University of São Paulo
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Wood properties of 17-year-old *Pinus taeda* L. trees under composted pulp-mill
sludge fertilization by tree-ring analysis

Daigard Ricardo Ortega Rodriguez

Dissertation presented to obtain the degree of Master in
Science. Area: Forest Resources. Option in: Silviculture
and Forest Management

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2018

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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DEDICATION

Para mis padres Rosa y David, gracias por sus oraciones.

For my parents Rosa and David, thank you for your prayers.

Para os meus pais Rosa e David, obrigado por suas orações.

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EPIGRAPH

"For me, trees have always been the most penetrating preachers. I revere them when they live in tribes and families, in forests and groves. And even more I revere them when they stand alone. They are like lonely persons. Not like hermits who have stolen away out of some weakness, but like great, solitary men, like Beethoven and Nietzsche. In their highest boughs the world rustles, their roots rest in infinity; but they do not lose themselves there, they struggle with all the force of their lives for one thing only: to fulfill themselves according to their own laws, to build up their own form, to represent themselves. Nothing is holier, nothing is more exemplary than a beautiful, strong tree. When a tree is cut down and reveals its naked death-wound to the sun, one can read its whole history in the luminous, inscribed disk of its trunk: in the rings of its years, its scars, all the struggle, all the suffering, all the sickness, all the happiness and prosperity stand truly written, the narrow years and the luxurious years, the attacks withstood, the storms endured. And every young farmboy knows that the hardest and noblest wood has the narrowest rings, that high on the mountains and in continuing danger the most indestructible, the strongest, the ideal trees grow. Trees are sanctuaries. Whoever knows how to speak to them, whoever knows how to listen to them, can learn the truth. They do not preach learning and precepts, they preach, undeterred by particulars, the ancient law of life. A tree says: A kernel is hidden in me, a spark, a thought, I am life from eternal life. The attempt and the risk that the eternal mother took with me is unique, unique the form and veins of my skin, unique the smallest play of leaves in my branches and the smallest scar on my bark. I was made to form and reveal the eternal in my smallest special detail. A tree says: My strength is trust. I know nothing about my fathers, I know nothing about the thousand children that every year spring out of me. I live out the secret of my seed to the very end, and I care for nothing else. I trust that God is in me. I trust that my labor is holy. Out of this trust I live. When we are stricken and cannot bear our lives any longer, then a tree has something to say to us: Be still! Be still! Look at me! Life is not easy, life is not difficult. Those are childish thoughts. Home is neither here nor there. Home is within you, or home is nowhere at all. A longing to wander tears my heart when I hear trees rustling in the wind at evening. If one listens to them silently for a long time, this longing reveals its kernel, its meaning. It is not so much a matter of escaping from one's suffering, though it may seem to be so. It is a longing for home, for a memory of the mother, for new metaphors for life. It leads home. Every path leads homeward, every step is birth, every step is death, every grave is mother. So the tree rustles in the evening, when we stand uneasy before our own childish thoughts: Trees have long thoughts, long-breathing and restful, just as they have longer lives than ours. They are wiser than we are, as long as we do not listen to them. But when we have learned how to listen to trees, then the brevity and the quickness and the childlike hastiness of our thoughts achieve an incomparable joy. Whoever has learned how to listen to trees no longer wants to be a tree. He wants to be nothing except what he is. That is home. That is happiness."

Hermann Hesse

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RESUMO

Propiedades do lenho do tronco das árvores de *Pinus taeda* L. submetidas a aplicação de resíduo celulósico através da análise de anéis de crescimento

Na aplicação de fertilizantes em plantações é fundamental o entendimento e a avaliação do efeito na produtividade e qualidade da madeira das árvores das espécies florestais. Os processos de monitoramento destas práticas são, geralmente, definidos pela mensuração contínua das dimensões das árvores em parcelas permanentes em inventários florestais periódicos. No geral, esta metodologia de monitoramento implica na disponibilidade de informações restritas a poucos anos. Por outro lado, a análise dos anéis de crescimento anuais constitui-se em eficiente metodologia alternativa ao uso de parcelas permanentes. Neste contexto, o presente estudo visa analisar o crescimento, densidade e concentração de nutrientes dos anéis de crescimento anuais do lenho de 60 árvores de *Pinus taeda* de 17 anos de plantação experimental submetida a 6 tratamentos com resíduo celulósico compostado (CPMS), sendo: 0, 20, 40, 80 e 100 t ha⁻¹. Foram selecionadas 10 árvores correspondentes a cada tratamento, cortados discos do lenho a 0, DAP, 25, 50, 75 e 100% da altura comercial do tronco (6 cm de diâmetro mínimo), delimitados e mensurados os anéis de crescimento na sua seção transversal e construído o crescimento radial-longitudinal do tronco das árvores. Amostras radiais gêmeas foram cortadas transversalmente do lenho dos disco do DAP do tronco, acondicionadas e analisadas por técnicas não destrutivas de densitometria de raios X e por fluorescência de raios X (μ -XRF), determinando-se a variação radial da microdensidade e dos nutrientes do lenho. As análises dendrocronológicas dos anéis de crescimento anuais propiciaram a construção das cronologias da largura, densidade, biomassa e concentração dos nutrientes, comparando-as com as variações sazonais de precipitação pluviométrica e de temperatura. Aplicou-se se a análise de componentes principais (PCA) para explorar a interação das variáveis dos anéis de crescimento, bem como a análise de variância (ANOVA) para verificar a diferença entre tratamentos. Os resultados das análises são apresentados em três capítulos atendendo ao objetivo do presente estudo. No **Capítulo I**, analisou-se a variação radial da microdensidade dos anéis de crescimento, e a integração dos dados de densidade e largura dos anéis de crescimento em equações alométricas para melhorar a estimativa da produção de biomassa de madeira. Ainda foi datada a resposta das árvores de *Pinus taeda* aos tratamentos com CPMS. No **Capítulo II** analisou-se o histórico da resposta do desenvolvimento do tronco das árvores de *Pinus taeda* às doses de CPMS, com incrementos de até 24, 37 e 127% no diâmetro, altura e volume do tronco, respectivamente. Propõe-se, ainda, a confecção de guia de manejo baseado na simulação da produção de madeira de *P. taeda* submetido a 84 t ha⁻¹ CPMS para rotação de 21 anos e desbastes ao 7º e 13º ano resultando em produtividade de 11,9 m³ ha⁻¹ ou 5,04 Mg ha⁻¹ por ano. No **capítulo III** quantificou-se a concentração de nutrientes (fósforo, enxofre, potássio, cálcio, manganês e ferro; P, S, K, Ca, Mn e Fe) nos anéis de crescimento anuais das árvores. Observou-se que Ca e Mn diminuem na direção medula-casca; K e S diminuem da medula até o 8-9º anel, em seguida, aumentam na direção da casca, enquanto Fe e P não apresentaram tendências radiais. Os resultados integrados do histórico do crescimento, densidade da madeira e concentração de nutrientes no lenho das árvores de *P. taeda* mostraram que a variabilidade anual esta correlacionada, principalmente, com a precipitação local e com o efeito da aplicação de CPMS até cerca do 5º ano. O presente estudo propiciou a avaliação histórica do crescimento, densidade e concentração de nutrientes do lenho das árvores de *Pinus taeda*. Evidenciando a aplicação dos anéis de crescimento anuais no monitoramento de plantações florestais submetidas a tratamentos silviculturais.

Palavras-chave: Produção florestal; Dendrocronologia; Métodos não destrutivos; Fertilizante orgânico

ABSTRACT

Wood properties of 17-year-old *Pinus taeda* L. trees under composted pulp-mill sludge fertilization by tree-ring analysis

The analysis of the fertilization effects on wood productivity and tree-wood quality is important in forest plantations management. The monitoring of these practices are generally defined by the continuous measurement of tree dimensions in permanent plots in periodic forest inventories. This methodology implies the availability of information restricted to a few years. On the other hand, the annual tree-ring analysis constitutes an efficient alternative methodology to the permanent plots uses. This study aimed to analyze the growth, wood density and nutritional concentration of 60 *Pinus taeda* trees treated with 6 different doses of composted pulp-mill sludge (CPMS): 0, 20, 40, 80 and 100 t ha⁻¹. Ten 17 year-old-trees for each treatment were selected, felled and wood cross sections (5 cm, thickness) were cut at the base, 1.30 m (DBH), 25, 50, 75 and 100% (6 cm, commercial trunk height). The annual tree-rings of the wood discs were delimited and radial-longitudinally synchronized, and the tree stems reconstructed. Twin radial thin wood slices were cut transversely (DBH, trunk position), conditioned and analyzed by non-destructive techniques of X-ray densitometry and X-ray fluorescence (μ -XRF), to obtain the microdensity and wood nutrients profile. Width, density, biomass and nutrient concentration chronologies were analyzed by dendrochronological methods and correlated to seasonal variations of rainfall and temperature. Principal Component Analysis (PCA) was applied to explore the interaction of tree-ring variables, as well as analysis of variance (ANOVA) to verify the difference between treatments. The results analysis are presented in three chapters aiming to reach the main objective of this study. In **Chapter I**, the radial variation of the tree-ring microdensity and the integration of tree-ring density and width data in allometric equations were analyzed in order to improve the estimation of wood biomass production. Also, the response of the *Pinus taeda* trees to the CPMS treatments was dated. In **Chapter II** the response of the stem *P. taeda* trees development to the CPMS doses was analyzed. Increments of up to 24, 37 and 127% in the stem diameter, height and volume, respectively were observed. It is also proposed a management guide based on the simulation of *P. taeda* wood production under 84 t ha⁻¹ CPMS for 21-year rotation and thinning at 7 and 13 years resulted in a wood productivity of 11.9 m³ ha⁻¹ or 5.04 Mg ha⁻¹ per year. In **Chapter III** the tree-ring nutrients concentration (phosphorus, sulfur, potassium, calcium, manganese and iron; P, S, K, Ca, Mn and Fe) were quantified. Ca and Mn decrease in the pith-bark direction; K and S decrease from the pith up to 8-9th annual tree-ring and, then, increase to the bark and Fe e P presented no distinguished radial trends. The results integration showed mainly correlations of *P. taeda* trees growth, wood density and nutrient concentration with local precipitation and significantly effects of CPMS soil fertilizer up to the 5th year after their application. This study provided an historical evaluation of growth, wood density and nutrient concentration of *P. taeda* trees. Evidencing the application of annual tree-rings in the monitoring of forest plantations under silvicultural treatments.

Keywords: Wood production; Dendrochronology; Non-destructive methods; Organic fertilizer

1. INTRODUCTION

Brazil has 7.7 Mha of planted forests (FAO, 2015) and 20.8% correspond to *Pinus* plantation (IBA, 2016). The *Pinus* silviculture was initiated between the 1960s and 1970s, mainly in southern Brazil due to favorable climatic and site conditions (Vasques et al., 2007). The *Pinus* forest plantations presented the highest productivity of 35 m³ ha⁻¹ year⁻¹. However, in 2015, 1.1% of the plantation areas decreased due to the substitution by *Eucalyptus* forests, currently the most cultivated genus in Brazil (IBA, 2016).

Pinus plantations were established as a development and sustainability strategy of the Brazilian productive chain of wood and wood products (Bacha and Barros, 2004; Ferreira et al., 2004; Vasques et al., 2007). Although they were considered as invasive alien species and changed the natural landscape (Fonseca et al., 2013). The genus maintains a 31 m³ ha⁻¹ year⁻¹ production and provides 22% of industrial wood from Brazil forest plantations, with the states of Paraná and Santa Catarina accounting for 76.6% of the *Pinus* planted area (IBA, 2016).

Pinus taeda, native to the United States, was introduced in 1960 and is the most planted *Pinus* species in Brazil (Vasques et al., 2007; Wrege et al., 2014). The wide use in Brazil is due to their higher 35 m³ ha⁻¹ year⁻¹ of growth rate in 18-year rotation cycles in forest plantations compared to the 7.4 m³ ha⁻¹ year⁻¹ in 40-year rotation cycles in natural US forests. Also to their economic potential, represented by a 24% internal return in forest plantations compared to 8% in natural forests (Cubbage et al., 2007). Therefore, the forest managers interest in *P. taeda* silviculture in forest plantations became important for the increase of their production, potentially more viable in Brazil (Vasques et al., 2007, Cardoso et al., 2013).

P. taeda silviculture as other forest tree species has been developed on nutrient-poor soils (Jokela et al., 2010). In Brazil, most forest plantations were installed on soils generally characterized as Yellow Red Latosols (IBA, 2016). These soils are considered as poor in nutrients and affected by weathering and leaching as a consequence of the continuous nutrients export by the different rotations of agricultural crops or forest plantations, and fertilization treatments are required (Gonçalves, 1995; Silva et al., 2013). Fertilization practices are related to the forest plantation productivity (Silva et al., 2013). It is a controlled procedure to increase nutrient availability from the early stages of forest plantation development (Gonçalves, 1995; Bellote and Neves, 2001). Furthermore, fertilization should contribute to high plantations production, at the lowest cost and without adversely affecting the environment (Silva et al., 2013). Therefore, the reuse of unconventional inputs such as industries wastes were tested with successful wood production increase results (Vance, 2000; Feldkirchner et al., 2003; Viera and Schumacher, 2010; Ferraz et al. 2016; Faria et al., 2015a).

One of these environmental and economic concern wastes is the pulp and paper biosolids (Henry et al., 1994, Moro and Gonçalves, 1995, Bellote et al., 1998, Altesor et al. 2008). It can reach 48 t of residues in the production of 100 t of cellulose (Arruda et al., 2011). In this context, several studies were developed seeking forest alternatives for their use and reduction of environmental impacts (Cabral et al., 1998, Andrade et al., 2003, Maeda and Bognola, 2013). Potential benefits were reported by the pulp-paper biosolid uses, such as improved nutrients availability, soil acidity correction and tree-wood productivity increases (Cabral et al., 1998; Jackson et al., 2000; Harrisson et al., 2003; Faria et al., 2015b). An attractive alternative for the biosolids recycling from pulp and paper companies (Feldkirchner et al., 2003). Successfully studies of US paper companies reported fertilization costs reductions and forest productivity increases (Vance, 2000). Nevertheless, the biosolid-uses in specific forest species, as well other fertilizers needs to be temporal and spatially monitored in distinct soil and climates (Moro et al., 2014;

Batista et al., 2015, Nisgoski et al. al., 2016). Mainly in forest areas where management techniques are intensified, such as *Pinus* forest plantations in Southern Brazil (Viera and Schumacher, 2010).

Monitoring methods can help forest managers to plan nutritional treatments and understand the tree-growth responses and physical-chemical wood properties changes (Barclay and Brix, 1985; Ferreira et al., 2004; Reissmann and Wisniewski, 2005). At this point, the annual tree-ring analysis is a potential alternative in the evaluation of these tree responses to fertilization treatments (Valinger, 1993; Henry et al., 1994; Love-Myers et al., 2009; Beauregard et al., 2010). In Brazil, despite the fertilization reports using pulp-mill biosolids in forest plantation, no consistent results were found in the literature reporting the effects on *P. taeda* trees growth, wood density and nutrient concentration in a complete rotation and with a forestry management approach.

The main objective of this study is to evaluate the growth responses and the wood density and nutritional concentration changes of *P. taeda* trees planted in soil amended with different doses of composted pulp-mill sludge. Thus, three chapters were developed to achieve the present goal.

1. **Radial wood density variability of 17-year-old *Pinus taeda* by X-ray densitometry to improve biomass estimation and to evaluate effects of pulp-mill sludge fertilizer.** This chapter seek analyze the wood density radial variability of *P. taeda* trees by non-destructive X-ray densitometry to estimate biomass increment using allometric equations and to monitor the CPMS effects. The interrelation of wood density, tree-ring thickness and biomass variation were also analyzed and correlated to local climate variables.
2. **Effect of pulp and paper mill sludge on the development of 17-year-old loblolly pine (*Pinus taeda* L.) trees in Southern Brazil.** Here, the annual tree ring features were the basis for analyzing the development of the complete rotation forest-plantation. The historic of the stand development, optimal CPMS dosage, and wood production simulation were used to propose a management guide for loblolly pine trees from plantations in Southern Brazil.
3. **Chemical nutrient concentrations in tree-rings of 17 years- *Pinus taeda* trees by X-ray fluorescence microanalysis (μ -XRF).** In this chapter the quantification of chemical element concentrations in the radial profile of wood cores of 24 *P. taeda* trees selected based on the higher tree-ring width chronology correlation were analyzed by μ -XRF techniques. Chemical element concentration trends, their correlation with local climate variables and the effects of CPMS soil treatments were also analyzed.

This document is presented in English to give scientific prominence to the study.

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2. RADIAL WOOD DENSITY VARIABILITY OF 17-YEAR-OLD *PINUS TAEDA* BY X-RAY DENSITOMETRY TO IMPROVE BIOMASS ESTIMATION AND TO EVALUATE EFFECTS OF PULP-MILL SLUDGE FERTILIZER

Abstract

Wood density, as the main property related to wood quality and application, is influenced by tree genetics, forest management and environmental conditions, being applied to monitoring tree development, wood production and silvicultural practices (e.g. fertilization). Nevertheless, few studies have evaluated the wood density relation at an inter-annual level of *Pinus taeda* trees in southern Brazil. The annual wood density variability of sixty 17-year-old-*Pinus taeda* trees was analyzed to improve biomass estimation, linked to local climate variables and to understand the growth responses to six composted pulp-mill sludge (CPMS) treatments. Ten trees for each treatment were selected and felled and from which wood discs were taken at different trunk heights. The tree-rings in the all disc cross-sections were synchronized and the annual volume measured. In the sequence, mean wood basic density of a quarter disc was determined and the annual biomass increment was calculated. Two scenarios for biomass estimation by allometric equations, including radial density variability obtained by X-ray densitometry, were tested and compared to the calculated biomass values. Also, tree-ring width (TRW), wood density (WD) and biomass series were constructed, and correlation to climate variables and CPMS treatment effects were analyzed. Both scenarios confirmed a good accuracy ($r^2 > 0.96$, standard error of the mean $< 16\%$) for *P. taeda* biomass increment estimation. The WD and TRW interrelations may exert opposing influences on biomass increments and their variation was correlated with precipitation. *P. taeda* wood density and resulting biomass was significantly affected by CPMS up to 6 years after soil application, promoting higher growth effect with CPMS 80 t ha⁻¹. The result confirms that wood density variability improved the biomass estimate, supporting tree monitoring development and the CPMS fertilizer effect, and also contributes to the *P. taeda* plantation management.

