University of São Paulo
"Luiz de Queiroz" College of Agriculture

# Availability of copper and zinc as affected by soil acidity in *Eucalyptus* plantation

Antonio Leite Florentino

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

Piracicaba
2021

**Antonio Leite Florentino**
**Forestry Engineer**

# Availability of copper and zinc as affected by soil acidity in *Eucalyptus* plantation

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **JOSÉ LEONARDO DE MORAES GONÇALVES**

Coadvisor:
Prof. Dr. **JOSÉ LAVRES JUNIOR**

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

**Piracicaba**
**2021**

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

Florentino, Antonio Leite

Availability of copper and zinc as affected by soil acidity in *Eucalyptus* plantation / Antonio Leite Florentino. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2021.

143 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura "Luiz de Queiroz".

1. Calagem 2. Fases do solo 3. Íons metálicos 4. Espécies reativas de oxigênio 5. Reflorestamento 6. Produção de madeira I. Título

# DEDICATION

To my parents Samuel Florentino de Souza (*in memoriam*) and Maria Elena Leite, brothers, sisters, nephews, nieces, brothers-in-law, sisters-in-law, and my lovely wife Ilizete Izabel Gasperin and daughter Luna Gasperin Florentino.

## ACKNOWLEDGEMENTS

I express my sincere gratitude to the following people and institutions who were fundamental to the achievement of this thesis:

- I would like to thank God for the countless blessings in my life.
- To my parents Samuel (*in memoriam*) and Elena, and all my lovely family for encouragement. Thanks very much!
- "Luiz de Queiroz" College of Agriculture, University of São Paulo (ESALQ/USP) and the Forest Resources Graduate Program for the opportunity and support provided to carry out my research.
- My advisers Prof. Dr. José Leonardo de Moraes Gonçalves (ESALQ/USP) and Prof. Dr. José Lavres Junior (Center for Nuclear Energy in Agriculture - CENA/USP) for their confidence in my potential as a student, and the strong guidance throughout this journey. I am grateful to have had the opportunity to work with you, thank!
- To Coordination for the Improvement of Higher Education Personnel (CAPES) - Finance Code 001 for the scholarship that sponsored my doctorate.
- To the Institute of Forest Research and Study (IPEF), through the Silviculture and Management Thematic Program (PTSM), especially Dr. Alexandre de Vicente Ferraz. Thanks for all support and encouragement.
- To Prof Dr. José L. Stape for the first teachings on forest science. Thanks very much!
- To Dr. Marcelo C. Alves (ESALQ) and Prof. Dr. Carlos T.S Dias (ESALQ) for the helping in the data analysis.
- To Prof. Dr. Luís R. F. Alleoni (ESALQ) for the opportunity to carry part of the soil chemical analyses, and the technicians Luiz, Marina Colzato, Ednéia, and all internships for supporting the activities.
- To all professors at ESALQ, especially from Department of Forest Science Antonio Natal, Fernando Seixas, Edson Vidal, José Garcia, and Luciana Duque for sharing your knowledge during my academic career. Thanks very much!
- To all professors at CENA, especially Prof. Dr. Cassio H. Abreu Junior, Prof. Dr. Takashi Muraoka, , and Prod. Dr. Francisco S. Linhares. Thanks!
- To Prof. Dr. Ricardo A. Azevedo (ESALQ) for the collaboration in conducting the biochemical analyzes at your laboratory and Dr. Salete A. Gaziola and Dr. Marcia E.A. Carvalho for your helping and teaching.

- To all employees at the CENA, especially Mônica Rossi, Cleusa, Everaldo, Inês, Aparecida de Fátima, Claudia, Mariana Belloti, Ana, Hugo, Pingin, Marcelo, Marília, Glauco, Cristina, and Suzineide for your helping.
- To my friend Dr. Izaias P. Lisboa for the discussions about work and the enjoyable moments shared.
- To all my friends at ESALQ, CENA, and the study groups of PTSM: José de Deus, Nikolas, Adam, Amanda, Ivanka, Liamara, Alvaro, Andressa, Gabriela, Leticia, José Rocha, Fábio, Robson, Yesid, Nicolas, Bruno, Jessica, Marco Antonio, Michel, Anderson, Pedro Garcia, Natalia, Fernanda, Paulo Cardoso, and Thais Mandu. Thanks!
- To all PTSM internships, especially Rafael, Lucas, Eder, Ana, Douglas, Gabriel, Gardenia, Oscar, Vitória, Joana, Pedro, João, Bruno, José Renato, and Renan for supporting the activities. Thanks!
- To all employees at the Department of Forest Sciences (ESALQ), especially Sabino, Natanael, Amarildo, Giovana Oliveira, Alba, Jefferson, Eliezer, Cido, Facco, Alex, Alexandre, and Udemilson. Thanks very much!
- To all employee at the Experimental Station of Forest Sciences of Itatinga (ESALQ/USP), especially Rildo Moreira, Lorival, and Elaine. Thanks all for support!
- To all friends at “Grupo Florestal Monte Olimpo” (GFMO/ESALQ), especially Isabel, Rafaela, Ricardo, Luiz, Carla, Fabricio, Felipe, Rodrigo, Eduardo, and Edimar. Thanks!
- To all friends from Piracicaba city, especially Jofre, Luciana, André, Frederico, Roger, Ellen, David, Kelly, Lucas, Pedro, Lucas, Gabriel, Luis, Diogo, Verònica, Daniel, Léo, and Luciano Zambonin. Thanks for the moments shared!
- To all professors at EE Prof. Maria L. Martins, especially Valdemir Leal da Rocha and Sandra Tadini Santos. Thanks very much!
- To all my old friends, especially Aldo Messias, José Carlos, André, Anderson, Ricardo, Luciano, Fábio, Elias, Renato, Odenório, Naldo, Wagner, Wellington. Thanks, boys!
- To all my family members for their patience and support in everything. I owe you a lot. Thanks all!
- My lovely wife Ilizete Gasperin for partnership, patience, and lovely moments. Thanks!

**Thank you all!**

"As grandes conquistas da humanidade foram obtidas conversando, e as grandes falhas pela falta de diálogo"

"Mankind's greatest achievements have come about by talking, and its greatest failures by not talking"

*Stephen William Hawking (1942 - † 2018)*

**CONTENTS**

## RESUMO


**Disponibilidade de cobre e zinco afetada pela acidez do solo em plantação de *Eucalyptus***

O Brasil é referência mundial no manejo de plantação de *Eucalyptus* para produção de madeira (e.g., celulose e produtos de madeira sólida) e aproximadamente 77% das áreas reflorestadas são com espécies deste gênero, principalmente em solos ácidos e de baixa fertilidade. A aplicação de calcário (calagem) é uma prática agrícola para reduzir a acidez do solo e fornecer teores de Ca e Mg (como recomendado para eucalipto) à nutrição das plantas. A calagem pode reduzir a biodisponibilidade de nutrientes metálicos no solo (e.g., Cu e Zn), e a recomendação de Cu e Zn para plantação de *Eucalyptus* não é um consenso na silvicultura brasileira. A aplicação desses micronutrientes é recomendada para suprir à quantidade removida nos produtos da colheita florestal. Nesse sentido, poucos pesquisadores avaliaram a aplicação combinada de calcário e Cu mais Zn em plantas de eucalipto manejadas em solos tropicais. Neste estudo, as implicações desses fatores nos atributos químicos do solo, desempenho nutricional e de desenvolvimento de *Eucalyptus grandis* foram analisadas. As variáveis avaliadas incluíram a área foliar e índice de área foliar, conteúdo de clorofila e flavonoides e desempenho do metabolismo antioxidante [peroxidação lipídica (teor de malondialdeído), teor de peróxido de hidrogênio, e atividade de enzimas antioxidantes (superóxido dismutase, catalase, glutationa redutase, ascorbato peroxidase, guaiacol peroxidase e glutationa peroxidase)]. A aplicação de calcário aumentou o pH do solo e as disponibilidades de Ca e Mg, enquanto reduziu as disponibilidades de Cu e Zn no solo. Em geral, a calagem reduziu os teores de Cu e Zn na fração residual (ligada aos minerais silicatados), porém aumentou na fração ligada à matéria orgânica (oxidável). A calagem aumentou a eficiência de uso de Cu, Fe, Mn e Zn, enquanto a fertilização com Cu mais Zn aumentou o desempenho antioxidante enzimático das plantas de eucalipto. As plantas cultivadas no solo corrigido com calcário apresentaram maior área foliar, índice de área foliar, pigmentos foliares e concentrações foliares de Ca e Mg, enquanto as concentrações de Cu e Zn foram reduzidas. A aplicação combinada de calcário e Cu mais Zn promove maior desenvolvimento das plantas de *Eucalyptus*, o que foi observado através do aumento da produtividade do eucalipto. Assim, a utilização da fertilização de Cu mais Zn associada com a aplicação de calcário pode ser uma estratégia útil para aliar nutrição balanceada e otimizar o crescimento das plantas a fim de aumentar a produção de madeira. Em conclusão, nosso estudo trouxe novas abordagens relacionadas a fertilidade do solo, produção de madeira, área foliar e índice de área foliar, características bioquímicas, metabolismo antioxidante e estado nutricional de plantas de *Eucalyptus* com a aplicação de calcário associada à fertilização com Cu e Zn.


Palavras-chave: Calagem, Fases do solo, Íons metálicos, Espécies reativas de oxigênio, Reflorestamento, Produção de madeira

## ABSTRACT


### Availability of copper and zinc as affected by soil acidity in *Eucalyptus* plantation

Brazil is a global reference regarding *Eucalyptus* management for wood production (e.g., pulp and solid wood products) and approximately 77% of the areas under reforestation is done with species of this genus, mainly in acidic and poorly fertile soils. Lime application (liming) is a consensus strategy to reduce soil acidity and to supply Ca and Mg (as recommended for *Eucalyptus*) contents to crops nutrition. Liming can reduce soil metallic micronutrients (e.g., Cu and Zn) bioavailabilities, and Cu and Zn recommendation for *Eucalyptus* plants is not a consensus in Brazilian silviculture. Applying these micronutrients is recommended to supply the amount removed within forest harvesting products. In this sense, few studies investigated the combined application of lime and Cu plus Zn rates for *Eucalyptus* grown under tropical soils. In this study, the implications of those factors on soil chemical attributes, plant nutritional status and plant growth performance of *Eucalyptus grandis* were investigated. Variables evaluated included leaf area and leaf area index, chlorophyll and flavonoid content, and antioxidant metabolism performance [lipid peroxidation (malondialdehyde content), hydrogen peroxide content, and activity of antioxidant enzymes (i.e., superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase, guaiacol peroxidase, and glutathione peroxidase)]. Lime application increased soil pH and Ca and Mg availabilities, while Cu and Zn availabilities bioavailability into the soil was reduced. Overall, liming reduced soil Cu and Zn contents in residual fraction (bound to silicate minerals) but increased in fraction bound to organic matter (oxidizable). Liming augmented Cu, Fe, Mn, and Zn use efficiency, while Cu plus Zn fertilization increased enzymatic antioxidant performance of *Eucalyptus* plants. *Eucalyptus* grown in soil amended with lime had higher leaf area, leaf area index, leaf pigments, leaf Ca and Mg concentrations, while Cu and Zn concentrations were reduced. *Eucalyptus* grown under lime application associated with Cu plus Zn fertilization leads to better plant performance, which is observed through crop productivity improvement. Thus, the adoption of Cu plus Zn fertilization combined with the lime can be promising strategy to link balanced nutrition and optimize plant growth aimed to higher wood yield. In conclusion, our study brought new insights into the soil fertility, wood yields, leaf area and leaf area index, biochemical traits, antioxidant metabolism and nutritional status of the *Eucalyptus* plants under lime application associated with Cu and Zn fertilization.


Keywords: Liming, Soil phases, Metallic ions, Reactive oxygen species, Reforestation, Wood production

# 1. GENERAL INTRODUCTION

The forest plantation for wood production (e.g., pulp and solid wood products) is largely adopted worldwide (Booth, 2013; Keenan et al., 2015) and Brazil is global reference in the management for *Eucalyptus* plantations (Alvares et al., 2013; Stape et al., 2004). *Eucalyptus* species occupy approximately 77% of the area of forests plantations devoted to wood products in Brazil (IBA, 2020). Brazilian *Eucalyptus* forest are predominantly established with varieties of clones (Gonçalves et al., 2008; Stape et al., 2010), planted mainly in acidic and poorly fertile soils (Gonçalves et al., 2020; Scolforo et al., 2019). These soils are characterized by high Al contents, which can impair plant growth (Eswaran et al., 1997; Silva et al., 2004), such as nutritional and biochemical disorders (von Uexküll and Mutert, 1995). Lime application is a well-known strategy widely adopted to reduce soil acidity and, then benefit plant nutrition (Crusciol et al., 2019). Liming has been long adopted for management of crops, which is directly associated with the improves of soil pH and the soil-nutrient availability (Ameyu, 2019; Caires et al., 2008, 2006; Mallarino et al., 2018). The main goal of lime recommendation rate for annual crops (e.g., *Triticum aestivum* L. and *Oryza sativa* L.) is to reduce the soil acidity and to increase the soil availabilities of Ca and Mg contents to crops uptake (Raij et al., 1997), while the use of lime in *Eucalyptus* plantation aims to supply only Ca and Mg contents for tree nutrition (Gonçalves, 2010; Smethurst, 2010).

The increasing Ca and Mg contents are reported in soil and plants amended with lime application (Holland et al., 2018). However, liming potentially have side effects in soil Cu and Zn availability for plant uptake (Havlin et al., 2013). This hypothesis is based on the fact that lime is rich in Ca and Mg contents (Rocha et al., 2019; Smethurst, 2010) and, once applied into the soil, $H^+$ and $Al^+$ availability is reduced (Gomes et al., 2019; Rocha et al., 2019). Accordingly, liming increases pH levels and contributes to higher precipitations of Cu and Zn bound to carbonates or adsorbed to compounds rich in the Ca (González-Alcaraz et al., 2013; Jiménez-Cárceles et al., 2008). In this sense, fertilization with Cu and Zn can enhance the bioavailability of these metals for plant uptake, however, the technical recommendation of Cu and Zn fertilization for *Eucalyptus* plantation is not a consistent practice (Gonçalves et al., 2013; Gonçalves and Valeri, 2001).

The lime application increases the adsorption of Cu and Zn and, subsequently reduces the availability of these micronutrients in the soil (Holland et al., 2018; Wisawapipat et al., 2017). Copper was more adsorbed as compared with Zn, however, only low levels of Cu and Zn were in the exchangeable form (Mesquita and Vieira e Silva, 1996). Plants grown under lime condition can uptake less content of Cu and Zn (Barman et al., 2014; Fageria et al., 2002). This effect can occur because liming increases soil pH and, consequently can reduce the bioavailability these metals for plant uptake (Aubert et al., 2010; Havlin et al., 2013; Kabata-Pendias, 2011). To avoid this implication, the fertilization with Cu and Zn may offset liming undesirable effects because Cu and Zn fertilization can improve morphophysiological responses by plant tissues. Clarifying our understanding of the combined application of lime (more Ca and Mg soil availability) and mineral Cu and Zn fertilization to benefit *Eucalyptus* growth is crucial to elucidate the associate mechanisms that influence the soil chemical attributes, nutritional, and morphophysiological performance of the *Eucalyptus* plants.

Copper and Zn are involved in plant metabolism processes, they can regulate the activity of antioxidant enzymes, e.g., superoxide dismutase - SOD (Soares et al., 2019). The activity of SOD and plasma membrane $Ca^{2+}$-ATPase was inhibited under strong rhizosphere acidification (Zhang et al., 2015). Metallic ions (e.g., $Cu^{2+}$, and $Zn^{2+}$) work as cofactors of several enzymes and are therefore essential for plant growth (Saito and Uozumi, 2020). For example, Mg ions exhibit fast ligand exchange kinetics, which means they can easily be replaced by other divalent metal ions, such as $Ca^{2+}$, $Cu^{2+}$, and $Zn^{2+}$ (Bang et al., 2021).

Because of their fast development rate and the high degree to alleviate the biotic and abiotic stresses (Gonçalves et al., 2013), the *Eucalyptus* species have become industrially important and is currently globally cultivated for wood production (Plett et al., 2015). Mineral fertilization is needed to guarantee higher plant development and avoid environmental stress associated with a nutritional deficiency (Smethurst, 2010). Plant cells when subjected to stresses factors, such as nutritional disorders, active the antioxidant metabolism to increase the production of compound enzymatic and non-enzymatic aims to guarantee plant homeostasis (Alexieva et al., 2001; Gratão et al., 2005; Kandziora-Ciupa et al., 2017; Noctor et al., 2016). The production of reactive oxygen species (ROS) plays a fundamental role in signaling molecules for the regulation of innumerable biological properties such as the processes of growth, and responses of antioxidant metabolism (Baxter et al., 2014). Lipid peroxidation is another key indicator of ROS in plants, in which one of the products formed is malondialdehyde (MDA), which is often used as a lipid peroxidation standard (Noctor et al., 2016). The biochemical responses closely associated with morphophysiological traits, such as leaf epidermis, photosynthesis, mesophyll conductance, and root growth could further benefit plants to tolerate environmental stress (Bertolino et al., 2019).

The comprehensive use of lime and mineral fertilization, such as Cu and Zn fertilizers, in modern agriculture has been key to achieve higher yields in the agroecosystems

(Pathak and Nedwell, 2001). However, few researchers evaluated the integrative effects of the combined application of these factors in *Eucalyptus* plantations. In this sense, our intention with this study is to show new insights on soil fertility and plant nutritional aspects, and production of the *Eucalyptus* plants. Thus, it can approach the mechanisms that respond to stress or nutrient depletion, and ultimately lead to resistance against unfavorable conditions (Saito and Uozumi, 2020). Several responses are attributed to the effect of lime to improves soil fertility and, then, crops growth (Allen et al., 2020; Bossolani et al., 2020; Carmeis Filho et al., 2017; Castro and Crusciol, 2013; Chatzistathis et al., 2015; Court et al., 2018; Crusciol et al., 2019; Gabriel et al., 2018; González-Alcaraz et al., 2013).

In this sense, the general hypotheses tested in our study were: i) liming may offset undesirable implications on soil Cu and Zn availability, and this strategy can lead to a lower content of these micronutrients in *Eucalyptus* plant; and ii) combined application of lime and Cu plus Zn would fulfill requirements of these micronutrients by *Eucalyptus* plantation, upregulating plant growth. To test the hypotheses, we evaluated the development of *Eucalyptus grandis* plantation, as well as chemical attributes of a Ferralsol, amended with different rates of limestone (lime) and fertilizers with Cu and Zn. Thus, three chapters are presented in this thesis, each chapter was written as a scientific article (**chapters 2, 3, and 4**).

The **second chapter** was knowledge gaps about the combined application of lime and Cu plus Zn fertilization for soil and plant responses under field conditions, evaluations for that chapter included: soil pH monitoring, soil chemical attributes, sequential extraction of Cu and Zn in soil, plant nutrition, leaf area index, and growth of the *Eucalyptus grandis* plantation. Within the **third chapter** was presented a new insight about the performance of *Eucalyptus* field established in soil fertilized with different rates of lime and Cu plus Zn application; which showed responses of soil chemical attributes, shoot biomass, nutrient accumulation and nutrient use efficiency, and biochemical traits, such as leaf pigments (i.e., chlorophyll and flavonoids) and the activity of antioxidant enzymes [i.e., superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), guaiacol peroxidase (GPOX), and glutathione peroxidase (GPX)] in the *Eucalyptus* trees. Finally, the **fourth chapter** showed a gist of current understandings about the effect of lime application and Cu plus Zn fertilization in *Eucalyptus* seedlings nutritional physiology under trial setup in a greenhouse. Parameters evaluated encompassed biomass production, leaf area, leaf pigments, and Cu and Zn concentration, accumulation, use efficiency, and distribution in *Eucalyptus grandis* seedlings.

**References**

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell Environ. 24, 1337–1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x

Allen, J., Maunoury-Danger, F., Felten, V., Danger, M., Legout, A., Guérold, F., 2020. Liming of acidified forests changes leaf litter traits but does not improve leaf litter decomposability in forest streams. For. Ecol. Manage. 475. https://doi.org/10.1016/j.foreco.2020.118431

Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorol. Zeitschrift 22, 711–728.

Ameyu, T., 2019. A Review on the Potential Effect of Lime on Soil Properties and Crop Productivity Improvements. J. Environ. Earth Sci. https://doi.org/10.7176/jees/9-2-03

Aubert, H., Pinta, M., Hooda, P., 2010. Trace elements in soils. Trace Elem. Soils 199–226. https://doi.org/10.1002/9781444319477.ch10

Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New Phytol. https://doi.org/10.1111/nph.17074

Barman, M., Shukla, L.M., Datta, S.P., Rattan, R.K., 2014. EFFECT OF APPLIED LIME AND BORON ON THE AVAILABILITY OF NUTRIENTS IN AN ACID SOIL. J. Plant Nutr. 37, 357–373. https://doi.org/10.1080/01904167.2013.859698

Baxter, A., Mittler, R., Suzuki, N., 2014. ROS as key players in plant stress signalling. J. Exp. Bot. https://doi.org/10.1093/jxb/ert375

Bertolino, L.T., Caine, R.S., Gray, J.E., 2019. Impact of stomatal density and morphology on water-use efficiency in a changing world. Front. Plant Sci. 10. https://doi.org/10.3389/fpls.2019.00225

Booth, T.H., 2013. Eucalypt plantations and climate change. For. Ecol. Manage. 301, 28–34. https://doi.org/10.1016/j.foreco.2012.04.004

Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375. https://doi.org/10.1016/j.geoderma.2020.114476

Caires, E.F., Barth, G., Garbuio, F.J., 2006. Lime application in the establishment of a no-till system for grain crop production in Southern Brazil. Soil Tillage Res. 89, 3–12. https://doi.org/10.1016/j.still.2005.06.006

Caires, E.F., Barth, G., Garbuio, F.J., Churka, S., 2008. Soil acidity, liming and soybean performance under no-till. Sci. Agric. 65, 532–540. https://doi.org/10.1590/s0103-90162008000500013

Carmeis Filho, A.C.A., Penn, C.J., Crusciol, C.A.C., Calonego, J.C., 2017. Lime and phosphogypsum impacts on soil organic matter pools in a tropical Oxisol under long-term no-till conditions. Agric. Ecosyst. Environ. 241, 11–23. https://doi.org/10.1016/j.agee.2017.02.027

Castro, G.S.A., Crusciol, C.A.C., 2013. Effects of superficial liming and silicate application on soil fertility and crop yield under rotation. Geoderma 195–196, 234–242. https://doi.org/10.1016/j.geoderma.2012.12.006

Chatzistathis, T., Alifragis, D., Papaioannou, A., 2015. The influence of liming on soil chemical properties and on the alleviation of manganese and copper toxicity in Juglans regia, Robinia pseudoacacia, *Eucalyptus* sp. And Populus sp. Plantations. J. Environ. Manage. 150, 149–156. https://doi.org/10.1016/j.jenvman.2014.11.020

Court, M., van der Heijden, G., Didier, S., Nys, C., Richter, C., Pousse, N., Saint-André, L., Legout, A., 2018. Long-term effects of forest liming on mineral soil, organic layer and foliage chemistry: Insights from multiple beech experimental sites in Northern France. For. Ecol. Manage. 409, 872–889. https://doi.org/10.1016/j.foreco.2017.12.007

Crusciol, C.A.C., Marques, R.R., Carmeis Filho, A.C.A., Soratto, R.P., Costa, C.H.M., Ferrari Neto, J., Castro, G.S.A., Pariz, C.M., Castilhos, A.M., Franzluebbers, A.J., 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutr. Cycl. Agroecosystems 115, 347–372. https://doi.org/10.1007/s10705-019-10017-0

Eswaran, H., Reich, P., Beinroth, F., 1997. Global distribution of soils with acidity, in: Plant-Soil Interactions at Low PH. pp. 159–164.

Fageria, N.K., Baligar, C., Clark, R.B., 2002. Micronutrients in crop production. Adv. Agron. https://doi.org/10.1016/s0065-2113(02)77015-6

Gabriel, C.A., Cassol, P.C., Simonete, M.A., Moro, L., Pfleger, P., Mumbach, G.L., 2018. Lime and gypsum applications on soil chemical attributes and initial growth of eucalyptus. Floresta 48, 573–582. https://doi.org/10.5380/rf.v48i4.57455

Gomes, S.D.S., Gonçalves, J.L.D.M., Rocha, J.H.T., Menegale, M.L.D.C., 2019. Tolerance of *Eucalyptus* and *Pinus* seedlings to exchangeable aluminium. Sci. Agric. 76, 494–500. https://doi.org/10.1590/1678-992x-2018-0011

Gonçalves, J., Stape, J., Laclau, J., Bouillet, J., Ranger, J., 2008. Assessing the effects of early silvicultural management on long-term site productivity of fast-growing eucalypt plantations: the Brazilian experience Assessing the effects of early silvicultural management on long-term site productivity of fast-growing eu. J. For. Sci. 70, 105–118. https://doi.org/10.2989/SOUTH.FOR.2008.70.2.6.534

Gonçalves, J.L. de M., 2010. Capítulo 8 - Livro do IPNI - Adubação do eucalipto.

Gonçalves, J.L. de M., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., Ferraz, S.F. de B., Lima, W. de P., Brancalion, P.H.S., Hubner, A., Bouillet, J.P.D., Laclau, J.P., Nouvellon, Y., Epron, D., 2013. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. For. Ecol. Manage. 301, 6–27. https://doi.org/10.1016/j.foreco.2012.12.030

Gonçalves, J.L. de M., de Vicente Ferraz, A., Tertulino Rocha, J.H., Peressin, M., Alcarde Alvares, C., 2020. Forest outgrower schemes in small and medium-sized farmers in Brazil. For. Ecol. Manage. 456. https://doi.org/10.1016/j.foreco.2019.117654

Gonçalves, J.L.D.M., Valeri, S.V., 2001. Eucalipto e pínus, in: Ferreira, M.E., Cruz, M.C.P., Raij, B., Abreu, C.A. (Eds.), Micronutrientes: Elementos Tóxicos Na Agricultura. CNPq/FAPESP/PATAFOS, Jaboticabal, Pp. 393–423. Jaboticabal (SP), pp. 393–423.

González-Alcaraz, M.N., Conesa, M., Álvarez-Rogel, J., 2013. When liming and revegetation contribute to the mobilisation of metals: Learning lessons for the phytomanagement of metal-polluted wetlands. J. Environ. Manage. 116, 72–80. https://doi.org/10.1016/j.jenvman.2012.11.044

Gratão, P.L., Polle, A., Lea, P.J., Azevedo, R.A., 2005. Making the life of heavy metal-stressed plants a little easier. Funct. Plant Biol. https://doi.org/10.1071/FP05016

Havlin, J.L., Tisdale, S.L., Nelson, W.L., Beaton, J.D., 2013. Soil fertility and nutrient management: An introduction to nutrient management, 8th Edition. Pearson, Upper Saddle River, New Jersey, USA.

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.08.020

IBA, 2020. Brazilian tree industry annual report - base year 2019. Assoc. Brasleira Árvores 160.

Jiménez-Cárceles, F.J., Álvarez-Rogel, J., Conesa Alcaraz, H.M., 2008. Trace element concentrations in saltmarsh soils strongly affected by wastes from metal sulphide mining areas. Water. Air. Soil Pollut. 188, 283–295. https://doi.org/10.1007/s11270-007-9544-4

Kabata-Pendias, A., 2011. References, in: Trace Elements in Soils and Plants, Fourth Edition. CRC Press, pp. 407–505. https://doi.org/10.1201/b10158-25

Kandziora-Ciupa, M., Nadgórska-Socha, A., Barczyk, G., Ciepał, R., 2017. Bioaccumulation of heavy metals and ecophysiological responses to heavy metal stress in selected populations of Vaccinium myrtillus L. and Vaccinium vitis-idaea L. Ecotoxicology 26, 966–980. https://doi.org/10.1007/s10646-017-1825-0

Keenan, R.J., Reams, G.A., Achard, F., de Freitas, J. V., Grainger, A., Lindquist, E., 2015. Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2015.06.014

Mallarino, A.P., Pagani, A., Sawyer, J.E., 2018. Corn and soybean response to soil pH level and liming. https://doi.org/10.31274/icm-180809-74

Mesquita, M.E., Vieira e Silva, J.M., 1996. Zinc adsorption by a calcareous soil. Copper interaction. Geoderma 69, 137–146. https://doi.org/10.1016/0016-7061(95)00058-5

Noctor, G., Mhamdi, A., Foyer, C.H., 2016. Oxidative stress and antioxidative systems: Recipes for successful data collection and interpretation. Plant Cell Environ. 39, 1140–1160. https://doi.org/10.1111/pce.12726

Pathak, H., Nedwell, D.B., 2001. Nitrous oxide emission from soil with different fertilizers, water levels and nitrification inhibitors. Water. Air. Soil Pollut. 129, 217–228. https://doi.org/10.1023/A:1010316215596

Plett, J.M., Kohler, A., Khachane, A., Keniry, K., Plett, K.L., Martin, F., Anderson, I.C., 2015. The effect of elevated carbon dioxide on the interaction between *Eucalyptus grandis* and diverse isolates of Pisolithus sp. is associated with a complex shift in the root transcriptome. New Phytol. 206, 1423–1436. https://doi.org/10.1111/nph.13103

Raij, B. van, Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1997. Recomendacoes da adubacao e calagem para o Estado de Sao Paulo 1–88.

Rocha, J.H.T., du Toit, B., Gonçalves, J.L. de M., 2019. Ca and Mg nutrition and its application in *Eucalyptus* and *Pinus* plantations. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2019.03.062

Saito, S., Uozumi, N., 2020. Calcium-Regulated Phosphorylation Systems Controlling Uptake and Balance of Plant Nutrients. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00044

Scolforo, H.F., McTague, J.P., Burkhart, H., Roise, J., Campoe, O., Stape, J.L., 2019. *Eucalyptus* growth and yield system: Linking individual-tree and stand-level growth models in clonal Eucalypt plantations in Brazil. For. Ecol. Manage. 432, 1–16. https://doi.org/10.1016/j.foreco.2018.08.045

Silva, I.R., Novais, R.F., Jham, G.N., Barros, N.F., Gebrim, F.O., Nunes, F.N., Neves, J.C.L., Leite, F.P., 2004. Responses of eucalypt species to aluminum: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism, Tree Physiology. Oxford Academic. https://doi.org/10.1093/TREEPHYS/24.11.1267

Smethurst, P.J., 2010. Forest fertilization: Trends in knowledge and practice compared to agriculture. Plant Soil 335, 83–100. https://doi.org/10.1007/s11104-010-0316-3

Soares, C., Carvalho, M.E.A., Azevedo, R.A., Fidalgo, F., 2019. Plants facing oxidative challenges—A little help from the antioxidant networks. Environ. Exp. Bot. 161, 4–25. https://doi.org/10.1016/j.envexpbot.2018.12.009

Stape, J.L., Binkley, D., Ryan, M.G., 2004. *Eucalyptus* production and the supply, use and efficiency of use of water, light and nitrogen across a geographic gradient in Brazil. For. Ecol. Manage. 193, 17–31. https://doi.org/10.1016/j.foreco.2004.01.020

Stape, J.L., Binkley, D., Ryan, M.G., Fonseca, S., Loos, R.A., Takahashi, E.N., Silva, C.R., Silva, S.R., Hakamada, R.E., Ferreira, J.M. de A., Lima, A.M.N.N., Gava, J.L., Leite, F.P., Andrade, H.B., Alves, J.M., Silva, G.G.C.C., Azevedo, M.R., 2010. The Brazil *Eucalyptus* Potential Productivity Project: Influence of water, nutrients and stand uniformity on wood production. For. Ecol. Manage. 259, 1684–1694. https://doi.org/10.1016/j.foreco.2010.01.012

von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic impact of acid soils. Plant Soil 171, 1–15. https://doi.org/10.1007/BF00009558

Wisawapipat, W., Janlaksana, Y., Christl, I., 2017. Zinc solubility in tropical paddy soils: A multi-chemical extraction technique study. Geoderma 301, 1–10. https://doi.org/10.1016/j.geoderma.2017.04.002

Zhang, Y.-K., Zhu, D.-F., Zhang, Y.-P., Chen, H.-Z., Xiang, J., Lin, X.-Q., 2015. Low pH-Induced Changes of Antioxidant Enzyme and ATPase Activities in the Roots of Rice (Oryza sativa L.) Seedlings. https://doi.org/10.1371/journal.pone.0116971

# 2. NUTRITIONAL STATUS OF *Eucalyptus* PLANTATION AND CHEMICAL ATTRIBUTES OF A FERRALSOL AMENDED WITH LIME AND COPPER PLUS ZINC

**Abstract**

Brazil is a global reference in forest management for wood production (e.g., pulp, wood charcoal, and solid wood products), and large areas are planted mainly in acidic and poorly fertile soils, with a predominance of *Eucalyptus* species. Liming is widely adopted in acidic soils in humid tropical regions planted with *Eucalyptus* to increase the availability of some nutrients and supply Ca and Mg. However, liming decreases the availability of some micronutrients, such as Cu and Zn, depending on the lime content. Copper and Zn fertilization for *Eucalyptus* is usually indicated to replace the amount of micronutrients extracted after wood harvesting. However, few studies have been carried out to investigate the combined effects of lime and Cu plus Zn application on forest plantations in tropical soils. Here, the effects of these factors on soil chemical attributes, as well as nutrition, and growth performance of *Eucalyptus grandis* plantations were evaluated on a medium-textured Ferralsol. The stemwood volume yield improved after liming, regardless of the rate of application, and after Cu plus Zn fertilization. Liming increased soil pH and Ca and Mg availability and reduced Cu and Zn availability. Overall, liming reduced soil Cu and Zn contents in the residual-fraction, while increasing the fraction bound to organic-matter. High contents of Cu and Zn were linked to the organic-matter fraction in the soil under the *Eucalyptus* plantation. *Eucalyptus* trees grown in soils amended with lime had higher values for leaf area index and leaf Ca and Mg concentrations but showed reduced Cu and Zn concentrations. The combined application of lime and Cu plus Zn improved *Eucalyptus* growth, with the higher contribution of lime compared to Cu and Zn. In conclusion, the combined application of lime and Cu plus Zn is a useful strategy to provide balanced nutrition and optimize stem volume yields.

**Keywords:** Soil fertility, Sequential extraction, *Eucalyptus grandis*, Forest nutrition and development, Leaf area index, Principal component analysis

## 2.1. Introduction

Brazil is a global reference in forest management for wood production (e.g., pulp, wood charcoal, wood panels and laminate, and solid wood products), with large areas of mainly *Eucalyptus* species planted in acidic and poorly fertile soils (Gonçalves et al., 2020; IBA, 2020; Scolforo et al., 2019). Soil acidity causes serious limitations in plant growth and productivity (FAO, 2015). Acidic soils occupy approximately 30% of the world's agricultural area, and 60% of these areas are covered by forests (von Uexküll and Mutert, 1995). In Brazil, approximately 50% of the soil is acidic and has a high content of available aluminum ($Al^{3+}$), which may cause plant-physiological and nutritional disorders (Eswaran et al., 1997; Silva et al., 2004).

Liming has been used as the main management practice, especially through dolomitic limestone [i.e., $CaMg(CO_3)_2$], to increase soil pH and base saturation (Fageria and Nascente, 2014; Holland et al., 2018). However, in soil under forest plantations, liming is not used to remedy soil acidity but to increase Ca and Mg availability and, consequently, plant growth (Rocha et al., 2019; Rodrigues et al., 2016).

Genetic variants of *Eucalyptus* that are more responsive to mineral fertilizer and have higher stemwood production potential have been widely planted, and micronutrient deficiency can appear in these plants. For example, Cu and Zn deficiency are generally associated with Brazilian Cerrado (savanna) soils (Rodrigues et al., 2012). These micronutrients are distributed in many forms in soils, such as solid phases, free ions in the solution, soluble organomineral complexes, or adsorbed on colloidal particles (Singh and Agrawal, 2008). The behavior and mobility of Cu and Zn in the soil are dependent on the pH, base saturation (BS), cation exchange capacity (CEC), particle size, soil organic matter content (SOM), hydroxides (especially Fe, Mn, and Al), and soil microbial activity (Kabata-Pendias, 2011).

Soil pH is one of the main factors controlling micronutrient availability. Increasing soil pH can reduce Cu and Zn content in the soil (Havlin et al., 2013), and lime is the factor that most influences the adsorption of metals (e.g., Cu and Zn) in the topsoil (Joris et al., 2012). Copper and Zn are transition metals predominantly found in soil solutions as $Cu^{2+}$ and $Zn^{2+}$ cations (Aubert and Pinta, 1980). Copper has low mobility in the soil (Oliveira and Mattiazzo, 2001), and the total Cu content in the soil is related to humified organic matter, in which occlusion and coprecipitation are involved in nonspecific adsorption of Cu (Aubert et al., 2010). On the other hand, Zn mobility depends on soil pH (Fageria and Nascente, 2014), and soil Zn content varies with the type of parent material, humus, and clay content.

There is yet to be a consensus on the recommended levels of micronutrient fertilization (in particular, Cu and Zn) in forest plantations. In Brazil, e.g, most forestry companies apply micronutrients, mainly B, Cu, and Zn, to a lesser extent, mixed with NPK fertilizers (Gonçalves and Valeri, 2001). Under these circumstances, studies regarding micronutrient fertilization are needed to evaluate the appropriate rates for plant growth in the long term, and to evaluate the effects of fertilizers on micronutrient availability and uptake by plants (Fageria et al., 2002).

