 34: 93-98. - doi: [10.52945/frac.v34i3.1251](https://doi.org/10.52945/frac.v34i3.1251)
- Kuzyakov Y, Horwath WR, Dorodnikov M, Blagodatskaya E (2019). Review and synthesis of the effects of elevated atmospheric  $CO_2$  on soil processes: no changes in pools, but increased fluxes and accelerated cycles. *Soil Biology and Biochemistry* 128: 66-78. - doi: [10.1016/j.soilbio.2018.10.005](https://doi.org/10.1016/j.soilbio.2018.10.005)

- Lee KH, Jose S (2003). Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. *Forest Ecology Management* 185 (3): 263-273. - doi: [10.1016/S0378-1127\(03\)00164](https://doi.org/10.1016/S0378-1127(03)00164)
- López-Poma R, Pivello VR, Brito GS, Bautista S (2020). Impact of the conversion of Brazilian woodland savanna (Cerradão) to pasture and *Eucalyptus* plantations on soil nitrogen mineralization. *Science of the Total Environment* 704: 135397. - doi: [10.1016/j.scitotenv.2019.135397](https://doi.org/10.1016/j.scitotenv.2019.135397)
- Lu T, Wang Y, Zhu H, Wei X, Shao M (2020). Drying-wetting cycles consistently increase net nitrogen mineralization in 25 agricultural soils across intensity and number of drying-wetting cycles. *Science of the Total Environment* 710: 135574. - doi: [10.1016/j.scitotenv.2019.135574](https://doi.org/10.1016/j.scitotenv.2019.135574)
- Manirakiza E, Ziadi N, Luce MS, Hamel C, Antoun H, Karam A (2019). Nitrogen mineralization and microbial biomass carbon and nitrogen in response to co-application of biochar and paper mill biosolids. *Applied Soil Ecology* 142: 90-98. - doi: [10.1016/j.apsoil.2019.04.025](https://doi.org/10.1016/j.apsoil.2019.04.025)
- Mckenzie J, Goldman R (1999). The student edition of Minitab for Windows manual. Addison-Wesley Longman, Inc., CA, USA, web site. [in Portuguese] [online] URL: <http://www.minitab.com/pt-br/>
- Miller AE, Schimel JP, Meixner T, Sickman JO, Melak JM (2005). Episodic rewetting enhances carbon and nitrogen release from chaparral soils. *Soil Biology and Biochemistry* 37: 2195-2204. - doi: [10.1016/j.soilbio.2005.03.021](https://doi.org/10.1016/j.soilbio.2005.03.021)
- Monz CA, Reuss DE, Elliott ET (1991). Soil microbial biomass carbon and nitrogen estimates using 2450 MHz microwave irradiation or chloroform fumigation followed by direct extraction. *Agriculture, Ecosystems and Environment* 34: 55-63. - doi: [10.1016/0167-8809\(91\)90093-D](https://doi.org/10.1016/0167-8809(91)90093-D)
- Moro L, Gatiboni LC, Simonete MA, Cassol PC, Chaves DM (2014). Response of one-, five-, and nine-year-old *Pinus taeda* to NPK fertilization in southern Brazil. *Revista Brasileira de Ciência do Solo* 38: 1181-1189. - doi: [10.1590/S0100-06832014000400014](https://doi.org/10.1590/S0100-06832014000400014)
- Norman RJ, Stucki JW (1981). The determination of nitrate and nitrite in soil extracts by ultraviolet spectrophotometry. *Soil Science Society of America Journal* 45: 347-353. - doi: [10.2136/sssaj1981.03615995004500020024x](https://doi.org/10.2136/sssaj1981.03615995004500020024x)
- R Development Core Team (2019). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [online] URL: <http://www.r-project.org/>
- Rodriguez DRO, Andrade GC, Jurado Bellote AFJ, Tomazello Filho M (2018). Effect of pulp and paper mill sludge on the development of 17-year-old loblolly pine (*Pinus taeda* L.) trees in Southern Brazil. *Forest Ecology and Management* 422: 179-189. - doi: [10.1016/j.foreco.2018.04.016](https://doi.org/10.1016/j.foreco.2018.04.016)
- Romeo F, Settineri G, Sidari M, Mallamaci C, Muscolo A (2020). Responses of soil quality indicators to innovative and traditional thinning in a beech (*Fagus sylvatica* L.) forest. *Forest Ecology and Management* 465: 118106. - doi: [10.1016/j.foreco.2020.118106](https://doi.org/10.1016/j.foreco.2020.118106)