Keywords: Loblolly pine; Tree-ring density; Biomass production; Allometric equation; Biosolid fertilizer

2.1. Introduction

Wood density is considered a main physical quality parameter (Bouriaud et al., 2015; Jacquin et al., 2017; Rosada de Oliveira et al., 2017; Moreno-Fernández et al., 2018) and considered a good predictor of several wood properties and utilizations (Zhang, 1997). The wood density is directly related to anatomical features (Hacke et al., 2001; Roque and Tomazelo-Filho, 2007; Pritzkow et al., 2014; Wilkinson et al., 2015; Salvo et al., 2017) and influences wood mechanical (Giroud et al., 2017) and chemical properties (Nakagawa et al., 2016). Furthermore, annual tree-ring microdensity is correlated to cambial activity and used in dendrochronology (Schweingruber et al., 1978; Tomazello et al., 2008; Hietz et al., 2013; Sousa et al., 2016). The density, as well the tree growth, varies seasonality according to the species, genetics, environment and forest management (Zhang, 1997; Rozenberg et al., 2001; Pompa-García and Venegas-González, 2016; Rosada de Oliveira et al., 2017; Moreno-Fernández et al., 2018).

The tree-ring width and density are considered good proxies to understand the volume and mass annually produced by tree species (Yu et al., 2014; Locosselli and Buckeridge, 2017). The tree-ring width chronology has been extensively used to provide information referring to climate effect, volume, forest yield and modeled biomass (Babst et al., 2014; Wagner et al., 2017). Although in most cases, the density was considered constant, ignored or poorly studied (Wutzler et al., 2008; Picard et al., 2012; Babst et al., 2014a), recently density was applied as a variable to improve biomass and carbon estimates (Babst et al., 2014; Knapic et al., 2014; Bouriaud et al., 2015; Chave et al., 2015; Pompa-García and Venegas-González, 2016).

The biomass increment of tree species is crucial to understand their productivity and to reconstruct and monitor the carbon cycle (Bouriaud et al., 2015). The tree-ring density and width, integrated in an allometric equation, improve the inter-annual quantification of biomass (Bouriaud et al., 2015; Pompa-García and Venegas-González, 2016) due to the annual tree-ring features being related to the climatic factors (Briffa et al., 2002) and also the biomass (Babst et al., 2014b).

In the tree growing process, the biomass can be monitored by forest inventories correlating volume estimation based on trunk DBH and height as reference variables (Hackenberg et al., 2015) and applied in allometric models (Zianis and Seura, 2005; Picard et al., 2012). Recently, the incorporation of density improves these functions (FAO, 2013). Concerning the *P. taeda*, it is the most planted species in southern Brazil (Vasques et al., 2007) with one biomass equation reported for the country, while there are 20 and 54 for Argentina and USA, respectively. Furthermore, to our knowledge, one study that used intra-annual microdensity analyzed *P. taeda* responses to crown thinning and harvest age in southern Brazil (Dobner et al., 2018), however, no studies were reported for monitoring biomass using microdensity analysis.

The *Pinus taeda* plantation management is carried out applying a combination of silvicultural methods (Antony et al., 2015) aiming the optimal productivity concerning tree-growth, volume and quality (Will et al., 2002). The fertilizer treatments applied in *P. taeda* plantations may affect tree growth features and wood properties, normally evaluated by their density (Zhang, 1995; Mörling, 2002). Most of the studies used a mean density of DBH or several trunk heights and there are no differences between treatments or such differences are small (Zobel and van Buijtenen, 2012; Antony et al., 2015). However, several studies applying X-ray densitometry have been able to detect differences in wood density (e.g. Cown and McConchie, 1981; Mörling, 2002; Jordan et al., 2008; Kimberley et al., 2015; Rosada de Oliveira et al., 2017; Dobner et al., 2018; Moreno-Fernández et al., 2018).

The tree-ring density is reduced after tree species soil fertilization due to a cambial activity effect, and the changes in the features and composition of xylem cells (Love-Myers et al., 2009). In several conifer species, the tree-ring density modification is directly related to tracheid cells dimension and late and earlywood thickness (Cown and McConchie, 1981; Valinger et al., 2000; Love-Myers et al., 2009; Zobel and van Buijtenen, 2012). However, density change associated to biomass has still been little explored (Cown and McConchie, 1981; Albaugh et al., 1998; Valinger et al., 2000; Larson et al., 2001).

Thus, this study aimed to analyze the *P. taeda* tree-ring microdensity variability to improve stem biomass estimation in two scenarios using allometric equations. Furthermore, the study also aims to provide understanding information regarding the effect of CPMS soil application on the wood density and biomass of 17-year-old *P. taeda* trees.

2.2. Material and methods

2.2.1. Study area and experimental design

The experimental area was located on the Matarazzo Farm, Jaguariaíva, Paraná state, Brazil (24°15' S, 49°42' W; 872 m a.s.l.; slope 3-8 %) (Figure 1A). The area is characterized by a subtropical moist mesothermic climate (Cfb under Köppen classification), with frequent and severe frost occurrence. The temperature is moderate, with an annual average of 18.1°C. The average annual rainfall is 1323 mm, with a dry season between July and August (Figure 1B). The soil is classified as dystrophic Red-Yellow Latosol, according to the Brazilian Soil

Classification System (EMBRAPA, 2006). It has a medium texture with a clay content of less than 35% and a sand content of more than 15%, strongly drained and extremely acidic ($\text{pH} < 4.3$), poor in macronutrients for plants, low organic matter content ($10.7\text{--}14 \text{ g dm}^{-3}$) and low cation exchange capacity ($5 - 5.7 \text{ cmol}_e \text{ dm}^{-3}$) (Rodrigues et al., 2005).

Figure 1. Location (A) and climate variability (1996-2013) of the region (B) (KNMI, 2017).

The experiment with 3.5 ha was planted in June 1996 in a complete randomized block design with six treatments and four replicates (24 blocks of 400 m²; 7 rows; 13 plants each). Five internal lines/block (12 trees/line/block and 60 trees/replicate) were selected to avoid the edge effect. Each treatment consisted of six different doses of CPMS applied as input for the chemical soil preparation (Table 1). After the establishment of *P. taeda* trees, a mortality index of 1.1 % (19 trees) was registered. Then, a selective thinning of 54.5% of the stand density (940 smaller trees) was conducted in the 9th year (June 2005). The final harvest was carried out in the 17th year (December 2013) (Table 1).

Table 1. CPMS treatments and 17 years-old *P. taeda* characteristics.

Treatment (t ha ⁻¹)	Chemical element concentration (t ha ⁻¹)					Number of trees		Characteristics of 17-years <i>P. taeda</i> trees					
	N	P	K	Ca	Mg	1 st year	17 th year	DBH (cm)	Height (m)	Volume (m ³)	Wood basic density (g cm ⁻³)		
0 (control)	0	0	0	0	0	301	137	18.77 (0.87)	d	14.99 (1.29)	c	0.22 (0.029)	d
20	42	148	18	96	26	247	117	19.90 (0.64)	c	17.78 (0.94)	b	0.29 (0.031)	c
40	84	296	36	192	52	312	146	21.54 (0.66)	b	19.08 (1.05)	a	0.39 (0.017)	b
60	126	444	54	288	78	306	143	21.87 (0.47)	b	19.76 (0.62)	a	0.39 (0.035)	b
80	168	592	72	384	104	298	132	23.25 (0.84)	a	20.54 (0.62)	a	0.50 (0.052)	a
100	210	740	90	480	130	296	126	21.94 (0.64)	b	18.38 (0.80)	ab	0.40 (0.026)	b

The nutrient concentration (N=2.1, P=7.4, K=0.9, Ca=4.8 and Mg=1.3 kg ha⁻¹) refers to the CPMS after two years of decomposition. Nutrient CPMS information described in Rodrigues et al. (2005) in a previous study at the same experimental area and forest plantation. Number of trees at the planting and harvest time, 1st and 17th year respectively. Pairwise comparisons (Tukey test): Treatments with different letters (a, b, c and d) indicate statistically significant differences at a confidence level of 0.05. Standard deviations are provided in brackets.

2.2.2. Wood sample collection and preparation

Sixty 17-year-old *Pinus taeda* trees (10 trees/treatment) classified as dominant were selected, based on the mean volume inventoried (Table 1). These trees were felled and wood cross sections (5 cm, thickness) were cut at

the base, 1.30 m (DBH), 25, 50, 75 and 100% (6 cm, commercial trunk height). The wood cross-sections were polished (sandpaper, 120-600 grains inch⁻²) and the tree-rings were delimited, their widths were measured and synchronized - radial and longitudinal trunk axis - to obtain the annual volume increment of trees (Courbet and Houllier, 2002). In the sequence, a diametrical band (1cm width, pith centered) was marked on all 60 wood discs cross sections (DBH, trunk position); the wood samples were cut, glued on wood supports and transversely cut (2 mm, thickness) in a parallel double circular saw equipment. The thin transverse wood slices were conditioned in a climatic chamber at 12% moisture (20°C, 60% RH, 24 hours) and radially scanned (pith-bark direction) in a collimated X-ray beam (80 µm, step) by X-ray densitometry equipment (Q'TRS-01X, Quintek Measurement System) to obtain the microdensity profile (Tomazello-Filho et al., 2008). Then, a quarter of each disc was cut and its wood basic density was determined by hydrostatic balance method (ABTCP, 1974).

2.2.3. Stem wood volume, density and biomass calculation

The volume increment (ΔV) of 60 trees trunk was obtained from the tree-ring thickness of all wood discs (radial-longitudinal axis) and by Equations (1), (2) and (3).

$$\Delta V = VT_t - VT_{t-1} \quad (1)$$

$$VT_t = \sum_{i=1}^n Vs_t \quad (2)$$

$$Vs = \frac{\pi}{40000} * \left[d_b * d_t + \frac{(d_b - d_t)^2}{2r+1} \right] * L \quad (3)$$

where: VT_t= volume inside bark (m³) in year t; and Vs_t= segment stem volume (m³) in year t; db and dt = diameter at the trunk base and top (cm), respectively (calculated based on the annual tree-ring thickness); L = segment stem length (m); r = taper factor defines the stem shape (from 0 to 1.5).

The weighted arithmetic mean wood basic density (WBD) of 17-year-old *P. taeda* trees was calculated by Equation (4) considering the basic density (Equations 5, 6) and the volume by Smalian's (Vts) (Formula 7).

$$WBD = \frac{\sum_{i=1}^n WBD_{adi} Vts_i}{\sum_{i=1}^n WBD_{adi}} \quad (4)$$

$$WBD_{ad} = \frac{WBD_{db} + WBD_{dt}}{2} \quad (5)$$

$$WBD_d = \frac{M_d}{M_s - M_i} \quad (6)$$

$$Vts = 0,00007854 \frac{d_b^2 + d_t^2}{2} * L \quad (7)$$

where: WBD_{ad}=mean wood basic density of the trunk segment (g cm⁻³); WBD_d= wood basic density of each quarter of disc at the trunk base base WBD_{db} and top WBD_{dt} ; Md= dry mass (g); Ms= saturated mass (g); Mi= immerse mass (g); db and dt = diameter at the trunk base and top (cm); L = segment stem length (m).

Biomass increment (ΔB) and accumulated (B) of each tree were obtained by the standard methods using the volume accumulation (VT) (Equation (2) and the weighted arithmetic mean wood basic density (WBD) (Equation (4) (from details see item 2.3). These were considered the observed biomass data (Picard et al., 2012) and calculated by Equations (8) and (9).

$$\Delta B = B_t - B_{t-1} \quad (8)$$

$$B_t = \sum_{i=1}^n VT_t * WBD \quad (9)$$

where: B_t = biomass inside bark (kg) in year t ; V_t = volume inside bark (m^3) in year t ; WBD = weighted arithmetic mean wood basic density by tree.

2.2.4. Testing biomass models

Two scenarios using allometric equations were tested to produce biomass estimative and to verify the potentiality of annual tree-ring independent variables.

In the 1st scenario [sc1: $\Delta B \sim f(\Delta V) * f(WBD)$] (Equation 10) the volume increment (ΔV) adjusted by the model propose by Bouriaud et al. (2015) (Equation 11) was multiplied by the calibrated wood basic density (WBD) (Equation 12). The WBD calibration consists of the regression between weighted arithmetic mean wood basic density (WBD) and the mean annual tree-ring density (WD) of 17-year-old *P. taeda* trees measured by X-ray densitometry (Hackenberg et al., 2015).

$$\Delta B = WBD . \Delta V \quad (10)$$

where,

$$\Delta V = a + b . DBH^c . TRW^d \quad (11)$$

$$WBD = a + b . WD \quad (12)$$

In the 2nd scenario [sc2: $B \sim f(DBH, MWD)$] accumulated tree trunk diameter (DBH) and annual mean wood density (MWD) from X-ray densitometry measurements were used to estimate the accumulated biomass by an adaptation of the model proposed by Navar, (2009) (Equation 13).

$$B = a . DBH^b . c^{MWD} \quad (13)$$

$$MWD = \frac{\sum_{t=1}^n WD_t}{t} \quad (14)$$

where WD_t = annual tree-ring density ($g\ cm^{-3}$) in year t .

Parameters a , b , c , and d for equations 11-13 were recalculated for *P. taeda* trees in both scenarios.

2.2.5. Chronologies and climate relationship

Annual increment data (TRW, WD and biomass) was cross-dated, independently of the CPMS treatment, and controlled by COFECHA program (Holmes, 1983). The non-climatic trends of tree growth of the measurement chronology series were removed by the ARSTAN program (Cook, 1985). The time series were detrended using a linear regression with 50% frequency-response with a cut-off at 5 years. Detrending was followed by transforming annual data to growth indexes centered on a mean of one, by dividing observed values by fitted values. Then, the chronologies of TRW, WD and biomass were correlated with total precipitation and monthly mean temperature from previous and current years (period 1997-2013) of Curitiba Meteorological Station (49°18'W, 25°24'S) (KNMI, 2017).

2.2.6. CPMS effects analysis

The effects of CPMS treatments were analyzed with respect to the *P. taeda* tree wood properties: WD, MWD, TRW and biomass. Also, the annual biomass increment profile and biomass accumulation curves for each CPMS treatment were evaluated.

2.2.7. Data and statistical analysis

The fitting and parameter recalculation analysis (see item 2.4) considered all *P. taeda* trees data, independently of CPMS treatments, and used annual tree-ring independent variables (DBH, TRW, WD; MWD) to produce biomass estimations. This process was performed using the Origin 2017 program. The fitting quality was tested applying the statistic parameters: coefficient of determination (r^2) and root mean square error (RMSE) and exploring the residual data plot. In the sequence, the two quality scenarios were tested for *P. taeda* biomass estimation by a regression with observed biomass data (see item 2.3).

The TRW, WD and biomass quality chronologies (see item 2.5) were tested applying statistics tools: the mean sensitivity (MS) and first-order autocorrelation (AR1), which measures tree-ring inter-annual variability and the association of annual tree-ring growth in two consecutive years, respectively (Schweingruber, 1996). Furthermore, the principal component analysis (PCA) of the correlation matrix was applied to explore the annual TRW, WD and biomass interaction trends. Also, significant correlation coefficient ($p < 0.05$) was applied in order to determine the relationship of three *P. taeda* chronologies and climate variables (period 1997-2013).

In addition, the repeated measurements ANOVA test was applied to detected annual WD (by X-ray densitometry), TRW and biomass differences between and within *P. taeda* trees growing under the effect of CPMS doses. Furthermore, a pairwise comparison (Tukey test) of trees MWD at different years was tested.

2.3. Results

2.3.1. Tree-ring wood density

The radial profile (bark to bark) of the wood density and the cross section of *P. taeda* tree corresponding to each CPMS treatment (obtained via digital X-ray equipment) presented distinct annual tree-rings due to their density (WD) and thickness (TRW) variability. In all CPMS-*P. taeda* first-years growth the tree-ring microdensity is lower and the width is higher, in relation to final years, characteristic of juvenile and adult wood of conifer trees. This radial profile indicates an increase up to 74% of WD and a 96% decrease of TRW and, also, a significant 134% increase of mean WD earlywood compared to latewood, of 0.38 and 0.93 g cm⁻³, respectively. A continued *P. taeda* tree trunk-diameter increase from the control up to CPMS 80 t ha⁻¹, and then a decrease of CPMS 100 t ha⁻¹, can also be observed (Figure 2).

In all CPMS treatments of *P. taeda* trees the annual TRW showed an increase from the 1st to the 3rd year followed by a significant drop to the 17th year with mean values of 1.65 to 0.25 cm, with differences in the early years among treatments. However trees WD decreases until the 3rd followed by a gradual increase up to the 17th year, from 0.47 to 0.82 g cm⁻³, with intra-annual variations. The mean WD related to the TRW profile presented a

tendency with a linear correlation of -0.77 ($t=-36.99$, $df=958$, $p<0.001$) for the trees of all CPMF treatments (Figure 3).

Figure 2. *P. taeda* tree-ring density profile by X-ray densitometry for the CPMS treatments.

Figure 3. Annual tree-ring width (A) and wood density (B) profile of *P. taeda* trees treated with five different CPMS-doses.

2.3.2. Wood biomass modeled

The Equations of both scenarios fit the data well, and in all cases well over 66% (r^2) of the observed dependent variable variation (ΔV , WBD and B) was explained by the independent variables (DBH, TRW, WD and MWD) (Table 2). The 1st scenario indicated as significant ($p < 0.01$) Equations 11 and 12 for all scaling parameters a, b, c, d and a, b, respectively; the root mean square error (RMSE) was 19 and 4% of the mean for Equations 11 and 12, respectively (Table 2). Also, the residual plot confirmed the models adequacy (Figure 4A; B). The 2nd scenario indicated that Equation 13 was highly significant ($p < 0.001$) for all parameters a, b, c. Furthermore, the root mean square error (RMSE) was 16% of the mean (Table 2) and the residual plot confirmed the model adequacy (Figure 4C).

Figure 4. Fitted models and residuals of *P. taeda* tree annual volume increment (A); regression between tree-ring density (TRWD) and wood basic density (WBD) (B) and biomass accumulation (C).

Table 2. Fit statistics and parameters for models to estimate biomass. Biomass increment (sc1) based on volume increment and wood basic density models; (sc2) accumulated biomass based on multivariate model using DBH and MWD. ANOVA P-values for parameters are provided in brackets.