There have been a number of studies of the use of lime to improve soil conditions for plant growth, mainly focusing on soil pH (Caires et al., 2011; Carmeis Filho et al., 2017; Holland et al., 2018). However, little emphasis has been placed on a strategy to combine the application of lime and Cu plus Zn fertilizer in highly weathered tropical soils, such as Ferralsol, to enhance plant development under field conditions, regardless of the crop. To our knowledge, few researchers have investigated the effect of lime and Cu plus Zn on the dynamics of nutrients between different soil fractions and, consequently, the effect of these fertilizer applications on nutritional status and plant growth on field-trial setup.

In this context, assessing only the available contents of nutrients linked to soluble fractions does not provide a correct assessment of the total levels of nutrients in the soil (including Cu and Zn) for plant uptake (Leite et al., 2020). To overcome this deficiency, the technique of sequential extraction can be applied to evaluate the effect of crop management practices on the distribution of nutrients between distinguished soil fractions (Silveira et al., 2006). In the present study, we investigated the combined application of dolomitic limestone (lime) and Cu plus Zn fertilizer on soil chemical attributes and its influence on nutrition and yield during the growth stages of the *Eucalyptus grandis* plantation. The hypotheses tested in our study were: i) lime may offset undesirable implications on Cu and Zn availability, and this strategy can lead to a lower concentration of these metals in eucalyptus-trees; and ii) combined application of lime and Cu plus Zn would fulfill requirements of *Eucalyptus* to improve their nutritional status with these micronutrients, and upregulating stem volume yield. To test the hypotheses and clearly interpret these effects in the soil-eucalyptus system, the following traits related to soil and plantation were evaluated after trial setup: soil pH monitoring, soil chemical attributes, sequential extraction of Cu and Zn in soil, plant nutrition, leaf area index, and growth of the *Eucalyptus grandis* plantation.

## 2.2. Materials and methods

### 2.2.1. Site description

The study was carried out in a medium texture soil which represents Rhodic Ferralsol (IUSS Working Group WRB, 2015) or "Latossolo Vermelho-Amarelo distrófico [Brazilian Soil Classification System (Santos et al., 2018)] in Itatinga, State of São Paulo, Brazil (23°03 S; 48°37'W). The topography was flat, and the main minerals were quartz, kaolinite, Fe, and Al oxyhydroxides. In this region, the climate classification (Köppen) is $Cf_a$, with a mean annual rainfall of ~1,300 mm, average temperature of ~20°C, in the dry season (from April to September) and in the wet season (from October to March) [Supplementary Fig. S1 (Alvares et al., 2013)].

Before setting up the experiment, physical (Camargo et al., 1986) and chemical attributes (Raij et al., 2001) were measured at different depths (Supplementary Table S1).

These analyses showed variation from 13 to 18% for clay, 2 to 3% for silt, and 79 to 83% for sand at different soil depths. In addition, the soil has low natural fertility (i.e., very low base saturation) for annual crops according to Raij et al. (1996), and low availability of P, K, Ca, Mg, Mn, and Zn, and high Cu and Fe levels for *Eucalyptus* plants, according to Gonçalves (2010).

### 2.2.2. Experimental design and treatments

An experiment was set up in a randomized complete block design, with four replicates, in a factorial scheme of 3 × 2, with three dolomitic limestone rates (lime) and two Cu plus Zn fertilizer rates (fert), for a total of six treatments. The treatments of lime were: no lime rate (NLR, 0.0 Mg $ha^{-1}$); low lime rate (LLR, 1.6 Mg $ha^{-1}$); and high lime rate (HLR, 3.7 Mg $ha^{-1}$). The rate of LLR was calculated using the equation: 20 - [Ca + Mg] /10 according to Gonçalves (2010) for *Eucalyptus* plantations and rate of HLR was calculated by base saturation up to 60% according to Raij et al. (1996) for annual crops, for example, soybean (*Glycine max* L.). Lime composition had 40% of calcium oxide (CaO), 10% magnesium oxide (MgO), and 85% effective calcium carbonate equivalent (ECCE)]. The treatments of fert were: no Cu plus Zn (-CuZn, 0.0 kg $ha^{-1}$); and 0.5 kg $ha^{-1}$ Cu plus 1.5 kg $ha^{-1}$ Zn (+CuZn), as recommended by Gonçalves (2010). The source of fertilizer was copper sulfate (25% Cu) and zinc sulfate (21% Zn).

### 2.2.3. Plant material and crop management

Soil preparation was performed with a forest subsoiler at 0.4 m depth, using a plow disk attached to a mechanical tractor on the planting range. Lime were added in the planting range of 1.0 m in width, with incorporation of 0.2 m in depth, on the fifteenth day before planting *Eucalyptus* seedlings. In Brazil, lime is usually added to the total area until three months before planting *Eucalyptus* seedlings and without soil incorporation. In this trial setup, we incorporated lime into the soil to accelerate the reaction and to increase soil pH, in order to assess the changes in Cu and Zn availability and to evaluate if this strategy leads to the reduction of these metals in plants.

Three-month old seedlings of full-sib progeny of *Eucalyptus grandis* Hill Ex Maiden (from Coff's Harbor), were planted in July 2016, in an experimental area of 1.2 ha at 3 × 2 m spacing (1,667 trees $ha^{-1}$). Each plot consisted of 9 × 9 trees, with 5 × 5 trees in inner plot, and with a double border (Supplementary Fig. S2). Fertilization with Cu and Zn (25% Cu and 21% Zn) was added immediately after planting, with a side hole close to the seedling. In addition, in all treatments, equal amounts of NPK [60 kg $ha^{-1}$ of N (ammonium sulfate, 21% N), 60 kg $ha^{-1}$ $P_2O_5$, (triple superphosphate, 46% $P_2O_5$), 140 kg $ha^{-1}$ $K_2O$ (potassium chloride, 60% $K_2O$), and 5 kg $ha^{-1}$ B (borax, 11% B)] were added manually to the soil, close to the seedling on planting and at 3 and 6 months after planting (Supplementary Table S2).

### 2.2.4. Soil pH monitoring

The pH was measured only in the soil samples without (NLR) and with lime (LLR, and HLR) rates, because we believe that the application of Cu (0.5 kg $ha^{-1}$) and Zn (1.5 kg $ha^{-1}$) in a small side hole cannot significantly modify the soil pH at the level of experimental plots. Soil samples were collected with a Dutch type auger at five sampling points on the main diagonal of inner plots (Supplementary Fig. S2). These soil samples were collected close to the planting line and 0.2 m from the trees, at soil depths of 0-0.05, 0.05-0.10, 0.10-0.20 m, at 2, 4, 6, 8, 12, 15 months after planting. At 18 and 30 months, soil samples were collected only at 0-0.20 m layer of the soil. These soil samples were homogenized, dried at 45°C, and sieved through a 2.0-mm mesh. Thereafter, the soil sample pH in 0.01 mol $L^{-1}$ $CaCl_2$ was determined according to the method of Raij et al. (2001).

### 2.2.5. Soil chemical attributes

Soil samples were collected using a Dutch auger at depths of 0-0.2 m at 6, 18, and 30 months after planting, and at a depth of 0.2-0.4 m at 18 and 30 months. Each soil sample was composed of nine subsamples distributed within the useful area of each plot, close to the trees in the planting lines and close to the places where lime and mineral fertilizers were added (Supplementary Fig. S2). Each sample was homogenized and dried at 45°C and sieved through a 2-mm mesh. Soil chemical attributes, namely pH, soil organic matter (SOM), P, Ca, Mg, K, Al, and total acidity (H+Al), were determined or calculated, as were the effective cation exchange capacity ($_{e}$CEC), base saturation (BS), and levels of B, Cu, Fe, Mn, and Zn. In these analyses, the pH was determined in a $CaCl_2$ solution (0.01 mol $L^{-1}$); H+Al was extracted with SMP buffer, SOM content was obtained by wet oxidation, P, Ca, Mg, and K were extracted by the ionic exchange resin method; Al was extracted by 1 mol $L^{-1}$ KCl; B by the hot water extraction method; and Cu, Fe, Mn, and Zn by the DTPA method, according to Raij et al. (2001).

### 2.2.6. Sequential extraction of Cu, Fe, Mn, and Zn

Soil samples were sequentially extracted only in treatments under lime (NLR, LLR, and HLR treatments) at depths of 0-0.2 m at 6 and 30 months after planting. Soil samples were composed of nine subsamples, which were distributed within the useful area of each plot and close to the trees (Supplementary Fig. S2). These soil samples were then homogenized, dried in fresh air, and sieved (particle diameter < 150 μm). Afterwards the distribution of Cu, Fe, Mn, and Zn in 2 g of soil samples was determined in exchangeable fractions: soluble (Exc or F1); bound to carbonate-soluble in acid (Carb or F2); bound to organic matter, oxidizable (OM or F3); bound to oxide-reducible (Oxi or F4); and residual bound to silicate minerals (Res or F5), following the method reported by Silveira et al. (2006) and modified by Colzato et al. (2018).

The Exc fraction was extracted with 15 mL 0.1 mol $L^{-1}$ $CaCl_2$ solution, shaken for 2 h at 180 rpm, and then centrifuged at 3,000 rpm for 10 min at room temperature; Carb with 30 mL 1.0 mol $L^{-1}$ $CH_3COONa$ at pH 5.0, shaken for 5 h at 180 rpm, and then centrifuged at 3,000 rpm for 10 min at room temperature; OM in solution with 5 mL NaOCl centrifuged at 3,000 rpm for 10 min with pH adjusted to 8.5, at 90°C for 30 min; Oxi using 40 mL 0.2 mol $L^{-1}$ ammonium oxalate, 0.2 mol $L^{-1}$ oxalic acid, and 0.1 mol $L^{-1}$ ascorbic acid was centrifuged at 3,000 rpm for 10 min, and pH was adjusted to 3.0 for 30 min at 90°C, the entire time in the dark to avoid degradation of oxalate. The Res fraction in a solution of 9 mL $HNO_3$ and 3 mL HCl was then digested in a microwave for 5.5 min until the temperature reached 175°C and maintained for an additional 4.5 min at 175°C.

Additionally, pseudo-total (PST) Cu, Fe, Mn, and Zn contents were determined by the same method used in the Res fraction ($HNO_3$ + HCl), which is used to estimate the recovery of each nutrient (Eq. (1) and Eq. (2)), according to Leite et al. (2020). The standard reference material 2709a San Joaquim Soil was used to ensure the accuracy and precision of the analytical methods. The extracts were quantified using plasma optical emission spectrometry (ICP-OES), with quantification limit of 0.1 mg $L^{-1}$ for Fe and 0.01 mg $L^{-1}$ for Cu, Mn, and Zn.

$$Xi_{SUM} = Xi_{Exc} + Xi_{Carb} + Xi_{OM} + Xi_{Oxi} + Xi_{Res} \quad (1)$$

$$\text{Recovery (\%)} = (X_{SUM}/X_{PST}) \times 100, \quad (2)$$

where: Xi is the specific nutrient, SUM is the sum of content from all fractions (Exc, Carb, OM, Oxi, and Res), and PST = pseudo-total content.

### 2.2.7. Nutritional performance

Leaves were obtained at different stages of *Eucalyptus* growth (6, 12, 18, 24, and 30 months after planting), alternating between the wet season (October to March) and dry season (April to September). Leaves were collected from five medium trees located preferably in the diagonal transect of the useful area of the plots (Supplementary Fig. S2), in the upper third of the tree canopy (16 leaves per plant and 80 leaves per plot). These leaves were removed between the third and fifth insertion from the tip of the branch (Gonçalves, 2010). Leaves were then grouped by plot and dried at 60°C, ground in a Willey Mill, and N, P, Ca, Mg, S, B, Cu, Fe, Mn, and Zn contents were obtained according to Malavolta et al. (1997). In these analyses, N extraction was determined using micro-Kjeldahl analytical method after sulphuric acid digestion of plant material, B extraction was performed by dry digestion and quantification of the extracts by calorimetry, and other nutrients were extracted with nitric perchloric digestion and quantified by ICP-OES.

### 2.2.8. Dendrometric measurements

The diameter of all trees in the plots were measured at breast height (DBH), at 1.3 m above ground level, and tree height (H) was measured at 12, 18, 24, and 30 months after planting. In the measurements at 12 and 30 months after planting, 24 trees situated at the border of the plots were cut—representative of field-trial set up (Supplementary Fig. S2)—for rigorous cubing, which allowed us to adjust the equations for the stemwood volume estimate of solid over-bark per individual tree (VOL, $m^3$ $tree^{-1}$). The sample trees were measured along the trunk, from the base (0 m) and each successive meter to the total tree height. The VOL was calculated using the Smalian formula (Scolforo and Thiersch, 2004) estimated for plantations using the model adjusted by Schumacher and Hall (1933). The estimated equation for stemwood volume with bark at 12 months after planting ($VOL_{12}$) was used to calculate the volume at 12 months after planting (Eq. (3)). The estimated volume obtained from plants 30 months after planting ($VOL_{30}$) was used to estimate the VOL at 18, 24, and 30 months after planting (Eq. (4)). To estimate the wood volume produced per hectare in each treatment ($m^3$ $ha^{-1}$), the average of individual stem volume by plot was multiplied by 1667 (number of trees per hectare according to planting spacing).

$$VOL_{12} = -9.789 \times DBH^{(1.516) \times} H^{(1.152)}; (R^2_{aj.} = 0.965; P < 0.0001) \quad (3)$$

$$VOL_{30} = -11.033 \times DBH^{(1.873)} \times H^{(1.408)}; (R^2_{aj.} = 0.985; P < 0.0001) \quad (4)$$

where VOL is the stemwood volume with bark ($m^3$ $tree^{-1}$), DBH is the diameter at breast height (cm), and H is tree height (m).

### 2.2.9. Leaf area index

The leaf area index (LAI) was estimated using a nondestructive method [LAI-2200c plant canopy analyzer (LI-COR, 2016)] at 18, 21, 24, 27, and 30 months after planting. The measurements were carried out in the useful area of the plots, with a total of 24 measurement points of diffuse illumination, located below the canopy (Supplementary Fig. S3). Reference readings with a remote sensor positioned in a clearing to capture the incident radiation above the canopy, next to the field experiment, consisting of undergrowth and grasses, with a measurement interval of 15 s. All readings were performed at an angle of 10° to reduce the effect of edge and other variables of the plot, with an angled opening facing the side opposite the operator. Measurements were always taken during sunrise, at approximately 6:30 until 8:00.

### 2.2.10. Data analysis

Data were subjected to a two-way analysis of variance (ANOVA), with a P-value of 10%. The significance at the 10% level ($P < 0.10$) was selected because the field factors are highly complex compared to trials established under greenhouse conditions, for example. The data were checked for variance homogeneity and normal distribution through Levene and Shapiro-Wilk tests, respectively. Means were compared using the LSD test ($P < 0.10$) when the effect was significant. In addition, Pearson correlation analysis and principal component analysis (PCA) (to characterize treatments in function at the combined application of lime and Cu plus Zn) were employed for some set variables. Data were processed using SAS version 9.4 (SAS Institute Inc, 2016) and R version 4.0.2 (R Core Team, 2016).

## 2.3. Results

The individual effect of lime application changed the responses of some variables of the soil chemical attributes [e.g., increased soil pH (Fig. 1 and Table 1), while reduced availability of Cu content (Table 1)], planta nutritional status [e.g., increased leaf concentration of Ca (Fig. 3B)], and increased LAI in different months after planting (Table 2). Individual Cu plus Zn fertilization did not have a significant effect on the variables of soil chemical features and plant traits. Nonetheless, plant under treatment with the combined application of lime × Cu plus Zn increased the stemwood volume (VOL) and mean annual stemwood volume increment (MAI) as compared with plant under without lime × without Cu plus Zn (Fig. 4B, D). In addition, PCA analysis revealed that plants grown under treatments with lime × Cu plus Zn application were closely associated with the LAI and VOL, whereas the plants grown under treatments with no lime, no Cu, and no Zn application were distantly associated with LAI and VOL (Fig. 5D).

### 2.3.1. Soil pH monitoring showed different implications after lime application in soil

Low lime rate (LLR) and high lime rare (HLR) increased soil pH as compared with no-lime rate (NLR), regardless of soil depths at 2 until 30 months after planting (Fig. 1). The soil pH was higher in the first soil depths (0-0.05–0.05-0.10 m), mainly in the first months after planting (Fig. 1A, B). In the 0-0.05 m depth, the increase in pH was very fast in the first two months, and generally decreased in the succeeding months after planting (Fig. 1A).

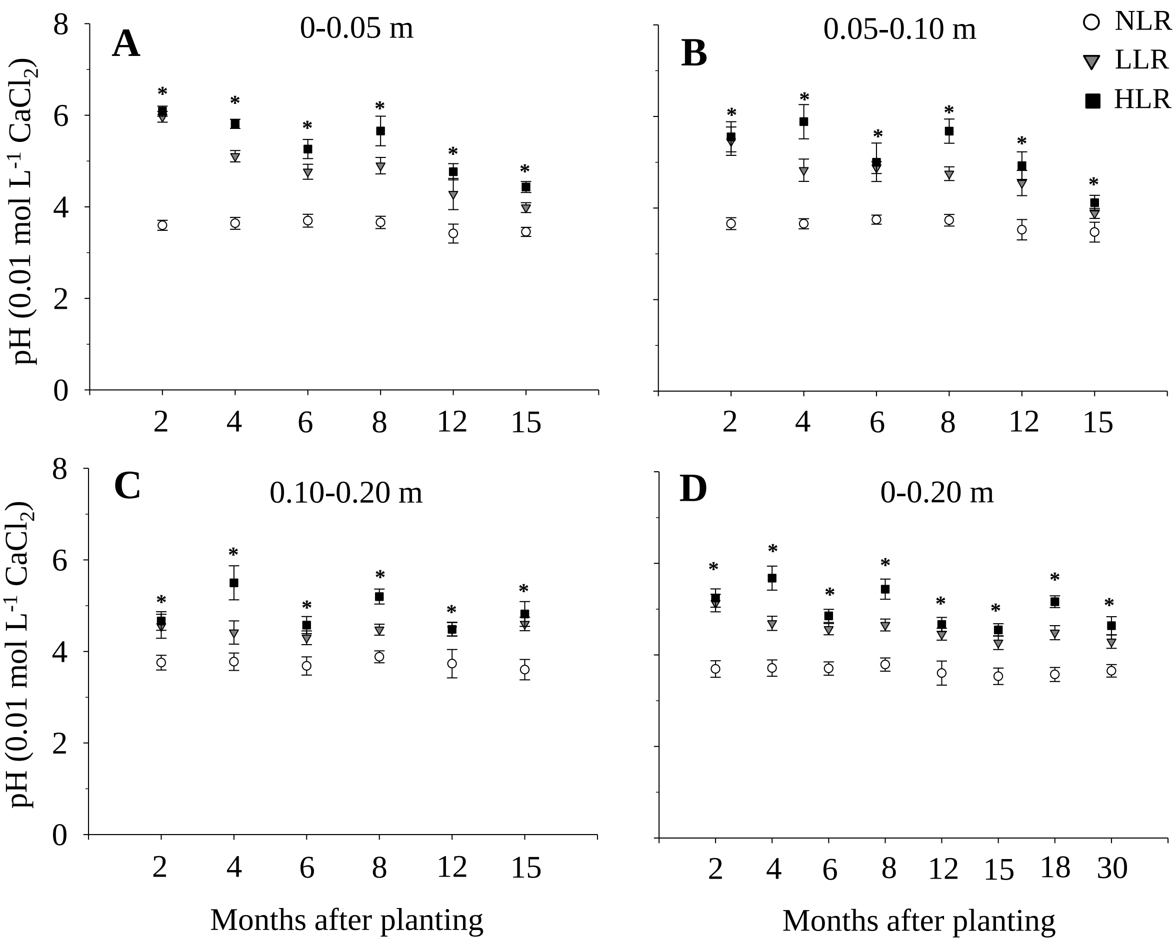



**Fig.1.** Soil pH at the depths (m) of 0-0.05 (A), 0.05-0.10 (B), 0.10-0.20 (C) and 0-0.20 (D), at the 2, 4, 6, 8, 12, 15, and 18 and 30 (only 0-0.20 m) months after planting *Eucalyptus grandis* seedlings, under dolomitic limestone application (lime). Treatment of lime: no lime rate - NLR (0.0 Mg $ha^{-1}$), low lime rate - LLR (1.6 Mg $ha^{-1}$, calculated by 20-[Ca+Mg]/10) and high lime rate - HLR (3.7 Mg $ha^{-1}$, calculated by base saturation up to 60%). Vertical bars stand for standard error and ***** to differences by *F*-test ($P < 0.10$) (n = 4).

In the depth range of 0-0.20 m, the soil pH varied from 3.5 to 3.8 in no-lime (NLR) and from 4.3 to 5.7 in soil with lime (i.e., LLR + HLR treatments) on different months after planting (Fig. 1D). In addition, the soil pH increased by 17 to 39% (0.6 to 1.4 units) under LLR treatment and by 27 to 53% (1.0 to 2.0 units) under HLR treatments as compared with NLR treatment, at 0-0.20 m soil depth among 2 to 30 months after planting (Fig. 1D). Overall, the maximum pH increase was obtained at 18 months for 0-20 cm depth (an increase of 0.9 units in LLR, and 1.6 units in HLR), and there was no difference in soil pH between the treatments under both lime rates (LLR and HLR treatments).

### 2.3.2. Soil chemical features were affected differently after lime application

Changes in soil chemical attributes (0-0.2 m depth) were more intense in the treatment under high lime rate (HLR) (Table 1). Lime (regardless of rate) increased pH, Ca, Mg, and Mn soil-availabilities, BS values, and decreased Al, Cu, Fe, and Zn soil-availabilities and H+Al values at 6, 18, and 30 (except for Zn) months after planting (Table 1). However, at 0.2-0.4 m depth at 18 and 30 months after planting, lime increased only soil pH, Ca and Mg availabilities, BS values, and decreased Al availability and H+Al values. At 0-0.2 m depth, treatments under high lime rates showed 63%, 62%, and 46% of BS at 0-0.2 m at 6, 18, and 30 months after planting, respectively. There was no difference in the SOM content as a function of lime treatment at all depths after planting. The treatment under high rate of lime increased available contents of soil P and K only in the 0-0.2 m depth; however, P contents enhanced only on 18-, K at 6 and 18-months after planting, whereas in this treatment (HLR), the available B content decreased at 6 and 18 months after planting relative to the LLR and NLR treatments (Table 1).

**Table 1**
Soil chemical attributes on different depths (0.0-0.2 and 0.2-0.4 m) at the 6 (only 0-0.2 m depth), 18, and 30 months after planting *Eucalyptus grandis* seedlings, under dolomitic limestone application – lime (mean±standard error, n=4).

| Lime | pH | SOM | P | Ca | Mg | K | Al | H+Al | $_e$CEC | BS | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.01 M $CaCl_2$ | g $kg^{-1}$ | mg $kg^{-1}$ | ---------- mmolc $kg^{-1}$ ---------- | | | | | | % | ---------- mg $kg^{-1}$ ---------- | | | | |
| | <u>6 months</u> | | | | | | | | | | | | | | |
| | *0-0.2 m* | | | | | | | | | | | | | | |
| NLR | 3.6±0.0 c | 21.8±0.7 a | 3.7±0.2 a | 0.7±0.0 c | 0.5±0.1 c | 0.19±0.02 b | 14.0±0.4 a | 60.2±2.4 a | 15.4±0.3 c | 2.2±0.2 c | 0.76±0.15 a | 0.71±0.03 a | 78.4±4.0 a | 0.97±0.10 a | 0.24±0.01 a |
| LLR | 4.3±0.1 b | 20.7±1.0 a | 3.5±0.1 a | 13.6±0.2 b | 8.4±0.4 b | 0.20±0.02 b | 7.2±0.4 b | 37.8±2.6 b | 29.5±0.5 b | 37.4±1.3 b | 0.66±0.09 a | 0.62±0.03 b | 51.4±2.4 b | 0.95±0.06 a | 0.21±0.02 b |
| HLR | 4.9±0.1 a | 20.8±1.2 a | 3.6±0.2 a | 32.3±0.6 a | 14.6±0.3 a | 0.26±0.01 a | 4.9±0.3 c | 28.2±2.6 c | 52.0±0.5 a | 63.0±2.1 a | 0.48±0.04 b | 0.58±0.02 b | 44.4±2.5 c | 0.91±0.04 a | 0.18±0.01 b |
| | <u>18 months</u> | | | | | | | | | | | | | | |
| | *0-0.2 m* | | | | | | | | | | | | | | |
| NLR | 3.6±0.0 c | 21.4±0.2 a | 4.8±0.3 b | 1.1±0.2 c | 0.4±0.1 c | 0.76±0.02 a | 10.7±0.5 a | 83.6±2.1 a | 12.9±0.7 c | 2.6±0.3 c | 0.81±0.48 a | 0.48±0.02 a | 62.4±2.6 a | 0.52±0.03 b | 0.15±0.01 a |
| LLR | 4.5±0.2 b | 21.5±0.5 a | 5.5±0.2 b | 12.5±1.9 b | 7.1±0.7 b | 0.70±0.02 a | 3.5±0.7 b | 36.9±3.6 b | 23.7±1.9 b | 35.9±4.9 b | 0.70±0.09 a | 0.43±0.02 b | 45.1±1.8 b | 1.05±0.15 a | 0.12±0.01 b |
| HLR | 5.2±0.1 a | 20.6±0.5 a | 6.7±0.5 a | 26.1±4.1 a | 11.9±1.8 a | 0.75±0.08 a | 0.7±0.1 c | 22.7±1.5 c | 39.5±5.6 a | 61.8±4.1 a | 0.57±0.02 b | 0.37±0.02 c | 29.2±1.7 c | 1.05±0.13 a | 0.12±0.01 b |
| | *0.2-0.4 m* | | | | | | | | | | | | | | |
| NLR | 3.8±0.0 b | 16.5±0.1 a | 3.6±0.2 a | 1.8±0.3 c | 0.5±0.1 c | 0.49±0.03 a | 7.8±0.2 a | 46.5±0.8 a | 10.5±0.3 a | 5.5±0.6 c | 0.56±0.05 a | 0.68±0.01 a | 42.6±2.1 a | 0.28±0.02 a | 0.10±0.01 a |
| LLR | 4.0±0.1 a | 17.0±0.3 a | 4.5±0.6 a | 2.8±0.3 b | 1.7±0.3 b | 0.50±0.03 a | 5.4±0.5 b | 36.6±1.3 b | 10.4±0.2 a | 12.1±1.1 b | 0.51±0.03 a | 0.65±0.03 a | 41.5±2.3 b | 0.33±0.03 a | 0.08±0.01 a |
| HLR | 4.1±0.1 a | 17.1±0.5 a | 3.9±0.5 a | 4.3±0.5 a | 2.4±0.2 a | 0.56±0.02 a | 4.6±0.3 b | 36.3±1.2 b | 11.9±0.7 a | 16.8±1.6 a | 0.44±0.02 a | 0.60±0.04 a | 31.8±2.1 b | 0.39±0.06 a | 0.09±0.01 a |
| | <u>30 months</u> | | | | | | | | | | | | | | |
| | *0-0.2 m* | | | | | | | | | | | | | | |
| NLR | 3.7±0.0 c | 20.5±0.4 a | 6.7±0.4 a | 0.8±0.4 c | 0.6±0.0 b | 0.60±0.03 a | 9.7±0.3 a | 66.6±2.5 a | 11.7±0.6 c | 3.0±0.6 c | 0.49±0.12 a | 0.67±0.02 a | 76.0±4.2 a | 0.46±0.04 b | 0.19±0.01 a |
| LLR | 4.3±0.1 b | 21.5±0.5 a | 6.4±0.5 a | 12.7±0.9 b | 7.7±1.1 a | 0.50±0.06 a | 3.7±0.7 b | 37.0±3.0 b | 24.6±1.4 b | 36.4±3.4 b | 0.48±0.01 a | 0.58±0.02 b | 66.1±4.7 a | 0.76±0.06 a | 0.18±0.01 a |
| HLR | 4.6±0.2 a | 20.8±0.7 a | 6.1±0.5 a | 18.5±0.3 a | 8.9±1.0 a | 0.48±0.03 a | 3.0±0.6 b | 33.6±3.7 b | 30.9±0.7 a | 46.1±3.1 a | 0.48±0.03 a | 0.51±0.03 c | 52.32.0 b | 0.72±0.04 a | 0.18±0.02 a |
| | *0.2-0.4 m* | | | | | | | | | | | | | | |
| NLR | 3.9±0.1 a | 16.4±0.1 a | 5.9±3.0 a | 0.6±0.0 b | 0.5±0.1 b | 0.40±0.05 a | 7.3±0.1 a | 44.2±1.1 a | 8.9±0.6 a | 3.3±0.3 b | 0.50±0.01 a | 0.79±0.02 a | 45.0±2.2 a | 0.39±0.09 a | 0.14±0.01 a |
| LLR | 3.9±0.0 a | 17.2±0.4 a | 6.7±2.6 a | 1.6±0.1 a | 1.2±0.2 a | 0.46±0.04 a | 6.8±0.4 a | 40.9±1.9 a | 10.1±0.7 a | 7.6±0.8 a | 0.41±0.04 a | 0.78±0.02 a | 49.0±2.9 a | 0.27±0.05 a | 0.17±0.02 a |
| HLR | 4.0±0.1 a | 17.2±0.5 a | 5.5±1.6 a | 2.1±0.6 a | 1.6±0.3 a | 0.41±0.04 a | 5.4±0.4 b | 35.7±1.9 b | 9.5±0.4 a | 10.3±2.2 a | 0.41±0.01 a | 0.74±0.01 a | 45.4±3.4 a | 0.24±0.02 a | 0.18±0.03 a |

Treatment of lime: Treatment of lime: no lime rate - NLR (0.0 Mg $ha^{-1}$ ), low lime rate - LLR (1.6 Mg $ha^{-1}$, calculated by 20-[Ca+Mg]/10) and high lime rate - HLR (3.7 Mg $ha^{-1}$, calculated by base saturation up to 60%). Chemical analysis according Raij et al. (2001), where: pH in 0.01 mol $L^{-1}$ $CaCl_2$; SOM = soil organic matter; H+Al = total acidity; $_e$CEC = effective cation exchange capacity, and BS = base saturation in percentage (%). Different letters in the columns and depths indicate differences by LSD test ($P < 0.10$).

### 2.3.3. Lime application changed the contents of Cu, Fe, Mn, and Zn in different soil fractions

Contents of Fe in the Carb, and Mn in the Carb and OM fractions were lower than the quantification limit (i.e., Fe 0.1 mg $L^{-1}$ and Mn 0.01 mg $L^{-1}$). The recoveries in the soil (average of all treatments at the 0-0.2 m depth) were Cu 160%, Fe 112%, Mn 124%, and Zn 167% at 6 and 30 months after planting. In surface horizon at 6 and 30 months after planting, the total contents for micronutrient were in the following order: Fe (16.6 to 21.1 g $kg^{-1}$) > Mn (58.5 to 63.6 mg $kg^{-1}$) > Cu (11.9 to 13.2 mg $kg^{-1}$) > Zn (8.2 to10.0 mg $kg^{-1}$) (Fig. 2).

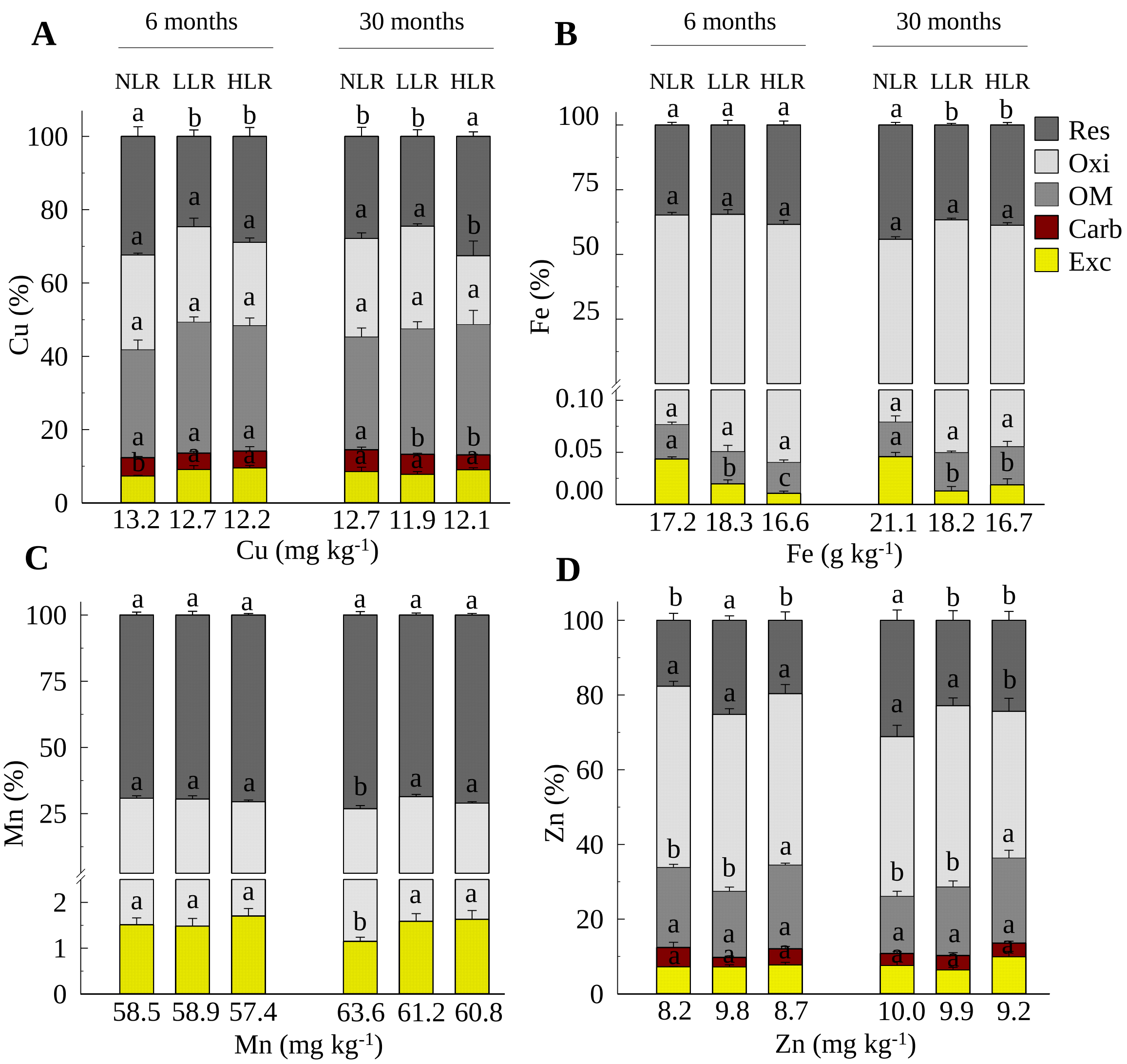



**Fig.2.** Relative values of Cu (A), Fe (B), Mn (C), and Zn (D) in soil fractions (0-0.2 m depth), on 6 and 30 months after planting *Eucalyptus grandis* seedlings, under dolomitic limestone application (lime). Fractions: exchangeable - soluble (Exc); bound to carbonates (Carb); bound to organic matter (OM); bound to oxides (Oxi); and residual - bound to silicate minerals (Res). Treatment of lime: no lime rate – NLR (0.0 Mg $ha^{-1}$), low lime rate – LLR (1.6 Mg $ha^{-1}$) and high lime rate – HLR (3.7 Mg $ha^{-1}$). In each month and fraction, means with standard error bars followed different letters indicate differences among treatments by LSD test ($P < 0.10$) (n=4).

Among the fractions, the concentrations followed the order: OM > Res > Oxi > Exc > Carb for Cu; Oxi > Res > Exc > OM > Carb for Fe; Res > Oxi > Exc > Carb ≈ OM for Mn; and Oxi > Res ≈ OM > Exc > Carb for Zn. In addition, among the treatments, the highest proportions of Cu were in the OM (ranged from 30 to 37%), Fe in Oxi (ranged from 56 to 66%), Mn in Res (ranged from 68 to 71%), and Zn in Oxi (ranged from 39 to 49%) (Fig. 2).

Lime decreased the content of Cu in the Res fraction at 6 months after planting and in Carb and Oxi fractions at 30 months, whereas it increased in Exc at 6 months and in the Res fraction at 30 months, mainly at higher rates of liming (Fig. 2A). The content of Fe decreased in the Exc fraction at 6 and 30 months after planting, and in the Res fraction at 30 months under both lime rates [i.e., LLR and HLR (Fig. 2B)]. Soil amended with lime improved the content of Mn in the Exc and Oxi fractions 30 months after planting (Fig. 2C). The higher rate of lime increased Zn content in the OM fraction at 6 and 30 months after planting, and in the Oxi fraction at 30 months, whereas the Zn content in the Res fraction was reduced 30 months after planting (Fig. 2D).

### 2.3.4. Individual lime application affected the nutritional status of *Eucalyptus* plantation

The leaf P concentration was higher in plants grown in limed treatments than in plants grown in unlimed treatment at 30 months after planting (Fig. 3A). During the stages of *Eucalyptus* growth (i.e., 6, 12, 18, 24, and 30 after planting), plants in the no-lime treatment (NLR) presented lower leaf concentrations of Ca and Mg than did plants in the limed treatments (LLR and HLR). In contrast, there were no differences in the leaf concentrations of these macronutrients between plants under LLR and HRL treatments, except at 6 months after planting, in which HLR had the highest Mg concentration (Fig. 3C). There was no effect of liming or Cu plus Zn fertilization on the N and S concentrations in the diagnostic leaves at 6, 12, 18, 24, and 30 months after planting; among these months, the leaves showed N concentrations from 26.0 to 28.9 g $kg^{-1}$ and S concentrations from 1.0 to 1.3 g $kg^{-1}$.