- Santos KF, Reichert JM (2022). Best tillage practices for *Eucalyptus* growth and productivity: a review on the Brazilian experience. *Revista Brasileira de Ciência do Solo* 46: e0210091. - doi: [10.36783/18069657rbcs20210091](https://doi.org/10.36783/18069657rbcs20210091)
- Samuelson L, Johnsen K, Stokes T (2004). Production, allocation and stemwood growth efficiency of *Pinus taeda* L. stands in response to 6 years of intensive management. *Forest Ecology and Management* 192: 59-70. - doi: [10.1016/j.foreco.2004.01.005](https://doi.org/10.1016/j.foreco.2004.01.005)
- Samuelson L, Mathew R, Stokes T, Feng Y, Aubrey D, Coleman M (2009). Soil and microbial respiration in a loblolly pine plantation in response to seven years of irrigation and fertilization. *Forest Ecology and Management* 258: 2431-2438. - doi: [10.1016/j.foreco.2009.08.020](https://doi.org/10.1016/j.foreco.2009.08.020)
- Sixel RMM, Arthur Junior JC, Gonçalves JLM, Alvares CA, Andrade GRP, Azevedo AC, Stahl J, Moreira AM (2015). Sustainability of wood productivity of *Pinus taeda* based on nutrient export and stocks in the biomass and in the soil. *Revista Brasileira de Ciência do Solo* 39: 1416-1427. - doi: [10.1590/01000683rbcs20140297](https://doi.org/10.1590/01000683rbcs20140297)
- Soil Survey Staff (1999). Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys (2<sup>nd</sup> edn). Agriculture Handbook Number 436, USDA Natural Resources Conservation Service, US Government Printing Office, Washington, DC, USA, pp. 886. [online] URL: <http://www.nrcs.usda.gov/sites/default/files/2022-06/SoilTaxonomy.pdf>
- Tulio RH, Rachwal MFG, Zanatta JA, Silva K, Kaschuk G (2022). Physical, chemical and microbiological soil attributes influence soil greenhouse gases fluxes in Atlantic Forest and pine (*Pinus taeda*) plantations in Brazil. *Soil Use and Management* 39: 183-197. - doi: [10.1111/sum.12864](https://doi.org/10.1111/sum.12864)
- Veloso MG, Cecagno D, Bayer C (2019). Legume cover crops under no-tillage favor organomineral association in microaggregates and soil C accumulation. *Soil and Tillage Research* 190: 139-146. - doi: [10.1016/j.still.2019.03.003](https://doi.org/10.1016/j.still.2019.03.003)
- Vose JM, Allen HL (1988). Leaf area, stemwood growth and nutrition relationships in loblolly pine. *Forest Science* 34: 547-563. - doi: [10.1093/forestscience/34.3.547](https://doi.org/10.1093/forestscience/34.3.547)
- Ward EJ, Domec JC, Laviner MA, Fox TR, Sun G, McNulty S, King J, Noormets A (2015). Fertilization intensifies drought stress: water use and stomatal conductance of *Pinus taeda* in a mid-rotation fertilization and throughfall reduction experiment. *Forest Ecology and Management* 355: 72-82. - doi: [10.1016/j.foreco.2015.04.009](https://doi.org/10.1016/j.foreco.2015.04.009)
- Waring SA, Bremner JM (1964). Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature* 201: 951-952. - doi: [10.1038/201951a0](https://doi.org/10.1038/201951a0)
- Yao X, Zheng W, Zeng H, Wang W (2020). Soil microbial attributes along a chronosequence of scots pine (*Pinus sylvestris* var. *mongolica*) plantations in northern China. *Pedosphere* 30 (4): 60329. - doi: [10.1016/S1002-0160\(17\)60329-1](https://doi.org/10.1016/S1002-0160(17)60329-1)
- Zucon A, Dominschek R, Motta ACV (2020). Can fertilization and liming affect the amount of litter and roots on *Pinus taeda* forest floor? *Scientia Forestalis* 48: (128) e3193. - doi: [10.18671/scifor.v48n128.21](https://doi.org/10.18671/scifor.v48n128.21)