Biomass function	Eq.	Model	Parameters	Fit statics	
				r ²	RMSE
(sc1) $\Delta B \sim f(\Delta V) * f(WBD)$	11	$\Delta V = a + b DBH^c TRW^d$	A	0.00152 (<0.01)	
			B	5.25E-05 (<0.001)	
			C	2.36628 (<0.001)	
			D	0.63378 (<0.001)	
(sc2) $B \sim f(DBH, MWD)$	12	$WBD = a + b WD$	A	0.12374 (<0.001)	
			B	0.52826 (<0.001)	
			A	0.00515 (<0.001)	
			B	3.1421 (<0.001)	
	13	$B = a DBH^b c^{MWD}$	A	0.00515 (<0.001)	
			C	3.22421 (<0.001)	

ΔV = volume increment; DBH=diameter; TRW= tree-ring width; WBD= wood basic density; WD, MWD= mean tree-ring density at 17-years *P. taeda* trees and annual mean wood density by X-ray; r²= coefficient of determination; RMSE= root mean square error. The models were fitted considering all trees data independent of CPMS treatments.

The coefficient of determination (r²) was more than 0.96 in both scenarios (sc1 and sc2) for the regression of estimated and observed biomass accumulation (Figure 5). The observed biomass was calculated using the annual values of the stem volume increment analysis and weighted arithmetic mean WBD for trees. The regression mean standard error was 15% (10.96 kg) and 16% (11.35 kg) of the mean for the sc1 and sc2, respectively. Furthermore, the accuracy for estimated values slightly decreases with higher accumulated biomass values (Figure 5)

Figure 5. Accumulated biomass estimations of *P. taeda* trees based on (sc1) modeled volume increment (ΔV) and modeled wood basic density (WBD) multiplication vs (sc2) multivariate model using trunk diameter (DBH) and annual mean wood density (MWD).

2.3.3. Chronologies and climate relationship

The ordination revealed interrelations of wood density (WD) raw values and biomass on one side (positive loadings); and on the other, tree-ring widths (TRW) (negative loadings) in first principal component (Figure 6A). This axis explain 72% of the total variance of the three variables (Biomass PC1= 0.65; TRW PC1= -0.96; WD PC1=0.89) (Figure 6B).

Figure 6. Ordination analyses described for *P. taeda* tree-ring width (TRW), tree-ring density (WD) and annual biomass values (A) and loading of variables correlation with PC1 (B). In B: values outside the dashed lines indicate significant correlation coefficients, $p < 0.05$.

The Similar trend of annual raw biomass, tree-ring width and density of all trees, independent of CPMF treatment, was confirmed for the high inter-correlation of the detrended series (Figure 7A) despite the three chronology lengths (16 years). The mean sensitivity of WD, TRW and biomass chronologies was 0.12, 0.25 and 0.27, respectively, showing a low (WD) to intermediate (TRW and biomass) of variability in response to the environment conditions. Residual chronology of all variables was chosen due to large 1st-order autocorrelation values (0.80, 0.68 and 0.96 for WD, TRW and biomass, respectively) observed before detrending the series. Also, negative Pearson correlation $r = -0.34$ ($p < 0.05$) between TRW and WD standardized chronologies was verified (Figure 7A) confirming the negative linear correlation between the raw values (Figure 3). While, a positive ($r = 0.49$, $p < 0.05$) and negative ($r = -0.28$, $p < 0.05$) Pearson correlation between biomass and TRW and WD was observed (Figure 3).

The relationship of the three chronologies with the current and previous year of 1997-2013 showed more influence of precipitation than temperature (Figure 7B, C). The precipitation of current and previous year showed a strong and positive correlation with TRW (pp: $r = 0.44$, pp-1: $r = 0.30$, $p < 0.05$) and strong negative correlation of WD with the current precipitation (pp: $r = -0.46$; $p < 0.05$) (Figure 7B). The temperature of the current year had a strong positive correlation with WD (T^o: $r = 0.30$; $p < 0.05$) (Figure 7C). Although TRW and WD presented significant correlation with climatic variables, no significant correlations for biomass were observed (Figure 7B, C).

Figure 7. *P. taeda* time series biomass, tree-ring width and wood density detrended (A) and correlation coefficients between chronologies and seasonal local climate for previous (t-1) and current (t) year for precipitation (in blue) (B) and mean temperature (in red) (C) in the 1997-2013 period. In A: Biomass chronology using observed increments “B”, and estimated increments “sc1” ($B \sim f(\Delta V) * f(WBD)$) and “sc2” ($B \sim f(DBH * MWD)$). Values in branch at the right of chronologies represent the inter-correlation of series. Annual biomass increment are in gray dots. In B and C: values outside the dashed lines indicate significant correlation coefficients, $p < 0.05$.

2.3.4. CPMS treatments effects

The repeated measurements ANOVA test (Table 3) shows significant difference of mean TRW, WD and biomass at one or more years of growth; although, only TRW and biomass were significantly different between CPMS treatments. The annual mean wood density (MWD) shows significant CPMS treatment differences up to their 6th year, and from the 1st to the 6th year growth period the mean maximum and minimum wood density decreased 30 and 10% after 0-100 and 0-80 t ha⁻¹ CPMS treatments, respectively (Figure 8).

Table 3. Repeated measurements ANOVA test for annual tree-ring width, wood density and biomass of 17 year-old-*P. taeda* trees.

Variable	Between trees (Treatment)	Within trees (Year)	Interaction (Year $\times$ Treatment)
	Prov>F		
TRW	<0.0001**	<0.0001**	0.0003**
WD	0.5244 ns	<0.0001**	0.68 ns
Biomass	<0.0001**	<0.0001**	0.0003**

Statistically significant differences at a confidence level of 0.05 is indicated by (*), confidence level of 0.01 by (**) and no statistically significant differences by (ns).

Figure 8. *P. taeda* trees mean annual wood density profile (MWD). Bottom right down, MWD pairwise comparison (Tukey test) at different years. Different letters (a,b,c) indicate statistically significant differences at a confidence level of 0.05.

During the 17-year growth *P. taeda* trees showed similar mean annual biomass trends between CPMS treatments, with the lowest and highest response values of 0.13-0.75 kg and 11.23-20.47 kg to 0 and 80 t ha⁻¹ CPMS, respectively (Figure 9A). Furthermore, increases up to 107% of biomass growth rate occur with 80 t ha⁻¹ CPMS and, in addition, these trees presented greater cumulative biomass than other CPMS doses. *P. taeda* trees at 17th years presented 224.1 kg cumulative biomass with CPMS 80 t ha⁻¹, 108% higher than the control treatment (Figure 9B).

Figure 9. *P. taeda* trees wood annual biomass increment (A) and biomass accumulation (B) with different CPMS treatments.

2.4. Discussion

2.4.1. Tree-ring wood density characteristics

The *P. taeda* annual tree-rings were clearly distinguishable in the radial wood density profile, due to differential X-ray attenuations in response to distinct features of their early and latewood densities of 0.38 and 0.93 g cm⁻³, respectively (increase of 143%) (Figure 2), similar to the reports in Daniels et al. (2002). Furthermore, the density variation of 0.47-0.82 g cm⁻³ (74%) from pith to bark at the DBH observed in Figure 2 and 3B are related to the increase from 0.40 to 0.70 g cm⁻³ (75%) for *P. taeda* trees reported in Daniels et al. (2002). Furthermore, they are slightly similar to the variation of 0.31-0.50 g cm⁻³ (58%) in wood basic density or 0.4-0.85 g cm⁻³ (112%) air-dry density observed in Melo (2015) and Mora and Schimleck (2009), respectively.

The high sensitivity and resolution of wood densitometric analysis were verified in several tree-species (e.g. Schweingruber et al., 1978; Tomazello-Filho et al., 2008; Bouriaud et al., 2015; Pompa-García and Venegas-González, 2016). It allows us to interpret, in the *P. taeda* trees analyzed, that early and late wood tracheids of juvenile wood present a greater width and less cell wall thickness than in the adult wood (Telewski et al., 1999; Dobner et al., 2018). Although microscopic anatomical analysis was not performed, the accuracy of this analysis is confirmed when comparing the intra and inter-annual tree-ring density variation and the corresponding wood digitalized cross section

X-ray (Figure 2). Thus, *P. taeda* trees presented representative characteristics of juvenile and mature wood; the first with larger width and lower density (up to the 8-10th year) followed by narrower width and denser tree-rings (Figure 2). A similar transition from juvenile to mature wood beginning in the 10th year was reported in Dobner et al., (2018).

In several tree species, like *Pinus taeda* (Love-Myers et al., 2009), *Picea abies* (Franceschini et al., 2013; Bouriaud et al., 2015), *Pinus thunbergii* (Taki et al., 2014) and *Pinus cooperi* (Pompa-García and Venegas-González, 2016), the tree-ring density increase with decreased ring width has been reported. Variations in WD related to TRW in our study presented a negative linear correlation of -0.75 of WD and TRW in *P. taeda* trees (Figure 3) and were similar to -0.77 observed in *Picea abies* (Bouriaud et al. 2015). The tree-ring annual resolution and the TRW and WD distinctness provide the annual biomass increment determination through their integration (Babst et al., 2014; Bouriaud et al., 2015; Pompa-García and Venegas-González, 2016), as was used in this study (item 3.2).

2.4.2. Biomass estimation

In the first scenario, the allometric Equation 11 (Table 2) was a satisfactory predictor of *P. taeda* volume increment (ΔV) since the total variation explained by the relationships was 88%. Also, the estimation was stronger for trees DBH $< \sim 20$ cm (Figure 4A) with a 19% quality prediction detected by an RMSE of 0.0043 m^3 (Table 2), similarly observed for *Picea abies* trees DBH $< \sim 30$ cm with a prediction uncertainty less than 10% (Bouriaud et al., 2015). This result is expected due to higher number of trees analyzed (60 compared to 22) with a greater dispersion of estimated values for trees with DBH > 20 cm. In addition, the calibrated WBD based on X-ray densitometry (Equation 12) presented good regression indicators explained by an $r^2 = 0.66$ and RMSE of 0.018 g cm^{-3} (4%) (Table 2). Although the coefficient of determination was less than that observed in other WBD estimations by non-destructive methods (Jones et al., 2005; Santos et al., 2012). It is possible that increases in the quantity of trees sampled at different ages cause greater r^2 and a decreasing of estimated values dispersion (Figure 4B). According to Henry et al. (2010) the wood density is a site-specific parameter with high accuracy in biomass calculations mainly if it represents all the trunk density (Hackenberg et al., 2015; Wassenberg et al., 2015). For this reason, the calibration between trees WBD (destructive method) and WD (X-ray densitometry) was performed.

On the other hand, in the second scenario Equation 13 (Table 2) was a satisfactory predictor of accumulated biomass since 96% of the total variation was explained by the relationships. The allometric equation to estimate *P. taeda* biomass was consistent with others that applied similar form functions with great results (Chave et al., 2005; Návar, 2009; Pompa-García and Venegas-González, 2016). Although the equations of other studies were performed for aboveground biomass estimation, the high significance ($p < 0.001$) for all parameters adjusted in this study confirmed the form function potential to estimate *P. taeda* biomass. Návar (2009), mentioned significances $p < 0.01$ for the statistical parameters of a similar biomass equation of *Pinus* species, and also observed that the stem biomass estimation line reflect higher variation in relation to the observed data, especially among large trees. Thus, the narrow range of measured diameters (5.2–32.6 cm) could explain smaller slopes in the estimation lines. This is confirmed in our results since the estimation was stronger for almost all tree diameters (DBH) (< 30 cm) (Figure 4C) with a 16% quality prediction detected by an RMSE of 11.38 Kg (Table 2).

Another *P. taeda* biomass equation reported (e.g. Zianis and Seura, 2005; FAO, 2013) considered the tree DBH the main calculation variable. At this point, for the first time we propose two scenarios using an allometric equation to estimate *P. taeda* biomass with the availability of width and wood density (Table 2) and with incorporation of wood basic density calibration to improve the biomass estimation. Both scenarios presented large

prediction intervals up to 96% (r^2) for the regression between the estimated and observed biomass data (Figure 5) similarly reported for *Pinus thunbergii* and *Picea abies* by Taki et al. (2014) and Bouriaud et al. (2015), respectively, which included wood density variation by X-ray densitometry methodology. Furthermore, the estimation errors at plot-level of 15% and 16% found for the sc1 and sc2, respectively (Figure 5), were similar to 10-20% observed by Bouriaud et al. (2015). Most of the prediction errors of *P. taeda* tree annual biomass increments come from the high between-tree variability of wood density and the local calibration is considered fundamental to reduce these errors (Bouriaud et al., 2015). Taki et al. (2014) cited, for *P. thunbergii* trees in Japan, an $\sim 8\%$ prediction, while Hackenberg et al. (2015) obtained prediction errors of $\sim 3\%$ for *P. massoniana* trees in China. Both these studies used less than 15 sampled trees.

2.4.3. Chronologies characteristics

The *P. taeda* WD and TRW inter-annual variability was lower than the biomass variability (Figure 6), as observed by Babst et al. (2014b). Also the WD ($r=-0.28$, $p<0.05$) and TRW ($r=0.49$, $p<0.05$) may exert opposite influence on biomass increment (Figure 6) according to raw value interrelations, similar to that observed by Babst et al. (2014b) and Pompa-García and Venegas-González (2016).

The tree-ring standardized chronologies evidenced good synchronized sequences for TRW, WD and biomass annual series (Figure 6), as observed by other authors, highlighting the application of X-ray densitometry methods to explore the WD variability and to contribute to annual biomass chronology constructions (Babst et al., 2014b; Bouriaud et al., 2015; Pompa-García and Venegas-González, 2016). Furthermore, the negative signal of standardized chronology correlations ($r=-0.34$, $p<0.05$) of *P. taeda* tree WD and TRW (Figure 7A) are related to the wood biology mechanism. Thus, there is greater cambium activity in terms of cambial division (larger TRW/lower WD tree-rings-juvenile wood) in earlier growth years followed by decreased cambium activity (smaller TRW/higher WD tree rings-mature wood) in the later years (Fritts et al., 1991; Ferreira and Tomazello Filho, 2009; Park and Spiecker, 2005; Franceschini et al., 2013).

The TRW and WD chronology trends variability are attributed to climatic factors (Briffa et al., 2002), and consequently also the biomass chronologies (Babst et al., 2014b). Although in the present study the chronologies were shorter (16 years), the annual tree growth parameters (TRW, WD and biomass) reacted according to climatic drivers (Figure 7B, C). The TRW (positive) and WD (negative correlation) are more related to annual precipitation variability (Figure 1, 7B). Other tropical and sub-tropical tree species – *Cedrella fissilis* in Mato Grosso state (Dünisch, 2005), tree species in Sao Paulo state (Lisi et al., 2008), *Araucaria angustifolia* in Parana state (Lorensi and Prestes, 2015) - presented similar TRW- September-March precipitation growth response. The WD-negative precipitation and WD-positive correlation temperature could be explained because high temperatures can contribute to latewood cell wall thickening, thus producing denser latewood (Fan et al., 2009). Meanwhile, precipitation in the growing season influences the number and size of earlywood cells, that often comprise the majority of annual tree-ring growth (Kerhoulas et al., 2013). On the other hand, the non-significant correlations between biomass variation and climate variables could be explained by the compensation between TRW and WD responses to climatic variables (Babst et al., 2014b), however, it is possible that our short analysis period has also influenced this result.

2.4.4. CPMS treatments, tree-growth and wood effects

Silvicultural treatments (e.g. fertilization) promote tree growth changes and therefore changes in wood density (Moreno-Fernández et al., 2018). Also, the management treatments affecting tree species growth (diameter, height and volume) may not significantly influence their wood density, especially when the mean wood density/tree species is compared (Table 1) (Zobel and van Buijtenen, 2012; Antony et al., 2015). In contrast, the X-ray densitometry allows to explore in detail the wood density radial variability profile as well as to monitor the trees growth in response to silvicultural treatments (Mörling, 2002; Moreno-Fernández et al., 2018). The WD differences for the 17-year-old trees were more significant between years than between CPMS doses, unlike TRW and biomass show differences between years and treatments (Table 3). These results are expected considering that the effect of fertilization treatments in conifer tree species generates changes in the mean WD usually over a 5-year period (Cown and McConchie, 1981; Mörling, 2002). Antony et al. (2015) reported a wood basic density decrease of *P. taeda* annual tree-rings after 3 to 4 years of fertilization, similar to the CPMS-6 year soil application in this present study (Figure 8). After this period, no significant CPMS effect (6%, $p > 0.05$) was observed (Figure 8) consistent to less than 5% reported for 16-year-old *Pinus radiata* (Cown and McConchie, 1981), 12-year-old *P. sylvestris* (Mörling, 2002) and 12-year-old *P. taeda* (Antony et al., 2015).

Also, the significant response ($p < 0.05$) observed in the 1st to 6th year-*P. taeda* maximum (30%) and minimum (10%) WD decrease after CPMS application (Figure 8) corroborated the reported 4% decrease ($p = 0.0055$) for *P. taeda* trees fertilized (Love-Myers et al. 2009); and 20 and 12 % for base and top of *P. radiata* trunk, one year after fertilization (Cown and McConchie, 1981). Among conifer tree species the wood density decrease due to fertilization effect is generally the result of a radial growth increase (or TRW increase) (Valinger et al., 2000; Love-Myers et al., 2009; Zobel and van Buijtenen, 2012) most pronounced in the tree trunk bases (Cown and McConchie, 1981). Also, volume and biomass increases are expected (Cown and McConchie, 1981; Valinger et al., 2000). Love-Myers et al. (2009) states that a *P. taeda* and *P. elliottii* tree-ring WD decrease, post-fertilization, is results from thin xylem cell wall thickness and larger lumen due to the effect of auxin hormones on meristem cambium produced during sprouting and in renewed foliar area (Larson et al., 2001). Albaugh et al. (1998) verified a leaf area index (LAI) increase in *P. taeda* trees up to four years after N and P fertilization with a corresponding increase in aboveground biomass and a decrease in root biomass. These references support the results of *P. taeda* tree annual growth and cumulative biomass increases of 107 and 108% respectively, in response to CPMS soil application (Figure 9).