In all nutritional stages until 30 months after planting, there were no differences in leaf B concentrations between treatments under lime and no-lime conditions (Fig. 3D). Lime application reduced leaf Cu, Fe, Mn, and Zn concentrations at different stages of plant growth (Fig. 3E-H). Overall, the leaf P, Ca, Mg, B, Cu, and Mn concentrations were lower in the dry season (12 and 24 months after planting), whereas leaf Zn and Fe concentrations were lower in the wet season [6, 18, and 30 months after planting (Fig. 3)].

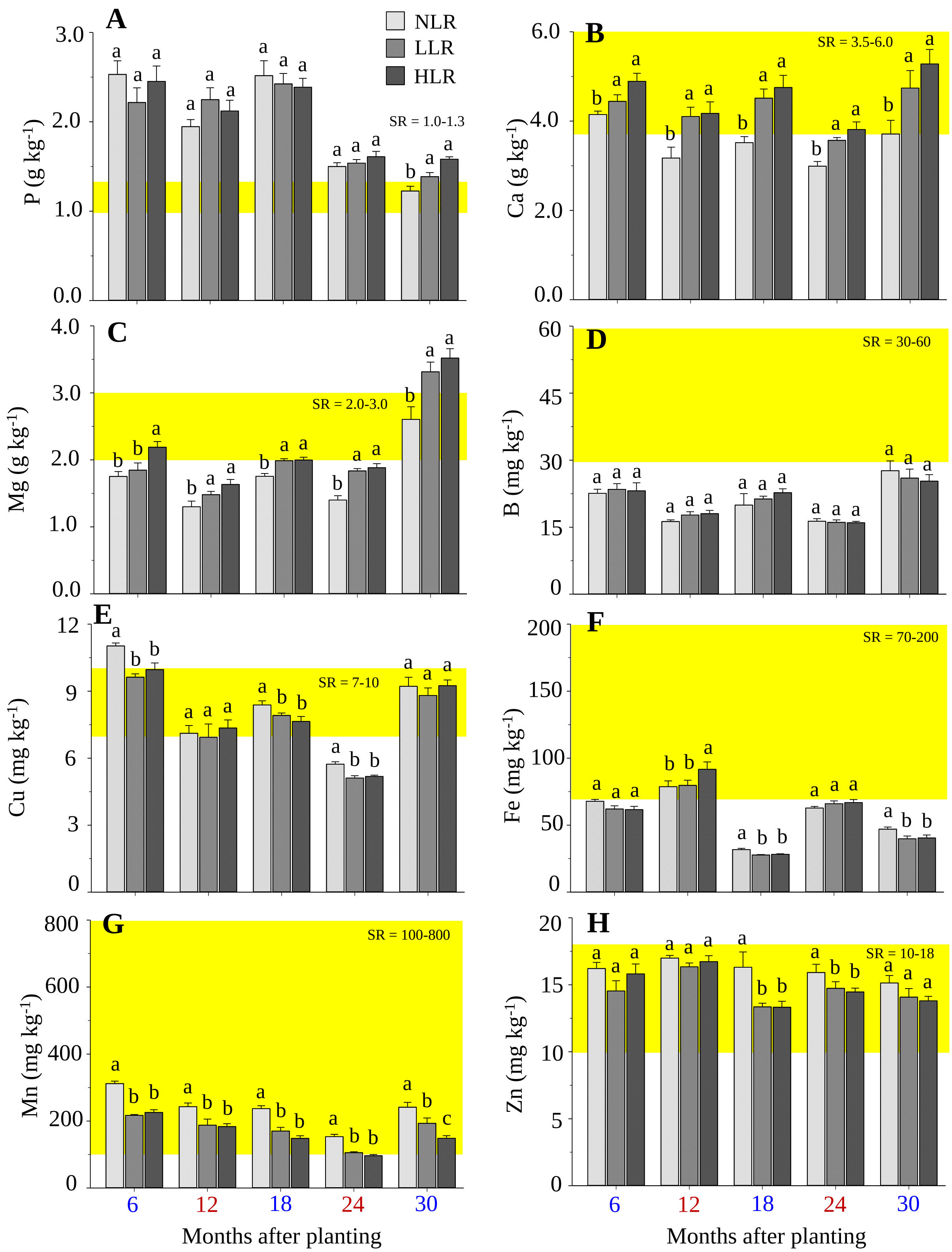



**Fig.3.** Leaf concentrations of P (A), Ca (B), Mg (C), B (D), Cu (E), Fe (F), Mn (G), and Zn (H) in *Eucalyptus grandis* at 6, 12, 18, 24 and 30 months after planting, under dolomitic limestone application (lime). Treatment of lime: no lime rate - NLR (0.0 Mg ha$^{-1}$ ), low lime rate - LLR (1.6 Mg ha$^{-1}$, calculated by 20-[Ca+Mg]/10) and high lime rate - HLR (3.7 Mg ha$^{-1}$, calculated by base saturation up to 60%). Sufficiency range – yellow color (SR): for *Eucalyptus* species more planted in Brazil (Gonçalves, 2010). In each month, means with standard error bars followed different letters indicate differences among treatments by LSD test ($P$ < 0.10) (n=4). In axis x, the blue numbers refer to wet season, while red numbers refer to dry season.

### 2.3.5. Lime application increased the leaf area index

Treatments with lime (LLR and HLR) enhanced the LAI in plants under rate recommendations for *Eucalyptus* plants (LLR) and annual crops (HLR) (Table 2). At 21, 24, 27, and 30 months after planting, the LAI variation in the no-lime treatment (NLR) was from 2.7 to 3.5 $m^2$ $m^{-2}$, whereas it varied from 3.1 to 4.0 $m^2$ $m^{-2}$ in the limed treatments. Plants treated with LLR and HLR did not show differences between themselves in the leaf area index. However, plant under these treatments increased LAI by 13% as compared with plant without lime treatment. Overall, there was a variation in the plant LAI among different months, with low LAI in plants in the dry season at 24 months after planting (Table. 2).

**Table 2**
Leaf area index (LAI) of *Eucalyptus grandis* plantation, at the 21, 24, 27, and 30 months after planting, under dolomitic limestone application – lime (mean ± standard error, n = 4).

| Treatments | 21 months | 24 months | 27 months | 30 months |
|---|---|---|---|---|
| | ------------------------------------------ $m^2$ $m^{-2}$ ------------------------------------------ | | | |
| NLR | 3.5±0.09 b | 2.7±0.10 b | 3.5±0.08 b | 3.0±0.12 b |
| LLR | 4.0±0.05 a | 3.1±0.09 a | 3.9±0.09 a | 3.3±0.10 a |
| HLR | 4.0±0.07 a | 3.1±0.08 a | 4.0±0.06 a | 3.4±0.08 a |

Treatment of lime: no lime rate - NLR (0.0 Mg $ha^{-1}$ ), low lime rate - LLR (1.6 Mg $ha^{-1}$, calculated by 20-[Ca+Mg]/10) and high lime rate - HLR (3.7 Mg $ha^{-1}$, calculated by base saturation up to 60%). Different letters in the columns indicate differences by LSD test ($P < 0.10$).

### 2.3.6. Combined application of lime and Cu plus Zn fertilizer improved the stem volume

The effect of Cu plus Zn fertilization (i.e., +CuZn treatments) within the liming treatments (i.e., NLR, LLR, and HLR) increased the volume of plants as compared with plant under treatments without Cu plus Zn fertilization (i.e., -CuZn treatments), regardless of the lime application rates at 12 months after planting (Fig. 4A). On the other hand, the effect of liming within the fertilizer treatment showed that the LLR and HLR treatment groups (both with lime) had higher volume than NLR (no-lime) under -CuZn and +CuZn treatments, except in the +CuZn treatment group at 12 months after planting (Fig. 4B). The stemwood volume yield ( $m^3$ $ha^{-1}$) varied from 5.4 to 7.4, from 30.0 to 38.6, from 59.7 to 71.5, and from 103.5 to 119.4 on trees at 6, 12, 18, 24, and 30 months after planting, respectively.

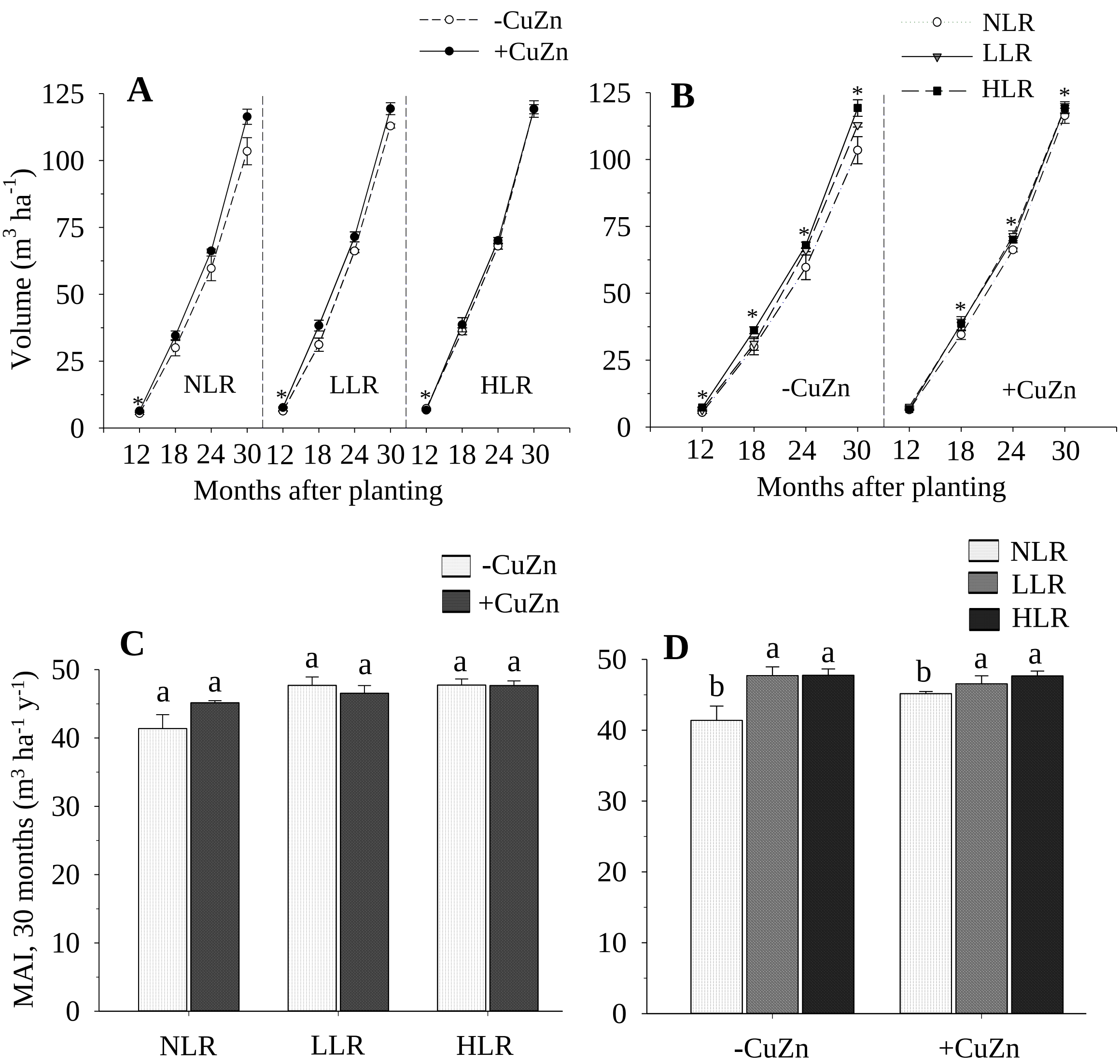



**Fig. 4.** Stemwood volume with bark and mean annual stemwood volume increment (MAI) of *Eucalyptus grandis,* under application of dolomitic limestone (lime) and Cu plus Zn fertilizer (fert), at the 12, 18, 24, and 30 for volume and MAI on 30 months after planting. Interaction effect of fert *inside* lime (A, C), and lime *inside* fert (B, D). In each level, means followed by * (A, B) represent differences by *F*-test ($P$ < 0.10) and different letters (C, D) represent differences by *LSD* test ($P$ < 0.10) (vertical bar = standard error, n = 4). Treatment of lime: no lime rate - NLR (0.0 Mg ha$^{-1}$ ), low lime rate - LLR (1.6 Mg ha$^{-1}$, calculated by 20-[Ca+Mg]/10) and high lime rate - HLR (3.7 Mg ha$^{-1}$, calculated by base saturation up to 60%). Treatment of fert: no Cu plus Zn (-CuZn), and 0.5 kg ha$^{-1}$ of Cu plus 1.5 kg ha$^{-1}$ of Zn (+CuZn).

The fertilizer effect within the lime treatments was not sufficient to increase the mean annual stemwood volume increment (MAI) of *Eucalyptus grandis* plants at 30 months after planting (Fig. 4C). During this period and the interaction of lime within the fertilizer treatment, there was an increase in the MAI in the plants grown under lime treatments as compared with plants grown under no-lime treatment, regardless of -CuZn and +CuZn treatments (Fig. 4D). In addition, under -CuZn, the plants grown under LLR and HLR increased MAI by 15% as compared with plants grown under NLR treatment, whereas under +CuZn, these treatments (LLR and HLR) showed an increase of 5% (i.e., 10% higher as compared with treatment under no-lime × no Cu plus Zn) of MAI as compared with the NLR-treated plants. Overall, plants amended under the higher lime rate (HLR; rate advised to annual crops) did not enhance the stemwood volume and MAI as compared with plants grown under the lower lime rate (LLR; rate advised for *Eucalyptus* plants), at 30 months after planting (Fig. 4D).

### 2.3.7. Correlation and PCA for some soil and plants responses with lime and Cu plus Zn application

Soil pH was correlated positively with Ca and Mg content, as well as to $_{e}$CEC and BS. But, for Cu and Fe soil-availabilities this correlation with soil pH was negative (Fig. 5A). In addition, the soil-availabilities of Cu, Fe, and Zn were correlated positively each other. In the *Eucalyptus* trees, the leaf concentration of P, Ca, and Mg, stem volume (VOL), and leaf area index (LAI) were correlated positively each other. On the other hand, the Mn concentration was correlated negatively with P, Ca, Mg, VOL, and LAI at 30 months after planting (Fig. 5A).

The first two components (PC1 and PC2) explained 98% of the total data variance; stronger explanatory power was shown in PC1 with respect to Exc, Carb and OM, and, at 30 months after planting, stronger explanatory power was evident in PC2 with respect to Oxi and Res (Fig. 5B). The copper contents were more associated with OM, Exc, and Carb fractions, Fe to Oxi, Mn to Res, and Zn to Oxi and Carb fractions. In addition, there was a positive correlation among Exc, Car, and OM, whereas these fractions presented a negative correlation as compared with the Oxi and Res fractions.

PC1 explained 58% and PC2 17% of the variation, with stronger prediction of pH, Ca, Mg, $_{e}$CEC, BS, and Fe in PC1, and stronger prediction of SOM, P, and B in PC2, at 0-0.2 m (Fig. *5C). The* treatment under no-lime rate (NLR) was characterized by higher values of B, Cu, Fe, Zn, and SOM, and lower values of pH, Ca, Mg, $_{e}$CEC, BS, and Mn. On the other hand, treatments under low lime rates (LLR) were characterized as having mean values for pH, P, Ca, Mg, $_{e}$CEC, BS, Mn, Cu, Fe, and Zn in LLR treatment, whereas under high lime rate (HLR), the treatments were characterized by higher values of pH, Ca, Mg, $_{e}$CEC, BS, and Mn and lower values of Cu, Fe, Mn, Zn, and SOM, at 6, 18, and 30 months after planting (Fig. 5C).

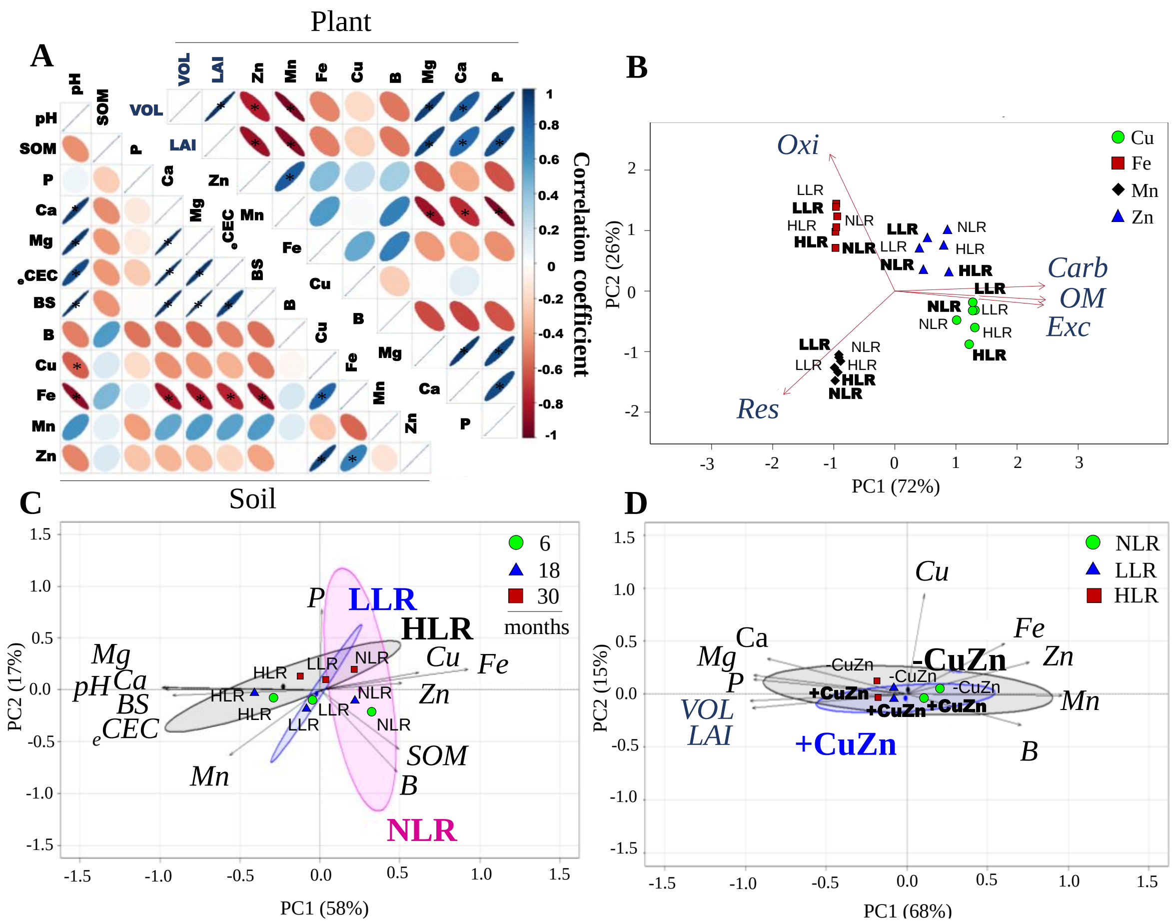



**Fig. 5.** Correlation ($P < 0.10$) for some soil chemical variables (0-0.2 m depth), leaf nutrient concentrations, volume with bark (VOL) and leaf area index (LAI) of *Eucalyptus grandis* (A) and principal component analysis (PCA): sequential extraction of Cu, Fe, Mn and Zn [fractions: exchangeable - soluble (Exc), bound to carbonates (Carb), bound to organic matter (OM), bound to oxides (Oxi), and residual - bound to silicate minerals (Res)] 30 months after planting (B), soil chemical attributes, evaluated on 6, 18 and 30 months after planting (C), and leaf nutrient concentrations, VOL and LAI at the 30 months after planting (D), under application of dolomitic limestone (lime) and Cu plus Zn fertilizer (fert). Treatment of lime: no lime rate - NLR (0.0 Mg $ha^{-1}$ ), low lime rate - LLR (1.6 Mg $ha^{-1}$, calculated by 20-[Ca+Mg]/10) and high lime rate - HLR (3.7 Mg $ha^{-1}$, calculated by base saturation up to 60%). Treatment of fert: no Cu plus Zn (-CuZn), and 0.5 kg $ha^{-1}$ of Cu plus 1.5 kg $ha^{-1}$ of Zn (+CuZn, highlighted in bold, B and D).

The plant's nutritional and productivity performance responses were explained by 68% in PC1 (stronger explication of P, Ca, Mg, Mn, LAI, and VOL) and 15% in PC2 (stronger weight of Cu), at 30 months after planting (Fig. 5D). In addition, the -CuZn treatments had greater variance than +CuZn, whereas higher VOL was obtained in plants with combined application of lime (i.e., LLR and HLR) and Cu plus Zn (i.e., +CuZn). With respect to the lime factor, plants under no-lime treatment were characterized by higher values of B, Cu, Fe, Mn, and Zn, and lower values of P, Ca, Mg, LAI, and VOL. The plants grown under low lime rate had lower values of B, Cu, Fe, Mn, and Zn and median values of P, Ca, Mg, LAI, and VOL, and plants grown under high lime rate had lower values of B, Cu, Fe, Mn, and Zn and larger values of P, Ca, Mg, LAI, and VOL (Fig. 5D).

## 2.4. Discussion

### 2.4.1. Soil chemical attributes was affected by lime application

The increase in soil pH after lime application (Fig. 1 and Table 1) is associated with a higher concentration of ions in the soil solution, which may reduce the exchangeable acidity (H+Al) and soil-availabilities of $Al^{3+}$ concentrations (Crusciol et al., 2019). Overall, soil pH is directly involved in soil macro- and micronutrient solubility, concentration, ionic form, and mobility. Consequently, soil pH directly influences nutrient uptake in plants (Holland et al., 2018). Liming potentially increased the dynamics of exchangeable cations in soils (e.g., Ferralsol) (Fageria and Nascente, 2014). Lime application triggered buffering processes that modified the dynamics of acidity, which was demonstrated by the increase in soil pH at different depths and over time, which was more remarkable within the upper soil layer during the first year after liming. These changes occur due to ligand exchange reactions with hydroxides associated with Al and Fe oxides, which displace $OH^-$ and promote a partial increase in soil pH (Bossolani et al., 2020).

Globally, there are few studies in which soil pH has been evaluated in detail under lime application in field conditions in *Eucalyptus* plantations during plant development (Rocha et al., 2019; Rodrigues et al., 2016). In the present study, we found that higher values of Ca, Mg, $_e$CEC, and BS were associated with the effect of lime at 6 months after planting, whereas the lower values of these variables were recorded in the months after, i.e., at 18 and 30 months after planting (Table 1). In this context, lime application increased soil pH, mainly in the most superficial layers (0-0.05 and 0.1-0.2 m depths) and in the first months after planting (Fig. 1). However, the soil pH in LLR was lower than in HLR, but in most months after planting, there was no difference in soil pH responses between the LLR and HLR treatments (Fig. 1). Considering only the pH values, the recommended lime rate for *Eucalyptus* plants (LLR treatments) probably enabled a pH range adequate to improve the Ca and Mg availability, however, it could reduce Cu and Zn soil-availabilities. The pH increased from 3.6 (NLR) to 4.3-4.5 (LLR) and 4.6-5.2 (HLR) (Table 1). The pH below 4.0 can locks most of the soil nutrients (e.g., P, K, Ca, and Mg; Table 1). The increase of about 0.8 points in pH for LLR could have been enough for improving the solubility and availability of the nutrients in the soil. Calcium and magnesium increased significantly because they were supplied with lime application. Copper and zinc content decreased in LLR and HLR, despite the increase in soil pH. In fact, they also decreased in NLR compared with the original soil

(1.1 mg kg$^{-1}$ of Cu and 0.4 mg kg$^{-1}$ of Zn for 0-0.2 m depth). So, they decreased in all lime treatments (NLR, LLR, HLR), although with a less decreased in NLR. Probably, fertilization with N and P will make Cu availability somewhat difficult (antagonism); the supply of P and Ca will make Zn availability somewhat difficult (antagonism); and fertilization with K slightly favored the availability of Mn (synergism) and, hence, that Mn did not cause the same problems as Cu and Zn.

The application of Cu and Zn did not influence the soil chemical attributes at 6, 18, and 30 months after planting, probably because of the low rate applied to the soil and probably also because they were not supplied in an efficient way. In this sense, it is recommended to study in the future their application through chelated fertilizer (Souri and Hatamian, 2019). On the other hand, lime application increased soil pH, Ca and Mg contents, $_{e}$CEC, and BS, whereas the values of Al, H+Al, Cu, Fe, and Zn decreased (Table 1). For some variables of the soil chemical attributes, there was no significant difference between treatments under application of low lime rate (LLR; 1.6 Mg ha$^{-1}$) and high lime rate (HLR; 3.7 Mg ha$^{-1}$) on soil depths. On the other hand, in cases where this effect was significant (e.g., pH, Ca, BS, and Cu), the HLR treatment was generally responsible for the higher contribution to changes in soil chemical attributes (Table 1).

Lime application directly interferes with soil physicochemical and biological attributes (González-Alcaraz et al., 2013). For example, lime potentially increased the availability of some macronutrients (e.g., Ca and Mg) in the soil, consequently benefiting the nutritional status of *Eucalyptus* plants, as it reduced the exchangeable Al concentration (Holland et al., 2018). Conversely, lime may reduce the availability of metallic micronutrients (e.g., Cu and Zn) (Aubert et al., 2010). In this context, Cu plus Zn fertilization can promote positive implications for increasing the Cu and Zn soil-bioavailabilities to benefit the nutritional status of *Eucalyptus* trees and improve plant development. Indeed, the effects of lime and mineral fertilization (such as Cu and Zn) are more intense during the initial stages of plant development (Gonçalves et al., 2013; Laclau et al., 2010).

Despite the lime effect being less remarkable within the depth range of 0.2-0.4 m, significant changes occurred mainly for Ca, Mg, Al, H+Al, and BS values (Table 1), probably because lime was incorporated in the surface horizon (0-0.2 m), and a gradual reaction of the lime within soil may have occurred in the transition depth with the subsurface horizon (0.2-0.4 m). Lime incorporation within soil is not a usual recommendation for the management of *Eucalyptus* plantations in Brazil (e.g., in Ferralsol). The incorporation of lime promotes mixing with the soil, resulting in a faster reaction between lime and the soil particles, while the lime application on the soil surface contributes to the longer reaction time of lime across the soil layers because of the alkalinization front (Joris et al., 2016). Another hypothesis may be the low mobility and solubility of carbonate in soil, and the dependence of the intensity of reaction on the soil physical-chemical-biological characteristics, as well as precipitation and the type of management (Crusciol et al., 2019).

The higher soil pH values and the lower Al, H+Al, Cu, Fe, and Zn (0-0.2 depth) contents in treatments with lime application (Table 1) can be explained by the increase in negative soil charges. In this hypothesis, the increase in soil pH accelerates the dissociation of $H^+$ ions bound to OH groups from MO and Fe and Al oxides, which will influence the increase in negative charges and greater adsorption of metallic cationic elements, such as Cu and Zn (Alleoni et al., 2005; Havlin et al., 2013). The availability of Cu, Fe, Mn, and Zn in soil is closely associated with the total content of these elements in the soil and available forms in soil solution and the values of soil pH, consequently lowering Al soil-availabilities (Fageria and Nascente, 2014). Soil adsorption reactions are important in determining the bioavailability of Cu, Fe, Mn, and Zn, which are not immediately available in solid phases. An increase in soil pH may have contributed to a decrease in the availability of Cu, Fe, and Zn contents and the interference in other variables of soil fertility, for example, reduced the values of Al and H+Al (Table 1). Lime application decreased the availability of Cu and Zn in the soil, however, the Cu content was reduced more significantly than that of Zn, and the lowest Cu content in the soil was found under HLR treatment (Table 1), thus, Cu was more adsorbed to fractions not immediately available to plants as compared with Zn. Copper has shown greater adsorption relative to Zn, and in the adsorption sites, Cu contributed to the reduction of the Zn bonds (Joris et al., 2012; Sipos et al., 2008).

Overall, lime management in *Eucalyptus* did not change the SOM, P, K, and B soil contents at different times after planting (Table 1). The absence of significant effects on these attributes, mainly for SOM, may result from interactions with the soil matrix, with emphasis on some chemical traits present in fatty acids (Yang et al., 2020). The SOM content in forest plantations can increase over time, and this trait contributes to enhancing soil moisture and improving microbial activity (Balland-Bolou-Bi et al., 2019; Pereira et al., 2019; Versini et al., 2014). These circumstances (mainly microbiological activity) may explain the higher Mn availability in soil under lime (Table 1), because the availability of Mn can be affected by the presence of organic ligands (Moreira et al., 2016; Pais and Jones, 1997). Indeed, changes in soil pH (Fig. 1) and its relationship with microbial activity are also relevant factors influencing Mn dynamics in soil, for example, root exudates can modify the Mn availability in soil (Alejandro et al., 2020).

As a rule, the availability of nutrients between treatments under low lime rate (LLR) and high lime rate (HLR) had contents within the same classes of interpretation of soil fertility for *Eucalyptus* plants according to Gonçalves (2010). Therefore, the recommended rate of lime for *Eucalyptus* plants [i.e., LLR treatment (Gonçalves, 2010)] potentially improved multiple benefits to plantations, such as supplying Ca and Mg levels to plants (Holland et al., 2018; Rocha et al., 2019).

### 2.4.2. The impact of lime application on soil chemical attributes regulated the Cu, Fe, Mn, and Zn contents in different soil fractions

Copper was more linked to OM and Res fractions, Fe to the Oxi and Res fractions, Mn to the Res and Oxi fractions, and Zn to the Oxi, Res, and OM fractions in soil amended with lime application and under *Eucalyptus grandis* plantation (Fig. 2). The contents of Cu, Fe, Mn, and Zn linked to the Res fraction may be associated with Mn and Fe oxides. However, changes in soil pH and redox potential affect the availability and mobility of these metals in the soil (Ahnstrom and Parker, 1999; Silveira et al., 2006; Tessier et al., 1979). The contents of Cu, Fe, Mn, and Zn are more related to more recalcitrant compounds or non-labile compounds in the soil, in which oxides predominate, such as Mn and amorphous oxides, and Fe crystalline oxides, especially in highly weathered tropical soils (Silveira et al., 2006). Although the contents of these micronutrients were more linked with the Res or Oxi fractions in all treatments, the higher Cu values (ranging from 30 to 37%) and Zn (ranging from 17 to 23%) linked to the OM fraction (Fig. 2A, D). It is possible that the Cu and Zn sources linked to this OM fraction, when mineralized, may supply nutritional plant performance (Cu and Zn organic sources). The high SOM content found in this study can be attributed to the historic area that has been planted with *Eucalyptus* for many years. Therefore, the harvest part of the forest residues was deposited over the soil and, then, can increase the content of Cu and Zn soil-availability and nutrient store in the ecosystem (Gonçalves et al., 2007).

Compounds rich in carbon contribute to an increase in the rate of decomposition in forest systems, which is characterized by a greater content of recalcitrant compounds (e.g., higher lignin content) compared to annual crops (Bachega et al., 2016; Hättenschwiler and Jørgensen, 2010). Higher quantities of organic carbon are obtained in the humin fraction, which accounts for 34%–48% of the total organic carbon in the organo-mineral complex (Giannetta et al., 2019). These recalcitrant compounds are integrated with the stabilization of reactions with organic matter, through the interaction of this fraction with clay particles and, to a lesser extent, with metal ions, such as Cu and Zn, which are more bound to the recalcitrant compounds of the soil (Sausen et al., 2014). In addition, limed soil may have enhanced microbial activity compared to no-limed soil. In this hypothesis, microbial communities improve the decomposition of labile compounds, which are strongly related to improved rates of organic matter mineralization (Pereira et al., 2019).

Lime application altered the contents of Cu, Fe, Mn, and Zn in the Exc, Carb, OM, Oxi, and Res soil fractions over time (Fig. 2), probably because of the increase in some soil chemical attributes, such as BS and pH, Ca, and Mg values (Table 1). The higher contents of Cu, Fe, Mn, and Zn are related to Mn oxides and amorphous and crystalline Fe oxides, and it is essential to identify the solid phases that control the availability of these micronutrients, mainly to understand, predict, and interpret mobility in soil phases (Silveira et al., 2006). The higher Cu percentage is bound to immobile fractions, more related to non-exchangeable fractions, formation of organometallic compounds, and low solubility (Nogueirol et al., 2010). The Zn content in soil is closely related to exchangeable, light organic matter, and carbonate-bound fractions (Liu et al., 2018). Liming directly influences the soil redox potential and, thereafter, Fe and Mn availability because these micronutrients are very sensitive to changes in soil pH (Silveira et al., 2006). In humid tropical soils, higher Zn contents are associated with non-labile fractions or crystalline forms of Fe, Mn, and Al oxide, and Zn in these forms is not readily available for plants (Leite et al., 2020). Therefore, the interaction among Cu, Fe, Mn, and Zn in different soil fractions can explain their bioavailability and uptake for better nutritional performance of *Eucalyptus* plants amended with lime and Cu plus Zn application.

### 2.4.3. Nutritional and growth performance during plants development were affected mainly by lime as compared with Cu plus Zn application

Liming contributed to plants absorbing higher contents of P, Ca, and Mg, but lower Fe and Mn, consequently influencing the nutritional status of the plants (Fig. 3). This occurred 6 to 30 months after planting, probably associated with lime incorporation into soil that increased soil pH and improved soil fertility (e.g., Ca, Mg, $_e$CEC, and BS (Fig.1 and Table 1)]. The lower leaf P, Ca, Mg, B, Cu, and Mn concentrations in the dry season (12 and

24 months after planting), and of Zn and Fe in the wet season [6, 18, and 30 months after planting (Fig. 3)] were related to changes in soil pH and soil availability of these nutrients.

Under these circumstances, the metallic micronutrients (i.e., Cu, Fe, Mn, and Zn) may have been adsorbed to the non-labile forms, mainly to the Res and Oxi fractions (Fig. 3). Lime application increases the enzymatic activity of soil microorganisms (e.g., β-glucosidase, synthesized for cellulose degradation), thus resulting in greater degradation of organic molecules and litter of forest soils (Chatzistathis et al., 2015; McCay et al., 2013). These nutrients are part of the cellular tissue of microorganisms and/or are bound to compounds that are not readily available. This may explain why plants uptake lower nutrient contents in the dry season than in the wet season.

Overall, the lime rate recommended for *Eucalyptus* plants (1.6 Mg $ha^{-1}$) and that recommended for annual crops (3.7 Mg $ha^{-1}$) did not differ in terms of macro-and micronutrient leaf concentrations (Fig. 3). These results evidenced that the recommendation rate of lime for *Eucalyptus* plants is sufficient for this species (Gonçalves et al., 2013), mostly because the content of Ca and Mg uptake through the plants was proportional between both rates (i.e., 1.6 and 3.7 Mg $ha^{-1}$). Thus, it is not justified to apply a higher rate of lime to improve Ca and Mg concentrations in *Eucalyptus* plantations.

The higher rate of lime can have contributed to reducing the availability of Cu and Zn in the soil (Table 1) and *Eucalyptus* plantations (Fig. 3E, H). Leaf B concentrations, in all treatments, and leaf Ca, Mg, Cu, and Fe concentrations in some months after planting presented values below the sufficiency range (SR) (Fig. 3) for *Eucalyptus* species planted in Brazil, according to Gonçalves (2010). However, in general, the characteristics of visual symptoms of macro-and micronutrient deficiencies were not observed in the different stages of *Eucalyptus* development. Indeed, plant nutrition at adequate sufficiency ranges depends on biotic and abiotic factors, highlighting the available concentrations of the soil solution and in the plants, supply and chemistry at root surfaces or in the rhizosphere, and interactions of one nutrient with another (Fageria et al., 2002).

From a practical point of view, the balance of the nutrients enhanced the nutritional plant performance in different stages of *Eucalyptus grandis* plantation after liming (Fig. 3), as evidenced by the higher leaf area index (Table 2) and stemwood volume (Fig. 4). Therefore, lime application changed the soil nutrient balance, and this effect was closely associated with a better nutritional status of plantation. For example, the higher leaf Ca and Mg concentrations (Fig. 3C, D) were probably implicated in the reduction of Mn (Fig. 3G) in plants of different ages. The Ca and Mg nutritional status of plants can interfere with Mn concentration and other nutrients (Pais and Jones, 1997). This hypothesis is related to the contribution of lime application, which increases the bioavailability of Ca and Mg for plant uptake (Bossolani et al., 2020; Holland et al., 2018; Paradelo et al., 2015).

Leaf area index (LAI) has been used frequently in ecophysiological models to predict forest management (Caldeira et al., 2020; White et al., 2010), as well as an indicator of forest productivity (le Maire et al., 2019; Mattos et al., 2020). The LAI in the plant grown under lime (i.e., LLR and HLR treatments) were 13% higher in comparation to plants grown under no-lime rate (NLR treatment), mainly under LLR (1.6 Mg $ha^{-1}$), because the LAI in this treatment had no difference as compared with HLR [treatment with 230% more lime rate (3.7 Mg $ha^{-1}$)]. Furthermore, applying a lime rate based on base saturation (i.e., BS up to 60% or HLR treatment) is not justified for *Eucalyptus*, as recommended by Raij et al. (1996) for annual crops. The lower LAI in dry seasons (21 and 24 months after planting) is closely related to the ecophysiological processes that occur in the soil-eucalyptus system, in which the dry seasons affected the LAI more negatively, probably due to the lower rate of sweating (Hakamada et al., 2020) and, consequently, lower photosynthetic rate (Han et al., 2016).

The application of lime and Cu plus Zn contributed to increased stemwood volume in *Eucalyptus grandis* plantation, mainly, as compared with plants without lime × without Cu plus Zn during plant growth (Fig. 4B, D). Also, this effect can was closely associated with the changes that occurred in soil chemical attributes (Fig. 1 and Table 1). Other associated effects may have influenced the yield of the *Eucalyptus* plantation, such as the alteration in soil microorganism enzymatic activity and their integrative effects after mineralization rates of organic compounds (Balland-Bolou-Bi et al., 2019; Bossolani et al., 2020; Pereira et al., 2019). This may interfere with yield and other ecophysiological processes in the soil-*Eucalyptus* system (Caldeira et al., 2020).

Regardless of lime rates (NLR, LLR, and HLR), the higher stemwood volume in plants under Cu plus Zn fertilization was probably associated with more uptake of Cu and Zn, mainly at 12 months after planting (Fig. 4A). Fertilization with Cu and Zn is usually recommended to replace the quantities extracted during the harvest of forest products. In addition, positive responses can frequently occur in areas with successive rotations, mainly in soils with highly weathered and nutrient-poor conditions (Gonçalves et al., 2013). The contribution of lime in increasing wood productivity tends to be more common in soils poor in nutrients, mainly when Ca and Mg contents are lower than 4 and 2 $mmol_c$ $L^{-1}$, respectively (Rocha et al., 2019). Indeed, our results revealed low availability of nutrients in the experimental area before liming and Cu plus Zn application (Supplementary Table 1). In this context, lime × Cu and Zn application contributed to improving wood yield, for example, plants grown under individual lime application increased stemwood volume by 15% as compared with plants grown under no-lime application (Fig. 4D and Fig. 5D), whereas plants grown under individual Cu plus Zn fertilizer and without liming enhanced absolute values of volume by 10% as compared with plants grown under no Cu plus Zn fertilizer and without liming (Fig. 4C and Fig. 5D).

Although the *Eucalyptus* species develop well in highly acidic tropical soils (Gabriel et al., 2018; Gonçalves and Valeri, 2001), our research showed that the combined application of lime and Cu plus Zn based on the recommended rate to *Eucalyptus* plantation (Gonçalves, 2010) was associated with the stem volume yield, mainly from 12 months after planting to the initial stage of complete canopy closure, at 30 months after planting. Moreover, the higher lime rate for *Eucalyptus* [i.e., base saturation up to 60% - HLR treatment (Raij et al., 1996)] may not be adequate from an economic point of view, because plants grown in plots that received the lower lime rate [i.e., LLR treatment (Gonçalves, 2010)] had stemwood volume yield similar to plants under higher lime rates. In addition, the Cu and Zn recommended rate for *Eucalyptus* plants can promote the growth of trees when associated with liming, mainly if there is a delay between liming and fertilization with these metals, considering the greater Cu and Zn availability due to the buffer effect within soil and the return of pH to original levels.

### 2.4.4. Correlation and multivariate analysis had a key role to investigate the soil and plants responses with lime and Cu plus Zn application

The correlation and PCA analysis showed the responses of soil chemical attributes and *Eucalyptus* trees under combined application of lime and Cu plus Zn, such as the strong effect of liming to reduce the Cu and Zn content in soil and trees (Fig 5). In these circumstances, Cu plus Zn fertilization can contribute to reducing this disadvantage, that is, increasing the availability of Cu and Zn in the soil to supply *Eucalyptus* plants. Liming recommendations for *Eucalyptus* plants aim to improve Ca and Mg soil-availabilities (Gonçalves and Valeri, 2001; Rocha et al., 2019) however, lime application can reduce the Cu and Zn bioavailability for plant uptake (Kabata-Pendias, 2011). Indeed, our data showed that liming reduced Cu and Zn availability at 0-0.20 depths (Table 1) and in *Eucalyptus* leaves (Fig. 3E, H). The presence of these micronutrients in the soil-eucalyptus system (e.g., forest residue management and litterfall (Masullo et al., 2020; Rocha et al., 2016)) and mineral Cu plus Zn fertilization may have been sufficient to meet the nutritional demand of the plants and to guarantee the expected wood productivity performance. Lime incorporation within soil before planting the seedlings may have contributed to improving the mineralization of wood residues, thus enhancing the bioavailability of Cu and Zn in soil-plant systems.

The PCA with 98% of data variance (PC1 + PC2) showed that the Cu content in the soil was closely associated with Exc, Carb, and OM fractions (labile fraction), while Zn was related to fractions of Carb and OM, and Oxi (no labile fraction), and Fe and Mn were closely associated with the Oxi and Res fractions, respectively (i.e., no labile fraction) (Fig. 5B). In addition, the Cu and Zn contents were positively correlated with the Exc, Carb, and OM fractions, which clearly interpreted the interactions of these micronutrients in different soil fractions, contributing to decision making relative to the management of *Eucalyptus* plantations, for example, increasing the bioavailability of these micronutrients to benefit nutrition and growth of *Eucalyptus* trees. The extraction of metallic micronutrients (e.g., Cu, Fe, Mn, and Zn) in tropical soils (e.g., Ferralsol) revealed a significant quantity of these micronutrients (mainly Cu and Zn) bound to organic matter and carbonate fractions, even with high contents of oxides, mainly of Fe and Mn (Colzato et al., 2018; Leite et al., 2020; Nogueirol et al., 2010; Silveira et al., 2006). However, these fractions are not bioavailable for plants, but can serve as a reservoir for metallic micronutrients. The differences in soil phases can be associated with distinct interactions (soil-plant systems) during plant development, in which the integrative effect of combined application of lime and Cu plus Zn fertilization enhanced the stemwood yield of *Eucalyptus* plantations.

PCA analysis explained the combined effects of lime and Cu plus Zn in some soil chemical variables, and thereafter in the nutrition and wood yield during the development of *Eucalyptus grandis* plantations (Fig. 5C, D). Thus, treatments with no lime and no Cu plus Zn were characterized by lower values of pH, Ca, Mg, $_e$CEC, BS, and Mn, and higher contents of B, Cu, Fe, Zn, and SOM in the Ferralsol. In addition, the same treatments (i.e., no lime × no Cu plus Zn) presented lower values of P, Ca, Mg, LAI, and VOL, and higher concentrations of Cu, Fe, Mn, and Zn in plants as compared with treatments with combined application of lime and Cu plus Zn. Furthermore, the changes that occurred in amended soil with both factors were positive for better nutritional performance and wood yield of *Eucalyptus* planting. The maximum lime rate of 2 Mg ha$^{-1}$ supplies the required Ca and Mg content to guarantee the expected wood yield of *Eucalyptus* plantations (Gonçalves, 2010; Rocha et al., 2019), mainly because lime sources are generally rich in Ca and Mg, which improves plant growth (Gabriel et al., 2018; Rodrigues et al., 2016; Silva and Coelho, 2010).

Copper and Zn fertilizers to *Eucalyptus* plantations have been usually recommended as a “maintenance fertilization,” aiming to replace the quantities exported with the harvest of the wood or losses due to edaphic processes (Gonçalves et al., 2013). However, Cu and Zn fertilization should preferably be carried out after liming (e.g., three months), so that the availability of these micronutrients is not significantly affected by the increase in pH, which is one of the implications of liming in soil (Rocha et al., 2019). Therefore, if the soil is amended with lime application, Cu and Zn fertilization can be beneficial for *Eucalyptus* development, in a sense guaranteeing an adequate supply of Cu and Zn to plant growth. Indeed, our findings are consistent with the implications of lime × Cu plus Zn application for improved stemwood volume productivity of *Eucalyptus grandis* plantation.

## 2.5. Conclusion

Dolomitic limestone application enhances soil fertility of Ferralsol, mainly by increasing the pH and thus the capacity of cation exchange and Ca and Mg availability. Individual lime application reduces Cu and Zn bioavailability for plants, mainly in the first months after planting. There are large levels of Cu and Zn bound to the organic matter fraction in soil under the *Eucalyptus* plantation. The results of our study show that lime application improved the leaf area index and nutritional performance of plants, mainly Ca and Mg concentrations, but decreased the leaf Cu and Zn concentrations in different stages after planting. The integrative effect of lime and Cu plus Zn application increased the growth performance of *Eucalyptus grandis* plantations, with higher yields in plants grown under individual lime application as compared with plants grown under individual Cu plus Zn fertilization. The combined application of lime rate and Cu plus Zn fertilizer for *Eucalyptus* plants is recommended to improving the degree of soil fertility and the nutritional and growth status of *Eucalyptus* plantation.

## References

Ahnstrom, Z.S., Parker, D.R., 1999. Development and assessment of a sequential extraction procedure for the fractionation of soil cadmium. Soil Sci. Soc. Am. J. 63, 1650–1658. https://doi.org/10.2136/sssaj1999.6361650x

Alejandro, S., Höller, S., Meier, B., Peiter, E., 2020. Manganese in Plants: From Acquisition to Subcellular Allocation. Front. Plant Sci. https://doi.org/10.3389/fpls.2020.00300

Alleoni, L.R.F., Iglesias, C.S.M., Mello, S.D.C., Camargo, O.A. De, Casagrande, J.C., Lavorenti, N.A., 2005. Atributos do solo relacionados à adsorção de cádmio e cobre em solos tropicais. Acta Sci. Agron. 27, 729–737. https://doi.org/10.4025/actasciagron.v27i4.1348

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L. de M., 2013. Modeling monthly mean air temperature for Brazil. Theor. Appl. Climatol. 113, 407–427. https://doi.org/10.1007/s00704-012-0796-6

Aubert, H., Pinta, M., 1980. Trace elements in soils, 1st ed, Elsevier Science.

Aubert, H., Pinta, M., Hooda, P., 2010. Trace elements in soils, Trace Elements in Soils. Blackwell Publishing Ltd Registered. https://doi.org/10.1002/9781444319477.ch10

Bachega, L.R., Bouillet, J.P., de Cássia Piccolo, M., Saint-André, L., Bouvet, J.M., Nouvellon, Y., Gonçalves, J.L. de M., Robin, A., Laclau, J.P., 2016. Decomposition of *Eucalyptus grandis* and *Acacia mangium* leaves and fine roots in tropical conditions did not meet the Home Field Advantage hypothesis. For. Ecol. Manage. 359, 33–43. https://doi.org/10.1016/j.foreco.2015.09.026

Balland-Bolou-Bi, C., Bolou-Bi, E.B., Alphonse, V., Giusti-Miller, S., Jusselme, M.D., Livet, A., Grimaldi, M., Bousserhine, N., 2019. Impact of microbial activity on the mobility of metallic elements (Fe, Al and Hg) in tropical soils. Geoderma 334, 146–154. https://doi.org/10.1016/j.geoderma.2018.07.044

Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375. https://doi.org/10.1016/j.geoderma.2020.114476

Caires, E.F., Joris, H.A.W., Churka, S., 2011. Long-term effects of lime and gypsum additions on no-till corn and soybean yield and soil chemical properties in southern Brazil. Soil Use Manag. 27, 45–53. https://doi.org/10.1111/j.1475-2743.2010.00310.x

Caldeira, D.R.M., Alvares, C.A., Campoe, O.C., Hakamada, R.E., Guerrini, I.A., Cegatta, Í.R., Stape, J.L., 2020. Multisite evaluation of the 3-PG model for the highest phenotypic plasticity *Eucalyptus* clone in Brazil. For. Ecol. Manage. 462. https://doi.org/10.1016/j.foreco.2020.117989

Camargo, O.A., Moniz, A.C., Jorge, J.A., Valadares, J.M., 1986. Methods of soil chemical, physical, and mineralogical analysis of the Agronomic Institute in Campinas.

Carmeis Filho, A.C.A., Penn, C.J., Crusciol, C.A.C., Calonego, J.C., 2017. Lime and phosphogypsum impacts on soil organic matter pools in a tropical Oxisol under long-term no-till conditions. Agric. Ecosyst. Environ. 241, 11–23. https://doi.org/10.1016/j.agee.2017.02.027

Chatzistathis, T., Alifragis, D., Papaioannou, A., 2015. The influence of liming on soil chemical properties and on the alleviation of manganese and copper toxicity in J*uglans regia, Robinia pseudoacacia, Eucalyptus sp. and Populus sp*. Plantations. J. Environ. Manage. 150, 149–156. https://doi.org/10.1016/j.jenvman.2014.11.020

Colzato, M., Alleoni, L.R.F., Kamogawa, M.Y., 2018. Cadmium sorption and extractability in tropical soils with variable charge. Environ. Monit. Assess. 190. https://doi.org/10.1007/s10661-018-6666-7

Crusciol, C.A.C., Marques, R.R., Carmeis Filho, A.C.A., Soratto, R.P., Costa, C.H.M., Ferrari Neto, J., Castro, G.S.A., Pariz, C.M., Castilhos, A.M., Franzluebbers, A.J., 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutr. Cycl. Agroecosystems 115, 347–372. https://doi.org/10.1007/s10705-019-10017-0

Eswaran, H., Reich, P., Beinroth, F., 1997. Global distribution of soils with acidity, in: Plant-Soil Interactions at Low PH. pp. 159–164.

Fageria, N.K., Baligar, C., Clark, R.B., 2002. Micronutrients in crop production. Adv. Agron. https://doi.org/10.1016/s0065-2113(02)77015-6

Fageria, N.K., Nascente, A.S., 2014. Management of soil acidity of South American soils for sustainable crop production, in: Advances in Agronomy. pp. 221–275. https://doi.org/10.1016/B978-0-12-802139-2.00006-8

FAO, 2015. Intergovernmental Technical Panel on Soils. Status of the World's Soil Resources., Intergovernmental Technical Panel on Soils.

Gabriel, C.A., Cassol, P.C., Simonete, M.A., Moro, L., Pfleger, P., Mumbach, G.L., 2018. Lime and gypsum applications on soil chemical attributes and initial growth of eucalyptus. Floresta 48, 573–582. https://doi.org/10.5380/rf.v48i4.57455

Giannetta, B., Plaza, C., Zaccone, C., Vischetti, C., Rovira, P., 2019. Ecosystem type effects on the stabilization of organic matter in soils: Combining size fractionation with sequential chemical extractions. Geoderma 353, 423–434. https://doi.org/10.1016/j.geoderma.2019.07.009

Gonçalves, J., Wichert, M., Gava, J., Masetto, A., Junior, A.J.C., Serrano, M., Mello, S., 2007. Soil fertility and growth of *Eucalyptu*s *grandis* in Brazil under different residue management practices. South. Hemisph. For. J. 69, 95–102. https://doi.org/10.2989/SHFJ.2007.69.2.4.289

Gonçalves, J.L. de M., 2010. Eucalipto. In: "Boas práticas para o uso eficiente de fertilizantes". Piracicaba: International Plant Nutrition Institute Brazil, V.3, pag. 309-369.

Gonçalves, J.L. de M., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., Ferraz, S.F. de B., Lima, W. de P., Brancalion, P.H.S., Hubner, A., Bouillet, J.P.D., Laclau, J.P., Nouvellon, Y., Epron, D., 2013. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. For. Ecol. Manage. 301, 6–27. https://doi.org/10.1016/j.foreco.2012.12.030

Gonçalves, J.L. de M., de Vicente Ferraz, A., Tertulino Rocha, J.H., Peressin, M., Alcarde Alvares, C., 2020. Forest outgrower schemes in small and medium-sized farmers in Brazil. For. Ecol. Manage. 456. https://doi.org/10.1016/j.foreco.2019.117654

Gonçalves, J.L.D.M., Valeri, S.V., 2001. Eucalipto e pínus, in: Ferreira, M.E., Cruz, M.C.P., Raij, B., Abreu, C.A. (Eds.), Micronutrientes: Elementos Tóxicos Na Agricultura. CNPq/FAPESP/PATAFOS, Jaboticabal, Pp. 393–423. Jaboticabal (SP), pp. 393–423.

González-Alcaraz, M.N., Conesa, M., Álvarez-Rogel, J., 2013. When liming and revegetation contribute to the mobilisation of metals: Learning lessons for the phytomanagement of metal-polluted wetlands. J. Environ. Manage. 116, 72–80. https://doi.org/10.1016/j.jenvman.2012.11.044

Hakamada, R.E., Hubbard, R.M., Moreira, G.G., Stape, J.L., Campoe, O., Ferraz, S.F. de B., 2020. Influence of stand density on growth and water use efficiency in *Eucalyptus* clones. For. Ecol. Manage. 466. https://doi.org/10.1016/j.foreco.2020.118125

Han, J., Chen, J., Miao, Y., Wan, S., 2016. Multiple Resource Use Efficiency (mRUE): A New Concept for Ecosystem Production. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep37453

Hättenschwiler, S., Jørgensen, H.B., 2010. Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. J. Ecol. 98, 754–763. https://doi.org/10.1111/j.1365-2745.2010.01671.x

Havlin, J.L., Tisdale, S.L., Nelson, W.L., Beaton, J.D., 2013. Soil fertility and nutrient management: An introduction to nutrient management, 8th Edition. Pearson, Upper Saddle River, New Jersey, USA.

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.08.020

IBA, 2020. Brazilian tree industry annual report - base year 2019. Assoc. Brasleira Árvores 160.

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106.

Joris, H.A.W., Caires, E.F., Scharr, D.A., Bini, Â.R., Haliski, A., 2016. Liming in the conversion from degraded pastureland to a no-till cropping system in Southern Brazil. Soil Tillage Res. 162, 68–77. https://doi.org/10.1016/j.still.2016.04.009

Joris, H.H.A.W., Fonseca, A. da, Da Fonseca, A.F., Asami, V.Y., Briedis, C., Borszowskei, P.R., Garbuio, F.J., 2012. Adsorção de metais pesados após calagem superficial em um Latossolo Vermelho sob sistema de plantio direto, Revista Ciencia Agronomica. https://doi.org/10.1590/S1806-66902012000100001

Kabata-Pendias, A., 2011. References, in: Trace Elements in Soils and Plants, Fourth Edition. CRC Press, pp. 407–505. https://doi.org/10.1201/b10158-25

Laclau, J.P., Ranger, J., Gonçalves, J.L. de M., Maquère, V., Krusche, A. V., M'Bou, A.T., Nouvellon, Y., Saint-André, L., Bouillet, J.P., de Cassia Piccolo, M., Deleporte, P., 2010. Biogeochemical cycles of nutrients in tropical *Eucalyptus* plantations. Main features shown by intensive monitoring in Congo and Brazil. For. Ecol. Manage. 259, 1771–1785. https://doi.org/10.1016/j.foreco.2009.06.010

le Maire, G., Guillemot, J., Campoe, O.C., Stape, J.L., Laclau, J.P., Nouvellon, Y., 2019. Light absorption, light use efficiency and productivity of 16 contrasted genotypes of several *Eucalyptus* species along a 6-year rotation in Brazil. For. Ecol. Manage. 449. https://doi.org/10.1016/j.foreco.2019.06.040

Leite, C.M.D.C., Muraoka, T., Colzato, M., Alleoni, L.R.F., 2020. Soil-applied Zn effect on soil fractions. Sci. Agric. 77, 2020. https://doi.org/10.1590/1678-992x-2018-0124

LI-COR, 2016. LAI-2200C Plant Canopz Analzyer Instruction Manual.

Liu, H., Zhao, P., Qin, S., Nie, Z., 2018. Chemical fractions and availability of zinc in winter weat soil in response to nitrogen and zinc combinations. Front. Plant Sci. 9, 1489. https://doi.org/10.3389/fpls.2018.01489

Malavolta, E., Vitti, G., Oliveira, S., 1997. Avaliação do estado nutricional das plantas: princípios e aplicações.

Masullo, L.S., Ferraz, A. de V., Gonçalves, J.L. de M., Camargo, L.E.A., de Ávila, P.A., Drago, J.R., Esteves, O., dos Santos Dias, C.T., 2020. Relationship between forest residue management and micronutrient fertilization with eucalyptus rust severity in *Eucalyptus grandis* plantations. For. Ecol. Manage. 475, 118443. https://doi.org/10.1016/j.foreco.2020.118443

Mattos, E.M., Binkley, D., Campoe, O.C., Alvares, C.A., Stape, J.L., 2020. Variation in canopy structure, leaf area, light interception and light use efficiency among *Eucalyptus* clones. For. Ecol. Manage. 463. https://doi.org/10.1016/j.foreco.2020.118038

McCay, T.S., Cardelús, C.L., Neatrour, M.A., 2013. Rate of litter decay and litter macroinvertebrates in limed and unlimed forests of the Adirondack Mountains, USA. For. Ecol. Manage. 304, 254–260. https://doi.org/10.1016/j.foreco.2013.05.010

Moreira, S.G., Prochnow, L.I., de Castro Kiehl, J., Pauletti, V., Ladislau, M.-N., Martin-Neto, L., 2016. Chemical forms in soil and availability of manganese and zinc to soybean in soil under different tillage systems. Soil Tillage Res. 163, 41–53. https://doi.org/10.1016/j.still.2016.05.007

Nogueirol, R.C., Alleoni, L.R.F., Nachtigall, G.R., de Melo, G.W., 2010. Sequential extraction and availability of copper in Cu fungicide-amended vineyard soils from Southern Brazil. J. Hazard. Mater. 181, 931–937. https://doi.org/10.1016/j.jhazmat.2010.05.102

Oliveira, F.C., Mattiazzo, M.E., 2001. Mobilidade de metais pesados em um Latossolo Amarelo distrófico tratado com lodo de esgoto e cultivado com cana-de-açúcar. Sci. Agric. 58, 807–812. https://doi.org/10.1590/S0103-90162001000400024

Pais, I., Jones, J.B.J., 1997. The Handbook of Trace Elements.

Paradelo, R., Virto, I., Chenu, C., 2015. Net effect of liming on soil organic carbon stocks: A review. Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2015.01.005

Pereira, A.P.A., Durrer, A., Gumiere, T., Gonçalves, J.L.M., Robin, A., Bouillet, J.P., Wang, J., Verma, J.P., Singh, B.K., Cardoso, E.J.B.N., 2019. Mixed *Eucalyptus* plantations induce changes in microbial communities and increase biological functions in the soil and litter layers. For. Ecol. Manage. 433, 332–342. https://doi.org/10.1016/j.foreco.2018.11.018

R Core Team, 2016. R Development Core Team, R: A language and environment for statistical computing, R Foundation for Statistical Computing. R A Lang. Environ. Stat. Comput.

Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1996. Recomendações de adubação e calagem para o Estado de São Paulo. Campinas: IAC, 1996. (IAC. Boletim 100). Banana.

Raij, B. van, Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Análise química para avaliação da fertilidade de solos tropicais, Campinas: Instituto Agronômico.

Rocha, J.H.T., du Toit, B., Gonçalves, J.L. de M., 2019. Ca and Mg nutrition and its application in *Eucalyptus* and *Pinus* plantations. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2019.03.062

Rocha, J.H.T., Gonçalves, J.L. de M., Gava, J.L., Godinho, T. de O., Melo, E.A.S.C., Bazani, J.H., Hubner, A., Arthur Junior, J.C., Wichert, M.P., 2016. Forest residue maintenance increased the wood productivity of a *Eucalyptus* plantation over two short rotations. For. Ecol. Manage. 379, 1–10. https://doi.org/10.1016/j.foreco.2016.07.042

Rodrigues, F.A.V., de Barros, N.F., Neves, J.C.L., Alvarez, V.H. V, Novais, R.F., 2012. Zinc availabity for eucalypt seedlings in cerrado soils. Rev. Bras. Cienc. do Solo. https://doi.org/10.1590/S0100-06832012000400019

Rodrigues, F.A.V., Víctor, H.A., De Barros, N.F., Da Silva, I.R., Neves, J.C.L., 2016. Productivity of eucalypt at 18 months of age, in Cerrado region, in response to application to application of calcium, by lime and to gypsum amendments. Sci. For. Sci. 44, 67–74. https://doi.org/10.18671/scifor.v44n109.06

Santos, H. dos, JACOMINE, P., Anjos, L. Dos, 2018. Sistema brasileiro de classificação de solos.

SAS Institute Inc, 2016. SAS Institute Inc., SAS Institute Inc.

Sausen, T.L., de Paula Schaefer, G.F., Tomazi, M., dos Santos, L.S., Bayer, C., Rosa, L.M.G., 2014. Clay content drives carbon stocks in soils under a plantation of *Eucalyptus saligna* Labill. In southern Brazil. Acta Bot. Brasilica 28, 266–273. https://doi.org/10.1590/S0102-33062014000200013

Schumacher, F.X., Hall, F.D.S., 1933. Logarithmic expression of timber-tree volume. J. Agric. Res. 47, 719–734.

Scolforo, H.F., McTague, J.P., Burkhart, H., Roise, J., Campoe, O., Stape, J.L., 2019. *Eucalyptus* growth and yield system: Linking individual-tree and stand-level growth models in clonal *Eucalyp*t plantations in Brazil. For. Ecol. Manage. 432, 1–16. https://doi.org/10.1016/j.foreco.2018.08.045

Scolforo, J.R.., Thiersch, C.., 2004. Scolforo: Biometria florestal: medição, volumetria, First. ed, UFLA/FAEPE, Lavras.

Silva, I.R., Novais, R.F., Jham, G.N., Barros, N.F., Gebrim, F.O., Nunes, F.N., Neves, J.C.L., Leite, F.P., 2004. Responses of eucalypt species to aluminum: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism, Tree Physiology. Oxford Academic. https://doi.org/10.1093/TREEPHYS/24.11.1267

Silva, J.C., Coelho, L., 2010. Lime and silicate applied in eucalypt: Effect on soil and plant. Biosci. J. 26, 919–924.

Silveira, M.L., Alleoni, L.R.F., O'Connor, G.A., Chang, A.C., 2006. Heavy metal sequential extraction methods-A modification for tropical soils. Chemosphere 64, 1929–1938. https://doi.org/10.1016/j.chemosphere.2006.01.018

Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. Waste Manag. 28, 347–358. https://doi.org/10.1016/j.wasman.2006.12.010

Sipos, P., Németh, T., Kis, V.K., Mohai, I., 2008. Sorption of copper, zinc and lead on soil mineral phases. Chemosphere 73, 461–469. https://doi.org/10.1016/j.chemosphere.2008.06.046

Souri, M.K., Hatamian, M., 2019. Aminochelates in plant nutrition: a review. J. Plant Nutr. https://doi.org/10.1080/01904167.2018.1549671

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851. https://doi.org/10.1021/ac50043a017

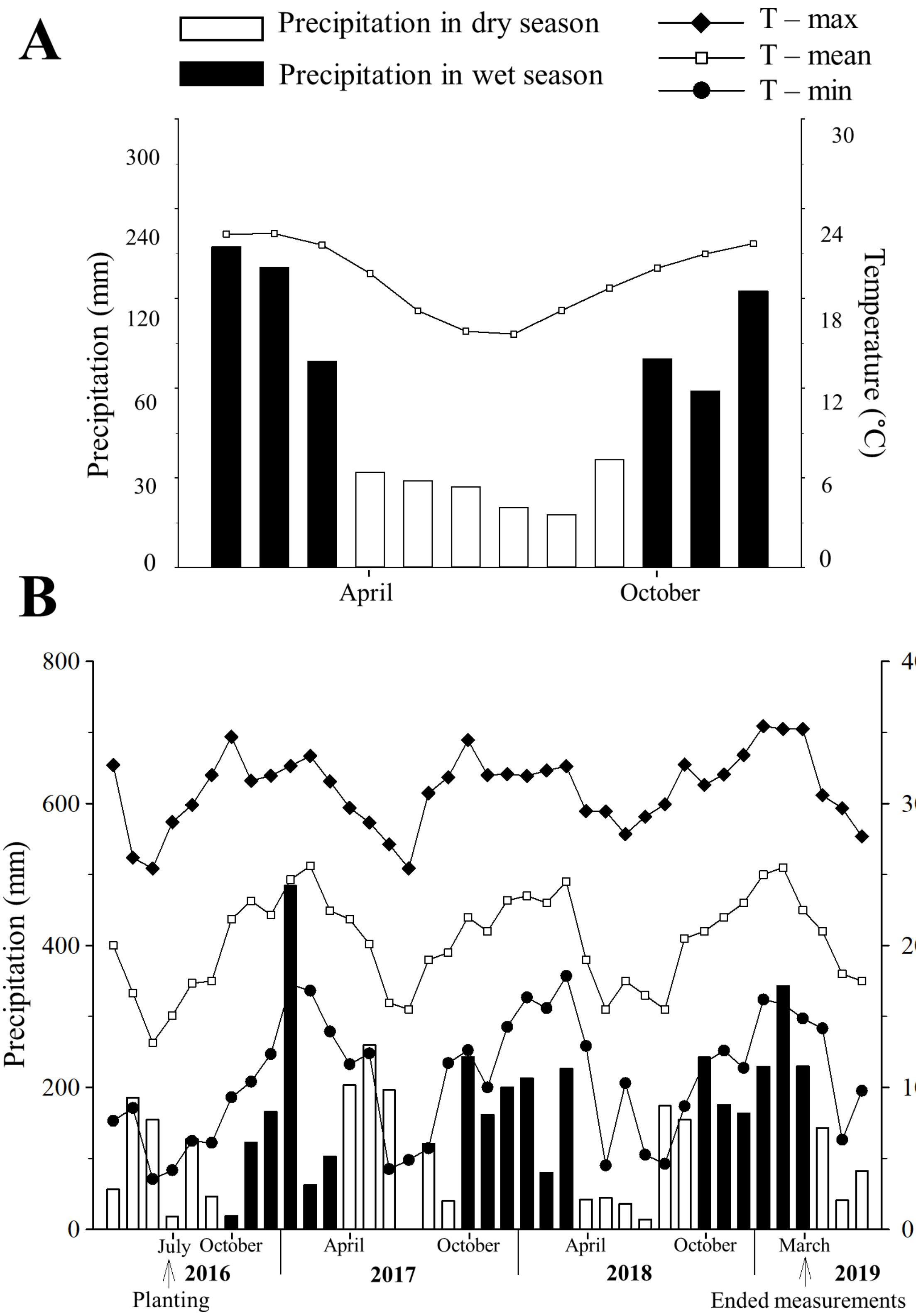
Versini, A., Mareschal, L., Matsoumbou, T., Zeller, B., Ranger, J., Laclau, J.P., 2014. Effects of litter manipulation in a tropical *Eucalyptus* plantation on leaching of mineral nutrients, dissolved organic nitrogen and dissolved organic carbon. Geoderma 232–234, 426–436. https://doi.org/10.1016/j.geoderma.2014.05.018

von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic impact of acid soils. Plant Soil 171, 1–15. https://doi.org/10.1007/BF00009558

White, D.A., Battaglia, M., Mendham, D.S., Crombie, D.S., Kinal, J., McGrath, J.F., 2010. Observed and modelled leaf area index in *Eucalyptus globulus* plantations: Tests of optimality and equilibrium hypotheses. Tree Physiol. 30, 831–844. https://doi.org/10.1093/treephys/tpq037

Yang, S., Jansen, B., Absalah, S., Kalbitz, K., Cammeraat, E.L.H., 2020. Selective stabilization of soil fatty acids related to their carbon chain length and presence of double bonds in the Peruvian Andes. Geoderma 373. https://doi.org/10.1016/j.geoderma.2020.114414

## Supplementary materials




**Supplementary Fig. S1**. Normal distribution (rainfall and mean temperature) according to the historical series from 1950-1990 (Alvares et al., 2013) (A) and mean monthly rainfall and temperature - T (maximum, mean, and minimum) during the field trial setup at the Itatinga, SP, Brazil (23º03'S; 48º37'W) (B).

**Supplementary Table S1**

Physical and chemical attributes of Rhodic Ferralsol, on different depths before planting *Eucalyptus grandis* seedlings, in Itatinga, State of São Paulo, Brazil (mean±standard error, n=3).

| Depth | Clay | Silt | Sand | pH | SOM | P | Ca | Mg | K | Al | H+Al | $_{e}$CEC | BS | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| m | --------------- % ----------- | | | 0.01 M $CaCl_2$ | g $kg^{-1}$ | mg $kg^{-1}$ | ---------------------------------- mmolc $kg^{-1}$ ------------------------------ | | | | | | % | ------------------------------ mg $kg^{-1}$ ---------------------------- | | | | |
| 0-0.1 | 13±0.7 | 3±0.9 | 83±0.3 | 3.5±0.1 | 26±2.1 | 3.4±0.1 | 2.48±0.1 | 1.9±0.1 | 0.48±0.0 | 15±0.2 | 67±5.4 | 22±0.3 | 5±0.2 | 0.3±0.0 | 1.0±0.0 | 117±9.8 | 0.6±0.0 | 0.5±0.1 |
| 0.1-0.2 | 15±0.7 | 2±0.1 | 83±0.7 | 3.6±0.1 | 16±0.5 | 2.6±0.2 | 1.59±0.1 | 1.4±0.1 | 0.43±0.0 | 13±0.1 | 44±6.4 | 17±0.1 | 5±0.1 | 0.3±0.0 | 1.1±0.0 | 74±7.8 | 0.5±0.0 | 0.3±0.0 |
| 0.2-0.4 | 14±0.1 | 3±0.3 | 83±0.3 | 3.7±0.1 | 12±0.7 | 1.5±0.3 | 0.3±0.1 | 0.4±0.4 | <0.1±0.0 | 12±0.3 | 52±3.2 | 12±0.5 | 1±0.7 | 0.3±0.0 | 1.1±0.0 | 37±1.8 | 0.4±0.0 | 0.3±0.0 |
| 0.4-0.6 | 16±0.1 | 2±0.3 | 82±0.3 | 3.9±0.1 | 10±0.7 | 1.3±0.3 | 0.4±0.1 | 0.1±0.1 | <0.1±0.0 | 11±0.3 | 42±3.2 | 11±0.5 | 1±0.7 | 0.3±0.0 | 1.1±0.0 | 25±1.8 | 0.4±0.0 | 0.3±0.0 |
| 0.6-0.8 | 17±0.1 | 3±0.3 | 80±1.0 | 3.7±0.1 | 9±0.8 | 1.1±0.2 | 0.5±0.2 | 0.1±0.1 | <0.1±0.0 | 10±0.3 | 36±3.3 | 11±0.2 | 1±0.8 | 0.3±0.0 | 1.0±0.1 | 15±1.3 | 0.5±0.0 | 0.3±0.0 |
| 0.8-1.0 | 18±0.1 | 3±0.1 | 79±0.1 | 3.7±0.1 | 8±0.4 | 1.1±0.1 | 0.4±0.2 | 0.1±0.1 | <0.1±0.0 | 10±0.6 | 37±1.3 | 10±0.3 | 1±0.7 | 0.3±0.0 | 1.0±0.0 | 11±1.5 | 0.6±0.0 | 0.3±0.0 |
| *0-0.2* | *14±0.1* | *3±0.4* | *83±0.4* | *3.6±0.1* | *21±1.2* | *3.0±0.2* | *2.0±0.1* | *1.7±0.1* | *0.46±0.0* | *14±0.1* | *56±2.5* | *20±0.2* | *5±0.1* | *0.3±0.0* | *1.1±0.0* | *96±8.3* | *0.6±0.0* | *0.4±0.0* |

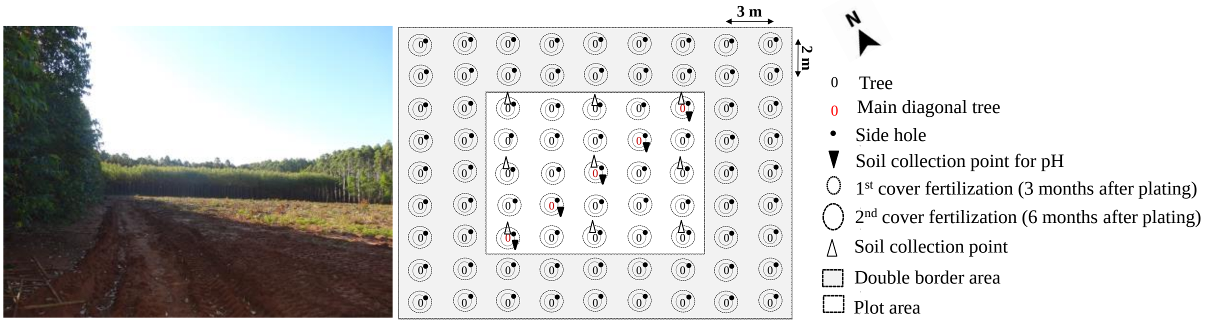
Physical analysis by method of Camargo et al (1986) and chemical by Raij et al (2001). where: pH in $CaCl_2$ (0.01 mol $L^{-1}$); SOM = soil organic matter; H+Al = total acidity; $_{e}$CEC = effective cation exchange capacity and BS= base saturation in percentage (%).