The *P. taeda* trees receiving 100 t ha⁻¹ CPMS compared with 80 t ha⁻¹ CPMS, presented lower mean trunk diameter-height (resulting in decrease volume-biomass) and higher wood density after the 4th growth year (Table 1, Figure 8, 9). This growth behavior can be explained by higher soil nutrient availability from CPMS fertilizer, stimulating an effective root system absorption and resulting in initial greater trunk diameter during first years (Valinger et al., 2000). The initial intensive nutrient uptake by the root system implies a soil availability reduction, resulting in another tree development period, characterized by a biomass decrease (Ingstad and Agren, 1991) and, also, affecting their wood density.

In the 7-year-old-*Pinus taeda* trees (Rodrigues, 2004) in the experimental area the organic matter content, pH, cation exchange capacity and soil base saturation levels were already significantly higher with 80 t ha⁻¹ than with 100 t ha⁻¹ CPMS. Possibly, due the 100 t ha⁻¹ nutrient cycling between the soil and trees having accelerated in the earliest years after CPMS application. Thus, to maintain tree-growth increases with 100 t ha⁻¹ CPMS treatment, re-fertilization would have been necessary (Barclay and Brix, 1985).

2.5. Conclusion

The radial wood density profile by X-ray densitometry provide a good approach to biomass calculation with more time sensitivity compared to unique-uniform wood density applications. This was confirmed with good accuracy of two scenarios applied to estimate the *P. taeda* biomass increment using allometric equations based on tree-ring independent variables (DBH, TRW, WD, MWD) to fit the observed data. The raw annual biomass variance was interrelated with tree-ring density and width as a positive and negative loading, respectively. High biomass, TRW and WD inter-correlation series confirm the environment effect on *P. taeda* trees growth, independent of the CPMS treatment. Furthermore, the precipitation was a principal climate variable related to TRW and WD with positive and negative correlation, respectively. Although no significant correlations for biomass were detected. The soil CPMS treatments significantly affected the *P. taeda* growth up to the 6th year after their application. The mean annual biomass trends along the 17-year-old *P. taeda* trees were significantly different in one or more years and CPMS treatments, and the cumulative biomass curves confirmed that 80 t ha⁻¹ CPMS induced a higher effect on *P. taeda* tree growth. Therefore, this work values the intra-annual wood density variability application to improve *P. taeda* tree growth monitoring in southern Brazil, with accuracy in estimating biomass and to understand the tree responses to climate and management treatment (such as CPMS fertilization).

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3. EFFECT OF PULP AND PAPER MILL SLUDGE ON THE DEVELOPMENT OF 17-YEAR-OLD LOBLOLLY PINE (*Pinus taeda* L.) TREES IN SOUTHERN BRAZIL*

Abstract

Pinus taeda is a principal conifer tree species and has long been used in forest plantations in Southern Brazil. At the same time, experiments have been carried out in order to determine the wood productivity concerning the economic, social and sustainable forest management practices. In that sense, the organic fertilizers, such as the biosolid residues from pulp and paper industries, are one of the alternatives to achieve this sustainability. Nevertheless, few studies addressing this objective have been reported in Brazil. This study aimed to analyze the growth responses of loblolly pine trees treated with different doses of composted pulp-mill sludge and to propose a management guide based on a wood production simulation. A randomized block design with four replicates for every six treatments - control, 20, 40, 60, 80 and 100 t ha⁻¹ of composted pulp-mill sludge - were carried out in an experimental plantation located in Arapoti, Paraná state, Brazil. Ten 17 year-old-trees for each treatment were selected, the stem variables were analyzed and the stand wood productivity for the optimal dosage was simulated. The trees were significantly influenced by the application of the composted pulp-mill sludge, increasing up to 24, 37 and 127% in stem diameter, height and volume, respectively, and the rotation of the pine plantation control and treated stands was 33 and 21 years, respectively. The forest management applied for a 21-year harvesting cycle (thinning at 7 and 13 years) with 84 t ha⁻¹ of composted pulp-mill sludge resulted in a wood productivity of 11.9 m³ ha⁻¹ or 5.04 Mg ha⁻¹ per year. The results confirm the potential application of pulp-mill sludge as a fertilizer to improve the wood productivity of forest plantations established in poor-nutrient sites. Also, the forest management plans, using the guide proposed, could be replicated in loblolly pine and other tree species plantations according to the end use of wood.

Keywords: Loblolly pine; Biosolid; Organic fertilizer; Management guide; Wood productivity

3.1. Introduction

In Southern Brazil, Paraná and Santa Catarina states have 76.6% of the pine plantations and contribute with 22% of the commercial timber from national forest plantations (IBA, 2016). *Pinus taeda* is the most planted species (Vasques et al., 2007) due to its higher growth rates (35 m³ ha⁻¹ year⁻¹; 18 year rotation) and economic importance represented by an internal return of 24% (Cubbage et al., 2007). Consequently, different studies to understand the effects of management practices on the development and productivity of *P. taeda* stands have been performed in this region (e.g. Bognola et al., 2010; IPEF, 2012; Cardoso et al., 2013; Kohler et al., 2015).

One of the management practices directly related to the wood productivity is fertilization (Will et al., 2002). This can be expensive due to the cost of inorganic fertilizers (Vance, 2000). Thus, the non-conventional fertilizers, such as mill residues, are an alternative to reduce the costs of this activity (Vance, 2000; Faria et al., 2015b; Ferraz et al., 2016).

Examples of these mill residues considered environmentally and economically important are the pulp and paper-mill biosolids that reach up to 48 tons of residues per 100 tons of cellulose (Bellote et al., 1998; Arruda et al., 2011). However, the high C:N ratio of pulp and paper-mill biosolids is a major limitation for their application in soils (Vance, 2000). Potential uses of pulp and paper-mill biosolids to improve the soil physical and chemical properties, especially the degraded soils, have been reported (Brockway, 1983; Bockheim et al., 1988; Bellote et al., 1998; Jackson et al., 2000; Faria et al., 2015ab; Corbel et al., 2016), principally, after practices to reduce the C:N ratio, such as composting and pre-conditioning (Cabral et al., 1998).

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Several studies have reported that pulp and paper-mill sludge (PMS) may benefit nutrient-limited forest plantations (Young et al., 1993; Henry et al., 1994; Feldkirchner et al., 2003). Furthermore, forest plantations are an attractive alternative for the paper companies for recycling pulp and paper-mill biosolids (Feldkirchner et al., 2003). This is supported by successful studies of forest companies in the United States demonstrating that fertilizer reduced costs and increased forest productivity (Vance, 2000). However, no consistent results for Brazil were found in the bibliography reporting the *P. taeda* tree growth responses to PMS application, including complete rotation and with a forestry management approach. Furthermore, it is necessary to contribute with examples of forest plantations under these residues that attend the national environmental regulations, such as the Brazilian Environmental National Council Resolution 481 (BRAZIL, 2017). On the other hand, there is information available on the growth response of *P. taeda* trees to nutrients incorporated in the soil with fertilizer treatments as an intensive forest management practice (Albaugh et al., 2004; Samuelson et al., 2004). Consequently, in this study, in addition to the references on PMS effects, other information about fertilization treatments in *P. taeda* plantations is discussed.

Thus, to contribute to decrease the mentioned gaps, we analyzed the development of *P. taeda* trees treated with doses of composted pulp-mill sludge as input for the soil chemical improvement, including the optimal dosage. In addition, a production simulation was performed to propose a management guide for *P. taeda* plantations using composted pulp-mill sludge.

3.2. Material and methods

3.2.1. Study area

The experimental *Pinus taeda* plantation was located on the Matarazzo Farm, Jaguariaíva, Paraná state, Brazil (24°15' S, 49°42' W; 872 m a.s.l.; slope 3-8 %) (Figure 1A). The area is characterized as having a subtropical moist mesothermic climate (Cfb) (Köppen classification); a mean annual rainfall of 1323 mm, with a dry season between July and August and a moderate temperature, the mean annual being 18.1°C (Figure 1B). The soil has a medium texture with less than 35% clay and more than 15% sand content, strongly drained and extremely acidic (pH <4.3) (Rodrigues et al., 2005), and it is classified as a dystrophic Red-Yellow Latosol (EMBRAPA, 2006).

Figure 1. Location (A) and climate variability (1996-2013) of the region (B) (KNMI, 2017).

3.2.2. Experiment design and treatments

An area of 3.5 ha of *Pinus taeda* planted at 3 x 2 m spacing was established in June 1996 in a complete randomized block design with six treatments and four replicates, for a total of 24 blocks (400 m²; seven rows; 13 plants each). Five internal lines/block (12 trees/line/block and 60 trees/replicate) were selected to avoid the edge effect. Each treatment consisted of different doses of composted pulp-mill sludge (CPMS) as input for the chemical soil preparation (Table 1). The CPMS corresponds to the thermochemical process disposal, the paper cleaning machines, discarded fibers and impurities (due to size heterogeneity) unsuitable for the production of cellulose and paper. The CPMS was composted for 2 years in the open air to reduce the high C:N ratio (initial 150:1 and final 9:1) and distributed on the soil surface at the planting period and incorporated into the soil with a rotary spade.

Table 1. Chemical element concentration of composted pulp-mill sludge for each treatment.

CPMS		Treatment (t ha ⁻¹)					
Chemical element	Concentration (g kg ⁻¹)	Control	20	40	60	80	100
		Concentration (kg ha ⁻¹)					
N	2.1	0	42	84	126	168	210
P	7.4	0	148	296	444	592	740
K	0.9	0	18	36	54	72	90
Ca	4.8	0	96	192	288	384	480
Mg	1.3	0	26	52	78	104	130

The nutrient concentration refers to the composted pulp-mill sludge after two years of decomposition, 9:1 C:N ratio. Table constructed based on the information described in Rodrigues et al. (2005) for a previous study at the same experimental area and forest plantation.

After the establishment of *P. taeda* trees, a mortality index of 1.1 % (19 trees) was registered and to reduce the competition the understory vegetation between the planting lines was cut in the 2nd year (July 1998). Then, a selective thinning of 53.4% of the stand density (940 smaller *P. taeda* trees) was conducted in the 9th year (June 2005), final stand density of 801 trees (Table 2). The final harvest was carried out in the 17th year (December 2013).

Table 2. Treatments of composted pulp-mill sludge doses, *P. taeda* trees before-after thinning and mortality.

Treatment (t ha ⁻¹)	Area of analysis (ha)	Number of trees		Mortality (%)
		Before thinning	After thinning	
0 (control)	0.2	301	137	0.2
20	0.16	247	117	0.4
40	0.2	312	146	0.2
60	0.2	306	143	0.1
80	0.2	298	132	0.1
100	0.2	296	126	0.1
Total	1.16	1760	801	1.1

3.2.3. Wood samples collection and preparation

Ten *P. taeda* trees classified as dominant and representative of each treatment (based on the mean wood volume inventoried) were selected; the number of *P. taeda* trees was established with a 95% confidence level ($Z\alpha = 1.96$) and with an acceptable limit of sampling error of 10% ($e = 0.1$) (Table 3). The *P. taeda* trees were felled and wood cross sections (5 cm thickness) were taken at the base, 1.30 m(DBH), 25, 50, 75 and 100% of the commercial trunk height (6 cm minimum diameter). The wood discs were dried and their cross sections polished with sandpaper (120-600 grains inch⁻²) to distinguish the early-latewood of the annual tree-ring boundaries by a stereoscopic microscope. In the sequence, the wood cross-sections were scanned, the area of the tree-ring boundaries was delimited and measured using the software Image Pro-plus v. 4.5 (0.001 mm accuracy).

Table 3. Sampling characteristics of *P. taeda* trees based on volume outside-bark from inventoried data (June 2013). Standard deviations are provided in brackets.

Treatment (t ha ⁻¹)	Inventoried data		n° sample trees (95%, e=0,1)	Sample data	
	N° trees	Volume (m3)		N° trees	Volume (m3)
0 (control)	137	0.31 (0.15)	8	10	0.31 (0.02)
20	117	0.41 (0.16)	9	10	0.40 (0.02)
40	146	0.51 (0.17)	11	10	0.50 (0.01)
60	143	0.52 (0.16)	10	10	0.52 (0.01)
80	132	0.62 (0.16)	10	10	0.61 (0.01)
100	126	0.53 (0.18)	11	10	0.53 (0.02)
Total	801	0.48 (0.20)	58	60	0.50 (0.09)

The number of sample trees was calculated by equation: $n = \frac{N \cdot SD^2 \cdot Z\alpha^2}{e^2(N-1) + SD^2 Z\alpha^2}$, where: n= n° sample trees; N= N° inventoried trees; SD= standard deviation; $Z\alpha$ = confidence level (95%); e= sampling error.

3.2.4. Stem analysis

The annual tree-rings of the wood discs were synchronized and cross-dated. After, the *P. taeda* tree stems were reconstructed by the thickness measurement of each annual tree-ring (radial-longitudinally) of all wood discs. In the sequence, the wood volume produced annually by each *P. taeda* tree was calculated by Equations (1) and (2) (Cancino, 2006). The stem diameter distribution, DBH growth curves, height and volume of *P. taeda* trees were also obtained for each treatment.

$$VT_t = \sum_{i=1}^n VS_t \quad (1)$$

where: VT_t = stem wood volume inside bark (m^3) in year t ; and VS_t = segment stem wood volume (m^3) in year t .

$$VS = \frac{\pi}{40000} * \left[d_b * d_t + \frac{(d_b - d_t)^2}{2r+1} \right] * L \quad (2)$$

where: db = diameter at the trunk base (cm); dt = diameter at the trunk top (cm); L = segment stem length (m); r = taper factor. The "r" value was calculated for each year as a parameter of the Equation (3) (Cancino, 2006). The fitting analysis for each treatment was tested for plotted data of radius width measured in each discs at different trunk heights.

$$Y = a (H - L)^r \quad (3)$$

where: Y = radius width in year t ; H = total trunk height in year t ; L = segment stem length in year t ; a = parameter that defines the rate of trunk radius increases for each unit of length increases; r = parameter that defines the stem shape formed (cylinder $r = 0$, paraboloid $r = 0.5$, cone $r = 1$, neiloid $r = 1.5$).

3.2.5. Stand analysis

Basal area and wood volume cumulative growth per tree of *P. taeda* for each treatment were fitted using the Richards nonlinear model (4) (Zeide, 1993) (Table 4).

$$GV_t = a(1 + (d - 1)e^{(-k(t-xc))})^{1/(1-d)} \quad (4)$$

where: GV_t = growth variable in year t ; GV = basal area (BA) ($cm^2 \cdot year^{-1}$), volume (V) ($m^3 \cdot year^{-1}$); a , xc , d and k = parameters.

Based on the modeled individual tree growth data, the current (CAI), mean (MAI) annual basal area increment and wood volume increment for each treatment of *P. taeda* trees were obtained by Equations (5) and (6).

$$CAI_GV_t = GV_t - GV_{t-1} \quad (5)$$

$$MAI_GV_t = GV_t / t \quad (6)$$

The biological rotation age (BRA) of *P. taeda* trees was estimated by intersecting the current and mean annual basal area and wood volume increment. Furthermore, other tree growth indicators were also interpreted using the growth increment curves.

3.2.6. Annual wood biomass production

Stem wood biomass (dry weight basis) was calculated from the wood apparent density of each annual tree-ring by X-ray densitometry equipment (QTRS-01X, Quintek Measurement System). DBH-radial wood samples (2 mm thickness) from each of ten *P. taeda* trees were cut, conditioned and scanned (pith-bark direction) in a collimated X-ray beam and the wood density profile and mean wood apparent density obtained (Tomazello-Filho et al., 2008). The regression Equation (7) of mean wood apparent density and the mean wood basic density of each 17-year- *P. taeda* tree was developed according to Hackenberg et al. (2015).

$$G_t = 0.1237 + 0.528 D12\%_t \quad (7)$$

where: G_t = mean wood basic density (g cm^{-3}) in year t ; and $D12\%_t$ = mean wood apparent density (g cm^{-3}) in year t .

Mean wood basic density of 17 year-*P. taeda*-trees was calculated according to Picard et al. (2012) using the wood discs cut at the tree trunk heights (see item 2.3.). With the value of mean wood basic density per year we determined the wood biomass per year of each tree by Equation (8) and scaled to hectare for each treatment of *P. taeda* trees.

$$B_t = \sum_{i=1}^{N-1} (G_t * VT_t) \quad (8)$$

where: B_t = wood biomass (kg) in year t ; G_t = wood basic density (g cm^{-3}) in year t ; VT_t = wood volume (m^3) in year t (from Equation 1).

Likewise, the current annual increment (CAI) of wood biomass per hectare was calculated by Equation (9).

$$CAI_Bh_t = 0.001(SD_{trees} \frac{m\%_t}{100} \frac{T\%_t}{100} (B_t - B_{t-1})) \quad (9)$$

where: CAI_Bh_t = wood biomass per hectare ($\text{Mg ha}^{-1} \text{ year}^{-1}$) in year t ; SD_{trees} = stand density (1.666 trees ha^{-1}); $m\%_t$ = % of stand density after mortality in year t ; $T\%_t$ = % of stand density after thinning in year t ; and B_t = wood biomass (kg) in year t .

3.2.7. Optimal dosage of CPMS and wood production simulation

A regression analysis was performed between CPMS treatment doses and, *P. taeda* trees volume and wood biomass, respectively. A quadratic equation was fitted and the parameters for the CPMS dosage model were calculated for the current and past ages of *P. taeda* trees. The CPMS of dosage curve of the *P. taeda* trees-past ages was fitted based on the cumulative growth data of volume and biomass per year obtained from the stem analysis. For the wood production simulation, the calculated tree wood volume and wood biomass per hectare data was used from the best dose of CPMS at all ages- *P. taeda* trees by readjusting the parameters of the Richards model (10).

$$PV_t = a(1 - (d - 1)e^{(-k(t-xc))})^{1/(1-d)} \quad (10)$$

where: PV_t = Production variable simulated from the CPMS best dose in year t ; PV = wood volume ($m^3 ha^{-1}$) (V_{bdt}), wood biomass ($Mg ha^{-1}$) (B_{bdt}).

Finally, the management guide of *P. taeda* trees was proposed, based on the best treatment of CPMS and on forest management practices applied in *P. taeda* plantations in southern Brazil (Cardoso et al., 2013), using, as parameters, an initial density ($1.666 trees ha^{-1}$); mortality (2,6 %), 7 year-first thinning (45 %); 13 year-second thinning (55 %); final density of $400 trees ha^{-1}$.

3.2.8. Statistical analysis

The analysis of variance (ANOVA) and pairwise comparison (Tukey test, $p=0.05$) were applied to verify the statistical differences among the means of DBH, height and wood volume cumulative growth of the 17 year-*P. taeda* trees from treatments at the end of the rotation. The statistical and fitting analysis in items 2.4, 2.5 and 2.7 were performed using the software Origin 2017.

3.3. Results

3.3.1. DBH, height and volume of stem *P. taeda* trees

More than a half of the stem diameter frequencies of *P. taeda* trees of all the CPMS treatments show a displaced distribution for diameters ≥ 25 cm, except for the treatment with $20 t ha^{-1}$ of CPMS. While in the control treatment, more than 50% of the stem diameter frequencies were ≤ 25 cm (Figure 2A). The cumulative DBH, height and wood volume curves of 17 year-old rotation of *P. taeda* trees show the growth response effect of the CPMS application (Figures 2B, C, D).

Figure 2. Stem diameter frequency (A) and cumulative DBH (B), height (C) and wood volume (D) of 17 year-old rotation of *P. taeda* trees treated with five different doses-CPMS.

The results of analysis of variance detected significant differences ($p < 0.05$) of 17 year-old *P. taeda* DBH, height and wood volume per tree showing increases of 6-24%, 19-37% and 31-127%, respectively, due to 5 different doses of CPMS-soil application. The 80 t ha⁻¹ CPMS treatment stood out among the others, and even higher in relation to the 100 t ha⁻¹ (Table 4).

Table 4. 17 year-old *P. taeda* trees stem DBH, height and wood volume in response to five different doses of CPMS-soil application. Standard deviations are provided in brackets.

Treatment	Stem DBH		Total height		Wood volume	
(t ha ⁻¹)	(cm)		(m)		(m ³)	
0	18.77 (0.87)	d	14.99 (1.29)	c	0.22 (0.029)	d
20	19.90 (0.64)	C	17.78 (0.94)	b	0.29 (0.031)	c
40	21.54 (0.66)	b	19.08 (1.05)	a	0.39 (0.017)	b
60	21.87 (0.47)	b	19.76 (0.62)	a	0.39 (0.035)	b
80	23.25 (0.84)	a	20.54 (0.62)	a	0.50 (0.052)	a
100	21.94 (0.64)	b	18.38 (0.80)	a b	0.40 (0.026)	b

Pairwise comparisons (Tukey test): Treatments with different letters (a, b, c and d) indicate statistically significant differences at a confidence level of 0.05.

3.3.2. Stand development

3.3.2.1. Stand characteristics

The *P. taeda* plantation shows differences of up 55% (10.54 m² ha⁻¹) and 122% (206.14 m³ ha⁻¹) in stand basal area and wood volume, respectively, comparing the control and 80 t ha⁻¹ of CPMS (Table 5, Figure 3). In relation to these 2 treatments, differences were also observed for the *P. taeda* growth parameters of mean annual increments of basal area and wood volume per hectare, up to 98 and 154%, respectively (Table 5, Figure 3).

Table 5. 17 year-old *P. taeda* plantation: stand basal area, wood volume, mean annual increments of basal area and wood volume in response to five different doses of CPMS-soil application. Standard deviations are provided in brackets.

	Treatments (t ha ⁻¹ of CPMS)					
	0	20	40	60	80	100
Stand Basal area (m ² ha ⁻¹)	21.06 (1.9)	22.68 (1.5)	25.03 (1.7)	27.50 (1.2)	31.60 (2.7)	27.63 (1.7)
Stand volume (m ³ ha ⁻¹)	168.95 (22.0)	219.93 (23.2)	293.38 (12.6)	293.84 (26.7)	375.09 (39.1)	293.84 (22.0)
MAI-BA (m ² ha ⁻¹ year ⁻¹)	1.27 (0.72)	1.51 (0.88)	1.93 (1.14)	1.94 (1.13)	2.52 (1.25)	2.21 (1.30)
MAI-V (m ³ ha ⁻¹ year ⁻¹)	6.98 (3.99)	9.70 (5.15)	12.48 (7.22)	13.10 (7.45)	17.78 (9.20)	13.10 (7.45)

Figure 3. 17 year-old *P. taeda* plantation: basal area (A) and wood volume per hectare (B) in response to five different doses of CPMS-soil application.

3.3.2.2. Current (CAI) and Mean (MAI) annual increment

The individual *P. taeda* tree Richards growth model (4) was significant ($p < 0.05$) for almost all parameters a , x_c , d and k (Table 6). Furthermore, the root mean square error (RMSE) of growth equations for basal area and volume was 8% (80 t ha⁻¹ CPMS) - 15% (and 20 t ha⁻¹ CPMS) and 11 (100 t ha⁻¹ CPMS) - 17% (0 t ha⁻¹ CPMS) of the mean, respectively.

The Biological Rotation Age (BRA) of the *P. taeda* trees, represented by the intersection of Current (CAI) and Mean (MAI) curves, showed a variation of 13-10 years for basal area per tree and 33-21 years for wood volume per tree (Figure 4). The 80 t ha⁻¹ CPMS induced a decrease of the BRA of *P. taeda* trees in relation to the other treatments, contrasting with the BRA of the control treatment (Figure 4E). Although the CAI and MAI intersection curves of the basal area and wood volume per tree of *P. taeda* of 80, 60 and 100 t ha⁻¹ of CPMS were reached at a similar age of 21 years, the highest wood growth increment was obtained for 80 t ha⁻¹ CPMS (Figure 4D, E, F). This treatment of 80 t ha⁻¹ CPMS showed an increase of 9 and 30% of the basal area (CAI-BA) and wood volume (CAI-V), respectively, compared to 60 and 100 t ha⁻¹ CPMS.

Figure 4. Current (CAI) and Mean (MAI) annual basal area and wood volume increments of *P. taeda* trees: control (A), 20 (B), 40 (C), 60 (D), 80 (E) and 100 t ha⁻¹ (F) CPMF soil treatments

Table 6. Fit statistics and growth parameters of basal area and volume model per tree of *P. taeda* for each treatment. ANOVA P-values for parameters are provided in brackets.

Growth variable	Treatment (t ha ⁻¹)	Parameters				RMSE	Adj. R ²
		a	xc	d	k		
Basal area (cm)	0	424.54 (<0.001)	7.11 (<0.001)	0.399 (0.1)	0.244 (0.02)	20.00	0.95
	20	732.45 (0.04)	4.81 (0.06)	0.114 (0.05)	0.189 (0.02)	23.80	0.94
	40	567.57 (<0.001)	4.30 (<0.001)	0.174 (0.02)	0.141 (<0.01)	21.32	0.97
	60	517.72 (<0.001)	4.82 (<0.001)	0.246 (0.01)	0.184 (<0.01)	24.54	0.95
	80	651.87 (<0.001)	4.65 (<0.001)	0.227 (0.04)	0.119 (<0.001)	18.40	0.98
	100	508.50 (<0.001)	3.79 (<0.001)	0.185 (0.02)	0.160 (<0.001)	24.85	0.96
Volume (m ³)	0	1.12 (0.05)	20.26 (0.01)	0.551 (0.04)	0.042 (0.05)	0.014	0.96
	20	1.01 (0.02)	16.54 (0.01)	0.597 (0.02)	0.057 (0.03)	0.018	0.97
	40	1.24 (0.07)	15.16 (<0.01)	0.554 (<0.001)	0.057 (<0.01)	0.019	0.98
	60	0.92 (<0.01)	12.58 (<0.001)	0.580 (<0.001)	0.575 (0.04)	0.018	0.98
	80	0.86 (<0.001)	11.60 (<0.001)	0.818 (<0.001)	0.124 (<0.01)	0.024	0.98
	100	1.08 (0.02)	13.33 (<0.001)	0.541 (<0.01)	0.063 (0.06)	0.018	0.98

GV_t: growth variable in year t. RMSE: root mean square error for Basal area (cm²) and Volume (m³).

The individual *P. taeda* tree growth models show significant differences in the management indicators among the CPMF treatments (Table 7). The control treatment reached the maximum basal area increment per tree (25.64 cm²) at 8 years and the maximum volume increment per tree (0.022 m³) at 19 years, and a DBH of 19.75 cm at the biological rotation age of 33 years. On the other hand, the *P. taeda* trees with 80 t ha⁻¹ CPMS were characterized as the best management treatment, with maximum basal area increment per tree (37.91 cm²) and maximum wood volume increment per tree (0.043 m³) reached at 7, 13 years, respectively. Furthermore, there was a stem DBH of 23.85 cm at the biological rotation age of 21 years (Table 7).

Table 7. *P. taeda* trees management indicators in response to five different doses of CPMS, with data modeled by growth equation (Table 6) and CAI and MAI (Equations 5, 6; item 2.5).

	CPMS doses (t ha ⁻¹)					
	0	20	40	60	80	100
Max. CAI-BA (cm ² year ⁻¹)	25.64	26.26	34.16	35.31	37.91	37.26
Age of max. CAI-BA (year)	8	8	7	7	7	6
Max. CAI-V (m ³ year ⁻¹)	0.0220	0.0264	0.0325	0.0338	0.0423	0.0332
Age of max. CAI-V (year)	19	16	15	13	13	13
Biological Rotation Age (BRA, year)	33	29	25	21	21	21
DBH at BRA (cm)	19.75	20.9	21.93	22.57	23.85	22

3.3.3. Stand productivity

3.3.3.1. Wood biomass per hectare

Stem wood biomass accumulation increases successively increased with the doses of CPMS, since treatment establishment (Figure 5A). Wood biomass increased up to 152% (170 Mg ha⁻¹) during the first 9 years before the thinning application. While in the 17th year, the total wood biomass increased up to 122% (207 Mg ha⁻¹). The most favorable CPMS treatment in stimulating the *P. taeda* wood production at the 9th (113.62 Mg ha⁻¹) and 17th (170.37 Mg ha⁻¹) consisted in the application of 80 t ha⁻¹ CPMS compared with control treatment at the same years (49.04 and 81.48 Mg ha⁻¹, respectively) (Figure 5A). Also, the CAI wood biomass of *P. taeda* trees increased up to 131% (7.18 Mg ha⁻¹ year⁻¹) until the 9th year-thinning application (Figure 5B) and up to 95% (7.36 Mg ha⁻¹ year⁻¹) between the 9-17th year (Figure 5C). The 80 t ha⁻¹ CPMS stands out in relation to the control, with 13.73 and 6.46 Mg ha⁻¹ year⁻¹, respectively (Figure 5B, C). The results also showed that the CAI decreased in all CPMS treatments when the basal area reach approximately 15 m² ha⁻¹, being more significant for the control treatment at 7 years (Figure 5B). While, after the thinning the CAI wood biomass curve decreased after the maximum value in the 15th year, except for 80 t ha⁻¹ CPMS in the 13th year (Figure 5C). The CAI wood biomass of *P. taeda* trees increases from the 10th year to the maximum value in all CPMS treatments, except for 80 t ha⁻¹ CPMS, where no significant increases, after thinning, were found.

Figure 5. Wood biomass per hectare in relation to tree age (A); relationship of CAI (Current Annual Increment) of wood biomass and basal area before (B) and after (C) thinning application of 17 year-*P. taeda* trees. Symbols represent years for each treatment.

3.3.3.2. Optimal CPMS dosage

The regression between the CPMS application and the mean wood volume and biomass of *P. taeda* trees shows good fit indicators with a quadratic equation (Figure 6). Significant regression coefficients ($p < 0.05$) and an acceptable residues distribution were also obtained. The optimal CPMS dosage, as input for the chemical preparation of soil, was 84 t ha^{-1} . However, 100 t ha^{-1} CPMS reduced the wood volume and biomass ($< 0.56 \text{ m}^3$ and $< 203.8 \text{ kg}$, respectively) of *P. taeda* trees (Figure 6).

Figure 6. Fitted curve and residuals for (A) wood volume and (B) biomass of 17 years-*P. taeda* trees and CPMS levels.

3.3.3.3. Wood production simulation

The production *P. taeda* tree per hectare model (10) was significant ($p < 0.01$) for all parameters a , xc , d and k (Table 8). Furthermore, the root mean square error (RMSE) of production equations was 1.5 and 1.3% of the mean for volume and biomass per hectare, respectively.

The *P. taeda* trees wood volume and biomass per hectare resulted from the proposed 21 year-plantation management guide were 441.4 m³ ha⁻¹ and 198.5 Mg ha⁻¹, respectively, and a site production capacity of 11.9 m³ ha⁻¹ and 5.04 Mg ha⁻¹ per year (Figure 7). *P. taeda* trees wood volume and biomass at final cutting were 223.4 m³ ha⁻¹ and 105.6 Mg ha⁻¹, including two partial productions of 71.4 m³ ha⁻¹ - 105.6 Mg ha⁻¹ and 146.6 m³ ha⁻¹ - 65.3 Mg ha⁻¹ at the 7th(45% stand density) and 13th(55% stand density) year-thinnings, respectively. Values calculated based on the best experimental conditions (item 2.7 and 3.3.2) and local management information (Cardoso et al., 2013).

Table 8. Fit statistics and parameters of volume and biomass production model of *P. taeda* trees per hectare based on the best experimental conditions. ANOVA P-values for parameters are provided in brackets.

Production variable	Parameters				RMSE	Adj. R ²
	A	xc	d	k		
Volume (m ³ ha ⁻¹)	0.878 (<0.01)	12.14 (<0.001)	0.704 (<0.01)	0.136 (<0.01)	0.003	0.99
Biomass (Mg ha ⁻¹)	408.79 (<0.001)	13.01 (<0.001)	0.797 (<0.001)	0.096 (<0.01)	1.021	0.99

RMSE: root mean square error for Volume (m³ ha⁻¹) and Biomass (Mg ha⁻¹).

Figure 7. *P. taeda* trees wood volume and biomass per hectare for the optimal dosage of 84 t ha⁻¹ CPMS modeled for a 21 year-cutting cycle (1.666 trees ha⁻¹ initial stand density; 2 thinnings at the 7th, and 13th year; 400 trees ha⁻¹ final stand density; 23.7 cm DBH-trees at cutting cycle).

3.4. Discussion

3.4.1. Stem growth

Pine tree growth stimuli have been reported in response to the soil application of mixed wood fiber (primary) and solid biological (secondary) sludge (Brockway, 1983; Bockheim et al., 1988; Jackson et al., 2000). Brockway (1983), reported an 11% increase of stem DBH of *P. resinosa* trees after the application of 16 t ha⁻¹ of paper-mill sludge. Also, Bockheim et al. (1988) reported that the application of 32 t ha⁻¹ of mixed primary and secondary sludge increased *P. resinosa* tree height and taper factor by 3%, although with no significance to the untreated stands. Jackson et al. (2000) described significant stem diameter increases of 40-60% of 3 year-*P. radiata* trees applying 20-60 t ha⁻¹ of primary pulp and paper mill sludge - as a nutrient-releasing mulch - compared to the control stand. Our results showed an increase of stem diameter, height and wood volume per tree for the 17 year-*P. taeda* of 24, 37 and 127%, respectively, by the 80 t ha⁻¹ CPMS application in the soil at planting time (Figure 2, Table 4). In addition, the stem diameter variation of *P. taeda* trees reflected the effect of the CPMS treatments, with an increase of the frequency towards the larger stem diameter class (Figure 2A) similar to that reported for 25 year- *P. taeda* trees in fertilizer treatments (Jokela et al., 2010).

Our results are similar to those described by Will et al. (2006) for the same 13 year old-tree species applying annual soil fertilization and reporting stem diameter and height increases of 33 and 30%, respectively. Other authors, namely Jokela et al. (2010), mentioned 49, 35 and 121% increases of *P. taeda* tree stem diameter, height, and wood volume due to macro and micronutrient fertilizers applied in the soil at the initial 10 year-plantation. Furthermore, it is expected that *P. taeda* trees should be more responsive to an intensive forest management regime (Borders et al., 2004) when the application of mineral nutrients was fractioned during the tree development stage (Albaugh et al., 2004; Samuelson et al., 2004) especially in soil with low natural nutrient levels (Alves et al., 2013; Cardoso et al., 2013).

3.4.2. Stand development

The acceleration of the stand development in forest plantations is dependent on the supply of natural or incorporated mineral nutrients to the soil (Will et al., 2002; Martin and Jokela, 2004; Albaugh et al., 2006). This similar effect was observed by the application of pulp-mill sludge as a nutrient supply in pine plantations (Brockway, 1983; Bockheim et al., 1988). Bockheim et al. (1988) reported, for *P. ponderosa* plantations, increases of $0.34 \text{ m}^2 \text{ ha}^{-1} \text{ year}^{-1}$ and $1.62 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ in basal area and wood volume, respectively, in stands treated with pulp-mill sludge application. In this study, increases of $1.25 \text{ m}^2 \text{ ha}^{-1} \text{ year}^{-1}$ and $10.8 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ for basal area and wood volume, respectively were founded (Table 5 and Figure 3).

According to Cardoso et al. (2013), stand basal area is a good indicator of the growth conditions of a study area and shows the ability of the stand to response to treatments. Bockheim et al. (1988) reported that the basal area per hectare of a *P. ponderosa* stand was up 10% higher with pulp-mill sludge compared with 55% registered in this study for the *P. taeda* stand of the 80 t ha^{-1} CPMS treatment (Table 5, Figure 3A). On the other hand, Albaugh et al. (2006) reported for fertilized 17 year-*P. taeda* stands that the basal area was $34.9 \text{ m}^2 \text{ ha}^{-1}$ compared with $19 \text{ m}^2 \text{ ha}^{-1}$ in control stands (density of $\sim 1115 \text{ trees ha}^{-1}$). While Will et al. (2002) reported a basal area, at 13 years, of $40.24 \text{ m}^2 \text{ ha}^{-1}$ in annual fertilized stands compared with $23.54 \text{ m}^2 \text{ ha}^{-1}$ in control stands (density of $1660 \text{ trees ha}^{-1}$). The results confirm that the *P. taeda*-stand (density of $1667 \text{ trees ha}^{-1}$) development with CPMS application was similar or even greater than the development with mineral fertilizers.