**Supplementary Table S2**

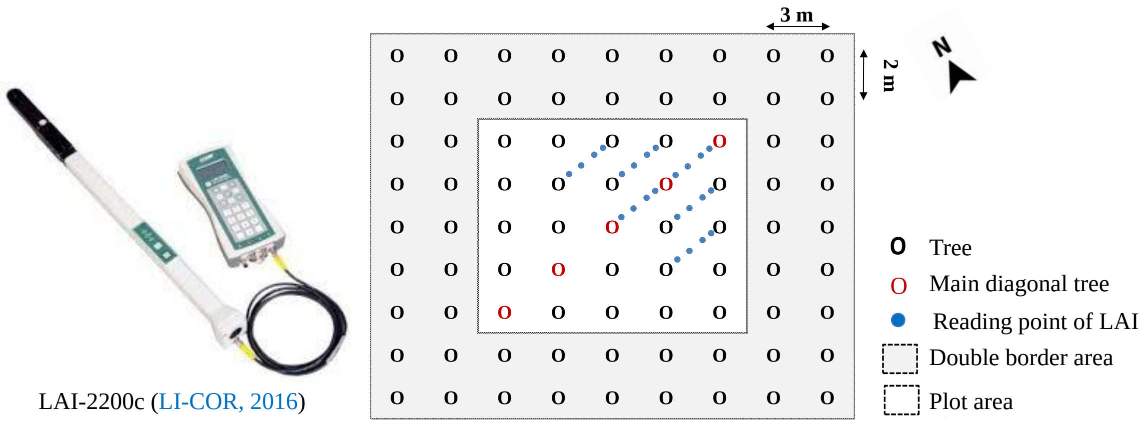
Quantity of nutrients (fertilizers), rates of dolomitic limestone (lime) and Cu plus Zn fertilizer (fert) in all conditions, according to fertilization phase of plant growth.

| Treatments | | Fertilization phases | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Planting | | | | | | 3 months after planting | | | 6 months after planting | |
| Lime | Fert | N | $P_2O_5$ | $K_2O$ | Cu | Zn | DL rates | N | $K_2O$ | B | N | $K_2O$ |
| | | kg $ha^{-1}$ | | | | | | | | | | |
| NLR | -CuZn | 10 | 60 | 10 | 0 | 0 | 0 | 20 | 50 | 5 | 30 | 80 |
| LLR | -CuZn | 10 | 60 | 10 | 0 | 0 | 1600 | 20 | 50 | 5 | 30 | 80 |
| HLR | -CuZn | 10 | 60 | 10 | 0 | 0 | 3700 | 20 | 50 | 5 | 30 | 80 |
| NLR | +CuZn | 10 | 60 | 10 | 0.5 | 1.5 | 0 | 20 | 50 | 5 | 30 | 80 |
| LLR | +CuZn | 10 | 60 | 10 | 0.5 | 1.5 | 1600 | 20 | 50 | 5 | 30 | 80 |
| HLR | +CuZn | 10 | 60 | 10 | 0.5 | 1.5 | 3700 | 20 | 50 | 5 | 30 | 80 |

Ammonium sulphate - 21% N; triple superphosphate - 46% $P_2O_5$; potassium chloride - 60% $K_2O$; borax - 11% B; copper sulfate - 25% Cu; zinc sulfate - 21% Zn; and DL - 40% CaO, and 10% MgO. Treatment of lime: no lime - NLR (0.0 Mg $ha^{-1}$ ), low lime rate - LLR (1.6 Mg $ha^{-1}$, calculated by 20-[Ca+Mg]/10) and high lime rate - HLR (3.7 Mg $ha^{-1}$, calculated by base saturation up to 60%). Treatment of fert: no Cu plus Zn (-CuZn), and 0.5 kg $ha^{-1}$ of Cu plus 1.5 kg $ha^{-1}$ of Zn (+CuZn).




**Supplementary Fig. S2.** Experimental plot, highlighting the locations of fertilization and soil samples.




**Supplementary Fig. S3.** LAI-20200c and samples points for the leaf area index (LAI) in the experimental plot.

# 3. WOOD PRODUCTION AND NUTRITIONAL AND ANTIOXIDANT STATUS OF FIELD-GROWN *Eucalyptus* UNDER A DIFFERENTIAL SUPPLY OF LIME AND COPPER PLUS ZINC

**Abstract**

The *Eucalyptus* plantations for industrial purposes (e.g., wood charcoal and pulp) are largely adopted worldwide, mainly in acidic and poorly fertile soils; and Brazil is global reference in the cultivation of *Eucalyptus*. The application of lime to soil is recommended for *Eucalyptus* plantation, but lime reduces the availability of metallic-micronutrients to plants. Therefore, the aim of this study was to investigate the performance of field-grown *Eucalyptus grandis* in Ferralsol amended with lime at different rates (0.0 and 1.6 and 3.7 Mg ha$^{-1}$) and copper plus zinc [0.0 and 0.0 kg ha$^{-1}$ (-CuZn) and 0.5 and 1.5 kg ha$^{-1}$ (+CuZn)]. The soil chemical attributes, shoot biomass, and nutrient accumulation were evaluated, while nutrient use efficiency, chlorophyll and flavonoid contents, and enzymatic antioxidant performance (superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase, guaiacol peroxidase, and glutathione peroxidase) were assayed to assess the nutritional status of the plants. Despite the lime application rate increasing Ca and Mg soil availabilities, effective cation exchange capacity (eCEC), base saturation (BS), and pH values, the available Cu, Fe, and Zn concentrations decreased at 6 and 30 months after planting. Conversely, Mn soil availability increased with lime application rates. Liming augmented Cu-, Fe-, Mn-, and Zn-use efficiency, while +CuZn supply increased enzymatic antioxidant performance in 30-month-old plant. Overall, the combined application of lime and +CuZn enhanced shoot biomass relative to that of non-treated plants. However, there was no observed difference in shoot biomass in 30-month-old limed and non-limed *Eucalyptus* trees that were supplied with +CuZn. This result can be partially explained by the improvement in soil chemical attributes, such as soil pH, eCEC, and BS. In conclusion, the use of integrative approaches and temporal evaluations provided new insights into wood yield, biochemical traits, antioxidant metabolism, and the nutritional status of field-grown *Eucalyptus* under lime and +CuZn application.

**Keywords:** *Eucalyptus grandis*, Limestone, Fertilization, Metallic micronutrients, Antioxidant metabolism, Biomass production

## 3.1. Introduction

The forest plantations for wood production (e.g., wood charcoal, pulp, and solid wood products) is largely adopted worldwide (Booth, 2013; Keenan et al., 2015; Perron et al., 2021) and Brazil is global reference in the management for *Eucalyptus* plantations (Alvares et al., 2013b; Stape et al., 2004). The management of *Eucalyptus* plantations is mainly carried out in acidic and poorly fertile soils (FAO, 2015; Gonçalves et al., 2020; IBA, 2020; Scolforo et al., 2019), whose plantations have expanded to meet the demand for wood products, owing its plasticity in relation to environmental conditions, rapid growth, diversity of uses, and wide knowledge concerning its silviculture (Freitas et al., 2020). *Eucalyptus* species occupy approximately 77% of the area of forest plantations devoted to wood products in Brazil (IBA, 2020).

The use of superior plant materials provides increased yield of high-quality products; however, such materials may have an elevated nutritional requirement (Lima Neto et al., 2020) that is often reflected as symptoms of mineral deficiency. In *Eucalyptus* plantations, for instance, the occurrence of Cu-and Zn–induced deficiencies has increased (Gonçalves and Valeri, 2001; Rodrigues et al., 2012, 2010). In addition to influence by diverse soil attributes (such as pH, cation exchange capacity, particle size, organic matter content, and microbial activity), the availability of metallic micronutrients to plants is influenced by crop management practices (Fageria et al., 2002; Kabata-Pendias, 2011). For example, the low recommended rate of lime (limestone) application for *Eucalyptus* plantations, as compared with the high recommended rate of lime application for annual crops [e.g., *Triticum aestivum* L. (wheat) and *Oryza sativa* L. (rice)], has been a strategy to increase the Ca and Mg availability to plants (Rocha et al., 2019a), and the recommended rate of lime application for annual crops aims to reduce the soil acidity (or to enhance the soil pH), mainly to increase the macronutrient (e.g., N and P) availability in the soil (Fageria and Nascente, 2014; Holland et al., 2018). However, lime application may reduce micronutrient soil availability (such as of Cu and Zn), which can potentially decrease plant development and wood yield (Eswaran et al., 1997; Silva et al., 2004), possibly under high rates of lime application.

Lime increases the activity of soil microorganisms that improve the mineralization of organic soil components (Chatzistathis et al., 2015; McCay et al., 2013), intensifying ecophysiological processes and enhancing the availability of nutrients for increased crop production (Caldeira et al., 2020). However, excessive lime rates may increase the soil pH to neutrality, thus indirectly affecting the reduction of micronutrient soil availability to plants (Eswaran et al., 1997; Silva et al., 2004). Therefore, a practical way of overcoming any reversible, undesirable effects of liming on the Cu and Zn soil unavailability is through the combined application of lime and fertilizers with Cu and Zn. In this sense, the recommendation of micronutrients for *Eucalyptus* plants is not yet consolidated, especially for Cu and Zn. In practice, most forestry companies (e.g., in Brazil) apply Cu and Zn fertilizers at a lower rate than that of fertilizers with B, mixed with NPK fertilizers to replace Cu and Zn contents removed by harvesting forest products (Gonçalves and Valeri, 2001). Deficiencies of Cu and Zn in *Eucalyptus* plantations are more frequently observed in the medium- or long-term and have been mainly reported in Brazilian Cerrado soils (Rodrigues et al., 2012).

Copper is a redox-active element with key roles in photosynthesis, respiration, antioxidant machinery, and C and N metabolism in plants (Bhatla and Lal, 2018; Broadley et al., 2012). In *Eucalyptus* plants, the visual damage caused by Cu deficiency includes the progressive death of branches, deformed young leaves, tortuous stems and branches, and reduced lignification rates, so that branches acquire a "fallen" appearance (Dell et al., 2003; Gonçalves and Valeri, 2001). Zinc is the second most abundant transition metal in organisms, and although Zn does not participate in redox reactions (Broadley et al., 2012), it is the only metal that is present in enzymes from all classes (i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases), thus playing highly diversified roles in plants (Sousa et al., 2009). The typical symptoms of Zn deficiency in *Eucalyptus* plants are characterized by the presence of lanceolate, narrow, and small young leaves, in addition to the loss of apical dominance and the subsequent overgrowth of buds (Dell et al., 2003; Gonçalves and Valeri, 2001). In addition, both Cu and Zn work together to facilitate the activity of Cu/Zn superoxide dismutase, a key enzyme of the plant antioxidant machinery (Soares et al., 2019). Despite the well-known potential impact of lime on Cu and Zn availability to plants, few studies have evaluated the combined application of lime and Cu plus Zn fertilization on *Eucalyptus* plantations.

In this study, the performance of field-grown *Eucalyptus grandis* in soil fertilized with different rates of application of dolomitic limestone and Cu plus Zn, along with tree development, was assessed. We hypothesized that the combined application of lime and Cu plus Zn may surpass some of the potential side-effects from lime application, thus improving the shoot biomass of *Eucalyptus* trees. To test this hypothesis, we evaluated the soil chemical attributes, shoot biomass, and nutrient accumulation, while nutrient use efficiency, chlorophyll (Chl) and flavonoid (Flav) contents, and enzymatic antioxidant performance [superoxide dismutase (SOD, EC 1.15.1.1), catalase (EC 1.11.1.6, CAT), glutathione reductase (EC 1.6.4.2, GR), ascorbate peroxidase (EC 1.11.1.11, APX), guaiacol peroxidase (EC 1.11.1.7, GPOX), and glutathione peroxidase (EC 1.11.1.9, GPX)] were assayed in order to assess the nutritional status of the plants.

## 3.2. Material and methods

### 3.2.1. Experimental area and plant materials

The research was carried out at one of the experimental stations of the University of São Paulo, in Itatinga, State of São Paulo, Brazil (23°03′ S and 48°37′ W, elevation of approximately 850 m). According to the Köppen classification, this region has a climate $Cf_a$, with a dry season from April to September, a wet season from October to March, a mean annual temperature of 20°C, and an average annual rainfall of 1200 mm (Alvares et al., 2013) (Supplementary Fig. S1). The topography of the experimental site is flat, and the soil is dominated by quartz, kaolinite, and oxyhydroxides, of medium texture, and is classified as Rhodic Ferralsol (IUSS Working Group WRB, 2015). The physical (Camargo et al., 1986) and chemical features of the soil (Raij et al., 2001) were determined at different depths before the experiment (Supplementary Table S1). Seminal seedlings of *Eucalyptus grandis* Hill Ex Maiden (from Coff's Harbor), 3 months old, were planted in an experimental area of 1.2 hectare at a 3 × 2 m spacing (1667 trees $ha^{-1}$) in July 2016. Each plot had 9 × 9 trees (with 5 × 5 trees as useful samples). Fertilizers were manually applied to the soil according to the phases of fertilization (Supplementary Table S2). A sulfate source of Cu and Zn (25% of Cu and 21% Zn) was added immediately after planting, with side holes at 0.2 m from the seedling and 0.10 m deep (Supplementary Fig. S2). In addition, the soil of all treatments received fertilizers containing NPK (60 kg $ha^{-1}$ of N, 60 kg $ha^{-1}$ of $P_2O_5$, 140 kg $ha^{-1}$ of $K_2O$) and B (5 kg $ha^{-1}$ of B, as borax) (Supplementary Table S2).

### 3.2.2. Treatments and crop management

A randomized block (four blocks) design was used in this experiment, in which three dolomitic limestone (DL) rates and two Cu plus Zn mineral fertilizer (MF) rates were applied according to a factorial scheme (3 × 2), totaling six treatments. The DL rates were identified as no lime rate or 0.0 Mg $ha^{-1}$ (NoR); low lime rate or 1.6 Mg $ha^{-1}$ (LoR), calculated by 20-[Ca+Mg]/10 as recommended for *Eucalyptus* plants by Gonçalves (2010); and high lime rate or 3.7 Mg $ha^{-1}$ (HiR), calculated by base saturation up to 60%, as recommended for annual crops (e.g., wheat and rice) by Raij et al. (1996). The MF rates were: 0.0 kg $ha^{-1}$ Cu plus 0.0 kg $ha^{-1}$ Zn (-CuZn), and 0.5 kg $ha^{-1}$ Cu plus 1.5 kg $ha^{-1}$ Zn (+CuZn), as recommended for *Eucalyptus* plantations by Gonçalves (2010). DL [(40% of calcium oxide (CaO), 10% of magnesium oxide (MgO), and 85% of effective calcium carbonate equivalent (ECCE)] was applied to the soil, which was previously prepared two weeks before planting using a forest subsoiler (up to 0.4 m) and a plow disk attached to a mechanical tractor in the planting range (at a 0.2 m depth of 1.0 m width). In Brazil, lime application for *Eucalyptus* is usually carried out in a total area without soil incorporation, approximately three months before planting. In our study, the incorporated soil-lime aimed to accelerate soil reactions and to increase pH, thus potentializing the reduction of the availability of Cu and Zn in the soil and plants at different times.

### 3.2.3. Soil chemical attributes

Soil samples (at depths of 0–0.20 m) were collected using a Dutch auger at 6 and 30 months after planting, both during the wet season. The soil samples at 6 months were analyzed to monitor soil fertility in the initial stage of the *Eucalyptus* plantation, and at 30 months to evaluate the soil fertility after complete canopy closure (Gonçalves et al., 2008). Each soil sample was composed of nine subsamples that were collected close to the trees in the planting lines and close to the place where the DL and MF were applied per plot (Supplementary Fig. S2). Each sample was homogenized, dried at 45°C, and sieved through a 2.0 mm mesh. Soil chemical attributes (i.e., pH, effective cation exchange capacity ($_e$CEC), base saturation (BS), and Ca, Mg, Cu, Fe, Mn, and Zn availability] were quantified according to Raij et al. (2001).

### 3.2.4. Plant biometry and leaf litter biomass

Both the stem diameter at breast height and tree height were measured at 12 and 30 months after planting. The measurements at 12 and 30 months were carried to represent the stages before and after the complete canopy closure of trees, respectively (Gonçalves et al., 2008, 2013). In each period, twenty-four trees (six per block, situated on the border of the plots) representative of the field-trial setup were selected for cubing by employing the Schumacher and Hall (1933) model, which yielded equations with $P < 0.0001$ (Supplementary Table S3).

Senescent leaves were collected monthly, from April 2018 to March 2019 (from 21 to 32 months after planting) using six quadrants (0.5 × 0.5 m) placed 0.5 m above the soil surface (Supplementary Fig. S3). The quadrants were arranged in the useful area of inner plots, according to the Voronoi diagram, to sample a quarter (¼) of the area occupied by a tree (Saint-André et al., 2005). The leaf litter biomass was grouped to estimate the dry weight accumulated in dry (April 2018 to September 2018 ) and wet (October 2018 to March 2019) seasons and the total sum of the leaf litter biomass of the two seasons.

### 3.2.5. Nutrient accumulation and nutrient use efficiency

Leaf litter and shoot organs (i.e., leaf, branch, bark, and stem) were dried in an oven at 60°C, and the dried samples were ground and subjected to nitric perchloric digestion (Malavolta et al., 1997). Then, the concentrations of Ca, Mg, Cu, Fe, Mn, and Zn were quantified using plasma optical emission spectrometry (ICP-OES equipment, Thermo Scientific iCAP 700). The accuracy and precision of the analytical method were confirmed using standard reference materials – SRM (NIST 1515 – apple leaves and NIST 1573a – tomato leaves). Elemental recovery from SRM ranged from 95 to 98%. The amount of nutrient accumulation (stock) in leaf litter and in different shoot compartments (from 12 and 30 months after planting) was obtained by multiplying the concentration of each nutrient by the dry biomass of each plant part. Nutrient use efficiency (NUE) was obtained by dividing the total biomass by the amount of accumulation of each nutrient in each plant compartment and leaf litter (Santos et al., 2020; Turner and Lambert, 2014).

### 3.2.6. Index of leaf pigments

The leaf Chl index, Flav index, and nitrogen balance index [NBI (Chl/Flav)] were evaluated at 24 and 30 months after planting using a Dualex Scientific meter (FORCE-A; Orsay, France (Cerovic et al., 2012)). The reading was obtained between 8:00 and 11:00h in leaves located in the upper third of the canopy, using the middle region of the adaxial and abaxial sides of mature leaves (and outside the leaf central rib). Twelve leaves from each of the five trees (i.e., 60 leaves) were located in the diagonal transect of the useful area of the experimental unit (Supplementary Fig. S2).

### 3.2.7. Malondialdehyde and hydrogen peroxide contents

Fully expanded leaves were harvested from the canopy upper third of the same 30-month-old trees (30 months after planting) that were felled to estimate biomass production and immediately stored in liquid nitrogen. Thereafter, the samples were stored at –80°C until biochemical analysis. Samples were ground to a fine powder in liquid nitrogen, and 0.2 g of leaf tissue from biological replicates were macerated in 2 mL of 0.1% trichloroacetic acid (TCA) containing approximately 20% polyvinylpolypyrrolidone (PVPP; w/v). Lipid peroxidation, expressed as malondialdehyde (MDA) content, was determined according to

Heath and Packer (1968), by reading the reaction mixture containing the sample extract, TCA, and 2-thiobarbituric acid in a spectrophotometer (Genesys 10S UV–VIS)at 532 and 600 nm. To determine the $H_2O_2$ content, the reaction mixture containing leaf extract, K phosphate buffer, and K iodide was quantified spectrophotometrically at 390 nm (Alexieva et al., 2001).

### 3.2.8. Enzymatic antioxidant performance

To extract proteins, ground leaves (0.25 g) were homogenized in a mortar and pestle, and phosphate buffer (2.5 mL) consisting 2% of Triton X-100 and 2% of PVPP (w/v) was added (Shvaleva et al., 2006). Superoxide dismutase (SOD, EC 1.15.1.1), activity was determined by reading the reaction mixture containing phosphate buffer (pH 7.8), nitroblue tetrazolium (NBT), riboflavin, methionine, ethylenediaminetetraacetic acid (EDTA), and protein extract in a spectrophotometer at 560 nm after irradiation with white light for 5 min (Cembrowska-Lech et al., 2015). Catalase (EC 1.11.1.6, CAT), activity was spectrophotometrically measured by evaluating $H_2O_2$ decomposition for 1 min in the reaction mixture (containing the leaf extract plus $H_2O_2$) at 240 nm (Azevedo et al., 1998). To determine glutathione reductase (EC 1.6.4.2, GR) activity, the reaction mixture containing oxidized glutathione (GSSG), dithiobis-nitrobenzoic acid (DTNB), nicotinamide adenine dinucleotide phosphate – NADPH, potassium phosphate buffer solution, and leaf extract) was spectrophotometrically assessed at 412 nm (Azevedo et al., 1998). The ascorbate peroxidase (EC 1.11.1.11, APX) activity was determined by reading the reaction mixture (containing the leaf extract, potassium phosphate buffer solution, ascorbate – AsA, EDTA, and $H_2O_2$) in a spectrophotometer at 290 nm (Nakano and Asada, 1981). The guaiacol peroxidase (EC 1.11.1.7, GPOX) activity was determined by reading the reaction mixture (containing the plant extract, phosphatocitrate buffer, guaiacol, and $H_2O_2$) in a spectrophotometer at 450 nm after incubation at 30°C for 15 min (Matsuno and Uritani, 1972; Monteiro et al., 2011). Glutathione peroxidase (EC 1.11.1.9, GPX) activity was determined in a reaction mixture containing potassium phosphate buffer, EDTA, GR, reduced glutathione (GSH), sodium azide, which was incubated at 37°C for 15 min. The protein (or leaf) extract was added and the reaction was started by adding NADPH and $H_2O_2$. The oxidation rate was monitored for 5 min at 340 mM (Anderson and Davis, 2004). The soluble protein concentration, which was measured according to Bradford (1976), was used to estimate the activity of all antioxidant enzymes.

### 3.2.9. Statistical procedures

A complete block design was a trial set up, which was carried out in a 3 × 2 factorial scheme (three limestone rates *vs.* two Cu plus Zn rates), totaling six treatments. The data were submitted to a two-way analysis of variance (ANOVA), with a *P*-value of 10%. All data were checked using the Levene test for homogeneity of variance and the Shapiro–Wilk test for normality of residuals. When the F test of ANOVA showed significance ($P < 0.10$), the means of the treatments were compared using the LSD test ($P < 0.10$). A P-value equal to or lower than 10% was selected owing to the high complexity of environmental factors in field trials, for example, as compared with those of the trial setup in greenhouse conditions. Principal component analysis (PCA) and hierarchical cluster analysis using the cluster Ward method were employed for some set of variables. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc, 2016) and R version 4.0.2 (R Core Team, 2016).

## 3.3. Results

### 3.3.1. Chemical attributes of the soil were only affected by lime application

Lime application increased pH, $_e$CEC, BS, and Ca and Mg availability, as observed at the 0–0.2 m soil layer at 6 and 30 months after planting (Fig. 1). Manganese availability was not altered, while Cu, Zn, and Fe availability were depressed at 6 months after planting (Fig. 1). Thirty months after planting, lime application did not significantly affect Zn availability among treatments. Meanwhile, Mn availability increased, whereas Cu and Fe soil availability decreased because of lime.

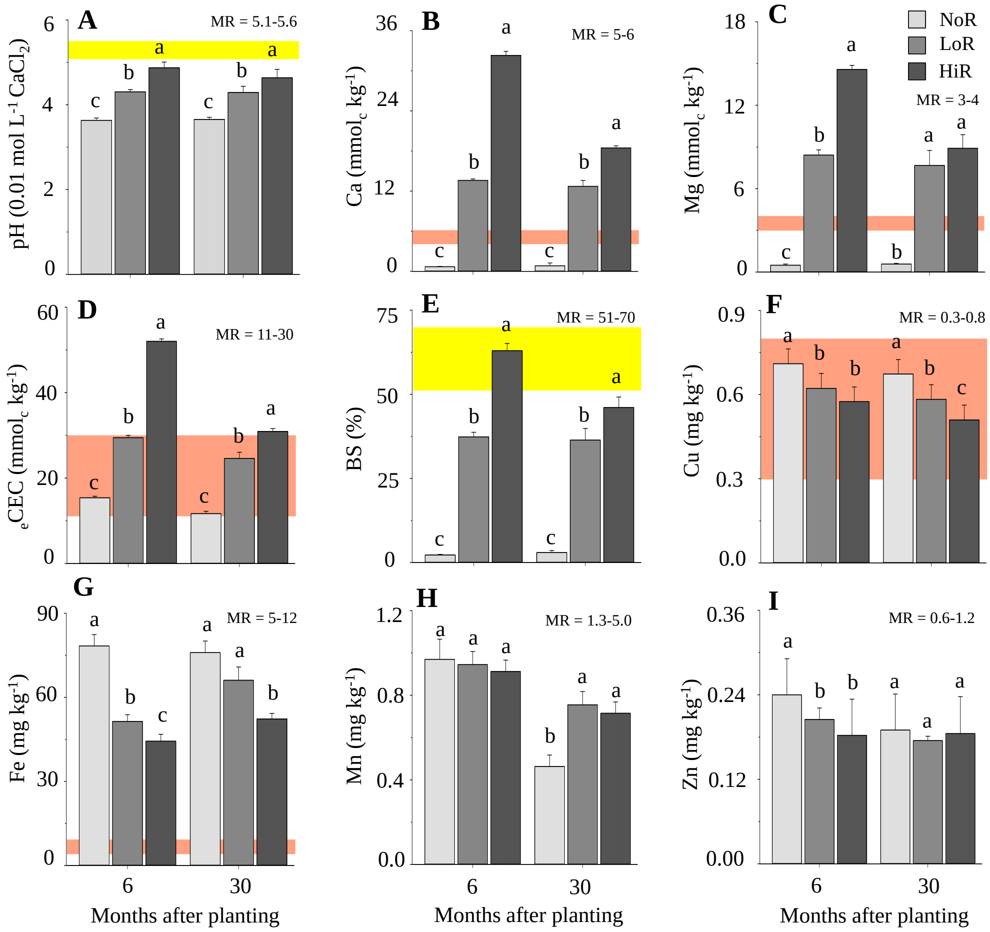


**Fig. 1**. Soil chemical attributes (0-0.2 m depth), at 6 and 30 months after planting *E. grandis*, under dolomitic limestone (DL). Treatments of DL: no lime rate - 0.0 Mg ha$^{-1}$ (NoR), low lime rate - 1.6 Mg ha$^{-1}$ (LoR), and high lime rate - 3.7 Mg ha$^{-1}$ (HiR). Chemical analysis according to Raij et al. (2001), where: pH in $CaCl_2$ (0.01 mol L$^{-1}$) (A); Ca (B) and Mg (C), effective cation exchange capacity - $_e$CEC (D), base saturation in percentage - BS (E) and available of Cu (F), Fe (G), Mn (H) and Zn (I). In each month, means with standard error bars followed different letters indicate differences among treatments by LSD test ($P$ < 0.10) (n=4). MR = mean range in the soil for *Eucalyptus* plants (Gonçalves, 2010; red) and relative to acidity in the soil (Raij, et al., 1996; yellow).

### 3.3.2. Lime × Cu plus Zn application improved wood production in *Eucalyptus* plantation

Liming increased the shoot biomass at 12 and 30-month-old plants (Table 1). The application of Cu plus Zn had a positive effect on leaf, branch, bark, and stem biomass at 12 months after planting in the no-lime treatment, whereas a negative effect was observed in the treatment with lime (Table 1). However, at 30-month-old plants, the application of Cu plus Zn did not affect lime-treated plants, while it was beneficial for the biomass of plants cultivated in lime-absent soil (Table 1). At 30 months after planting, the total biomass yield increased by 8% in plants treated with lime (i.e., LoR + HiR), 2% in plants under only treatment with Cu plus Zn (i.e., +CuZn), and 13% in plants under the combined application of lime and Cu plus Zn as compared with plants treated without lime × without Cu plus Zn fertilization.

**Table 1**
Biomass production in *Eucalyptus grandis* compartments, at 12 and 30 months after planting under dolomitic limestone application (DL) and Cu plus Zn mineral fertilizer (MF) (mean±standard error, n=4).

| Treat | -CuZn | +CuZn | -CuZn | +CuZn | -CuZn | +CuZn | -CuZn | +CuZn | -CuZn | +CuZn |
|---|---|---|---|---|---|---|---|---|---|---|
| | ------------------- Mg $ha^{-1}$ ------------------- | | | | | | | | | |
| | Leaf | | Branch | | Bark | | Stem | | Total biomass | |
| | 12 months | | | | | | | | | |
| NoR | 1.7±0.1 Bb | 2.1±0.1 Aa | 3.1±0.1 Bb | 3.5±0.0 Aa | 0.46±0.03 Bb | 0.54±0.01 Aa | 2.3±0.1 Bb | 2.6±0.0 Aa | 7.6±0.3 Bb | 8.7±0.2 Aa |
| LoR | 2.7±0.2 Aa | 2.1±0.1 Ab | 4.2±0.1 Aa | 3.6±0.2 Ab | 0.64±0.03 Aa | 0.54±0.02 Ab | 3.1±0.2 Aa | 2.6±0.1 Ab | 10.7±0.5 Aa | 8.9±0.4 Ab |
| HiR | 2.7±0.1 Aa | 2.3±0.1 Ab | 4.0±0.1 Aa | 3.7±0.1 Ab | 0.64±0.02 Aa | 0.56±0.02 Ab | 3.1±0.1 Aa | 2.7±0.1 Ab | 10.5±0.4 Aa | 9.3±0.2 Ab |
| | 30 months | | | | | | | | | |
| NoR | 5.9±0.4 Bb | 6.8±0.2 Aa | 5.4±0.4 Bb | 6.2±0.2 Aa | 6.2±0.3 Bb | 6.9±0.2 Aa | 35.7±1.7 Bb | 39.9±0.9 Aa | 53.2±2.7 Bb | 59.8±1.6 Aa |
| LoR | 7.1±0.2 Aa | 6.6±0.3 Aa | 6.5±0.2 Aa | 6.0±0.3 Aa | 7.1±0.2 Aa | 6.9±0.2 Aa | 40.9±1.0 Aa | 40.0±0.9 Aa | 61.6±1.6 Aa | 59.6±1.6 Aa |
| HiR | 7.1±0.2 Aa | 7.0±0.2 Aa | 6.5±0.2 Aa | 6.4±0.2 Aa | 7.1±0.1 Aa | 7.0±0.1 Aa | 41.0±0.7 Aa | 40.2±0.8 Aa | 61.6±1.2 Aa | 60.6±1.3 Aa |

Treatments (Treat) of DL: no lime rate - 0.0 Mg $ha^{-1}$ (NoR); low lime rate - 1.6 Mg $ha^{-1}$ (LoR); and high lime rate - 3.7 Mg $ha^{-1}$ (HiR). Treat of MF: no Cu plus Zn (-CuZn, 0.0 kg $ha^{-1}$), and with 0.5 kg $ha^{-1}$ of Cu plus 1.5 kg $ha^{-1}$ Zn (+CuZn). Different uppercase letters in the same period (column) indicate differences between treatments of DL, and different lowercase letters in the same compartment (line) indicate differences between treatments of MF by LSD test ($P < 0.10$).

### 3.3.3. The accumulated leaf litter biomass increased after lime application

Only lime application significantly influenced the accumulated leaf litter biomass from 21- to 32-month-old plants. Plants under lime treatments (i.e., LoR + HiR) increased the accumulated leaf litter biomass by 17% (19% in dry and 15% in wet seasons) relative to that of the no-lime treatment (Table 2). However, there was no difference in biomass yield accumulated in leaf litter between treatments with lime application by seasons (i.e., dry and wet) or the total accumulated biomass for 12 months.

**Table 2**
Biomass in the leaf litter accumulated over Apr-2018 to Mar-2019 (from 21 to 32 months after planting), under dolomitic limestone application (DL), in dry and wet season (mean±standard error, n=4).

| Treat | Dry season (Apr-2018 to Sep-2018) | Wet season (Oct-2018 to Mar-209) | Total sum (Apr-2018 to Mar-2019) |
|---|---|---|---|
| | ------------------------------------------- Mg $ha^{-1}$ ------------------------------------------- | | |
| NoR | 2.1±0.1 b | 2.3±0.2 b | 4.4±0.3 b |
| LoR | 2.6±0.1 a | 2.6±0.1 a | 5.1±0.2 a |
| HiR | 2.5±0.1 a | 2.7±0.1 a | 5.1±0.1 a |

Treatments (Treat) of DL: no lime rate - 0.0 Mg $ha^{-1}$ (NoR); low lime rate - 1.6 Mg $ha^{-1}$ (LoR); and high lime rate - 3.7 Mg $ha^{-1}$ (HiR). Different lowercase letters in the same period (column) indicate differences between treatments by LSD test ($P < 0.10$).

### 3.3.4. Lime application changed the dynamics of nutrient accumulation and nutrient use efficiency in plants and leaf litter

An increased total accumulation of Ca, Mg, Cu, Fe, and Zn was observed in the shoots of 12-month-old tree that were grown under a high lime rate of application, when compared with that of plants not treated with lime (Fig. 2). In 12-month-old plants, Ca, Mg, and micronutrients (i.e., Cu, Fe, Mn, and Zn) were mainly accumulated in leaves and branches, whereas in 30-month-old plants, Ca, Mg, and Mn accumulated mainly in barks and Cu, Fe, and Zn in stems (Supplementary Table S4). In addition, in 30-month-old plants, the total Cu and Zn accumulation did not differ significantly between treatments of different lime rates of application (Fig. 2A, B). Alternatively, differences were significant between lime-treated and non-limed plants in Ca and Mg contents (higher in plants subjected to lime) and Mn accumulation (lower in plants from lime treatment). In addition, the total Fe accumulation was higher in 30-month-old *Eucalyptus* trees grown in soil without lime than in plants grown in soil with lime. Plants under treatments with lime application decreased the use efficiency of Ca and Mg relative to that of plants under no-lime treatment at 12 and 30 months after planting, whereas improved Mn use efficiency was observed in both months after planting, and improved Fe use efficiency was observed only at 30 months after planting (Supplementary Fig. S4). In addition, among the nutrients, the use efficiency in shoots followed the order: Mg > Ca > Cu > Zn > Fe > Mn.

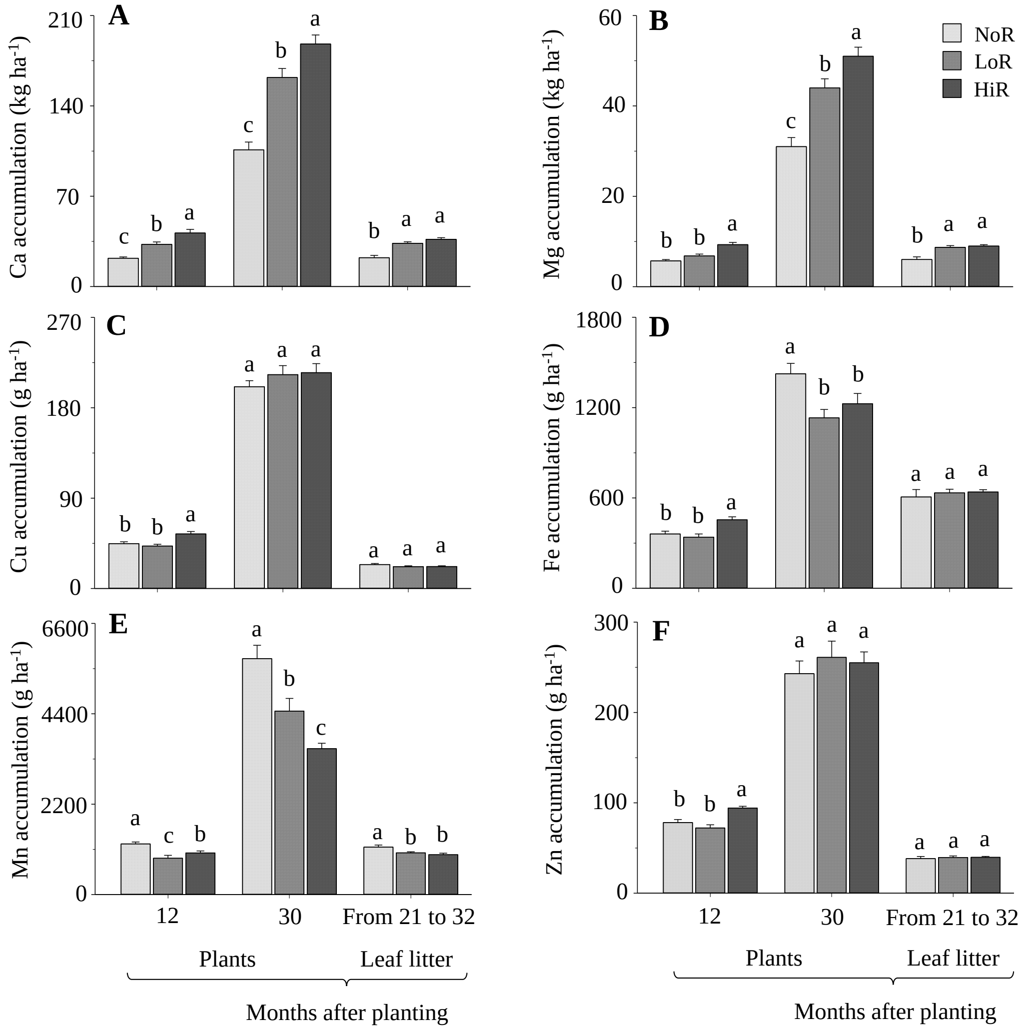



**Fig. 2.** Total accumulation of Ca (A), Mg (B), Cu (C), Fe (D), Mn (E) and Zn (F) in the plant (12 and 30 months after planting) and leaf litter (accumulated during 12 months after planting) at the *Eucalyptus grandis* plantation, after dolomitic limestone application (DL). Treatments of DL: no lime rate - 0.0 Mg ha$^{-1}$ (NoR), low lime rate - 1.6 Mg ha$^{-1}$ (LoR), and high lime rate - 3.7 Mg ha$^{-1}$ (HiR). Standard error bars with different lowercase letters in the same month indicate differences between treatments by LSD test ($P$ < 0.10) (n = 4).