Similarly, treated stands progress more rapidly along the growth curve and reach specified growth points, such as maximum MAI, maximum CAI, and biological rotation age earlier than untreated stands (Martin and Jokela, 2004). Our results indicated that the maximum CAI and MAI in volume were reached up to 12 years earlier in the treated stands than in the control stand (Figure 4). This result is better than the difference of 5 years to reach the maximum CAI and MAI found between fertilized and control stands (density of $1495 \text{ trees ha}^{-1}$) reported by Martin and Jokela (2004). The site indexes in both studies are similar (based on the $\sim 4 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ MAI-V of the control stands), however the 7 years-difference would be related to higher stand density ($172 \text{ trees ha}^{-1}$) increasing the CPMS treatment effect. As states by Sullivan and Sullivan (2016) the high stand density ($1670 \text{ trees ha}^{-1}$) in relation to the medium ($1190 \text{ trees ha}^{-1}$) accelerate the forest succession process, which is more significant when fertilizers are applied.

Significant changes in the *P. taeda* tree-growth trends were also verified by the CPMS application. The CAI curves of the basal area and wood volume of the *P. taeda* stand treated with 80 t ha^{-1} CPMS declined rapidly after it reached its maximum peak at the 6th and 10th year, respectively (Figure 4E). A similar peak at the 8th year - *P. taeda* trees, followed by a CAI curve decline in fertilizer treatment, was described by Martin and Jokela (2004), probably associated with the onset of inter-tree competition. Furthermore, the rapid declination of the increment curves in treated stands accelerates the intersection of MAI and CAI growth curves named BRA - Biological Rotation Age (Jokela and Martin, 2000, 2004). Martin and Jokela (2004) observed that for *Pinus taeda* trees the biological rotation age of fertilized stands reached 5 years before compared with control treatment trees. In this sense, the BRA of *P. taeda* wood volume per tree with 80 t ha^{-1} CPMS was reached 12 years before in relation to control stand trees (Figure 4E, Table 7). The *P. taeda* trees development acceleration observed is not unexpected due to fertilizer treatments stimulus, improving the inherent soil site quality (Martin and Jokela, 2004). This also means that the stem growth of *P. taeda* trees treatment control never reach the magnitude achieved of *P. taeda* trees-CPMS treatments due to the stand dynamic alterations induced of the CPMS-treated sites (Albaugh et al., 2004). Also, the

increases of carrying site capacity are expected by increases of soil fertilizer treatments (Martin and Jokela, 2004), as observed in *P. taeda* trees with 80-100 t ha⁻¹ CPMS (Figure 5B, C). The significant soil nutrient supply alterations is characterized mainly by the apparent shifts of the structure and function of *P. taeda* trees stands, while partially by the stand development changes (Martin and Jokela, 2004).

On the other hand, the pine trees growth response due to fertilizer treatments at a given site could be improved by other environmental factors, like increase of water availability in growing season, management practices (e.g. thinning) and control of understory vegetation (Borders et al., 2004). In our study, the thinning application nine years after the CPMS application presented no significant effects on the stand development (Figure 5C). Henry et al. (1994) mentioned that the wood-biomass increment effects of thinning-treatments are improved by the subsequent application of pulp and paper biosolids.

3.4.3. Optimal dosage

The response of growth rates and wood biomass in seedlings and pine tree species due to the paper-mill biosolids application have been investigated by several authors (Brockway, 1983; Bockheim et al., 1988; Young et al., 1993; Henry et al., 1994; Jackson et al., 2000). Some reported no significant effects in the first year on aboveground tree biomass (Young et al., 1993; Feldkirchner et al., 2003). However, 21 month-*P. ponderosa* trees presented wood biomass increases of 20, 61 and 82% in response to 10, 20 and 40 t ha⁻¹ CPMS (Young et al. 1993). In our study, the increase of 122% was observed with 80 t ha⁻¹ CPMS for 17 year-*P. taeda* trees (Figure 5). Jokela and Martin (2000) state that intensive fertilization, also considering pulp-mill sludge, maintains higher wood biomass annual increments at any level of stand basal area, as observed in our study (Figure 5B, C). In contrast, Feldkirchner et al. (2003) mentioned non-significant wood-biomass increments for the application of 33 and 64 t ha⁻¹ pulp-mill sludge compared with 16 t ha⁻¹ and 32 t ha⁻¹ PMS applied in two different hardwood forests. According to Vance (2000), pulp and paper-mill residues are potential nutrient-soil enrichments and the N concentration is often the limiting factor. Vance (2000) also indicated that there is no *P. taeda* wood-production benefit by adding 390 kg N ha⁻¹. In our study, the *P. taeda* tree wood biomass was 16% greater for 80 t ha⁻¹ CPMS (168 kg N ha⁻¹) in relation to 100 t ha⁻¹ CPMS (210 kg N ha⁻¹) (Figure 6). Cabral et al. (1998) mentioned that the CPMS high C:N ratio (equal or greater than 150:1) is the major limitation to its soil application (surface or incorporated) due to N immobilization and mineralization required by the plants and the need to reduce the C:N. In the study, the CPMS decomposition, 2 years previous and its superficial soil incorporation significantly reduce the N concentration (C:N 9:1 ratio).

The chemical elements concentration with 100 t ha⁻¹ CPMS incorporated into the soil at planting time was greater than with 80 t ha⁻¹ CPMS (Table 1). However, the soil properties analysis in the 7th year of *Pinus taeda* trees (Rodrigues, 2004) indicated greater pH, organic matter and cation exchange capacity values with 80 than with 100 t ha⁻¹ CPMS, from 4.2 to 4, 22.5 to 15.1 g kg⁻¹ and 8.8 to 6.3 cmol_c dm⁻³, respectively. It is possible that with 100 t ha⁻¹ CPMS the improvement of soil properties and the nutrient uptake occurred in the first few years after the application of CPMS (Rodrigues, 2004; 2005). In that sense, is reported that soils enriched by the incorporation of CPMS tend to equate their fertility in the 2nd year of tree species forest plantation (Guerrini and Moro, 1994). Our results show similar and higher values of *P. taeda* trees CAI wood biomass (~8 Mg ha⁻¹ year⁻¹) with 80 and 100 t ha⁻¹ CPM compared to the other treatments maintained up to the 4th year of *P. taeda* trees growth (Figure 5B). Then, higher *P. taeda* trees CAI wood biomass (>2 Mg ha⁻¹ year⁻¹) with 80 t ha⁻¹ CPMS compared to 60-100 t ha⁻¹ CPMS was observed. It is reported that increased soil mineral fertilization results in stimulus of tree species early years, where

wood volumetric growth supported by the greater and temporary availability of soil nutrients (Valinger, 1993). However, the trees wood growth and crown leaf area decrease are a result of the exhaustion soil nutrients replenishment necessary for the forest plantations sustainability (Ingestad and Agren, 1991).

On the other hand, when the fertilizer treatments are applied in poor soil conditions, the nutrients added tend to promote the competition between seedling (trees) and understory vegetation (Jokela et al., 2010), these better adapted plant species to absorb nutrients and water (Young et al., 1993). Thus, the tree species seedlings strategy in the first year's growth is uptake nutrients (from fertilizers) and priority producing more foliar than root volume to compete with understory vegetation. However, if the soil chemical properties cannot maintain the nutrient demands, a shoot/root ratio trees imbalance will not maintain the biomass increment growth (Donoso et al., 2009).

Thus, the *P. taeda* trees with 100 t ha⁻¹ CPMS used efficient and rapidly the available nutrients soil incorporated and reacted to their scarcity at their 4th year, with wood growth reduction, demanding the nutrients repositioning in case of trees growth rate maintenance (Barclay and Brix, 1985).

3.4.4. Management guide

The proposed 21 year- *P. taeda* trees management rotation (Figure 7) is in line with that generally practiced for this tree species in the region for 20-year rotation (Scolforo et al., 2001; Cardoso et al., 2013; Kohler et al., 2015). Our guide was simulated with an initial density of 1666 trees ha⁻¹, according to the density of 1500-2000 trees suggested for sites with nutrient-poor soils (Cardoso et al., 2013). Also, Cardoso et al. (2013) and Kohler et al. (2015) mentioned that at 7-10 years the first thinning be conducted for initial tree-spacing of forest stands of less than 2000 trees ha⁻¹. The management guide proposed a first thinning at 7 years for *P. taeda*, based on the occurrence of maximum CAI-BA for the best condition (Figure 4E, Table 7). Kerr and Haufe (2011) consider that at the maximum CAI-BA, the plantation needs the first thinning to increase space and decrease competition between *P. taeda* trees. The 2nd thinning is suggested for 13 year-*P. taeda* trees based on the maximum CAI-V for the best condition (Figure 4E, Table 7) and Kohler et al. (2015) recommended thinning at 10-12 years for *P. taeda* trees for producing commercial wood with volume and quality in southern Brazil.

The expected production of a 21 year rotation- *P. taeda* plantation is 223.4 m³ ha⁻¹ or 105.6 Mg ha⁻¹ (Figure 7) is considered less compared to low-medium wood productivity (~335 m³ ha⁻¹ wood) reported by Cardoso et al. (2013) in the southeast region. Indeed, the area site index could be classified as of low productivity (21 year-*P. taeda* trees; MAI-V 21.01 m³ ha⁻¹ year⁻¹) compared to that reported by Kronka et al. (2005) (20 year-*P. taeda* trees; MAI-V 18-40 m³ ha⁻¹ year⁻¹). However, 21 year-*P. taeda* MAI-B of 9.45 Mg ha⁻¹ year⁻¹ is slightly high than 18 year-*P. taeda* MAI-B of 6 Mg ha⁻¹ year⁻¹ reported in fertilizer poor-nutrient soils in the USA (Martin and Jokela, 2004).

3.5. Conclusions

The CPMS-composted pulp-mill sludge is recommended as input to improve the nutrient-poor soil chemical properties destined for the implantation of *P. taeda* forest stands in southern Brazil. The results showed a positive and significant response of *P. taeda* trees, applying CPMS doses of 80 and 90 t ha⁻¹ increasing the wood volume per tree by 127% and reducing the plantation rotation age from 33 to 21 years. Also, the analysis enables the management of *P. taeda* tree plantations with CPMS application as a mineral input and soil conditioner and could be

used for potentially replicable commercial plantations. In this sense, we propose an initial density of 1666 trees ha⁻¹ (expected mortality of 1-3%), applying a first thinning, in the 7th year, of 45% and a second thinning in the 13th year of 55% of the stand density, leaving about 400 trees ha⁻¹ for final harvest. With the proposed management practice it is expected to obtain a *P. taeda* tree wood production year of 11.9 m³ ha⁻¹ or 5.04 Mg ha⁻¹ and in the 21st year a production of 223.4 m³ ha⁻¹ or 105.6 Mg ha⁻¹. Studies testing other tree spacings, ages, and thinning intensities, associated with the application of pulp-mill sludge is also suggested. Finally, it is expected that the results provide an efficient tool to understand the loblolly pine growth and management of sustainable forest plantations that use mill residues, and include environmental, economic and social purposes.

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4. NUTRIENT CONCENTRATIONS IN TREE-RINGS OF 17-YEAR-OLD-*PINUS TAEDA* UNRAVELED BY X-RAY FLUORESCENCE MICROANALYSIS

Abstract

Tree-rings register the nutrients seasonally taken up from the soil. X-ray fluorescence microanalysis (μ -XRF) can reveal the elemental distribution pattern along these rings. However, the report of quantitative μ -XRF methods targeted to wood analysis are scarce. This study aimed at analyzing Iron (Fe), Manganese (Mn), Calcium (Ca), Potassium (K), Sulfur (S) and Phosphorus (P) in annual tree-rings of wood cores cut from 24 trees of 17 year-old *Pinus taeda* planted in soil amended with six doses of composted pulp-mill sludge (CPMS). The nutrient concentrations were accessed using calibration curves built with spiked wood pellets. The analysis detected that Ca and Mn decrease in the pith-bark direction; K and S decrease from the pith up to 8-9th annual tree-ring and, then, increase to the bark and Fe e P presented no distinguished radial trends. Also, the precipitation showed a strong and positive correlation with Ca, S and low frequency interaction of Ca, Mn, Fe, P and S, K ($r > 0.25$, $p < 0.05$). The presence of CPMS increased the concentration of Ca, K, Fe and S and decreased Mn and P in the wood of *P. taeda*. Furthermore, the annual tree-ring ratios of Ca/Mn and K/Ca were good indicators of soil-pH and *P. taeda* trees wood formation. The μ -XRF methodology as non-destructive method of nutrient concentration analysis in tree-ring revealed potential uses in monitoring soil fertilizer treatments.

Keywords: Loblolly pine; Dendrochemistry; X-ray fluorescence microanalysis; Non-destructive method; Organic fertilizer

4.1. Introduction

Chemical elements are uptake and loaded in tree compartments comprising the foliage and bark with higher concentration compared to xylem, although this is the main contributor to the mass productivity (Rubilar et al., 2005; Garcia Villacorta et al., 2015). In the xylem, chemical elements are translocated by apoplastic and symplastic process (Meerts, 2002). This depends to the element nature, solubility and ionic radius ratio; and also to the tree xylem matrix, sap pH and sapwood-heartwood equilibrium; varying within and between species and site conditions (Cutter and Guyette, 1993; Peterson and Anderson, 1990; Smith and Shortle, 1996; Hevia et al., 2018). Thus, the annual tree-rings being a precise tool to detect and measure the element variability inside the xylem (Balouet et al., 2009).

The dendrochronology supported by tree-ring analysis from a significant number of species in different forest biomes of the world has been extensively applied to record environmental variables (Dobbertin and Grissino-Mayer, 2004). Dendrochemistry, a branch of dendrochronology, monitors the chemical composition of annual tree-rings yielding information on the soil and water chemistry (Watmough, 1997). For example, one could uncover environmental contamination of soil and groundwater (Balouet et al., 2009; 2012; Balouet and Chalot, 2015). In some cases, contaminants benefited growth trees due to the incorporation of nutrients in the soil and their absorption is detected in wood tissues (e.g. MaClauchlan et al., 1987; McClenahan et al., 1989; Vroblesky and Yanosky, 1990; Vroblesky et al., 1992; Kashuba-Hockenberry and DeWalle, 1994; Beauregard et al., 2010; Smith et al., 2014; Balouet and Chalot, 2015). Conversely, the application dendrochemistry intending to evaluate the effectiveness of fertilizer and nutritional treatments is less common (Watmough, 1997; Hevia et al., 2018).

The dendrochemical methodologies comprises the application of Atomic Absorption Spectroscopy (AAS) as a standard method to determine the trees-elements concentrations (Malavolta et al., 1989; Welz and Sperling,

1999). However, it is limited to smaller temporal precision and sensitivity to identify certain intra-annual element trends and analyze their mobilization inside the xylem (MaClauchlan et al., 1987). Actually, the X-rays methods have been applying to avoid this aspect and are able to monitoring the chemical elements with inter-annual sensitivity (Balouet et al., 2009). Several studies report the advantages of X-rays Fluorescence (XRF) to investigate the trace elements contained in tree-rings in relation to analytical techniques as Neutron Activation Analysis (NAA) or Proton-Induced X-rays Fluorescence (PIXE) (Maclauchlan et al., 1987; Vives et al., 2005; Scharnweber et al., 2016).

The main advantages offered by X-ray fluorescence microanalysis regards: i) the straightforward preparation, since sample grinding and digestion are not required the sample preserved; ii) high lateral spatial resolution, X-ray spot size varying from tens of micrometers to the millimeter range; iii) all chemical elements with atomic number > 11 sodium can be simultaneously evaluated; iv) the analysis does not requires gases or chemical reactants which makes it relatively cheap and certainly environmentally friend (Maclauchlan et al., 1987; Smith et al., 2008; Balouet et al., 2012; Hevia et al., 2018). A disadvantage of the technique lies on the limits of detection, typically few mg kg^{-1} for direct analysis, which are higher than those exhibited by destructive methods such as AAS in the range of tens of $\mu\text{g kg}^{-1}$ (MacDonald et al., 2011).

Thus, this study aimed to develop a non-destructive method using $\mu\text{-XRF}$ to quantify Iron (Fe), Manganese (Mn), Calcium (Ca), Potassium (K), Sulfur (S) and Phosphorus (P) in xylem of annual tree-rings of *P. taeda* planted in soil amended with six doses of composted pulp-mill sludge (CPMS). We also explored annual nutrients concentration linked to climate precipitation and temperature variables. Furthermore, the study also aims to provide understanding information regarding the effect of CPMS soil application on the radial nutrient concentration trends and Ca/Mn, K/Ca ratio variation.

4.2. Material and methods

4.2.1. Sample collection, preparation and measurements

17 years-old *Pinus taeda* plantation located on the Matarazzo Farm, Jaguariáiva, Paraná state ($50^{\circ}43'W$; $25^{\circ}15'S$) was selected. The area is characterized by a subtropical moist mesothermic climate (Cfb) as a Köppen classification. The mean annual rainfall is 1323 mm, with a dry season between July and August and the temperature is moderate, with an annual mean of 18.1°C . The soil is classified as dystrophic Red-Yellow Latossol, medium texture, less than 35% of clay, more than 15% of sand, strongly drained and extremely acidic ($\text{pH} < 4.3$), poor in macronutrients, low organic matter content and low cation exchange capacity (Table 1).

Twenty-four trees (4 trees/treatment) were selected from the plots of 6 CPMS soil treatments applied as fertilizer (0, 20, 40, 60, 80 and 100 t ha^{-1}) (100 t ha^{-1} of CPMS = N, P, K, Ca, Mg; 210, 740, 90, 480, 130 kg ha^{-1} , respectively) (Table 1). The selection criteria were based on higher tree-ring width chronology correlation with the master chronology of 60 trees previously analyzed ($r > 0.6$, $p < 0.05$) (according to Hevia et al., 2018). The trees were felled, wood disc cross sections cut at 1.30 m (DBH); a thin transverse radial wood sample (1.5 mm, thickness) cut in a parallel double circular saw and conditioned (60°C , 24 hours in a climatic chamber).

Table 1. Soil characteristics and composted pulp-mill sludge (CPMS) applied in the experimental *P. taeda* trees forest plantation.

		Parameter								
		pH	O.M.	Al	H+Al	Ca	Mg	Ca+Mg	K	P
			g kg ⁻¹	cmolc dm ⁻³						mg dm ⁻³
Soil										
	to a deep 0-20 cm	3.9	10.7	0.6	5.1	0.1	0.5	0.4	0.03	1
	to a deep 20-40 cm	4	14	0.4	4.4	0.1	0.5	0.2	0.02	1
		pH	O.M.	N	P	K	Ca	Mg	Na	C/N
			g kg ⁻¹							
CPMS		7.9	88.2	2.1	7.4	0.9	4.8	1.3	0.3	9/1

* Table constructed based on the information described in Rodrigues et al. (2005) for the same experimental area and forest plantation.

The wood slices were radially scanned (pith-bark direction) follow a line scan area (spot of 1 mm in diameter) in an X-ray fluorescence microanalysis spectrometer (μ -XRF), Orbis PC model, EDAX, Rh tube, silicon drift chamber detector - SDD detector (30 keV, 600 μ A), vacuum condition and 20 seconds/measurement point (Figure 1). The tree-ring chemistry profiles of trees were built with the X-ray transmission intensity values and were selected chemical elements (Ca, Fe, K, Mn, P, S) for dendrochemistry analysis.

Figure 1. Line scan area in pith-bark direction and chemistry profiles of one radial sample of *P. taeda*.

4.2.2. Quantitative analysis

The intensity counts of Ca, Fe, K, Mn, P and S was analyzed to distinguish the detected signal from the background with a reasonable certainty for a quantification analytical process. A concentration equal to 8.485 standard deviations of the blank was selected to decide whether an element is presented or not using the Equation 1 (Kadachi and Al-Eshaikh, 2012).

$$LQ = 8.485 \sqrt{\frac{BG}{s}} \quad (1)$$

where: LQ = limit of quantification (cps); BG = background; and s = time at each measurement point.

The elements detected with a LQ $\text{Ca} > 4.84$, $\text{Fe} > 10.96$, $\text{K} > 4.47$, $\text{Mn} > 10.02$, $\text{P} > 2.14$ and $\text{S} > 2.99$ cps were quantified using a multi-elemental standard solutions (Geraldo et al., 2014).

Intending to build the calibration curves wood powder were spiked with the elements of interest and pelletized. A quarter of each *P. taeda* DBH wood discs were dried (60 °C, air circulation), grounded (Retsch ZM200 mill, 0.2 mm mesh), five portion of wood powder of 0.5 g separated to apply pure elements solution, dried (60° C air circulation fan) and pressed (X-Press 3624B) manufacturing wood pellets ($\sim 0.93 \text{ g cm}^{-3}$, density) (Table 2). Five measurements per pellet were obtained by the μ -XRF spectrometer applying the same condition than wood samples, following the determination of the mean intensity counts and the linear equations for each element (Figure 2). The calibration equations allow to obtain the Ca, Fe, K, Mn, P and S content in the thin wood samples based on μ -XRF intensity counts due to the high value of Pearson correlation coefficients ($R^2 \geq 0.99$).

Table 2. *P. taeda* wood features and chemical elements applied to wood pellets manufacture

	Standard pellet (*)			Element content (ppm) (**)					
	Wood powder mass (g)	Pellet volume (cm ³)	Pellet density (g cm ⁻³)	Ca	Fe	K	Mn	P	S
P0	0.468	0.583	0.80	0	0	0	0	0	0
P1	0.476	0.498	0.96	250	25	250	50	175	175
P2	0.481	0.511	0.94	500	50	500	100	350	350
P3	0.478	0.490	0.97	750	75	750	150	525	525
P4	0.482	0.488	0.99	1000	100	1000	200	700	700

* Manufacturing condition: 1 ton press in 1.77 cm² area for 10 seconds. ** Ca: solution for AAS 1 mg/mL in 0.5 N (HNO₃), ACROS. Fe, Mn and P: solution 1000 mg/L, Spec Sol. K: solution 1 mg/mL K in 2% HNO₃, ACROS. S: solution 1015 µg/mL in H₂O, Aldrich.

Figure 2. Nutrient content and mean intensity counts in standard *P. taeda* wood pellet ($\sim 0.93 \text{ g cm}^{-3}$, density).

4.2.3. Tree-ring chemical analysis

Intensity counts of Ca, Fe, K, Mn, P and S detected in wood samples was transformed in concentration (g kg^{-1}) and quantified by the equations in Figure 2. Intensity counts that not reach the LQ was indicated as “zero” (chemical element not detected). The mean nutrient concentration of each annual tree-ring from the six CPMS treatments was determined with the detected points and the “zero values” and the radial time series of nutrients constructed.

4.2.4. Data and statistical analysis

The repeated measurements ANOVA test was applied to detected annual tree-ring nutrient concentration differences between and within *P. taeda* trees growing under the effect of soil CPMS doses. The principal component analysis (PCA) of the correlation matrix was applied to explore nutrients interaction trends and aggrupation by annual tree-ring data in specific growth periods. Tree-ring width (TRW) and wood density (WD) of the radial samples determined by the X-ray densitometry measurement (QTRS-01X, Quintek Measurement System) (Tomazello-Filho et al. 2008) were also included in the PCA.

The cross-dating quality of nutrient series for all trees (independent of the treatment) at high (element concentration) and low (first two principal components) frequency was also checked using the COFECHA program (Holmes, 1983). Fe and P were checked at low frequency because registered many of “zero values” in some tree-rings. The cross-dating evaluation was carried out fitting a flexible spline curve (50% frequency cut-off at 5 years) to the nutrient series at high and low frequency (Scharnweber et al., 2016; Hevia et al., 2018), obtaining a mean index curve. Also, significant correlation coefficient ($p < 0.05$) was applied in order to determine the relationship of nutrients, TRW and WD *P. taeda* chronologies and climate variables (period 1997-2013) from Curitiba Meteorological Station (49°18'W, 25°24'S) (KNMI, 2017).

Furthermore, a second PCA on the correlation matrix was applied to explore differences between CPMS treatments described by the variance of the scores of the PCs in the specific growth periods obtained by the first PCA. The difference between treatments applying the scores and the TRW mean was analyzed by ANOVA test. The molar ratios of Ca/Mn and K/Ca were also determined for the same propose. The regression of tree-ring nutrient ratios with TRW and WD was analyzed in order to compare the effects of CPMS treatments. Statistical computations were carried out using the software OriginLab 2017.

4.3. Results

4.3.1. Trace nutrients signal

The counts of Ca, K, Mn and S are distinguished by a signal from the background in all annual tree-ring (Figure 3, Table 3). While Fe and P are presented in specific annual tree-rings, formed in the youngest (near the pith) and oldest (near the bark) tree-rings, respectively. Despite Fe and P are not distinguished in all annual tree-rings, the element concentration trends in the radial profile are detected with reasonable certainty for applying a quantification analytical process.

Figure 3. Net intensity counts above (reasonable certainty of detection) and below (unreliable detection) the limit of quantification (LQ) for the nutrients in the annual tree-rings of DBH *P. taeda* trees samples with CPMS treatments.

Table 3. Detection and quantification limits of nutrients for *P. taeda* tree-rings by μ -XRF. Zi: atomic number.

Element (Z_i)	Ca (20)	Fe (26)	K (19)	Mn (25)	P (15)	S (16)
Limit of detection						
cps	1.61	3.65	1.49	3.34	0.71	0.99
g kg^{-1}	0.040	0.013	0.057	0.017	0.037	0.027
Limit of quantification						
cps	4.84	10.96	4.47	10.02	2.14	2.99
g kg^{-1}	0.12	0.04	0.17	0.05	0.11	0.08

4.3.2. Nutrient distribution along the annual tree-rings

The intra-annual time series of Ca and Mn show a decrease trend in radial direction in all treatments (Figure 4). K and S show a decrease trend from trend from the pith up to 8-9th annual tree-ring and, then, increase to the bark. No distinguished trends for Fe and P are observed.

Figure 4. Nutrients concentration series of *P. taeda* annual tree-rings for six CPMS treatments.

The repeated measurements ANOVA test (Table 4) shows significant difference of mean Ca, K, Mn, P and S concentration together with TRW and WD at one or more years of growth; although, only mean Ca and Mn concentrations together with TRW were significantly different between CPMS treatments. Mean Fe concentration was not significantly different between tree-rings or treatments. The analysis of variance, therefore, shows that the variability of nutrient concentrations is principally affected by the annual tree-ring formation.

Table 4. Repeated measurements ANOVA test for annual tree-ring nutrient concentration of 17 year-old-*P. taeda* trees under CPMS treatments.

Nutrients	Between trees (Treatment)	Within trees (Tree-ring)	Interaction (Treatment x Tree-ring)
	Prov>F		
Ca	0.0387*	<0.0001**	0.1181 ^{ns}
Fe	0.4946 ^{ns}	0.2458 ^{ns}	0.9670 ^{ns}
K	0.3324 ^{ns}	<0.0001**	0.1566 ^{ns}
Mn	0.0003**	<0.0001**	0.0003**
P	0.5135 ^{ns}	<0.0001**	0.1726 ^{ns}
S	0.4187 ^{ns}	<0.0001**	0.9676 ^{ns}
TRW	<0.0001**	<0.0001**	0.0003**
WD	0.5411 ^{ns}	<0.0001**	0.6621 ^{ns}

Statistically significant differences at a confidence level of 0.05 is indicate by (*), confidence level of 0.01 by (**) and not statistically significant differences by (ns).

4.3.3. Nutrients interaction in tree-rings of *P. taeda*

The ordination revealed interrelations of Ca, Fe, Mn and P together with tree-ring width (TRW) on one site (positive loadings); and on the other, tree-ring wood density (WD) (negative loadings) in the first principal component (Figure 5). This axis explains 72% of the total variance and the second axis additionally 17% of the variance captures the S and K concentration with a positive loading (Figure 5). The first PC is dominated by a steadily decreasing trend of inter-annual Ca, Fe, Mn, P concentration and TRW and WD in opposite trend (Ca PC1= 0.96; Fe PC1= 0.85; Mn PC1= 0.85; P PC1= 0.89; TRW PC1= 0.93; WD PC1= -0.95). While the second PC mainly carries the K and S concentration trends increasing in radial direction (K PC2= 0.62; S PC2= 0.88). Furthermore, two *P. taeda* trees growth periods (2nd-5th and 11th-17th years), related to the element concentration in tree-rings, are distinguished (Figure 5).

Figure 5. Ordination analyses described for *P. taeda* nutrients concentration, tree-ring width (TRW) and tree-ring density (WD). Interrelations of Ca, Fe, Mn, P, TRW and WD in PC1 and of K and S in PC2. Principal component scores are shown in black curves.

4.3.4. Nutrient chronologies and climate relationship

Nutrients-chronologies presented inter-correlation of 0.33 (PC2: S and K interaction)-0.41 (Ca), while TRW and WD chronologies an inter-correlation of 0.66 and 0.74, respectively (Figure 6A). All index patterns of nutrient-chronologies of *P. taeda* trees decrease at first-6 years (1998-2002). The mean sensitivity of Ca, S, K, Mn, TRW and WD chronologies was 0.13, 0.29, 0.3, 0.1, 0.22 and 0.1, respectively, showing a low (Ca, Mn and WD) to intermediate (K, Mn and TRW) of variability in response to the environment conditions. While, PC1 and PC2 chronologies show high sensibility to environment conditions, 0.71 and 1.11, respectively.

The precipitation presented higher correlation with nutrients and TRW than temperature (Figure 6B and C). Ca, S, PC1 (low frequency interaction of Ca, Mn, Fe and P) and PC2 (low frequency interaction of S and K) showed a strong and positive correlation with annual precipitation during the current year, while S, K, PC1 and TRW during the previous year (Figure 6B). No significant correlations were observed between chemical elements and temperature, however, WD showed a strong and positive correlation with annual mean temperature during the current year, while TRW showed significant negative correlations during the current year, (Figure 6C).

Figure 6. *P. taeda* annual nutrient concentration at high and low (principal component) frequency, tree-ring width and density cross-dating, and climate variables (A). Correlation coefficients calculated between chronologies and annual local climate variables precipitation (B) and temperature (C) for previous (t-1) and current (t) year in the period 1997–2014. In A: values in branch at the right of element-chronologies represent the inter-correlation and number of series (trees). In (B) and (C): values outside the dashed lines indicate significant correlation coefficients, $P < 0.05$.

4.3.5. CPMS fertilizer analysis

4.3.5.1. Tree-ring nutrient concentration and CPMS-treatments relationship

The ordination analysis in each growth period (2nd-5th and 11nd-17th years) shows differences between CPMS treatments described by the scores variance of the PCs, related to annual tree-ring nutrient concentrations (Figure 7A, B).

In the 2nd-5th years, based on the pairwise score comparisons in first axis (57% of the total variance), tree-rings Ca concentrations increase from control and 20 t ha⁻¹ to the others CPMS treatments; while Mn and P shows an opposite trend (Figure 7C). In the second axis (30% of the total variance), tree-rings Fe, K and S concentrations are higher in the treatment with 40 t ha⁻¹ (Figure 7E). While TRW was significant higher with 80 and 100 t ha⁻¹ CPMS ha⁻¹ compared to control and 20 t CPMS ha⁻¹ (Figure 7G).

In the 11nd-17th years, tree-ring Ca concentrations increase from control to the others CPMS treatments greater with 60 t ha⁻¹ CPMS; while Fe, K, Mn and P show an opposite trend in the first component (60% of the total variance). In the second component (20% of the total variance) tree-ring S concentration was higher with 40 t ha⁻¹ CPMS as a similar in the 2nd-5th years, (Figure 7F). While TRW was significant smaller with 100 t ha⁻¹ CPMS than other treatments (Figure 7G).

Figure 7. Ordination analyses of CPMS treatments (dots) described by tree-ring nutrients concentration series, and box plots with pairwise comparison of mean scores of PCs and mean TRW. In the 2nd-5th years (A, C, E, G), and 11nd-17th years (B, D, F, H) of *P. taeda* tree growth. Pairwise comparison (Tukey test): treatments with different letters (a, b and c) indicate statistically significant differences at a confidence level of 0.05.

4.3.5.2. Nutrient ratios comparison

The mean comparison of Ca/Mn and K/Ca ratios between treatments show significantly difference at more than one CPMS treatments (Figure 8). The CPMS applied as potential fertilizer increase the Ca/Mn, related to soil-pH improves, and decrease the K/Ca ratio, related to cambium activity, in tree-rings at different growth periods (Figure 8A, B). Significantly greater Ca/Mn values were obtained with 60 t ha⁻¹ CPMS; also the Ca/Mn ratio were higher between the 2nd and 5th year than between the 11th and 17th year, independently of the treatment; in contrast to the values within treatments and between growth periods observed for the K/Ca ratio (Figure 8A, B). In addition, increases in the Ca/Mn ratio in tree-rings are correlated with increases of the TRW and a decrease of the WD, which means that improvements in soil pH by CPMS produce wider and less dense rings. While increases in the K/Ca are correlated with, TRW decrease and WD increase, which means that CPMS increase the cell expansion (cambial osmotic potential) reducing the cell differentiation (Figure 8A, B) (Fromm, 2010).

Figure 8. Tree-ring nutrient ratios comparison between CPMS treatments at different growth periods, and ratios regression with TRW and WD for: Ca/Mn ratio, pH indicator (A) and K/Ca ratio, cambial activity indicator (B). Pairwise comparison (Tukey test): treatments with different letters (a, b and c) indicate statistically significant differences at a confidence level of 0.05.

4.4. Discussion

4.4.1. Chemistry detection by μ -XRF

The extraction of robust nutrient signals (Figure 3) are consistent to other studies using X-ray techniques (*e.g.* Injuk et al., 1987; Maclauchlan et al., 1987; Nagj et al., 1987; Peterson and Anderson, 1990; Vives et al., 2005; Pearson et al., 2009). Although, some mobile nutrients such as S and P present uncertainties reports or are not mentioned due to the post-depositional translocation (Nagj et al., 1987; Smith and Shortle, 1996; Barrelet et al., 2006). In this study, S and P signals were distinguished using a μ -XRF system (Figure 3 and Table 3), possible by the

effect of CPMS applications. A number of authors mentioned that S and P is rarely determined in the xylem, being detected when trees are fertilizing, as an evidences of pollution or by changes in soil acidity (Delwiche, 1983; Herschbach and Rennenberg, 2001; Fairchild et al., 2009; Hevia et al., 2018). Thus, some advantages to measure low energy X-ray as P ($Z_i=15$) and S ($Z_i=16$) and to reach low detection limits of these elements were reported with XRF spectrometers, principally since the detectors were improvement (Osmic et al., 2003). S and P detection using XRF spectrometers were reported by Fairchild et al. (2009) in a Sulfur fixation study in *Picea abies* (L.) Karst and *Abies alba* Miller and by Scharnweber et al. (2016) and Hevia et al. (2018), that study element-trends including S and P in tree-rings of *Pinus sylvestris* L. and *Pinus uncinata*.

The analytical μ -XRF technique offers relative element concentrations or count rates (Scharnweber et al., 2016). Thus, to nutrient quantifications in specific annual tree-ring, the determination of the limit of detection (LD) is necessary to demonstrate certainly detections (Thomsen et al., 2003; Balouet et al., 2009; Kadachi and Al-Eshaikh, 2012). In this study, the 20 s X-ray exposure and the 1 mm in diameter of beam size detected LD-values that decrease with increase Z_i (Table 3). K and S LD-values of 0.057 and 0.027 g kg⁻¹, respectively, were quite similar to 0.08 and 0.015 g kg⁻¹ described in Smith et al. (2008) for *Liriodendron tulipifera* samples. While, Ca, Fe, K, Mn and S LD-values of 0.00091, 0.00176, 0.0011, 0.0004 and 0.0064 g kg⁻¹, respectively, were significant different to the reports for *Pinus* sp. samples using 60 s X-ray exposure (Vives et al., 2005). According to Smith et al. (2008), the LD can be reduced by increasing the counting time or X-ray exposure and enlarge sample size.

Furthermore, in nutrient concentration analysis are generally agreed to initiate at a concentration equal to 10 standard deviations of the blank, called limit of quantification (LQ) that is 3-3.3 times the LD (Thomsen et al., 2003; Balouet and Chalot, 2015). At this point, few studies were found in bibliography reported similar control process or methodology for quantify nutrient concentrations in tree-rings using XRF techniques (Balouet and Chalot, 2015). Fairchild et al. (2009) describe a control process using S/O count ratios to analysis outlying points in S signals in *Picea abies* and *Abies alba* trees, although the study was performed for count rates.