The most remarkable effects of lime on shoot nutrients were reflected in leaf litter: increased total accumulation of Ca and Mg, whereas decreased total accumulation of Mn, were observed after the application of lime to the soil (Fig. 2). Nonetheless, in the wet season, the accumulated leaf litter was reduced only in Cu and Mn contents in the plants treated with lime as compared with that of plants without lime treatment (Supplementary Table S5). Meanwhile, plants under lime treatment decreased the use efficiency of Ca and Mg in dry season (38% Ca and 28% Mg) and wet season (31% Ca and 30% Mg) and increased the use efficiency of Cu (21% dry and 47% wet season), Fe (30% only wet season), Mn (31% dry and 46% wet season), and Zn (12% dry and 10% wet season) relative to that of plants under no-lime application (Supplementary Fig. S4).

### 3.3.5. Chlorophyll and flavonoid contents and several antioxidant system components were modulated by lime and/or Cu plus Zn application

Combined application of lime and Cu plus Zn improved Chl and Flav contents in 24-month-old plants (Table 3). This influence was maintained for the Chl index in 30-month-old plants. However, only lime enhanced the Flav index in 30-month-old plants. NBI was unaltered by these factors in 24-month-old plants, but it was increased in lime-treated 30-month-old plants compared to no-limed plants (Table 3).

**Table 3**
Chlorophyll (Chl), flavonoids (Flav), and nitrogen balance index (NBI or Chl/Flav ration) in the leaf of *Eucalyptus grandis*, in dry (24 months old plants) and wet seasons (30 months old plants), after dolomitic limestone application (DL) and Cu plus Zn mineral fertilizer (MF) (mean±standard error, n=4).

| Treat | -CuZn | +CuZn | -CuZn | +CuZn | -CuZn | +CuZn |
|---|---|---|---|---|---|---|
| | --------------------------------------- units (Dualex index) ------------------------------------ | | | | | |
| | Chlorophyll | | *Flavonoid* | | *Nitrogen Balance Index* | |
| | 24 months (dry season) | | | | | |
| NoR | 36.9±1.4 Bb | 39.5±1.4 Ba | 3.1±0.0 Bb | 3.4±0.1 Ba | 11.8±0.3 Aa | 11.5±0.3 Aa |
| LoR | 42.3±1.2 Ab | 43.5±1.0 Aa | 3.5±0.1 Ab | 3.6±0.1 Aa | 12.0±0.5 Aa | 12.5±0.4 Aa |
| HiR | 41.7±1.0 Ab | 43.0±1.5 Aa | 3.4±0.1 Ab | 3.6±0.1 Aa | 12.4±0.3 Aa | 12.1±0.6 Aa |
| | 30 months (wet season) | | | | | |
| NoR | 31.2±0.7 Ca | 31.6±0.3 Ca | 3.1±0.1 Ba | 3.1±0.1 Ba | 9.4±0.3 Ba | 10.1±0.4 Ba |
| LoR | 34.7±0.7 Ab | 38.2±1.6 Aa | 3.4±0.1 Aa | 3.5±0.1 Aa | 10.6±0.5 Aa | 10.9±0.5 Aa |
| HiR | 32.7±0.9 Bb | 33.7±0.7 Ba | 3.2±0.1 Ba | 3.2±0.1 Ba | 10.4±0.1 Aa | 10.6±0.5 Aa |

Treatments (Treat) of DL: no lime rate - 0.0 Mg ha$^{-1}$ (NoR), low lime rate - 1.6 Mg ha$^{-1}$ (LoR), and high lime rate - 3.7 Mg ha$^{-1}$ (HiR). Treat of MF: no Cu plus Zn (-CuZn, 0.0 kg ha$^{-1}$), and with 0.5 kg ha$^{-1}$ of Cu plus 1.5 kg ha$^{-1}$ Zn (+CuZn). Different uppercase letters in the same period (column) indicate differences between treatments of DL, and different lowercase letters in the same pigment (line) indicate differences between treatments of MF by LSD test ($P < 0.10$).

The MDA content was increased by increasing the lime content in soil, but the Cu plus Zn application reduced lime-induced MDA production to normal levels (Fig. 3A). The lime-treated plants also exhibited higher $H_2O_2$ content than no-limed plants, but the use of Cu plus Zn did not affect it (Fig. 3B). Total protein concentration and CAT in leaves were unaltered by the combined application of lime and Cu and Zn (Fig. 3C, E), while CAT activity increased only in plants without lime and that were fertilized with Cu and Zn (Fig. 3E). The SOD and GPOX activities were only improved in limed plants that received Cu and

Zn (Fig. 3D, H), whereas GR, APX, and GPX activities were synergistically improved by the combined application of lime and Cu plus Zn (Fig. 3F, G, I).

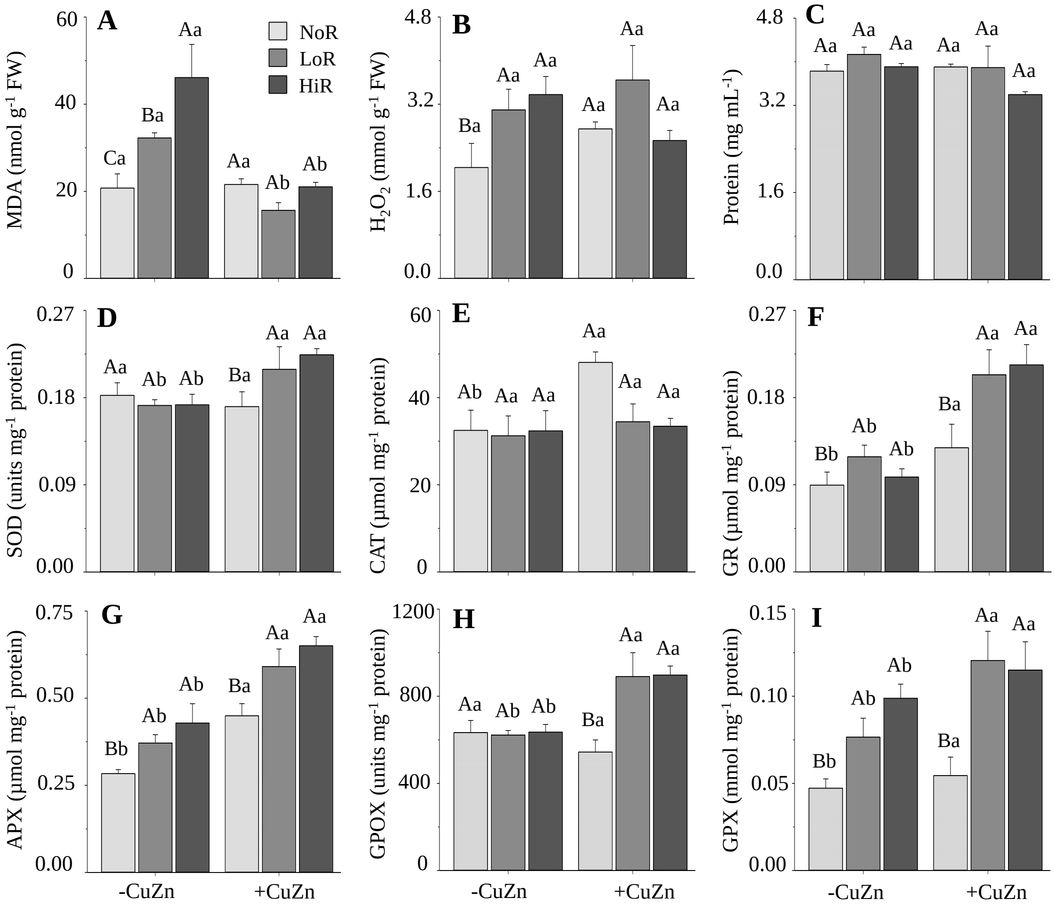



**Fig. 3.** Lipid peroxidation (MDA) (A), hydrogen peroxide ($H_2O_2$) (B), proteins (C) and activity of superoxide dismutase (SOD) (D), catalase (CAT) (E), glutathione reductase (GR) (F), ascorbate peroxidase (APX) (G), guaiacol peroxidase (GPOX) (H), and glutathione peroxidase (GPX) (I) in the leaf of *Eucalyptus grandis* plantation, at 30 months old, under application of dolomitic limestone (DL) and Cu plus Zn fertilizer (MF). Treatments of DL: no lime rate - 0.0 Mg $ha^{-1}$ (NoR), low lime rate - 1.6 Mg $ha^{-1}$ (LoR), and high lime rate - 3.7 Mg $ha^{-1}$ (HiR). Treatments of MF: no Cu plus Zn (-CuZn, 0.0 kg $ha^{-1}$), and with 0.5 kg $ha^{-1}$ of Cu plus 1.5 kg $ha^{-1}$ Zn (+CuZn). Standard error bars with different uppercase letters indicate differences between treatments of DL, and different lowercase letters indicate differences between treatments of MF by LSD test ($P < 0.10$) (n = 4).

### 3.3.6. PCA and cluster analysis described combined implications of lime and Cu plus Zn in crop

Through PCA, the relationship between total biomass (TB) and use efficiency of Ca, Mg, Cu, Fe, Mn, and Zn was evaluated within the plant compartments (Fig. 4A) and leaf litter (Fig. 4B). In plants, these variables plus Chl and Flav index, $H_2O_2$ and MDA contents, and SOD activity were investigated through PCA (Fig. 4C) and hierarchical cluster analysis (Fig. 4D). TB was positively correlated with micronutrient use efficiency (i.e., Cu, Fe, Mn, and

Zn). In addition, the relationship between the use efficiency of these micronutrients was of positive correlation with Chl, Flav, $H_2O_2$, and SOD in plants.

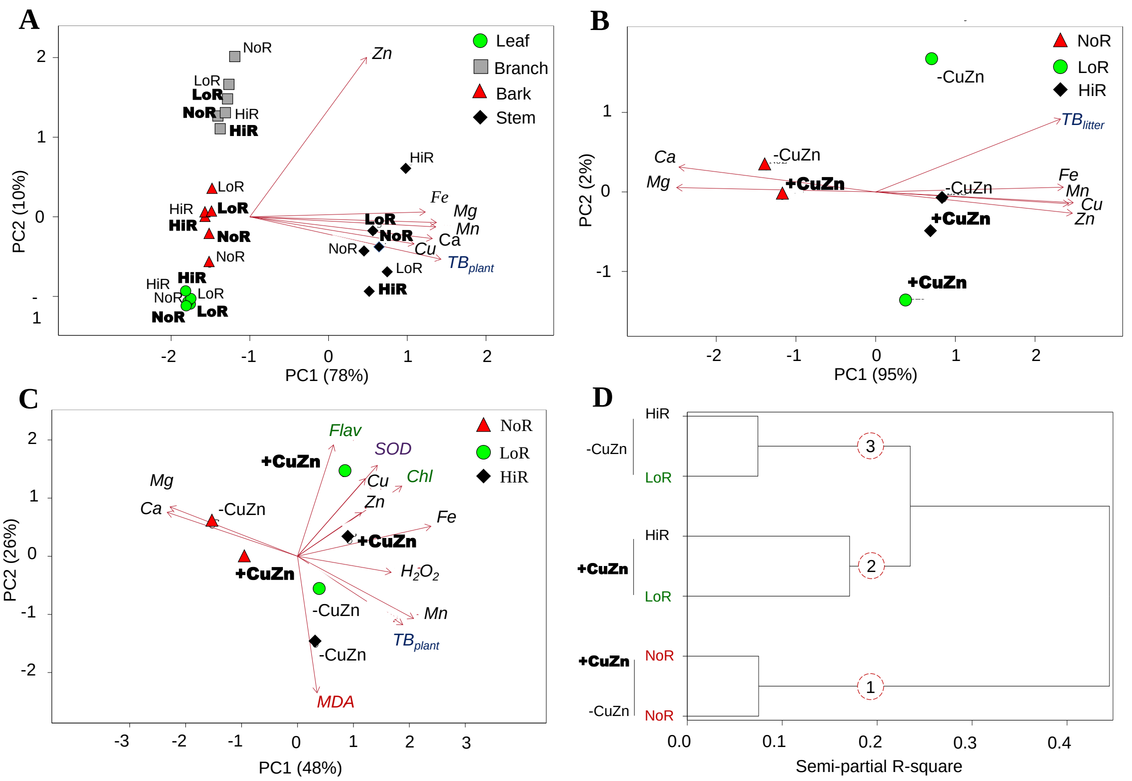


**Fig. 4.** Principal component analysis (PCA) among total biomass (TB) and use efficiency of Ca, Mg, Cu, Fe, Mn, and Zn in *Eucalyptus grandis* compartments 30 months after planting (A), leaf litter accumulated at 21 to 32 months after planting (B) and these previous variables plus content of chlorophyll (Chl), flavonoids (Flav), hydrogen peroxide ($H_2O_2$) and lipid peroxidation [expressed by malondialdehyde(MDA)], and activity of superoxide dismutase (SOD) (C) in trees at 30 months after planting, and variables of letter C for cluster analysis (D), under rates of dolomitic limestone (DL) and Cu plus Zn mineral fertilizer (MF). Treatments of DL: no lime rate - 0.0 Mg ha$^{-1}$ (NoR), low lime rate - 1.6 Mg ha$^{-1}$ (LoR), and high lime rate - 3.7 Mg ha$^{-1}$ (HiR). Treatments of MF: no Cu plus Zn (-CuZn, 0.0 kg ha$^{-1}$), and with 0.5 kg ha$^{-1}$ of Cu plus 1.5 kg ha$^{-1}$ Zn (+CuZn).

The first two components explained 88% of the data variance, and the leaf followed by bark and branch was characterized by lower TB and Ca, Mg, Cu, Fe, Mn, and Zn use efficiency, whereas higher values were observed in stem (Fig. 4A). In leaf litter, combined treatments with lime and Cu plus Zn were characterized by higher TB and Cu, Fe, Mn, and Zn use efficiency and lower Ca and Mg use efficiency (Fig. 4B). Overall, plants under lime (i.e., LoR and HiR) + Cu plus Zn (i.e., +CuZn) treatment had higher Chl, Flav, and SOD and lower MDA (Fig. 4C). These previous outcomes were supported by cluster analysis (Fig. 4D). Indeed, approximately 0.2 semi-partial R-square values were obtained for three different groups: group 1) treatments no-lime + with or without Cu plus Zn (i.e., NoR × -CuZn or +CuZn), group 2) treatments with-lime + Cu plus Zn (i.e., LoR or HiR × +CuZn), and group 3) treatments with-lime + no Cu plus Zn (i.e., LoR or HiR × -CuZn).

## 3.4. Discussion

Increased *Eucalyptus* wood yield has been achieved owing to advances in plant breeding and improvements in silvicultural practices, including enhanced nutritional and fertilization management of forest plantations (Lima Neto et al., 2020). However, there are several gaps in silvicultural management that need to be solved to improve further *Eucalyptus* wood productivity. Our study showed that the combined application of lime and Cu and Zn increased the shoot biomass production of eucalyptus-trees. This effect was seen by the increased values of Chl index, Flav index, antioxidant enzyme activity, Ca and Mg accumulation, and Cu and Zn use efficiencies in *Eucalyptus grandis* trees. In addition, different rates of lime treatments (i.e., LoR and HiR) increased the pH, Ca, Mg, $_e$CEC, and BS in soil relative to those of no-lime treatments (i.e., NoR).

### 3.4.1. Combined application of lime and Cu plus Zn altered soil fertility and then enhanced tree growth

The soil in which *Eucalyptus* trees were grown had naturally low levels of Ca, Mg, Mn, and Zn, medium $_e$CEC levels, and high Cu and Fe levels (Supplementary Table S1) according to Gonçalves (2010) for *Eucalyptus* species more planted in Brazil. The application of Cu and Zn fertilizers was not significant for responses of the soil chemical attributes at 6 and 30 months after *Eucalyptus* planting,  probably because of the low rate applied to the soil, which in this study was applied in a side hole and close to plant. However, lime application increased Ca and Mg availability to the plants (Fig. 1), validating previous reports *on* the lime-induced improvement of basic cation levels (Court et al., 2018; Crusciol et al., 2019; Nguyen et al., 2021). This outcome was a consequence of Ca and Mg input (as CaO and MgO, respectively) and elevation of the soil pH from below 4 to 5 (Fig. 1), which made Ca and Mg more available to plants. However, an increase in soil pH may decrease the solubility, concentration, and mobility of Cu, Fe, Mn, and Zn (Barman et al., 2014; Fageria et al., 2002). Accordingly, Cu, Fe, and Zn availabilities were reduced, and such reductions had an increased and prolonged effect when the soil was amended with a high lime rate (Fig. 1). Another hypothesis is that increases in pH depress the redox potential (Eh), favoring the precipitation of these micronutrients by certain minerals. This hypothesis is based on the fact that the most mobile fractions of Cu, Fe, Mn, and Zn occur at pH values lower than 5 and at low Eh

(Kabata-Pendias, 2011), when these metals are easily precipitated in carbonates or linked to compounds of calcium (González-Alcaraz et al., 2013; Jiménez-Cárceles et al., 2008). However, Mn availability had an inverse behavior, which is potentially explained by modifications in the soil biological properties. According to Jones (1957), both microbial inhibition and rapid reduction of Mn oxide may favor slow Mn oxidation at pH below 6, thus increasing Mn availability to plants. Moreover, as kaolinite has two surface exchange sites for $Mn^{2+}$ and demonstrates slightly higher Ca desorption than Mn adsorption at pH 5.2 (Wang et al., 1993), another hypothesis is related to Mn release from kaolinite through desorption.

The demand for soil nutrients by forest plantations is high in the first years after planting (Snowdon, 2002), mainly during the canopy closure stage (Gonçalves et al., 2008; Pereira et al., 2019). Specifically in Brazil, approximately 50% of the soil is acidic and has a high content of available aluminum ($Al^{3+}$), which may cause nutritional disorders (Eswaran et al., 1997; Silva et al., 2004). The lime-enhanced availability of several nutrients was reflected as increased nutrient accumulation in *Eucalyptus* trees the first year after planting (Fig. 2). Exceptions were observed for Mn accumulation in leaves, branches, bark, and stems, despite increments in soil Mn availability after lime application, indicating that Mn acquisition is limited by either mechanism actively modulated by root plants or by competition among nutrients for transporters located in the root system. Accordingly, the Mn uptake is reduced by Mg (Le Bot et al., 1990), and the combination of both Ca and Mg is even more inhibitory to Mn absorption than Mg alone (Maas et al., 1969). In this context, evidence indicated that antagonism between Mn and Ca and Mg at root levels may decrease uptake of Mn by *Eucalyptus*. This may have occurred because of the strong contribution of lime in increasing the Ca and Mg levels in plants (Bossolani et al., 2020; Crusciol et al., 2019; Holland et al., 2018; Paradelo et al., 2015) and leaf litter (Allen et al., 2020; Court et al., 2018; Rocha et al., 2019b).

An increased balance of plant nutrition improved shoot biomass at 12-month-old plants (Table 1) and enhanced leaf litter biomass (Table 2). In addition, the data indicated that reduced initial development of non-limed plants, when compared to limed plants, may be potentially related to antagonism among Mn, Ca, and Mg in *Eucalyptus grandis* (Tables 3 and 4) and probably associated with excessive Mn accumulation in plants (Fig. 2 and Supplementary Table S4). Reduced plant growth was also associated with excessive Mn levels in leaves and linked to disturbances in the cambium region and secondary phloem, which were completely phenolized and collapsed (Leite et al., 2014).

The higher use efficiency of Ca and Mg than those of Cu, Zn, Fe, and Mn (Supplementary Fig. S4) confirmed that these macronutrients have a stronger effect on *Eucalyptus* growth. Indeed, nutrients with high use efficiency, in general, are mainly responsible for limiting yield (Santana et al., 2002; Smethurst, 2010). The higher Cu and Zn use efficiency in plants grown with lime than that of those grown without lime (Supplementary Fig. S4) is explained because leaves have higher metabolic activity than the woodiest tissue (i.e., stemwood). Thus, these micronutrients are redistributed to other plant tissues (Lambers et al., 2008). Accordingly, the data showed that Cu and Zn use efficiency is related to the changes that occurred in the soil-eucalyptus system and the enhanced yield of shoot biomass after planting (Fig. 1 and Table 1).

### 3.4.2. Wood yield reduced in the absence of lime, but supply of Cu plus Zn enhanced plant development

The differences in biomass produced by limed or non-limed *Eucalyptus* at 12 months after planting disappeared in 30-month-old plants under Cu and Zn fertilization. This suggests that plants can reach the maximum potential for biomass production, although this process is slower in the absence of lime but can be achieved with an additional supply of Cu and Zn. It is likely that the lime-induced rearrangement of nutrient partitioning and allocation to counteract the side effects from the high internal Mn accumulation (Tables 3 and 4) allowed fast growth (a kind “compensatory behavior”) in the last stages before canopy closure. Some of these counteraction mechanisms are probably linked to the modulation of leaf pigments (Table 3) and the antioxidant metabolism (Fig. 3). Plant pigments belong to diverse classes of compounds with different structures, properties, and biological functions that can vary from photosynthesis performance and oxidative stress protection to pollinator/disperser attraction, among others (Carvalho et al., 2021; Soares et al., 2019; Solovchenko and Neverov, 2017). Indeed, the lower content of Chl, Flav, and NBI in the no-lime and no Cu plus Zn treatments (Table 3) can be explained by the lower Chl content and photosynthetic rate (Cerovic et al., 2012, 2005), and Flav is directly related to antioxidant functions (Pinkard et al., 2006), and NBI is affected by the nutritional status and the dry leaf mass per area (Marenco et al., 2009).

The green color of leaves mostly depends on Chl content, which generally increases by elevating Mg supply to the plants, and one of the outcomes from reduced Mg concentrations in leaf tissues is the induction of photo-oxidative stress primarily by limiting $CO_2$ assimilation (Jaghdani et al., 2021). Any significant effect on plant growth can be observed only if photosynthesis is affected (Carvalho et al., 2021). Our data indicated that improved Mg accumulation in leaves due to the lime-induced elevation of Mg availability may boost plant growth by enhancing $CO_2$ assimilation. Flavonoid content was also improved by lime, which exhibited a synergistic effect with Cu and Zn (Table 3). Flavonoids are secondary metabolites exclusively produced by plant organisms (Agati and Tattini, 2010; Pollastri and Tattini, 2011) and are classified as anthoxanthins, flavanones, flavanonols, flavans, and anthocyanidins (Gill and Tuteja, 2010) according to their chemical structures and are capable of directly interacting with reactive oxygen species (such as $^1O_2$ and $H_2O_2$). In addition, they can serve as substrates for different peroxidases (Soares et al., 2019).

However, there was no unique pattern to explain wood production as affected by lime and the supply of Cu and Zn. Leaf pigments have been successfully used in agriculture to reveal the relationship between the physiological mechanisms of non-enzymatic antioxidant stress and plant growth (Kalaji et al., 2017). For example, Flav act as efficient antioxidants with different biological functions owing to their antimutagenic principles (Sakihama et al., 2002), as well as reducing agents in the elimination of free radicals (Saxena et al., 2012), with emphasis on the *Eucalyptus* species, which have great potential for producing this pigment content under stress (Bhuyan et al., 2016).

### 3.4.3. The benefits from Cu plus Zn fertilization on growth of plants are partially related to differential modulation of the antioxidant metabolism

An additional supply of Cu and Zn fertilizers modified the activity of several antioxidant enzymes in *Eucalyptus* trees (Fig. 3). In the presence of oxygen ($O_2$), the metallic micronutrients (e.g., Cu and Zn) catalyze the redox cycling of phenolic compounds, thus contributing to combat the production of ROS and other organic radicals, which can damage DNA, lipids, and other organic molecules (Sakihama et al., 2002). Indeed, the modulation of antioxidant machinery is crucial for plants to cope with challenging environments (Soares et al., 2019). The increased enzymatic activity in treatments with Cu plus Zn fertilizers may have occurred because these micronutrients reduced the production of organic acids and the low efficiency of the antioxidant system (Matsumoto and Motoda, 2013). When the data set was divided into groups of plants that either received or did not receive Cu plus Zn, different responses were observed in plants that were treated with these micronutrients. For example, GR, APX, and SOD are more important in protecting against ROS than CAT (Shvaleva et al., 2006). However, during plant development, the supply of Cu and Zn can change CAT activity to the detriment of these peroxidases by affecting GR activity. This hypothesis is supported by the fact that GR is directly and indirectly necessary for the suitable functioning of distinct enzymes; for example, GR is responsible for the reoxidation of GSH, which is (i) an electron donor for GPX, and is (ii) also used to recover ascorbate, which serves as an electron donor for APX (Gratão et al., 2005; Soares et al., 2019).

Moreover, lipid peroxidation (measured as MDA content) was reduced in plants that received Cu plus Zn, most likely owing to the increased activity of GR, APX, GPX, GPOX, and SOD enzymes (Fig. 3). However, Cu and Zn did not reduce $H_2O_2$ content in plants grown with lime, indicating that (i) the modulation of lipid peroxidation depended on the action of GR, APX, GPX, GPOX, and SOD, and (ii) the content of peroxidized lipid was affected by other challenging compounds (e.g., superoxide anion) rather than $H_2O_2$. Through the production of antioxidant enzymes (e.g., SOD), plant defense mechanisms of plants act against reactions that may cause metabolic imbalances, avoiding the activation of different signals through the production of ROS at the apoplast by a different respiratory burst oxidase homolog (RBOH) (Miller et al., 2010). The overproduction of superoxide anions was linked to the increase in MDA content because high SOD activity was detected in all plants with increased lipid peroxidation (Fig. 3). SOD is considered the first enzymatic defense line against oxidative stress that catalyzes $O_2^-$ dismutation in $H_2O_2$ and $O_2$ (Gratão et al., 2005; Soares et al., 2019). Future research should give more attention to the role of distinct SOD isoenyzmes especially due to the fact that they contain metallic ions such as Cu and Zn and that total SOD activity was enhanced after lime and Cu plus Zn application (Fig. 4D).

### 3.4.4. Multivariate analysis indicated that lime and/or Cu plus Zn improve the shoot biomass of trees

The positive correlation between TB and NUE of Cu, Fe, Mn, and Zn confirmed that when the biomass increased, the NUE also increased (Fig. 4A-C). The NUE contributes to explaining the links associated with nutrient balance and wood productivity; for example, significant imbalances in a given nutrient in the soil-plant system can cause structural changes in the development of plants (Turner and Lambert, 2014). The positive correlation among TB, use efficiency of metallic micronutrients (i.e., Cu, Fe, Mn, and Zn), Chl and Flav indexes, $H_2O_2$ content, and SOD activity indicate that these responses were enhanced after lime and Cu plus Zn application (Fig. 4C). These results are associated with high photosynthetic rates and high metabolic activity in plants (Cerovic et al., 2012, 2005). In addition, plants need to activate the antioxidant system to combat ROS (Soares et al., 2019), thereby improving their development (Carvalho et al., 2020). The high contents of Chl, Flav, and SOD and low MDA contents in plants with lime and Cu plus Zn application (Fig. 4C) may indicate the benefit of these factors in plant growth. Accordingly, deficiency of nutrients accelerates the increase of ROS production and, consequently, can modify plant growth. For example, a decrease in the contents of Cu, Fe, Mn, and Zn can influence the activity of SOD, because these micronutrients are cofactors of this enzyme as discussed above (Sharma et al., 2012).

The PCA characterized different treatments under lime × Cu plus Zn rates (Fig. 4A-C), and this outcome was supported by the formation of three groups by cluster analysis (Fig. 4D). Indeed, treatments under combined application of lime and Cu plus Zn rates (group 2) were in different groups as compared with treatments under no-lime and no Cu plus Zn rates (group 1) or only separate lime application (group 3). Group 2 was related to the combined application of lime and Cu plus Zn that improved nutritional status, Chl and Flav contents, the activity of SOD, and wood production and, therefore, benefitted plant growth (Fig. 4C). These responses are associated with a strategy adopted by plants under stress conditions, which may vary according to the nutritional status and plant performance (Pimentel et al., 2005). In addition, because in some cases production can be disproportionately increased, plants need to increase the activity of antioxidant enzymes to reduce ROS, thereby aiming to prevent damage to plant growth (Soares et al., 2019).

## 3.5. Conclusion

The combined application of dolomitic limestone (DL) and Cu plus Zn improved the shoot biomass of *Eucalyptus grandis* plantations. Trees under these two conditions increased Chl and Flav contents, antioxidant enzyme activities; and under DL increased Ca and Mg accumulation, and Cu and Zn use efficiencies. Soil amended with DL increased the values of pH, Ca, and Mg contents, eCEC, and BS, while decreasing Cu and Zn availabilities, mainly in the first months after liming. In conclusion, the use of integrative approaches and temporal evaluations provided new insights into the wood production, biochemical traits, antioxidant metabolism, and nutritional status of field-grown *Eucalyptus* under different lime and Cu plus Zn application rates.

**References**

Agati, G., Tattini, M., 2010. Multiple functional roles of flavonoids in photoprotection. New Phytol. https://doi.org/10.1111/j.1469-8137.2010.03269.x

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell Environ. 24, 1337–1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x

Allen, J., Maunoury-Danger, F., Felten, V., Danger, M., Legout, A., Guérold, F., 2020. Liming of acidified forests changes leaf litter traits but does not improve leaf litter decomposability in forest streams. For. Ecol. Manage. 475. https://doi.org/10.1016/j.foreco.2020.118431

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L. de M., 2013a. Modeling monthly mean air temperature for Brazil. Theor. Appl. Climatol. 113, 407–427. https://doi.org/10.1007/s00704-012-0796-6

Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G., 2013b. Köppen's climate classification map for Brazil. Meteorol. Zeitschrift 22, 711–728.

Anderson, J. V., Davis, D.G., 2004. Abiotic stress alters transcript profiles and activity of glutathione S-transferase, glutathione peroxidase, and glutathione reductase in Euphorbia esula. Physiol. Plant. 120, 421–433. https://doi.org/10.1111/j.0031-9317.2004.00249.x

Azevedo, R.A., Alas, R.M., Smith, R.J., Lea, P.J., 1998. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol. Plant. 104, 280–292. https://doi.org/10.1034/j.1399-3054.1998.1040217.x

Barman, M., Shukla, L.M., Datta, S.P., Rattan, R.K., 2014. Effect of applied lime and boron on the availability of nutrients in an acid soil. J. Plant Nutr. 37, 357–373. https://doi.org/10.1080/01904167.2013.859698

Bhatla, S.C., Lal, M.A., 2018. Plant Physiology, Development and Metabolism, Plant Physiology, Development and Metabolism. https://doi.org/10.1007/978-981-13-2023-1

Bhuyan, D.J., Vuong, Q. V., Chalmers, A.C., Van Altena, I.A., Bowyer, M.C., Scarlett, C.J., 2016. Investigation of phytochemicals and antioxidant capacity of selected *Eucalyptus* species using conventional extraction. Chem. Pap. 70, 567–575. https://doi.org/10.1515/chempap-2015-0237

Booth, T.H., 2013. Eucalypt plantations and climate change. For. Ecol. Manage. 301, 28–34. https://doi.org/10.1016/j.foreco.2012.04.004

Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375. https://doi.org/10.1016/j.geoderma.2020.114476

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., 2012. Function of Nutrients: Micronutrients, Marschner's Mineral Nutrition of Higher Plants: Third Edition. https://doi.org/10.1016/B978-0-12-384905-2.00007-8

Caldeira, D.R.M., Alvares, C.A., Campoe, O.C., Hakamada, R.E., Guerrini, I.A., Cegatta, Í.R., Stape, J.L., 2020. Multisite evaluation of the 3-PG model for the highest phenotypic plasticity *Eucalyptus* clone in Brazil. For. Ecol. Manage. 462. https://doi.org/10.1016/j.foreco.2020.117989

Camargo, O.A., Moniz, A.C., Jorge, J.A., Valadares, J.M.A.S., 1986. Methods of Chemical, Mineralogical and Physical Analysis of Soils of the Agronomic Institute of Campinas.

Carvalho, M.E.A., Castro, P.R.C., Azevedo, R.A., 2020. Hormesis in plants under Cd exposure: From toxic to beneficial element? J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2019.121434

Carvalho, M.E.A., Gaziola, S.A., Carvalho, L.A., Azevedo, R.A., 2021. Cadmium effects on plant reproductive organs: Physiological, productive, evolutionary and ecological aspects. Ann. Appl. Biol. 178, 227–243. https://doi.org/10.1111/aab.12612

Cembrowska-Lech, D., Koprowski, M., Kepczyński, J., 2015. Germination induction of dormant Avena fatua caryopses by KAR1 and GA3 involving the control of reactive oxygen species (H2O2 and O2-) and enzymatic antioxidants (superoxide dismutase and catalase) both in the embryo and the aleurone layers. J. Plant Physiol. 176, 169–179. https://doi.org/10.1016/j.jplph.2014.11.010

Cerovic, Z.G., Cartelat, A., Goulas, Y., Meyer, S., 2005. In-field assessment of wheat-leaf polyphenolics using the new optical leaf-clip Dualex, in: Precision Agriculture 2005, ECPA 2005. pp. 243–250.

Cerovic, Z.G., Masdoumier, G., Ghozlen, N. Ben, Latouche, G., 2012. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiol. Plant. 146, 251–260. https://doi.org/10.1111/j.1399-3054.2012.01639.x

Chatzistathis, T., Alifragis, D., Papaioannou, A., 2015. The influence of liming on soil chemical properties and on the alleviation of manganese and copper toxicity in Juglans regia, Robinia pseudoacacia, *Eucalyptus sp*. and *Populus sp.* Plantations. J. Environ. Manage. 150, 149–156. https://doi.org/10.1016/j.jenvman.2014.11.020

Court, M., van der Heijden, G., Didier, S., Nys, C., Richter, C., Pousse, N., Saint-André, L., Legout,

A., 2018. Long-term effects of forest liming on mineral soil, organic layer and foliage chemistry: Insights from multiple beech experimental sites in Northern France. For. Ecol. Manage. 409, 872–889. https://doi.org/10.1016/j.foreco.2017.12.007

Crusciol, C.A.C., Marques, R.R., Carmeis Filho, A.C.A., Soratto, R.P., Costa, C.H.M., Ferrari Neto, J., Castro, G.S.A., Pariz, C.M., Castilhos, A.M., Franzluebbers, A.J., 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutr. Cycl. Agroecosystems 115, 347–372. https://doi.org/10.1007/s10705-019-10017-0

Dell, B., XU, D., ROGERS, C., HUANG, L., 2003. Micronutrient disorders in eucalypt plantations: causes, symptoms, identification, impact and management. World Scientific Pub Co Pte Lt, pp. 241–252. https://doi.org/10.1142/9789812704504_0020

Eswaran, H., Reich, P., Beinroth, F., 1997. Global distribution of soils with acidity, in: Plant-Soil Interactions at Low PH. pp. 159–164.

Fageria, N.K., Baligar, C., Clark, R.B., 2002. Micronutrients in crop production. Adv. Agron. https://doi.org/10.1016/s0065-2113(02)77015-6

Fageria, N.K., Nascente, A.S., 2014. Management of soil acidity of South American soils for sustainable crop production, in: Advances in Agronomy. pp. 221–275. https://doi.org/10.1016/B978-0-12-802139-2.00006-8

FAO, 2015. Intergovernmental Technical Panel on Soils. Status of the World's Soil Resources., Intergovernmental Technical Panel on Soils.

Freitas, E.C.S. de, Paiva, H.N. de, Neves, J.C.L., Marcatti, G.E., Leite, H.G., 2020. Modeling of eucalyptus productivity with artificial neural networks. Ind. Crops Prod. 146, 112149. https://doi.org/10.1016/j.indcrop.2020.112149

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. https://doi.org/10.1016/j.plaphy.2010.08.016

Gonçalves, J., Stape, J., Laclau, J., Bouillet, J., Ranger, J., 2008. Assessing the effects of early silvicultural management on long-term site productivity of fast-growing eucalypt plantations: the Brazilian experience Assessing the effects of early silvicultural management on long-term site productivity of fast-growing eu. J. For. Sci. 70, 105–118. https://doi.org/10.2989/SOUTH.FOR.2008.70.2.6.534

Gonçalves, J.L. de M., 2010. Capítulo 8 - Livro do IPNI - Adubação do eucalipto.

Gonçalves, J.L. de M., de Vicente Ferraz, A., Tertulino Rocha, J.H., Peressin, M., Alcarde Alvares, C., 2020. Forest outgrower schemes in small and medium-sized farmers in Brazil. For. Ecol. Manage. 456. https://doi.org/10.1016/j.foreco.2019.117654

Gonçalves, J.L.D.M., Valeri, S.V., 2001. Eucalipto e pínus, in: Ferreira, M.E., Cruz, M.C.P., Raij, B., Abreu, C.A. (Eds.), Micronutrientes: Elementos Tóxicos Na Agricultura. CNPq/FAPESP/PATAFOS, Jaboticabal, Pp. 393–423. Jaboticabal (SP), pp. 393–423.

González-Alcaraz, M.N., Conesa, M., Álvarez-Rogel, J., 2013. When liming and revegetation contribute to the mobilisation of metals: Learning lessons for the phytomanagement of metal-polluted wetlands. J. Environ. Manage. 116, 72–80. https://doi.org/10.1016/j.jenvman.2012.11.044

Gratão, P.L., Polle, A., Lea, P.J., Azevedo, R.A., 2005. Making the life of heavy metal-stressed plants a little easier. Funct. Plant Biol. https://doi.org/10.1071/FP05016

Gullón, B., Gullón, P., Lú-Chau, T.A., Moreira, M.T., Lema, J.M., Eibes, G., 2017. Optimization of solvent extraction of antioxidants from *Eucalyptus globulus* leaves by response surface methodology: Characterization and assessment of their bioactive properties. Ind. Crops Prod. 108, 649–659. https://doi.org/10.1016/j.indcrop.2017.07.014

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. https://doi.org/10.1016/0003-9861(68)90654-1

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.08.020

IBA, 2020. Brazilian tree industry annual report - base year 2019. Assoc. Brasleira Árvores 160.