Although, several laboratories are currently using and testing different equipment, analysis and interpretations for tree-ring chemical studies, not yet address an application of standard analytical method (Balouet et al., 2009; Balouet and Chalot, 2015). According to Smith and Shortle (1996) and Balouet and Chalot (2015), studies can carry out several steps to minimize the effect of the analytical techniques when are analyzed tree-ring chemical trends. For example, collect more than one sample for tree and more than one tree, take care whit the chemical contamination of the sample, at process of detection verify the effect of noises (LD), and consider to calculate the frequency of the net change in results analysis trough correlations between tree-ring series of elements. At this last point, different authors suggested apply traditional dendrochronoly analysis for cross-dating accuracy (e.g. Balouet et al., 2009; Scharnweber et al., 2016; Hevia et al., 2018). In this study, therefore, LD and LQ analysis, nutrient counts calibration and tree-ring-nutrient series cross-dating analysis were performed for nutrient quantification (Table 3; Figure 2; item 2.4).

4.4.2. Tree-ring-nutrients of *P. taeda*-trees

4.4.2.1. Intra-annual chemical element trends

The nutrients wood-concentrations in *P. taeda* trees (Table 4) indicate, as other studies (Balouet et al., 2009; Scharnweber et al., 2016), a nutrient radial variability by seasonal cambial activity of trees. Although, the

nutrients trend varies among the elements analyzed in single trees (Injuk et al., 1987; Nagj et al., 1987; Vives et al., 2005; Smith et al., 2014). Consistent radial trends of nutrients distribution is obtained when sampling trees is expanded (Peterson and Anderson, 1990; Scharnweber et al., 2016; Hevia et al., 2018). Ca, Mn, and Fe (raw values) and P (low frequency) common decrease trend in the pith-bark direction were observed (Figure 4 and 5). Similar to declining trend in Ca, Mn and Fe reported for other *Pinus* (Nagj et al., 1987; Vives et al., 2005; Scharnweber et al., 2016). Although, different to the not clear trends in Mn and Fe reported for *P. contorta*, *P. albicaulis* and *P. uncinata* (Peterson and Anderson, 1990; Hevia et al., 2018). While for P, reports for *P. sylvestris* and *P. uncinata* showed increasing trends in the pith-bark direction (Scharnweber et al., 2016; Hevia et al., 2018). Furthermore, not clear S and K trends, despite to the higher concentration close to the pith and to the bark, were observed (Figure 4 and 5), similar in other *Pinus* trees (Nagj et al., 1987; Vives et al., 2005). Another studies, however, report K trend increases in the pith-bark direction (Peterson and Anderson, 1990; Hevia et al., 2018).

Nutrients concentrations and trends into the tree vary according to their features and soil site conditions and even for same species inconsistent results can be reported (Meerts, 2002). After the soil nutrients absorption, however, these are usually distributed to the same plant compounds or structure and fulfills the same functions, regardless of the species (Robson and Pitman, 1983; Baligar et al., 2001). Ca is generally soil abundant and is a critical plant macronutrient; it is important in the formation of the middle lamella of cell walls and in the metabolism of the cell (Peterson and Anderson, 1990). Mn is involved in catalytic roles, in the Krebs cycle and other respiratory processes, nitrogen metabolism, and photosynthesis; also, affect the availability of Fe (ibid). Fe is the most required plant micronutrient, probably because their low solubility; it is also part of the catalytic site of important oxidation-reduction enzymes, and is involved in the chlorophyll synthesis (ibid). P is a structural component of many plant compounds, plays a critical role in energy metabolism and wood formation (ibid). K is important in the catalytic role, the osmotic process of maintaining the opening of the cambium and the transport of synthesized photosynthesis through the phloem (ibid). S is an essential macronutrient for the growth and development of the plant, present in a variety of forms in plant tissues and plays an important physiological role in regulating the redox state (Fairchild et al., 2009).

In the xylem tissues, nutrients mobility is related to the atomic mass and ion charge (Baligar et al., 2001). Ca, Mn and Fe are considered immobile elements, fixed in xylem cell walls and incorporated into the woody tissue, while P, K and S are mobile elements, easily translocated among different tissue types in trees (Peterson and Anderson, 1990; Baligar et al., 2001; Muhammad et al., 2017). Penninckx et al. (2001) observed in *Fagus sylvatica* trees decreases of less mobile nutrients and increases of mobile nutrients in pith-bark direction. Similar for Ca and Mn and slightly for Fe observed in *P. taeda* trees (Figures 3, 4). In xylem tissues, the decline mobile elements concentration with age are related to their declining binding capacities (Momoshima and Bondietti, 1990; Momoshima et al., 1995; Prohaska et al., 1998). For P, no trends were detected in the 10th-15th tree-ring, following a slightly nutrient increase in the last 2 tree-rings (Figures 3, 4). For K and S, a slight increase from the 10th tree-ring was observed (Figure 3, 4 and 5). The K, S and P increase in tree-rings close to the bark are attributed to the low xylem affinity for these nutrients and their internal leaching (Arp and Manasc, 1988). In addition, the translocation processes of these high mobility nutrients take place from older to younger tissues, creating an age trend (Lim and Cousens, 1986; Helmisaari and Siltala, 1989; Colin-Belgrand et al., 1996; Fairchild et al., 2009).

4.4.2.2. Dendrochemical approach and climate association

Representative master-series sites usually use 15–20 trees in dendrochronological studies; in contrast, dendrochemical series can be constructed with less samples (Peterson and Anderson, 1990; Martins et al., 1999; Balouet and Chalot, 2015; Scharnweber et al., 2016; Hevia et al., 2018). However, the previously verification of the inter-annual tree-ring width correlation of the sampled trees with the site chronology of more samples is necessary (Smith and Shortle, 1996; Scharnweber et al., 2016; Hevia et al., 2018). In this study, the nutrients chronologies of 17-year-old *P. taeda* trees were constructed with 16–24 series (trees) (Figure 6A), after the verification of the master tree-ring series of 60 trees. Thus, tree-ring nutrient inter-correlation of 0.33–0.41 were obtained, even though lower than TRW and WD inter-correlation of 0.66 and 0.74 respectively (Figure 6A). Poor detrending inter-annual nutrient-counts correlations between *Pinus sylvestris* trees (natural forest, ~80 years series) less than 0.3 and TRW (0.49) and WD (0.39) inter-correlation were reported in Scharnweber et al. (2016). However, the authors mention a common high signal trends among nutrient series considering the cambial age and not a calendar year aligned, although the environmental conditions cannot be linked at this level. In addition, for potential good proxy, possible environmental drivers should be separated from any age-related trend (Smith and Shortle, 1996; Balouet et al., 2009; Scharnweber et al., 2016). The provenance of samples (forest plantation) in our study homogenizes the age-related trend and allows obtaining common nutrient inter-correlations, which can be correlated with environmental fluctuations (e.g. climate conditions or silvicultural-management-practices).

Inter-annual nutrient concentrations are more related to annual precipitation variability than temperature (Figure 6B and C). Few dendrochemistry studies report historical changes of nutrient uptake associated to the climate variables or seasonality (Watmough, 1997; Poussart et al., 2006; Hevia et al., 2018). Our results show significant correlations of nutrients (together with TRW and WD) with the precipitation (Figure 6B). According to Robson and Pitman (1983), some elements has been uptake by the plant as function of the stimulate leaching by precipitation increases. For example, tree-ring Ca concentration is described as indicative of tree-growth associate to increases in the rainfall season (Poussart et al., 2006; Hevia et al. (2018), similar observed in this study. While S concentration in storage tissues (phloem) of deciduous trees of temperate zones, is loaded during spring (beginning growing season), while in summer and autumn not exchanged between the phloem and the xylem are seeming (Herschbach and Rennenberg, 2001; Fairchild et al., 2009; Hevia et al., 2018). Furthermore, S exportation increases into stem tissues are correlated with biomass production increases (Ibid). The correlation of tree-ring S concentration and TRW with precipitation, could be constitutes an indication of their xylem loaded during the growing season (Figure 6B).

The temperature variability and nutrient concentration show poor negative correlations, different to the stronger positive correlations with WD (Figure 6C). Hevia et al. (2018) mentioned that high temperatures increase the wood density which could lead less mobile elements accumulation as Mn (poor negative correlation in the current year, Figure 6C). Also, temperature increases affect the trees metabolism to recycle or transport other elements to other storage plant tissues, different to the xylem (Herschbach and Rennenberg, 2001; Hevia et al., 2018). However, complementary anatomical, physiological and phenological seasonality studies within an annual tree-growth period and their relation to xylem storage to understand this nutrient concentrations and climate variability correlations are necessary (Schupp et al., 1991; Herschbach and Rennenberg, 2001; Poussart et al., 2006; Hevia et al., 2018).

4.4.3. CPMS effects analysis by dendrochemical approach

Similar to in environmental studies (Balouet et al., 2009; Balouet and Chalot, 2015; Hevia et al., 2018), the nutrient-chronologies analysis in the present study allowed to monitoring and dating CPMS-treatments effects. The ordination analysis (Figure 5) and the decreased nutrient concentrations and interactions trends allowed to observed CPMS effects until the 6th year, and then they are minimized (Figure 6A). While, in the 11th-17th years (Figure 7D, F) the difference is possible not direct due to the CPMS inputs, but rather to a recharge effect by the nutrient cycle of the plantation (Switzer and Nelson, 1972; Jorgensen et al., 1975; Robson and Pitman, 1983; Baligar et al., 2001). In addition, after the application of the CPM, the trees could have stored excess nutrient-concentrations because the soil was deficient in nutrients (Vroblecky and Yanosky, 1990; Vroblecky et al., 1992; Balouet et al., 2009). The latosols in Brazil are generally characterized by a deficient of N, P, Ca, Mg and Mo, and a toxicity of Al, Mn and Fe (Davies, 1997; Baligar et al., 2001). While CPMS adds N, P, K, Ca, Mg, Na (Table 1), S, Zn, B, Mn, Fe, Cu, Al and Mo to the soil and reduces its acidity (Young et al., 1993; Jackson et al., 2000; Vance, 2000).

High tree-ring Ca and less Mn and P concentrations associated with TRW increases in both periods of growth were also observed, mainly distinguishable in the 2nd-5th years (Figure 7C, G). According to Fromm (2010), soil Ca increases are associated to the element cambial concentration caused the cell differentiation and increasing the TRW. In addition, Ca can affect the absorption of anions and cations in trees (e.g. Mn and P) (Robson and Pitman, 1983). In contrasting to Ca, Mn is considering an important growth-limiting factor, within and among species, in acid soils (Kogelmann and Sharpe, 2006), also their leach may be less susceptible than other cations in the soil, while in xylem may being replaced on exchange sites by increased presence of Ca (Robson and Pitman, 1983; McClenahen et al., 1989). On the other hand, the P availability for plants on neutral and alkaline soils may be controlled by Ca phosphates, thus increases of pH in presence of Ca will decrease the P concentration (Robson and Pitman, 1983).

Not clear associations between tree-ring K, Fe or S concentrations and CPMS doses were observed (Figure 7). Possible because their nutrient cycle within the tree causes its storage in another plant compounds or wood tissues or may not be sensitive indicators of soil chemical changes (Robson and Pitman, 1983). K for example, is highly stored in parenchymatic tissue (e.g. rays) (Fromm, 2010). While Fe and S are not good indicators or respond retroactively to treatments that reduce soil acidity, such as liming (McClenahen et al., 1989).

On the other hand, works whit element ratios is suggested to compare results of site condition changes, and they have been reported as better compared to single elements (Kuang et al., 2008; Scharnweber et al. 2016; Hevia et al., 2018). Indeed, xylem ratios as Mg/Mn, Ca/Al and Ca/Mn are good indicators of soil-pH (Kogelmann and Sharpe, 2006; Poussart et al., 2006; Kuang et al., 2008) while K/Ca of the cambium activity (Fromm, 2010). In addition, some of them show trends similar to comparable ratios in the soil and foliage (Kogelmann and Sharpe, 2006).

In the 7-year-old-*Pinus taeda* trees (Rodrigues, 2004) in the experimental area the pH, cation exchange capacity and soil base saturation levels were higher (but not significant) in CPMS treated plots than control plot. Our results show an increase of xylem Ca/Mn ratio after CPMS application (mainly with 60 t ha⁻¹), higher in the 2nd-5th years than in the 11th-17th years (Figure 8A). Different authors mention the potential of the CPMS as a physical-chemical soil modifier that increases the soil pH (e.g. Cabral et al., 1998; Vance, 2000; Corbel et al., 2016), principally in acids soil as the case of the study (Table 1) (Kuang et al., 2008).

High xylem K/Ca ratios was observed with less CPMS doses (mainly in control treatment) and in the 11th-15th year (Figure 8B). In *Sequoia sempervirens* high K/Ca ratios were detected in youngest tissues (Boulay, 2012), this may explain the higher values of K/Ca ratios in the last tree-rings growth close to the cambium in all treatments. However, high K/Ca values in poor or no CPMS doses, it could be due to the deficiency of Ca in the soil of the site which causes a greater storage of K that fulfills the same cell formation function (Fromm, 2010).

Furthermore, high xylem Ca/Mn and less K/Ca ratios in tree-rings are correlated with TRW increases and WD decreases (Figure 8). Similar in TRW of *Pinus* sp. observed by Vives et al. (2005) for tropical plantation samples. However different to the results for *P. uncinata* of temperate forest, which present high xylem K/Ca ratios, associated to high TRW and WD but lower correlations with Ca/Mn values (Hevia et al., 2018). It is possible that the relations between chemical elements or molar ratios and tree-ring variables could be closely associated with a common controlling climate factor (Hevia et al., 2018), varying within temperate and tropical regions even for the same species.

Finally, despite the detected relations in this study are consistent with the literature, it should be taken with caution. According to Robson and Pitman (1983), the complex nutrient interactions involve several processes in the external media and within the tree, and one nutrient may affect the absorption and utilization of another depending on the plant tissues. At this point, the results show that not always high doses of fertilizers (such as CPMS) applied in the soil are associated with higher concentrations of nutrients stored in the xylem or greater wood formation (Figure 7; 8). In addition, this type of studies should be complemented with foliar, bark and root chemical analyses, since they do not always show similar associations (Kogelmann and Sharpe, 2006).

4.5. Conclusions

The developed non-destructive μ -XRF method to quantify Iron (Fe), Manganese (Mn), Calcium (Ca), Potassium (K), Sulfur (S) and Phosphorus (P) in annual tree-rings revealed potential uses in monitoring and dating historical soil fertilizer treatments effects in forest plantations. The formation of *P. taeda* annual tree-rings induce the variability of nutrient concentration in the radial trends. The precipitation was the main climate factor for the annual nutrient (Ca, S and low frequency Mn, Fe, P and K interaction, positive correlation) and the tree-ring thickness (positive correlation) and density (negative correlation) variation. CPMS treatments affected the *P. taeda* wood formation (<K/Ca ratios) and improved the soil-pH (>Ca/Mn ratios) up to the 5th year, after their application.

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5. MAIN RESULTS INTEGRATION AND GENERAL CONCLUSION

5.1. Trunk wood characteristics at 17-years *P.taeda* trees

At 17-year-old *P. taeda* trees, the mean trunk growth (diameter and height) and wood biomass production with 80 t ha⁻¹ CPMS were significant higher in relation to other treatments (Figure 1). Whereas the wood basic density decreased slightly (7%) on *P. taeda* trees 80 t ha⁻¹ CPMS compared to the control treatment (Figure 2). However, no significant differences between CPMS-treatments were observed. Furthermore, the N, K and Ca concentrations *P. taeda* wood 60 t ha⁻¹ CPMS were greater; also the Ca concentrations increased in relation to higher CPMS-doses; P and Mg concentrations did not differ significantly among *P. taeda* wood CPMS treatments (Figure 3).

Thus, it is evident that the only CPMS application at the time of the forest plantation establishment promoted growth responses of *P. taeda* trees that remained until their 17th year (Figures 1, 2 and 3). This effect was also observed at 7 years of age (Rodrigues, 2004, 2005). However, one of the limiting factors of these results is the lack of historical monitoring of *P. taeda* trees response to CPMS application, especially when their effect was not monitored in successive years. In the present work, the lack of continuous monitoring and precise response of *P. taeda* trees growth, in relation to CPMS treatments, motivated the application of annual growth tree-rings as indispensable methodology.

Figure 1. Average *Pinus taeda* trees trunk growth and wood production (A); wood basic density determined by hydrostatic balance method (ABTCP, 1974) (B); stemwood nutrients determined by Malavolta et al. (1989) (C) at 17-years *P.taeda* trees treated with five doses of composted pulp-mill sludge (CPMS). In B: values up histogram indicate weighted arithmetic mean wood basic density for each CPMS-treatment; similar lower-case letters indicate no significant differences between treatments, respectively ($p < 0.05$). In C: pairwise comparisons (Tukey test) in upper right-hand corner; treatments with different letters (a, b and c) indicate statistically significant differences ($p < 0.05$).

5.2. Evaluating wood trunk *P. taeda* trees responses to the CPMS application

By applying methodologies based on the *P. taeda* trees annual tree-ring characteristics, it was possible to distinguish the CPMS effect application until the 5th years of trees growth and wood physical-chemical properties (Table 1). The treatment 80 t ha⁻¹ CPMS showed the greatest increase of *P. taeda* trees development with a significant wood density reduction. While the treatment 60 t ha⁻¹ CPMS showed higher interactions between detected nutrients and a significant improvement in soil pH related to a higher Ca/Mn ratio interaction found in *P. taeda* wood.

Table1. Main results integration of different analysis using tree-ring methods.

Stem wood	Methodology	Unit	Main CPMS treatments with significant difference	Up to year
Growth	Stem analysis	Mg ha ⁻¹ year ⁻¹ and m ² ha ⁻¹ interaction	0 and 80-100 t ha ⁻¹	4
Mean annual density	X-ray densitometry analysis	g cm ⁻³	0 and 80 t ha ⁻¹	6
Nutrients (P, K, Ca, Mn, Fe, S)	X-ray fluorescence microanalysis	Low frequency nutrient and Ca/Mn ratio interaction	0 and 60 t ha ⁻¹	5

5.3. General conclusion and final coments

The present study allowed presenting an alternative vision in the historical evaluation of the trees growth, and the wood physical-chemical properties. Evidenciating the potential of the application of methodologies that use the annual tree-ring characteristics for studies of forest plantations monitoring submitted to silvicultural treatments.

In future works that apply these methodologies must be integrated information of the soli characteristics in different plantation stages, as well as the evaluation of other tree species components. Furthermore, to promote the CPMS aplication, economic feasibility studies should be carried out.

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