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106.

Jaghdani, S.J., Jahns, P., Tränkner, M., 2021. Mg deficiency induces photo-oxidative stress primarily by limiting CO2 assimilation and not by limiting photosynthetic light utilization. Plant Sci. 302, 110751. https://doi.org/10.1016/j.plantsci.2020.110751

Jiménez-Cárceles, F.J., Álvarez-Rogel, J., Conesa Alcaraz, H.M., 2008. Trace element concentrations in saltmarsh soils strongly affected by wastes from metal sulphide mining areas. Water. Air. Soil Pollut. 188, 283–295. https://doi.org/10.1007/s11270-007-9544-4

Jones, L.H.P., 1957. The effect of liming a neutral soil on the cycle of manganese. Plant Soil 8, 315–327. https://doi.org/10.1007/BF01666321

Kabata-Pendias, A., 2011. References, in: Trace Elements in Soils and Plants, Fourth Edition. CRC Press, pp. 407–505. https://doi.org/10.1201/b10158-25

Kalaji, H.M., Dąbrowski, P., Cetner, M.D., Samborska, I.A., Łukasik, I., Brestic, M., Zivcak, M., Tomasz, H., Mojski, J., Kociel, H., Panchal, B.M., 2017. A comparison between different chlorophyll content meters under nutrient deficiency conditions. J. Plant Nutr. 40, 1024–1034. https://doi.org/10.1080/01904167.2016.1263323

Keenan, R.J., Reams, G.A., Achard, F., de Freitas, J. V., Grainger, A., Lindquist, E., 2015. Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2015.06.014

Lambers, H., Chapin, F.S., Pons, T.L., 2008. Plant physiological ecology: Second edition, Plant Physiological Ecology: Second Edition. https://doi.org/10.1007/978-0-387-78341-3

Le Bot, J., Goss, M.J., Carvalho, M.J.G.P.R., van Beusichem, M.L., Kirkby, E.A., 1990. The significance of the magnesium to manganese ratio in plant tissues for growth and alleviation of manganese toxicity in tomato (Lycopersicon esculentum) and wheat (Triticum aestivum) plants, in: Plant Nutrition — Physiology and Applications. Springer Netherlands, pp. 223–228. https://doi.org/10.1007/978-94-009-0585-6_38

Leite, F.P., Novais, R.F., Silva, I.R., Barros, N.F., Neves, J.C.L., Medeiros, A.G.B., Ventrella, M.C., Villani, E.M. de A., 2014. Manganese accumulation and its relation to" eucalyptus shoot blight in the Vale do Rio Doce". Rev. Bras. Cienc. do Solo 38, 193–204. https://doi.org/10.1590/S0100-06832014000100019

Lima Neto, A.J. de, Neves, J.C.L., Martinez, H.E.P., Sousa, J.S., Fernandes, L.V., 2020. Establishment of critical nutrient levels in soil and plant for eucalyptus. Rev. Bras. Cienc. do Solo 44, 1–16. https://doi.org/10.36783/18069657RBCS20190150

Maas, E. V., Moore, D.P., Mason, B.J., 1969. Influence of calcium and magnesium on manganese absorption. Plant Physiol. 44, 796–800. https://doi.org/10.1104/pp.44.6.796

Malavolta, E., Vitti, G., Oliveira, S., 1997. Avaliação do estado nutricional das plantas: princípios e aplicações.

Marenco, R.A., Antezana-Vera, S.A., Nascimento, H.C.S., 2009. Relationship between specific leaf area, leaf thickness, leaf water content and SPAD-502 readings in six Amazonian tree species. Photosynthetica 47, 184–190. https://doi.org/10.1007/s11099-009-0031-6

Matsumoto, H., Motoda, H., 2013. Oxidative stress is associated with aluminum toxicity recovery in apex of pea root. Plant Soil 363, 399–410. https://doi.org/10.1007/s11104-012-1396-z

Matsuno, H., Uritani, I., 1972. Physiological behavior of peroxidase isozymes in sweet potato root tissue injured by cutting or with black rot. Plant Cell Physiol. 13, 1091–1101. https://doi.org/10.1093/oxfordjournals.pcp.a074815

McCay, T.S., Cardelús, C.L., Neatrour, M.A., 2013. Rate of litter decay and litter macroinvertebrates in limed and unlimed forests of the Adirondack Mountains, USA. For. Ecol. Manage. 304, 254–260. https://doi.org/10.1016/j.foreco.2013.05.010

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell Environ. 33, 453–467. https://doi.org/10.1111/j.1365-3040.2009.02041.x

Monteiro, C.C., Carvalho, R.F., Gratão, P.L., Carvalho, G., Tezotto, T., Medici, L.O., Peres, L.E.P., Azevedo, R.A., 2011. Biochemical responses of the ethylene-insensitive Never ripe tomato mutant subjected to cadmium and sodium stresses. Environ. Exp. Bot. 71, 306–320. https://doi.org/10.1016/j.envexpbot.2010.12.020

Nakano, Y., Asada, K., 1981. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. Plant Cell Physiol. 22, 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Nguyen, B.T., Le, L.B., Pham, L.P., Nguyen, H.T., Tran, T.D., Van Thai, N., 2021. The effects of biochar on the biomass yield of elephant grass (Pennisetum Purpureum Schumach) and properties of acidic soils. Ind. Crops Prod. 161, 113224. https://doi.org/10.1016/j.indcrop.2020.113224

Paradelo, R., Virto, I., Chenu, C., 2015. Net effect of liming on soil organic carbon stocks: A review. Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2015.01.005

Pereira, A.P.A., Durrer, A., Gumiere, T., Gonçalves, J.L.M., Robin, A., Bouillet, J.P., Wang, J., Verma, J.P., Singh, B.K., Cardoso, E.J.B.N., 2019. Mixed *Eucalyptus* plantations induce changes in microbial communities and increase biological functions in the soil and litter layers. For. Ecol. Manage. 433, 332–342. https://doi.org/10.1016/j.foreco.2018.11.018

Perron, T., Mareschal, L., Laclau, J.P., Deffontaines, L., Deleporte, P., Masson, A., Cauchy, T., Gay, F., 2021. Dynamics of biomass and nutrient accumulation in rubber (Hevea brasiliensis) plantations established on two soil types: Implications for nutrient management over the immature phase. Ind. Crops Prod. 159, 113084. https://doi.org/10.1016/j.indcrop.2020.113084

Pimentel, C., Davey, P.A., Juvik, J.A., Long, S.P., 2005. Gene loci in maize influencing susceptibility to chilling dependent photoinhibition of photosynthesis. Photosynth. Res. 85, 319–326. https://doi.org/10.1007/s11120-005-5738-z

Pinkard, E.A., Patel, V., Mohammed, C., 2006. Chlorophyll and nitrogen determination for plantation-grown *Eucalyptus nitens* and *E. globulus* using a non-destructive meter. For. Ecol. Manage. 223, 211–217. https://doi.org/10.1016/j.foreco.2005.11.003

Pizzaia, D., Nogueira, M.L., Mondin, M., Carvalho, M.E.A., Piotto, F.A., Rosario, M.F., Azevedo, R.A., 2019. Cadmium toxicity and its relationship with disturbances in the cytoskeleton, cell cycle and chromosome stability. Ecotoxicology 28, 1046–1055. https://doi.org/10.1007/s10646-019-02096-0

Pollastri, S., Tattini, M., 2011. Flavonols: Old compounds for old roles. Ann. Bot. https://doi.org/10.1093/aob/mcr234

R Core Team, 2016. R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing. R A Lang. Environ. Stat. Comput.

Raij, B. van, Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1996. Recomendações de adubação e calagem para o Estado de São Paulo. Campinas: IAC, 1996. (IAC. Boletim 100).

Raij, B. van, Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Análise química para avaliação da fertilidade de solos tropicais, Campinas: Instituto Agronômico.

Rocha, J.H.T., du Toit, B., Gonçalves, J.L. de M., 2019a. Ca and Mg nutrition and its application in *Eucalyptus* and Pinus plantations. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2019.03.062

Rocha, J.H.T., Gonçalves, J.L. de M., Ferraz, A. de V., Poiati, D.A., Arthur Junior, J.C., Hubner, A., 2019b. Growth dynamics and productivity of an *Eucalyptus grandis* plantation under omission of N, P, K Ca and Mg over two crop rotation. For. Ecol. Manage. 447, 158–168. https://doi.org/10.1016/j.foreco.2019.05.060

Rodrigues, F.A.V., de Barros, N.F., Neves, J.C.L., Alvarez, V.H., Novais, R.F., 2010. Copper availabity for eucalyptus seedlings in Cerrado soils. Rev. Bras. Cienc. do Solo 34, 1923–1932. https://doi.org/10.1590/s0100-06832010000600017

Rodrigues, F.A.V., de Barros, N.F., Neves, J.C.L., Alvarez, V.H. V, Novais, R.F., 2012. Zinc availabity for eucalypt seedlings in cerrado soils. Rev. Bras. Cienc. do Solo. https://doi.org/10.1590/S0100-06832012000400019

Saint-André, L., M'Bou, A.T., Mabiala, A., Mouvondy, W., Jourdan, C., Roupsard, O., Deleporte, P., Hamel, O., Nouvellon, Y., 2005. Age-related equations for above- and below-ground biomass of a *Eucalyptus* hybrid in Congo. For. Ecol. Manage. 205, 199–214. https://doi.org/10.1016/j.foreco.2004.10.006

Sakihama, Y., Cohen, M.F., Grace, S.C., Yamasaki, H., 2002. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. Toxicology 177, 67–80. https://doi.org/10.1016/S0300-483X(02)00196-8

Santana, R.C., Barros, N.F. de, Neves, J.C.L., 2002. Eficiência de utilização de nutrientes e sustentabilidade da produção em procedências de *Eucalyptus grandis* e *Eucalyptus saligna* em sítios florestais do estado de São Paulo. Rev. Árvore 26, 447–457. https://doi.org/10.1590/s0100-67622002000400007

Santos, E.F., Mateus, N.S., Rabêlo, F.H.S., Macedo, F.G., Lavres, J., 2020. Diagnosing early disorders in *Jatropha curcas* to calcium, magnesium and sulfur deficiency. J. Plant Nutr. 43, 1604–1616. https://doi.org/10.1080/01904167.2020.1730899

Santos, S.A.O., Vilela, C., Domingues, R.M.A., Oliveira, C.S.D., Villaverde, J.J., Freire, C.S.R., Neto, C.P., Silvestre, A.J.D., 2017. Secondary metabolites from *Eucalyptus grandis* wood cultivated in Portugal, Brazil and South Africa. Ind. Crops Prod. 95, 357–364. https://doi.org/10.1016/j.indcrop.2016.10.044

Santos, S.A.O., Villaverde, J.J., Freire, C.S.R., Domingues, R.M.M., Neto, C.P., Silvestre, A.J.D., 2012. Phenolic composition and antioxidant activity of *Eucalyptus grandis*, *E. urograndis* (*E. grandis*×*E. urophylla*) and *E. maidenii* bark extracts. Ind. Crops Prod. 39, 120–127. https://doi.org/10.1016/j.indcrop.2012.02.003

SAS Institute Inc, 2016. SAS Institute Inc., SAS Institute Inc.

Saxena, M., Saxena, J., Pradhan, A., 2012. Flavonoids and phenolic acids as antioxidants in plants and human health. Int. J. Pharm. Sci. Rev. Res.

Scolforo, H.F., McTague, J.P., Burkhart, H., Roise, J., Campoe, O., Stape, J.L., 2019. *Eucalyptus* growth and yield system: Linking individual-tree and stand-level growth models in clonal Eucalypt plantations in Brazil. For. Ecol. Manage. 432, 1–16. https://doi.org/10.1016/j.foreco.2018.08.045

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. J. Bot. 2012. https://doi.org/10.1155/2012/217037

Shvaleva, A.L., Costa, F., Breia, E., Jouve, L., Hausman, J.F., Almeida, M.H., Maroco, J.P., Rodrigues, M.L., Pereira, J.S., Chaves, M.M., 2006. Metabolic responses to water deficit in two *Eucalyptus globulus* clones with contrasting drought sensitivity. Tree Physiol. 26, 239–248. https://doi.org/10.1093/treephys/26.2.239

Silva, I.R., Novais, R.F., Jham, G.N., Barros, N.F., Gebrim, F.O., Nunes, F.N., Neves, J.C.L., Leite, F.P., 2004. Responses of eucalypt species to aluminum: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism, Tree Physiology. Oxford Academic. https://doi.org/10.1093/TREEPHYS/24.11.1267

Smethurst, P.J., 2010. Forest fertilization: Trends in knowledge and practice compared to agriculture. Plant Soil 335, 83–100. https://doi.org/10.1007/s11104-010-0316-3

Snowdon, P., 2002. Modeling type 1 and type 2 growth responses in plantations after application of fertilizer or other silvicultural treatments. For. Ecol. Manage. 163, 229–244. https://doi.org/10.1016/S0378-1127(01)00582-5

Soares, C., Carvalho, M.E.A., Azevedo, R.A., Fidalgo, F., 2019. Plants facing oxidative challenges—A little help from the antioxidant networks. Environ. Exp. Bot. 161, 4–25. https://doi.org/10.1016/j.envexpbot.2018.12.009

Solovchenko, A., Neverov, K., 2017. Carotenogenic response in photosynthetic organisms: a colorful story. Photosynth Res 3, 31–47. https://doi.org/10.1007/s11120-017-0358-y

Sousa, S., Lopes, A., Fernandes, P., Ramos, M., 2009. The Zinc proteome: a tale of stability and functionality. Dalt. Trans.

Stape, J.L., Binkley, D., Ryan, M.G., 2004. *Eucalyptus* production and the supply, use and efficiency of use of water, light and nitrogen across a geographic gradient in Brazil. For. Ecol. Manage. 193, 17–31. https://doi.org/10.1016/j.foreco.2004.01.020

Turner, J., Lambert, M.J., 2014. Analysis of nutrient use efficiency (NUE) in *Eucalyptus pilularis* forests. Aust. J. Bot. 62, 558–569. https://doi.org/10.1071/BT14162

Wang, J., Evangelou, V.P., Creech, B., 1993. Characteristics of Manganese-Calcium Exchange Behavior on Kaolinite and Illite and pH Influence. J. Environ. Sci. Heal. Part A Environ. Sci. Eng. Toxicol. 28, 1381–1391. https://doi.org/10.1080/10934529309375948

## Supplementary materials

**Supplementary Table S1**

Physical and chemical traits of Rhodic Ferralsol (IUSS Working Group WRB, 2015), before planting *Eucalyptus grandis* seedlings, in Itatinga city, State of São Paulo, Brazil (mean±standard error, n=3).

| Depth | Clay | Silt | Sand | pH | SOM | P | Ca | Mg | K | Al | H+Al | $_e$CEC | BS | B | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| m | --------------- % --------------- | | | $CaCl_2$ | g $kg^{-1}$ | mg $kg^{-1}$ | --------------------------------- $mmol_c$ $kg^{-1}$ ---------------------------- | | | | | | % | ----------------------------- mg $kg^{-1}$ --------------------------- | | | | |
| 0-0.10 | 13±0.7 | 3±0.9 | 83±0.3 | 3.5±0.1 | 26±2.1 | 3.4±0.1 | 2.48±0.1 | 1.9±0.1 | 0.48±0.0 | 15±0.2 | 67±5.4 | 22±0.3 | 5±0.2 | 0.3±0.0 | 1.0±0.0 | 117±9.8 | 0.6±0.0 | 0.5±0.1 |
| 0.1-0.2 | 15±0.7 | 2±0.1 | 83±0.7 | 3.6±0.1 | 16±0.5 | 2.6±0.2 | 1.59±0.1 | 1.4±0.1 | 0.43±0.0 | 13±0.1 | 44±6.4 | 17±0.1 | 5±0.1 | 0.3±0.0 | 1.1±0.0 | 74±7.8 | 0.5±0.0 | 0.3±0.0 |
| 0.2-0.4 | 14±0.1 | 3±0.3 | 83±0.3 | 3.7±0.1 | 12±0.7 | 1.5±0.3 | 0.3±0.1 | 0.4±0.4 | <0.1±0.0 | 12±0.3 | 52±3.2 | 12±0.5 | 1±0.7 | 0.3±0.0 | 1.1±0.0 | 37±1.8 | 0.4±0.0 | 0.3±0.0 |
| *0-0.20* | *14±0.1* | *3±0.4* | *83±0.4* | *3.6±0.1* | *21±1.2* | *3.0±0.2* | *2.0±0.1* | *1.7±0.1* | *0.46±0.0* | *14±0.1* | *56±2.5* | *20±0.2* | *5±0.1* | *0.3±0.0* | *1.1±0.0* | *96±8.3* | *0.6±0.0* | *0.4±0.0* |

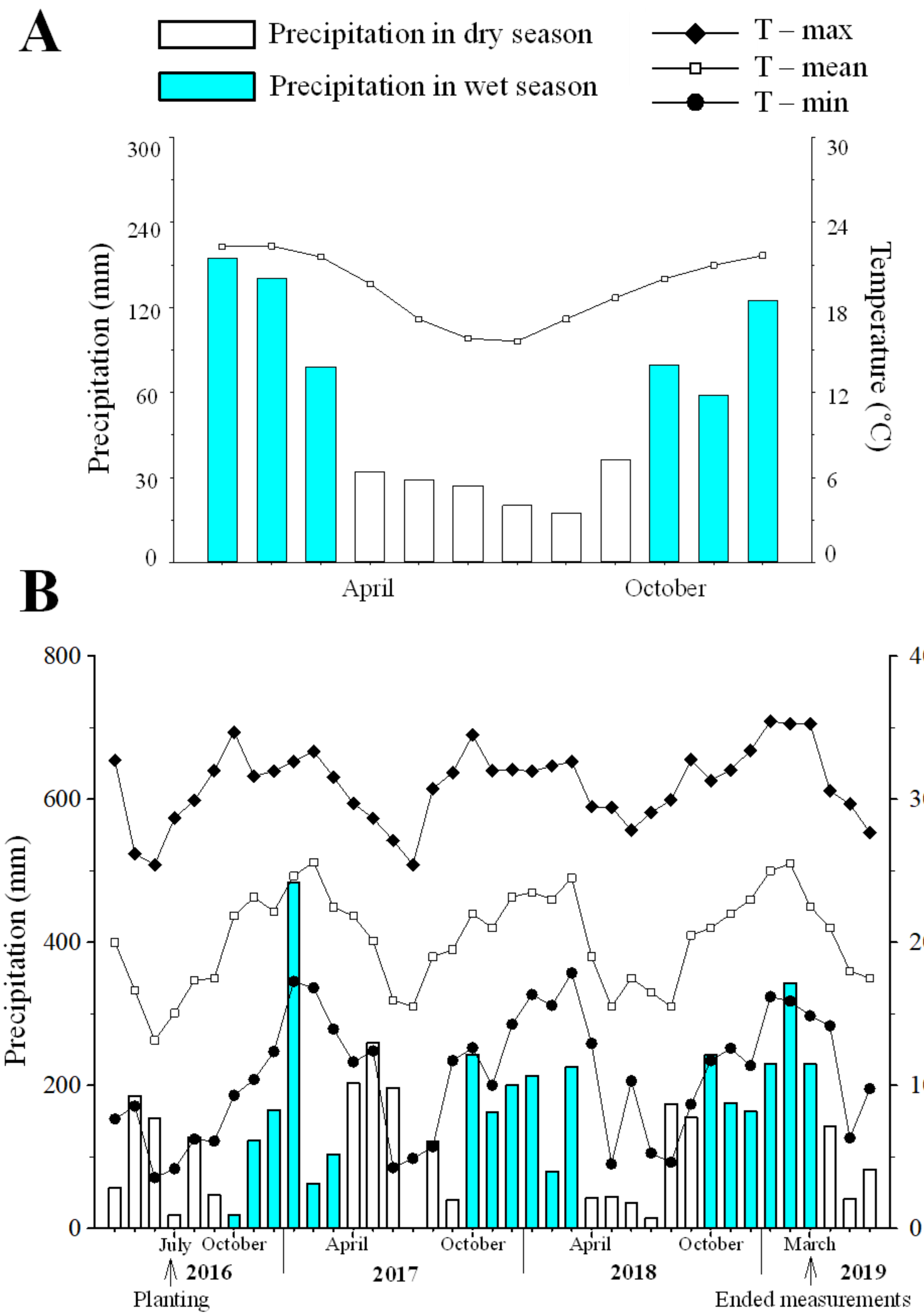
Physical analysis by method of Camargo et al (1986) and chemical by Raij et al (2001). where: pH in $CaCl_2$ (0.01 mol $L^{-1}$); SOM = soil organic matter; H+Al = total acidity; $_e$CEC = effective cation exchange capacity and BS= base saturation in percentage.

**Supplementary Table S2**

Application of dolomitic limestone (DL) and Cu plus Zn mineral fertilizer (MF) to development of *Eucalyptus grandis* plantation according to fertilization phases.

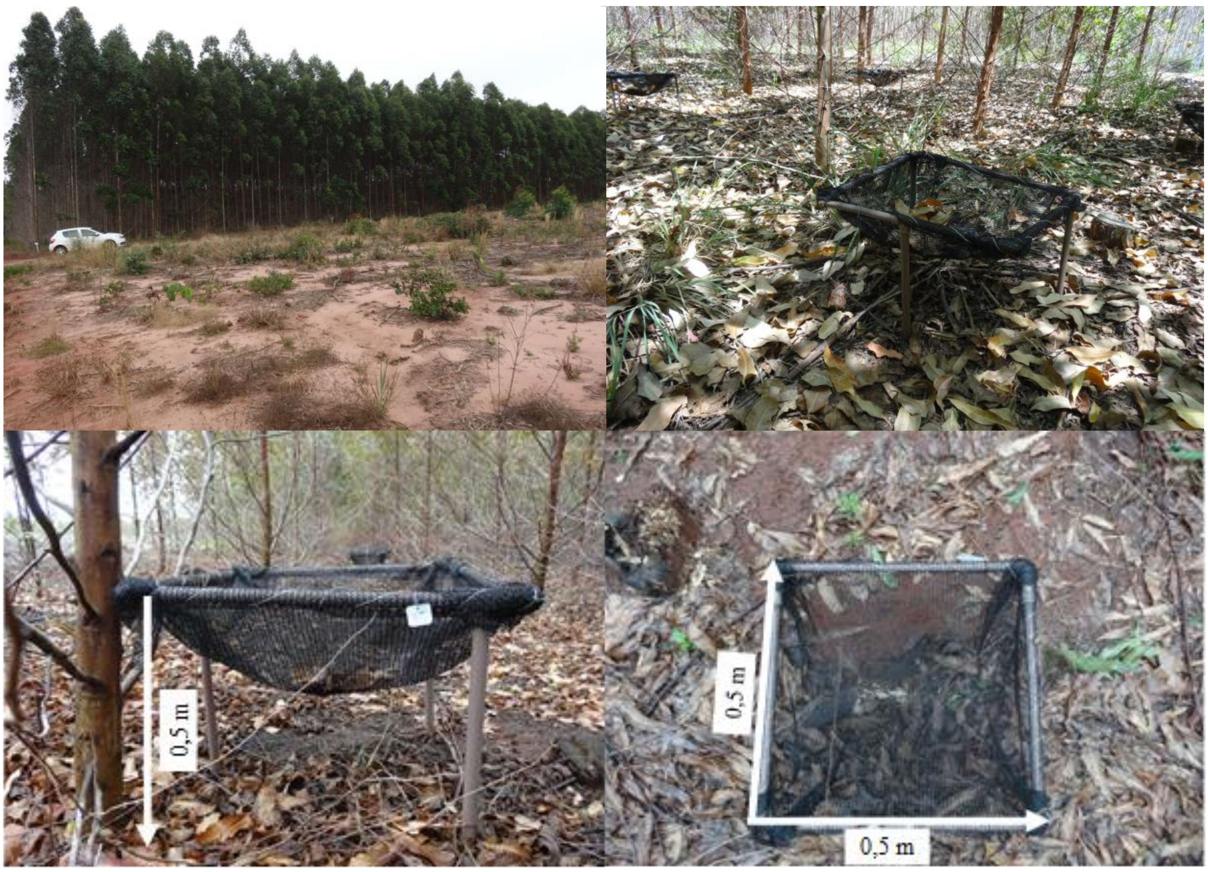
| Treatments | | Fertilization phases | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Planting | | | | | | 3 months after planting | | | 6 months after planting | |
| DL | MF | N | $P_2O_5$ | $K_2O$ | Cu | Zn | DL rates | N | $K_2O$ | B | N | $K_2O$ |
| | | ----------------------------------------------------------- kg $ha^{-1}$ ----------------------------------------------------------- | | | | | | | | | | |
| NoR | -CuZn | 10 | 60 | 10 | 0 | 0 | 0 | 20 | 50 | 5 | 30 | 80 |
| LoR | -CuZn | 10 | 60 | 10 | 0 | 0 | 1600 | 20 | 50 | 5 | 30 | 80 |
| HiR | -CuZn | 10 | 60 | 10 | 0 | 0 | 3700 | 20 | 50 | 5 | 30 | 80 |
| NoR | +CuZn | 10 | 60 | 10 | 0.5 | 1.5 | 0 | 20 | 50 | 5 | 30 | 80 |
| LoR | +CuZn | 10 | 60 | 10 | 0.5 | 1.5 | 1600 | 20 | 50 | 5 | 30 | 80 |
| HiR | +CuZn | 10 | 60 | 10 | 0.5 | 1.5 | 3700 | 20 | 50 | 5 | 30 | 80 |

Ammonium sulphate - 21% N; triple superphosphate - 46% $P_2O_5$; potassium chloride - 60% $K_2O$; borax - 11% B; copper sulfate - 25% Cu; zinc sulfate - 21% Zn; and DL - 40% CaO, and 10% MgO. Treatments of dolomitic limestone application (DL): no lime rate - 0.0 Mg $ha^{-1}$ (NoR), low lime rate - 1.6 Mg $ha^{-1}$ (LoR), and high lime rate - 3.7 Mg $ha^{-1}$ (HiR). Treatments of Cu plus Zn mineral fertilizer (MF): 0.0 kg $ha^{-1}$ of Cu plus 0.0 kg $ha^{-1}$ Zn (-CuZn), and with 0.5 kg $ha^{-1}$ of Cu plus 1.5 kg $ha^{-1}$ Zn (+CuZn).




**Supplementary Fig. S2.** Normal distribution (precipitation and mean temperature) according to the historical series from 1950-1990 (Alvares et al., 2013) (A) and mean monthly rainfall and temperature - T (maximum, mean, and minimum) during the field trial setup at the Itatinga, SP, Brazil (23º03'S; 48º37'W) (B).


**Supplementary Fig. S2.** Visual design of experimental plot and identification of some activities.




**Supplementary Fig. S3.** Visual aspect of leaf litter (senescent leaves) collectors attached to the soil of an experimental plot of *Eucalyptus grandis* plantation.

**Supplementary Table S3**
Equations used to estimate the dry biomass of leaf, branch, bark (stembark), stem (stemwood), and total biomass of *Eucalyptus grandis* plantation, at 12 and 30 months after planting under dolomitic limestone application (DL) and Cu plus Zn mineral fertilizer (MF), in Itatinga city, State of São Paulo, Brazil (n=24).

| Description | Equations | $R^2ij^{(1)}$ |
|---|---|---|
| **12 months** | | |
| Volume (VOL) ($m^3$ $tree^{-1}$) | VOL = -9.789 DBH $^{(1.516)}$ H $^{(1.152)}$ | $0.966^*$ |
| Leaf biomass (Lb) (Mg $tree^{-1}$) | Lb = -4.961 DBH $^{(2.677)}$ H $^{(0.645)}$ | $0.950^*$ |
| Branch biomass (Bbr) (Mg $tree^{-1}$) | Bbr = -0.95901 DBH $^{(3.067)}$ H $^{(-1.760)}$ | $0.878^*$ |
| Bark biomass (Bba) (Mg $tree^{-1}$) | Bba = -4.74215 DBH $^{(1.663)}$ H $^{(0.657)}$ | $0.970^*$ |
| Stem biomass (Sb) (Mg $tree^{-1}$) | Sb = -3.15652 DBH $^{(1.663)}$ H $^{(0.657)}$ | $0.970^*$ |
| Total biomass (TB) (Mg $tree^{-1}$) | TB = -1.28162 DAP $^{(2.342)}$ H $^{(-0.360)}$ | $0.922^*$ |
| **30 months** | | |
| Volume (Vol) ($m^3$ $tree^{-1}$) | Vol = -11.03280 DBH $^{(1.873)}$ H $^{(1.408)}$ | $0.987^*$ |
| Leaf biomass (Lb) (Mg $tree^{-1}$) | Lb = -9.86491 DBH $^{(2.468)}$ H $^{(1.936)}$ | $0.910^*$ |
| Branch biomass (Bbr) (Mg $tree^{-1}$) | Bbr = -4.20532 DBH $^{(3.548)}$ H $^{(-1.096)}$ | $0.871^*$ |
| Bark biomass (Bba) (Mg $tree^{-1}$) | Bba = -6.61512 DBH $^{(1.740)}$ H $^{(1.406)}$ | $0.935^*$ |
| Stem biomass (Sb) (Mg $tree^{-1}$) | Sb = -7.40936 DBH $^{(1.422)}$ H $^{(2.607)}$ | $0.968^*$ |
| Total biomass (TB) (Mg $tree^{-1}$) | TB = -6.73395 DBH $^{(1.804)}$ H $^{(2.172)}$ | $0.960^*$ |

(1)Adjusted coefficient of determination. *Significance of model ($P$ < 0.0001). Model proposed of Shumacher and Hall (1933). Where: DBH (cm) = diameter at breast height, and H (m) = tree height.

**Supplementary Table S4**

Nutrient accumulations in the aerial components of *E.grandis* plantation, at 12 and 30 months old, after dolomitic limestone application (DL) (mean±standard error, n=4).

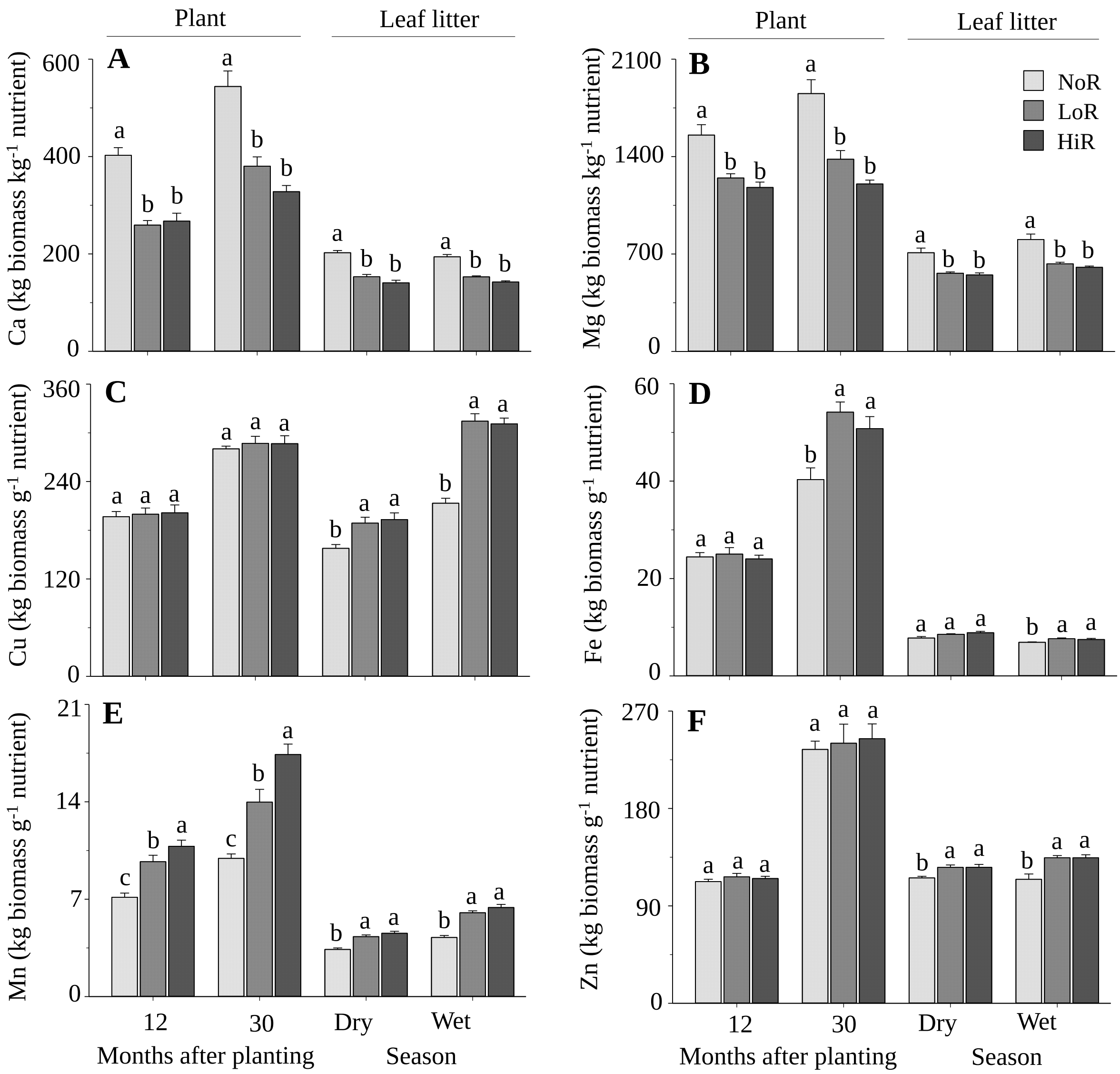
| Treat | Ca | Mg | Cu | Fe | Mn | Zn | Ca | Mg | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ------ kg ha$^{-1}$ -------- | | ------------------------ g ha$^{-1}$ ------------------------ | | | | --------- kg ha$^{-1}$ --------- | | ----------------------- g ha$^{-1}$ ----------------------- | | | |
| | 12 months | | | | | | 30 months | | | | | |
| | Leaf | | | | | | | | | | | |
| NoR | 6.7±0.6 b | 2.7±0.2 b | 14.4±0.3 b | 159±6 b | 538±29 a | 35.6±1.3 b | 23.5±2.1 b | 16.6±0.8 b | 58.6±3.8 a | 313±12 a | 1577±90 a | 95.3±2.6 a |
| LoR | 8.4±1.0 b | 3.0±0.2 b | 14.8±0.8 b | 160±13 b | 384±51 b | 33.9±1.7 b | 32.4±2.8 a | 22.7±1.0 a | 60.3±3.5 a | 290±11 a | 1359±75 b | 96.5±6.2 a |
| HiR | 11.8±1.1 a | 4.6±0.3 a | 20.6±1.2 a | 241±9 a | 472±45 b | 46.7±1.1 a | 37.2±2.7 a | 24.7±1.0 a | 64.9±2.2 a | 294±12 a | 1045±62 c | 96.9±2.7 a |
| | Branch | | | | | | | | | | | |
| NoR | 9.8±0.6 b | 1.7±0.1 b | 23.3±1.7 a | 81±3 a | 448±19 a | 27.5±1.9 a | 12.6±1.4 c | 2.5±0.3 c | 46.1±2.2 a | 151±20 a | 731±41 a | 13.6±1.5 a |
| LoR | 17.9±1.3 a | 2.3±0.1 a | 20.7±1.1 a | 68±7 a | 297±17 b | 23.1±1.2 b | 17.9±1.1 b | 3.7±0.4 b | 37.7±3.3 b | 179±16 a | 530±45 b | 15.2±1.8 a |
| HiR | 20.7±1.9 a | 2.6±0.1 a | 25.1±1.9 a | 71±6 a | 306±38 b | 29.0±1.5 a | 24.2±1.7 a | 5.2±0.6 a | 40.3±2.2 b | 142±5 a | 494±28 b | 16.8±1.2 a |
| | Bark | | | | | | | | | | | |
| NoR | 4.1±0.2 b | 0.8±0.1 b | 1.3±0.1 b | 75±12 a | 198±17 a | 5.0±0.3 b | 48.9±5.9 b | 7.2±1.0 c | 19.6±1.0 b | 260±20 b | 2330±125 a | 35.7±3.9 a |
| LoR | 4.6±0.4 b | 0.9±0.0 b | 1.2±0.1 b | 71±9 a | 162±15 b | 5.4±0.4 b | 82.9±4.6 a | 11.3±0.7 b | 21.1±0.9 b | 337±39 b | 1869±193 b | 25.8±1.3 b |
| HiR | 6.3±0.4 a | 1.4±0.1 a | 1.6±0.1 a | 94±10 a | 176±5 b | 6.3±0.1 a | 94.2±7.3 a | 14.6±0.9 a | 23.1±0.8 a | 505±50 a | 1408±167 c | 29.1±1.7 b |
| | Stem | | | | | | | | | | | |
| NoR | 1.4±0.1 b | 0.5±0.0 b | 5.8±0.2 b | 46±4 a | 80±7 a | 10. 20.8± a | 20±2 c | 4.0±0.5 c | 77±4 a | 692±67 a | 1107±131 a | 99±9 a |
| LoR | 1.8±0.1 b | 0.5±0.0 b | 5.6±0.5 b | 41±3 a | 42±5 b | 9.9±0.8 a | 28±1 b | 5.5±0.3 b | 94±4 a | 325±32 b | 710±40 b | 123±13 a |
| HiR | 2.7±0.3 a | 0.7±0.1 a | 7.1±0.4 a | 49±5 a | 41±1 b | 12.3±1.0 a | 32±1 a | 6.5±0.3 a | 87±8 a | 331±40 b | 603±57 b | 112±12 a |

Treatments (Treat) of DL: no lime rate - 0.0 Mg ha$^{-1}$ (NoR), low lime rate - 1.6 Mg ha$^{-1}$ (LoR), and high lime rate - 3.7 Mg ha$^{-1}$ (HiR). Different lowercase letters in the same compartment (column) indicate differences between treatments by LSD test ($P$ < 0.10).

**Supplementary Table S5**

Nutrient accumulations in the leaf litter accumulated of *E.grandis* plantation on 21-32 months old (Apr-2018 to Mar-2019), in the dry and wet season, after dolomitic limestone application (DL) (mean±standard error, n=4).

| Treatments | Ca | Mg | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|
| | ----------- kg ha$^{-1}$ ----------- | | ------------------------------ g ha$^{-1}$ ------------------------------ | | | |
| | Dry season (Apr-2018 to Sep-2018) | | | | | |
| NoR | 10.6±0.7 b | 3.1±0.3 b | 13.5±0.6 | 278±23 a | 625±24 a | 18.4±1.0 a |
| LoR | 16.7±0.5 a | 4.6±0.2 a | 13.7±0.8 | 299±10 a | 593±12 a | 20.4±0.8 a |
| HiR | 17.9±1.0 a | 4.6±0.2 a | 13.1±0.7 | 284±14 a | 553±29 a | 19.9±0.9 a |
| | Wet season (Oct-2018 to Mar-2019) | | | | | |
| NoR | 11.8±1.1 b | 2.9±0.3 b | 10.3±0.6 | 329±26 a | 530±33 a | 19.8±1.4 a |
| LoR | 16.7±1.0 a | 4.1±0.3 a | 8.0±0.4 b | 335±17 a | 424±21 b | 19.1±1.3 a |
| HiR | 18.7±0.7 a | 4.4±0.2 a | 8.7±0.2 b | 356±14 a | 417±17 b | 19.7±0.5 a |




**Supplementary Fig. S4.** Nutrients use efficiencies (NUE) of Ca (A), Mg (B), Cu (C), Fe (D), Mn (E), and Zn (F) in the plant (12 and 30 months after planting) and leaf litter (accumulated on dry and wet seasons from 21 to 32 months after planting) at the *Eucalyptus grandis* plantation, after dolomitic limestone application (DL). Treatments of DL: no lime rate - 0.0 Mg ha$^{-1}$ (NoR), low lime rate - 1.6 Mg ha$^{-1}$ (LoR), and high lime rate - 3.7 Mg ha$^{-1}$ (HiR). Standard error bars with different lowercase letters in the same month or season indicate differences between treatments by LSD test ($P$ < 0.10) (n = 4).

# 4. METALLIC MICRONUTRIENTS STATUS IN *Eucalyptus* SEEDLINGS MODULATED BY LIMING AND COPPER PLUS ZINC FERTILIZATION: AN INTEGRATION OF LEAF TRAITS, NUTRITIONAL PARAMETERS, AND PLANT GROWTH

**Abstract**

Soil lime application can reduce metallic micronutrient (e.g., Cu and Zn) availability in soil and thereby impair plants nutritional physiology. The copper and zinc fertilization to *Eucalyptus* plant is not a consistent practice worldwide; however, application of these micronutrients is recommended to restore the amount extracted from plant harvesting products. We investigated the effects of lime and Cu plus Zn application on developmental plant performance through biomass production, leaf area, leaf pigments contents, as well as Cu and Zn concentrations and accumulations, their nutritional use efficiency, and its distribution among roots, stem, and leaves in *Eucalyptus grandis* seedlings. A greenhouse experiment was conducted with seedlings under Ferralsol in five randomized blocks, in a 3 × 4 factorial scheme, with three lime rates and four fertilizers rates of Cu and Zn. The seedlings grown under lime had increased leaf area and flavonoid contents. Application of lime and Cu plus Zn improved biomass production relative to plants grown under no-lime and without those micronutrients supply. Despite of liming decreased the Cu and Zn contents in plants, Cu- and Zn-use efficiencies were increased. In contrast, Cu plus Zn fertilization enhanced Cu and Zn contents in *Eucalyptus* seedlings, whereas decreasing its use efficiency. The copper plus zinc fertilization improves the nutritional status of *Eucalyptus* seedlings, leading to enhance accumulation of Cu and Zn, leaf pigments, leaf area and with the addition of lime could prove a crucial strategy for increased plants growth and, consequently, boosting biomass yield of *Eucalyptus* seedlings.

**Keywords:** Plant physiology, Heavy metals, Leaf area, Chlorophyll and flavonoid, Nutritional status, Biomass production

## 4.1. Introduction

Soil lime application as a strategy to increase soil pH (Li et al. 2019; Morton 2020) has long been adopted in agroecosystem (Ameyu 2019; Caires et al. 2006, 2008; Mallarino et al. 2018). This management is closely associated with high soil microbial activity, which improves nutrient cycling and availability for plant uptake, leading to better nutritional homeostasis of plant tissue (Alloway 2008; Joris et al. 2012; Kirkham 2006). Therefore, the application of lime as a soil amendment to enhance plant growth and crop yield is well-established (Caires et al. 2011; Lukin and Epplin 2003; Osman 2013). Despite the aforementioned benefits associated with lime on annual crops [e.g., wheat (*Triticum aestivum* L.) and soybean (*Glycine max* L.)], for tree cultivation such as *Eucalyptus* crop, lime application has been adopted as a strategy for increase soil calcium and magnesium availability for plants (Rocha et al. 2019). In addition, lime application can reduce soil aluminum ($Al^{3+}$) availability, soil acidity and enhances nutritional status of plant (Holland et al. 2018; Li et al. 2019).

The undesirable implications of liming have also been reported in the literature (Barman et al. 2014; Holger and Eskilsson 2010). Overall, under high rates of lime application, soil metallic micronutrient (e.g., Cu and Zn) availability is reduced (Valentinuzzi et al. 2015), which is associated with increased soil pH (Barnard et al. 1992; González-Alcaraz et al. 2013). The presence of calcium carbonate ($CaCO_3$) dissociated under lime application favors Cu and Zn adsorption and subsequent precipitation (Holland et al. 2018; Wisawapipat et al. 2017). Due to the higher soil pH, Cu and Zn poorly bound to crystalline Fe oxides are precipitated, which also reduces their availability for plant uptake (Alloway 2008; Moreno-Lora and Delgado 2020; Wisawapipat et al. 2017). In this sense, a practical way of overcoming most undesirable implications of liming on soil Cu and Zn availabilities is through the combined addition of lime and Cu plus Zn fertilizer. For *Eucalyptus* plant, supplying Cu and Zn through fertilizer is not a well-established practice, but fertilization with these micronutrients is recommended to replace Cu and Zn contents removed within tree harvesting products (Gonçalves and Valeri 2001).

Deficiency of micronutrients (mainly B, Cu, Fe, Mn, and Zn) impairs *Eucalyptus* metabolism and, thereafter, its growth (Masullo et al. 2021; Rogers et al. 2001). Copper and Zn are transition metals that are essential for plant development, as they act as cofactors for numerous enzymes (Broadley et al. 2012) . In addition, Cu and Zn are required for the balance of metal ions required for plant to acquire tolerance against various environmental toxins (Saito and Uozumi 2020). Studies have shown that plant requirement of metallic ions usually contrasts with the low bioavailability of these ions in various soil types, as a strategy to overcome low soil metallic micronutrients availability, plant optimizes their use and facilitating their relocation from one cellular compartment to another (Barnard et al. 1992; Roschzttardtz et al. 2019). The contribution of lime plus fertilizers containing Cu and Zn rates to enhancing *Eucalyptus* yield could be determined by investigating the relative contents of metallic micronutrients in soil and their allocation in the plant compartments (e.g., leaf, stem, and root).

Management changes directly affect plant metabolic activity, and responses to new conditions occur by optimization of their performance under the new environment, which can either speed up and/or slow down plants development and biomass production (Verdaguer et al. 2017). Leaf pigments such as chlorophyll and flavonoids are indicators of plant nutritional status and metabolic activity (Croft and Chen 2017; Kalaji et al. 2017; Li et al. 2020), and these molecules are crucial for regulating radiation effects and plant homeostasis (Kreslavski et al. 2016; Verdaguer et al. 2017). The nutrient accumulation and use efficiency can vary within the same plant species because of the differences in the genotype-environment interaction, and high nutrient use efficiency is not always linked to high productivity (Santana et al. 2002). The responses of plants to regulate the concentration, accumulation, nutrient use efficiency, and distribution of metallic micronutrients are dependent on soil fertility, and mineral fertilization (such as Cu and Zn application) aimed to enhance the nutritional status and productivity of the crop. An understanding of the relative contribution of these nutritional parameters is necessary to advance studies on metallic micronutrient homeostasis in plants and for successful strategy development (Sperotto et al. 2014).

The absence of suitable fertilization for crop growth may lead to reduced soil nutrient availability, and under such conditions, biomass production can be impaired (Resquin et al. 2020). Copper and Zn play a crucial role in redox systems in cells and in various enzymes (Broadley et al. 2012), and these micronutrients present low mobility and redistribution *via* phloem (Alloway 2008). The use efficiency of phloem-mobile nutrients increases with decreasing nutrient availability, whereas the opposite occurs for phloem-low or -immobile nutrients (Turner and Lambert 2014). For *Eucalyptus* plant, nutrients with high use efficiency(e.g., Ca and K) can are mainly responsible for limiting wood production (Santana et al. 2002; Smethurst 2010). In this sense, despite of plant physiological responses due to better nutritional performance through macronutrients fertilization is well documented in the literature, there is a knowledge gap with metallic micronutrients fertilization. Plants can shape its biochemical and morphophysiological state to combat nutritional disorders, increasing their nutrient use efficiency as well as to optimize growth and yields related parameters (Battie-Laclau et al. 2014a, b; Mateus et al. 2021; Santos et al. 2021). The amount of nutrients accumulated in plant tissues is a key factor to achieve sustainable biomass production by effectively determining which plant compartments will export fewer nutrients (Resquin et al. 2020; Srivastava and Malhotra 2017).

Few studies have investigated the implications of the combined application of lime and Cu plus Zn fertilizers for *Eucalyptus* plant growth. In this sense, it was hypothesized that (i) the well-known benefits of lime application for *Eucalyptus* seedlings can offset the undesirable implications of this management on soil-Cu and -Zn availability, and (ii) a strategy to overcome the undesirable implications of soil lime application in the Cu and Zn availability is through the combined application of lime and Cu plus Zn fertilizer, which leads to higher plant biomass production than lime or Cu plus Zn fertilizer application separately. To test the hypotheses, we investigated the effects of lime application and Cu plus Zn fertilizer on developmental plant performance through biomass production, leaf area, leaf pigments contents, as well as Cu and Zn concentrations and accumulations, their nutritional use efficiency, and its distribution among roots, stem, and leaves in *Eucalyptus grandis* seedlings.

### 4.1.1. Materials and methods

### 4.1.2. Experimental setup and conditions

The experiment was conducted in a greenhouse at the Center for Nuclear Energy in Agriculture (CENA-USP) in Piracicaba, São Paulo State, Brazil (22°43´04″ S and 47°36´55″ W) from December 2018 to February 2019, with seminal seedlings from monoprogeny of *Eucalyptus grandis*. The seedlings were produced in individual plastic tubes of 50 $cm^3$, filled with substrate, and previously fertilized with macro- and micronutrients. After 100 days of sowing, the seedlings were transplanted into individual plastic pots (5 L) (Supplementary Fig. S1). The soil for seedling cultivation was obtained from the upper 0–0.2 m soil layer from the Itatinga Experimental Station at the University of São Paulo, Brazil (23° 03′ S and 48° 37′ W, approximate elevation 850 m), and classified as Rhodic Ferralsol (IUSS Working Group WRB, 2015), with medium textured and 1.3 g $dm^{-3}$ density . The soil was sieved in 6.0 mm mesh and physical characterization was done according to Camargo et al. (1986), revealing 800 g $kg^{-1}$ of sand, 21 g $kg^{-1}$ of silt, and 180 g $kg^{-1}$ of clay. Soil chemical attributes were determined according to Raij et al. (2001), as 3.9 for pH in $CaCl_2$ , 22.5 g $kg^{-1}$ for organic matter, 0.37 $mmol_c$ $kg^{-1}$ for K, 0.76 $mmol_c$ $kg^{-1}$ for Ca, 0.39 $mmol_c$ $kg^{-1}$ for Mg, 17.3 $mmol_c$ $kg^{-1}$ for Al, and 65 $mmol_c$ $kg^{-1}$ for H+Al (total acidity), as well as 0.72 mg $kg^{-1}$ for Cu, 86.5 mg $kg^{-1}$ for Fe, 0.91 mg $kg^{-1}$ for Mn, and 0.24 mg $kg^{-1}$ for Zn. According to Raij et al. (2001), the soil pH was determined with 0.01 mol $L^{-1}$ $CaCl_2$, total acidity in the same soil suspension that determined the pH. The organic matter content was estimated by the Walkley-Black method, K, Ca, and Mg contents were extracted using the ion-exchange resin method, and metallic micronutrients with 0.005 mol $L^{-1}$ DTPA solution at pH 7.3.

The experimental design was randomized in five blocks, in a 3 × 4 factorial scheme, with three lime rates (lime) and four fertilizer rates of Cu and Zn (fert). The lime treatments were: no lime rate (NoL); low lime rate (LoL), with application of 0.77 g $CaCO_3$ $kg^{-1}$ soil and 0.17 g $MgCO_3$ $kg^{-1}$ soil (based on the calculation: 20-[Ca+Mg]/10 for *Eucalyptus* plants according to Gonçalves (2010)); and high lime rate (HiL), with the application of 1.42 g $CaCO_3$ $kg^{-1}$ soil and 0.30 g $MgCO_3$ $kg^{-1}$ soil (base saturation method – BS, to raise BS to 60% for annual crops, e.g., soybean and wheat according to Raij et al (1996)) (Supplementary Table S1). The fert treatments were: no Cu plus Zn fertilizer (-CuZn); only 1.72 mg Cu $kg^{-1}$ soil (+Cu); only 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn), represented by 6.8 mg copper sulfate $kg^{-1}$ soil ($CuSO_4.5H_2O$) and 22.8 mg zinc sulfate $kg^{-1}$ soil ($ZnSO_4.7H_2O$) (Supplementary Table S1). The amounts and sources of nutrients for the fertilizer of Cu and Zn and the other nutrients (N, P, K, S, and B) were calculated according to Novaes et al. (1991).

The soil was placed in individual pots, homogenized, and moistened with deionized water until reaching 70% of the total water-holding capacity (WHC total), and incubated for 100 days. After the incubation, treatments with different rates of Cu and Zn fertilizers were established; and were applied the rates of N, P, K, S, and B in all pots, as recommended by Novaes et al. (1991). Five days later, the *Eucalyptus* seedlings were transplanted to individual pots, where they were grown for 80 days. Across the experimental period, the temperature in the greenhouse ranged between 24°C (minimum) and 35°C (maximum) and averaged 28°C. The average relative air humidity was 65%, and the maximum photosynthetic photon flux density (sunlight) was approximately 1600 μmol $m^{-2}$ $s^{-1}$.

### 4.1.3. Measurements, harvesting, and analyses

Before harvesting, chlorophyll (Chl) and flavonoid (Flav) contents and the nitrogen balance index (NBI or Chl/Flav ratio) were measured in leaves at 45, 60, and 80 days after planting. The values of these variables were estimated by the non-destructive method using a Dualex Scientific meter (FORCE-A, Orsay, France) (Cerovic et al., 2012, 2005; Li et al., 2020). Data were collected from four fully developed leaves located in the upper third of the *Eucalyptus* canopy. The reading was conducted from 8:00 a.m. to 10:00 a.m. from a reading point in the middle region of the adaxial and abaxial parts of mature leaves, and in the region outside the central rib.

At the end of the trial, the leaves, stems, and roots of each plant were harvested. Immediately after harvest, the fresh phytomass of leaves was determined and digitized using the LI-COR electronic leaf area integrator, Model LI-3100c (LI-COR, 2004). Fresh phytomass from the stems, roots, and leaves was oven-dried under forced air circulation at 60°C for dry biomass determination. Thereafter, dry biomass samples were ground and chemically analyzed for Cu, Fe, Mn, and Zn concentrations, according to Malavolta et al. (1997). In these analyses, the extraction was performed with nitric perchloric digestion and quantified using plasma optical emission spectrometry (ICP-OES equipment, Thermo Scientific iCAP 700). A standard reference (1515 Apple Leaves) from the National Institute of Standards and Technology (Gaithersburg, Maryland) was used to check the quality control of the analytical procedures.

Soil samples were also collected from individual pots, homogenized and dried at 45°C, sieved through a 2.0 mm mesh for determination of pH in $CaCl_2$ (0.01 mol $L^{-1}$), H+Al (SMP buffer solution), and availability of Cu, Fe, Mn, and Zn by the DTPA solution according to Raij et al. (2001).

#### 4.1.4. Specific leaf area calculation

Specific leaf area (SLA) was calculated by dividing leaf area (LA) by leaf dry biomass (Eq. (1)).

$$SLA\ (m^2\ kg^{-1}) = LA\ (m^2\ plant^{-1})\ /\ leaf\ dry\ biomass\ (kg\ plant^{-1}), \quad (1)$$

#### 4.1.5. Nutritional parameters calculation

Specific leaf area (SLA) was calculated by dividing leaf area (LA) by leaf dry biomass (Eq. (2)).

$$SLA\ (m^2\ kg^{-1}) = LA\ (m^2\ plant^{-1})\ /\ leaf\ dry\ biomass\ (kg\ plant^{-1}), \quad (2)$$

Nutrient accumulation (Accu) was calculated from the product of each nutrient concentration in specific plant tissues ($C_i$) and dry biomass from specific plant tissues ($B_j$) (Eq. (3)):

$$Accu\ (mg\ plant^{-1}) = C_i\ (mg\ kg^{-1}) \times B_j\ (g\ plant^{-1}), \quad (3)$$

Nutrient use efficiency (Effi) was calculated by dividing the sum of dry biomass in specific plant tissues ($B_j$) by the Accu of each nutrient in the same plant tissues, according to Turner and Lambert (2014) (Eq. (4)).

$$Effi\ (g\ biomass\ mg^{-1}\ nutrient) = B_j\ (g\ plant^{-1})/Accu\ (mg\ plant^{-1}), \quad (4)$$

Nutrient distribution (Dist) was calculated by dividing the amount Accu in the specific tissues ($D_{tissue}$) by total Accu in the plant ($D_{plant}$) and multiplying by 100, according to Lavres et al (2012) (Eq. (5)).

$$Distr\ (\%) = D_{tissue}\ (mg\ plant^{-1})/D_{plant}\ (mg\ plant^{-1})\ x\ 100 \quad (5)$$

### 4.1.6. Data analysis

Two-way analysis of variance (ANOVA) was performed to test the main effects of lime application and fertilizer with Cu and Zn at the 5% significance level ($P < 0.05$). The means were compared using the LSD test ($P < 0.05$), and data were checked using the Levene test for homogeneity of variance and the Shapiro–Wilk test for normality of residuals. The data were also subjected to multivariate analysis, through principal component analysis (PCA), to characterize the treatments under liming and Cu plus Zn fertilization. Statistical analysis was conducted using SAS version 9.4 (SAS Institute Inc, 2016) and R version 4.0.2 (R Core Team, 2016).

## 4.2. Results

### 4.2.1. Soil lime and Cu plus Zn applications: implications on soil acidity and metallic micronutrients availabilities

A significant effect was found from individual lime application on the pH values, H+Al, and the content of Fe, and significant responses in soil under individual Cu and Zn fertilization (fert) on soil availability of Cu, Fe, Mn and Zn (Table 1).

**Table 1**
Soil chemical attributes at 80 days after planting *Eucalyptus* seedlings, under lime application (lime) and fertilizers with Cu and Zn (fert) (mean±standard error, n=5).

| Treat | pH | H+Al | Cu | Fe | Mn | Zn |
|---|---|---|---|---|---|---|
| | 0.01 mol $L^{-1}$ $CaCl_2$ | $mmol_c$ $kg^{-1}$ | ------------------------------ | mg $kg^{-1}$ | ------------------------------ | |
| Lime | | | | | | |
| NoL | 3.71±0.04 b | 57.3±1.9 a | 1.13±0.08 a | 148±3 a | 1.56±0.28 a | 1.23±0.17 a |
| LoL | 3.69±0.03 b | 53.3±1.2 a | 1.11±0.10 a | 128±2 b | 1.05±0.27 a | 1.03±0.30 a |
| HiL | 4.12±0.05 a | 40.7±1.4 b | 1.02±0.08 a | 116±7 c | 0.98±0.07 a | 0.84±0.25 a |
| Fert | | | | | | |
| -CuZn | - | - | 0.83±0.03 b | 133±5 a | 1.44±0.37 a | 0.30±0.01 b |
| +Cu | - | - | 1.34±0.07 a | 139±4 a | 1.29±0.33 a | 0.31±0.02 b |
| +Zn | - | - | 0.84±0.03 b | 128±8 a | 0.95±0.10 a | 1.74±0.15 a |
| +CuZn | - | - | 1.33±0.08 a | 123±8 a | 1.10±0.22 a | 1.78±0.25 a |

Lime treatments (Treat): no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%). Fert treat: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu $kg^{-1}$ soil (+Cu); 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn). Chemical analysis according to Raij et al. (2001). Different lowercase letters in the same column indicate differences between treatments by LSD test ($P < 0.05$) (n=5).

The mean values from all treatments ranged from 3.71–4.12 for pH and 40.7–57.3 $mmol_c$ $kg^{-1}$ for H+Al. The soil micronutrient contents (nutrients availability) varied from 0.83–1.33 mg $kg^{-1}$ for Cu, 116–148 mg $kg^{-1}$ for Fe, 0.95–1.56 mg $kg^{-1}$ for Mn, and 0.30–1.78 mg $kg^{-1}$ for Zn. Among these metals in soil, the mean values from all treatments were found in the following sequence: Fe (131 mg $kg^{-1}$) > Mn (1.19 mg $kg^{-1}$) > Cu (1.09 mg $kg^{-1}$) > Zn (1.03 mg $kg^{-1}$). The high lime rate (HiL) increased pH and reduced the H+Al , while within the low lime rate (LoL) and HiL treatments, soil Fe availability was reduced significantly ($P < 0.05$). At 80 days after planting *Eucalyptus* seedlings, the soil-Cu, -Mn, and -Zn contents were unaffected by the different treatments either with or without lime application (Table 1). In contrast, Cu and Zn application increased its availability in soils fertilized with these micronutrients (+Cu, +Zn, and +CuZn treatments) (Table 1).

### 4.2.2. The effect of liming and Cu plus Zn fertilization on biomass production

At the end of the trial, the total biomass (TB) of *Eucalyptus* seedlings under LoL and HiL treatments (with lime) and +Cu, +Zn, and +CuZn treatments (with fert) ranged from 107.4 to 123.0 g (c.a. of 15%), whereas for plants grown at NoL treatment (without lime) and -CuZn treatment (without fert) the TB ranged from 94.8 and 103.5 g (c.a of 9%) (Table 2), with no interaction effect observed for biomass production between factors of lime and fertilization with Cu and Zn. Nonetheless, treatments with lime and with fert application increased biomass production in plants and in different tissues (i.e., leaves, stems, and roots). In addition, no difference was found in biomass production between plants treated with LoL and those treated with HiL.

The root:shoot ratio (R:S) of plants ranged from 0.40 to 0.47 in lime treatments and from 0.41 to 0.44 in fert treatments, with higher R:S ratio in the treatment without lime application (Table 2). *Eucalyptus* seedlings under lime (LoL and HiL treatments) application had 28% more TB than seedlings grown without lime application (NoL treatment). Seedlings fertilized with Cu plus Zn (+CuZn treatment) had 19% more TB than seedlings grown without Cu plus Zn fertilizers (-CuZn treatment); seedlings under Cu condition (+Cu treatment) had 13% more TB relative to -CuZn treatment; and seedlings under +Zn treatment had 4% more TB relative to -CuZn treatment (Table 2).

**Table 2**
Biomass production in *Eucalyptus grandis* seedling at 80 days grown under lime application (lime) and fertilizers with Cu and Zn (fert) (mean±standard error, n=5).

| Treat | Leaf | Stem | Root | Shoot | Root:Shoot | Total biomass |
|---|---|---|---|---|---|---|
| | ------------------------------------------------- g plant$^{-1}$ ------------------------------------------------- | | | | | |
| Lime | | | | | | |
| NoL | 22.6±1.0 b | 41.8±1.2 b | 30.4±1.7 b | 64.4±2.0 b | 0.47±0.02 a | 94.8±3.2 b |
| LoL | 33.0±1.2 a | 54.1±1.1 a | 35.3±1.4 a | 87.1±1.9 a | 0.41±0.01 b | 122.5±2.9 a |
| HiL | 33.3±1.3 a | 52.9±1.5 a | 34.5±1.2 a | 86.2±2.5 a | 0.40±0.01 b | 120.7±3.2 a |
| Fert | | | | | | |
| -CuZn | 26.9±2.0 b | 46.3±2.1 b | 30.2±1.8 b | 73.2±3.8 b | 0.42±0.02 a | 103.5±5.2 b |
| +Cu | 29.6±1.8 b | 51.7±1.8 a | 35.4±1.5 a | 82.3±3.4 a | 0.44±0.02 a | 116.8±4.1 a |
| +Zn | 29.0±1.6 b | 47.6±2.1 b | 30.9±1.7 b | 74.5±3.3 b | 0.41±0.02 a | 107.4±4.4 b |
| +CuZn | 33.1±2.0 a | 52.8±1.9 a | 37.0±1.3 a | 86.0±3.6 a | 0.44±0.02 a | 123.0±4.1 a |

Lime treatments (Treat): no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%). Fert treat: no Cu and Zn (-CuZn); 1.72 mg Cu kg$^{-1}$ soil (+Cu); 5.16 mg Zn kg$^{-1}$ soil (+Zn); and 1.72 mg Cu kg$^{-1}$ soil plus 5.16 mg Zn kg$^{-1}$ soil (+CuZn). Different lowercase letters in the same column indicate differences between treatments by LSD test ($P$ < 0.05).

Plants grown under fert (i.e., +Cu, +Zn, and +CuZn) and lime (i.e., LoL and HiL) had higher visual aspects than those grown under no fert (-CuZn) and no lime (NoL) (Fig. 1). Higher plant performance was also observed in *Eucalyptus* seedlings under lime × fert (i.e., LoL × +CuZn and HiL× +CuZn) relative to treatment without lime × without fert (i.e., NoL × -CuZn), as seen through the better development of branches and roots and plant growth (Fig. 1 and Supplementary Fig. S2).

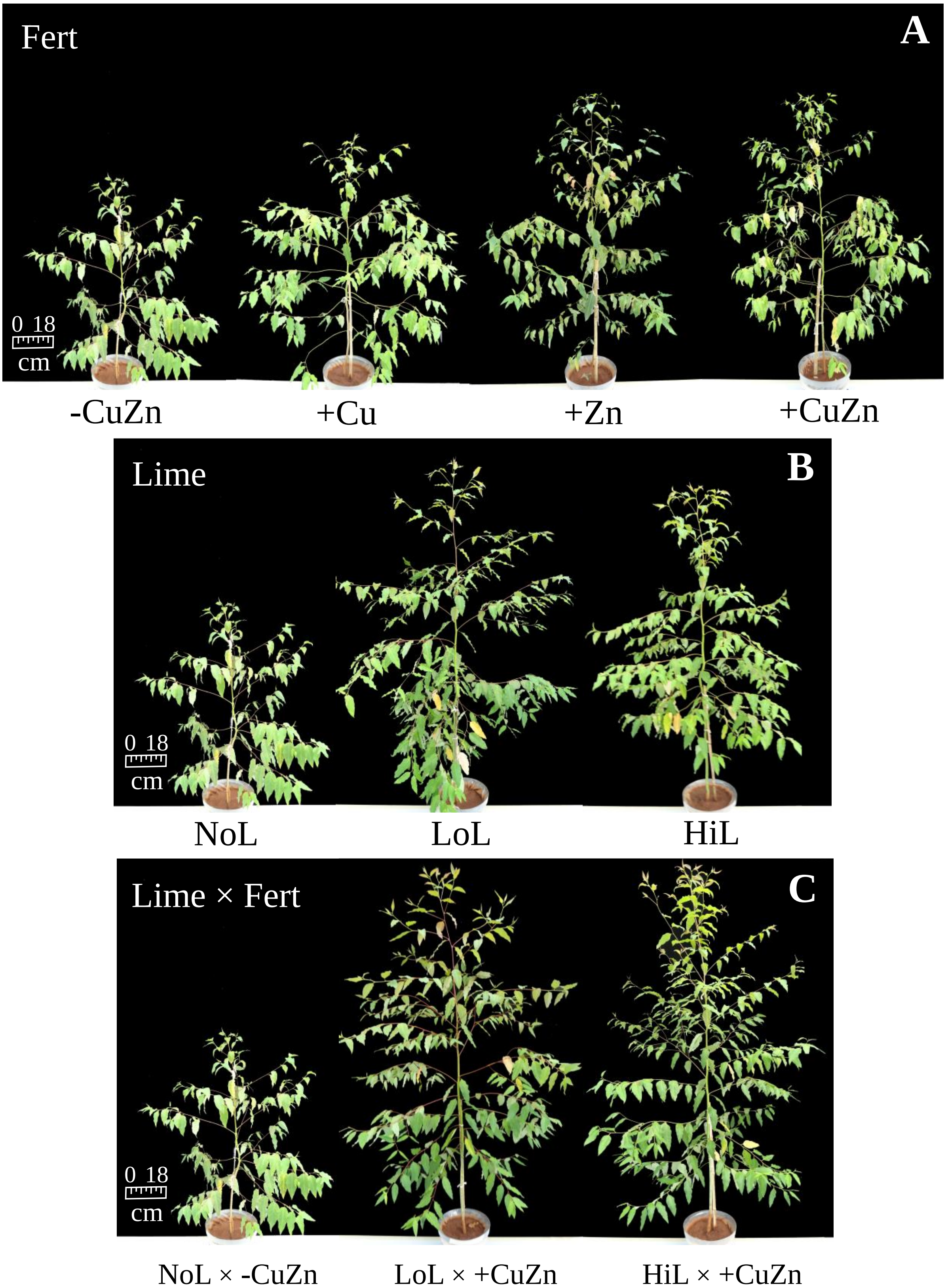



**Fig. 1.** Visual aspect of *Eucalyptus grandis* seedlings at 80 days grown under separate application of Cu and Zn fertilizers (Fert) (A) and lime (Lime) (B) and Lime × Fert (C). Fert treatments: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu $kg^{-1}$ soil (+Cu); 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn). Lime treatments: no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%).

### 4.2.3. Lime application influence on leaf area and specific leaf area

Leaf area and SLA in *Eucalyptus* seedlings were significantly increased only in treatments under individual lime application (i.e., LoL and HiL) (Table 3). Using these rates of lime, the LA and SLA were enhanced by 60% and 11% relative, respectively, to control (no lime; NoL); however, increasing the lime rates did not increase LA and SLA. Under Cu and Zn fertilizer rates (fert), LA and SLA were unaffected; however, in contrast to plants grown without Cu plus Zn (-CuZn), the *Eucalyptus* seedlings fertilized with Cu plus Zn (+CuZn) increased both LA (8%) and SLA (5%).

**Table 3**
Leaf area (LA) and specific leaf area (SLA) in *Eucalyptus grandis* seedlings, at 80 days grown under lime application (lime) and fertilizers with Cu and Zn (fert) (mean±standard error, n=5).

| Treat | Leaf area (LA) | Specific leaf area (SLA) |
|---|---|---|
| | ------------------------ $m^2$ $plant^{-1}$ ------------------------ | ------------------------ $m^2$ $kg^{-1}$------------------------ |
| Lime | | |
| NoL | 0.39±0.02 b | 17.1±0.4 b |
| LoL | 0.63±0.02 a | 19.0±0.5 a |
| HiL | 0.62±0.02 a | 18.9±0.3 a |
| Fert | | |
| -CuZn | 0.52±0.4 a | 17.6±0.4 a |
| +Cu | 0.56±0.4 a | 19.0±0.6 a |
| +Zn | 0.53±0.3 a | 18.5±0.5 a |
| +CuZn | 0.56±0.4 a | 18.4±0.4 a |

Lime treatments (Treat): no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%). Fert treat: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu $kg^{-1}$ soil (+Cu); 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn). Different lowercase letters in the same column indicate differences between treatments by LSD test ($P < 0.05$).

### 4.2.4. Soil lime and Cu plus Zn fertilizer implications on leaf pigments

The interaction between lime and fertilization with Cu and Zn (fert) was not significant for chlorophyll (Chl) and flavonoid (Flav) content or for the nitrogen balance index - NBI (Chl/Flav ratio) in *Eucalyptus grandis* at 45, 60, and 80 days after the seedlings were transplanted (DAT). However, the individual effect was observed for flavonoid under fert treatment and chlorophyll under lime treatment (Fig. 2). The chlorophyll content was higher only for seedlings under fert (+Cu, +Zn, and +CuZn treatments) at 45 and 60 DAT relative to seedlings grown without fert (-CuZn treatment) (Fig. 2A). Seedlings under lime (i.e., LoL and HiL) showed higher flavonoid content relative to seedlings grown without lime (NoL) at 45 and 80 DAT (Fig. 2B).

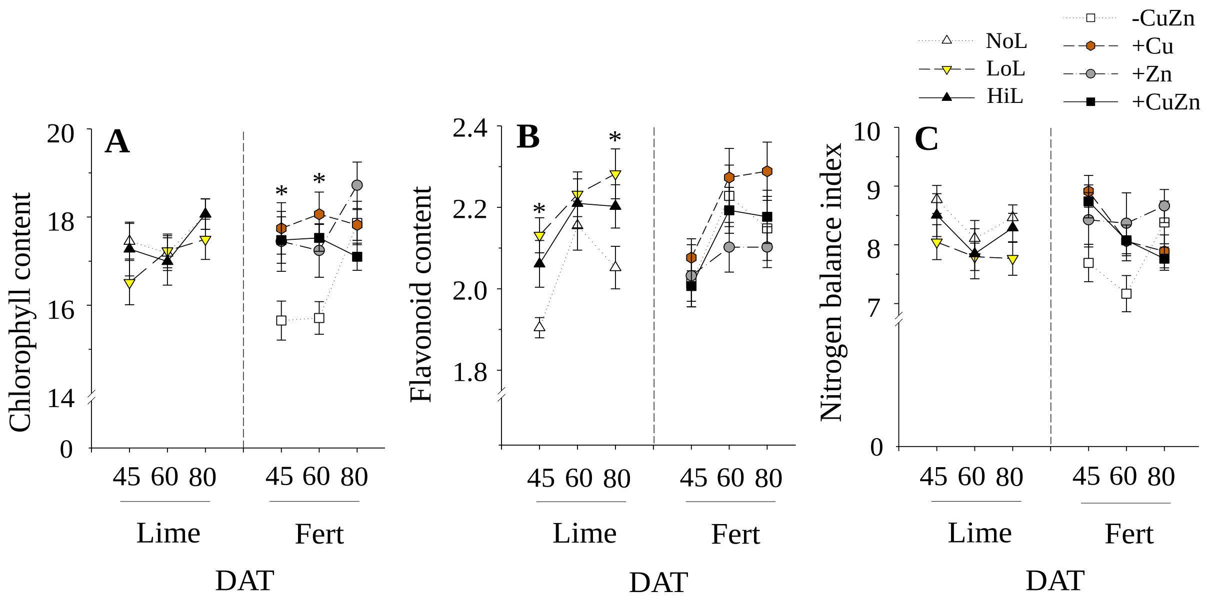



**Fig. 2.** Chlorophyll (A) and flavonoids contents (B) and nitrogen balance index (C) in *Eucalyptus grandis* seedlings, at 45, 60, and 80 days grown under lime application (lime) and fertilizers with Cu and Zn (fert). Lime treatments: no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%). Fert treatments: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu $kg^{-1}$ soil (+Cu); 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn). Standard error bars with asterisks (*) in the same condition (lime or fert) indicate differences between treatments by *F*-test ($P < 0.05$) (n = 5).

The nitrogen balance index was unaffected by the treatments used in this study (Fig. 2C). During *Eucalyptus* seedlings growth (45, 60, and 80 DAT), the mean values ranged from 10% for chlorophyll, 21% for flavonoid, and 13% for NBI under lime rates conditions, and from 21% for chlorophyll, 15% for flavonoid, and 24% for NBI in *Eucalyptus* under fert rates conditions. In general, seedlings grown without lime had lower flavonoid contents, whereas seedlings grown without fert had lower chlorophyll contents and NBI.

### 4.2.5. Soil lime and Cu plus Zn application influence on metallic micronutrient in *Eucalyptus* tissue

No interaction effect was observed between lime (NoL, LoL, and HiL) and fert (-CuZn, +Cu, +Zn, and +CuZn) application for metallic micronutrient (i.e., Cu, Fe, Mn, and Zn) concentrations (Conc), accumulation (Accu), use efficiency (Effi), and distribution (Dist) in *Eucalyptus* seedlings grown for 80 days. However, these parameters were affected by lime or fert application separately (Fig. 3).

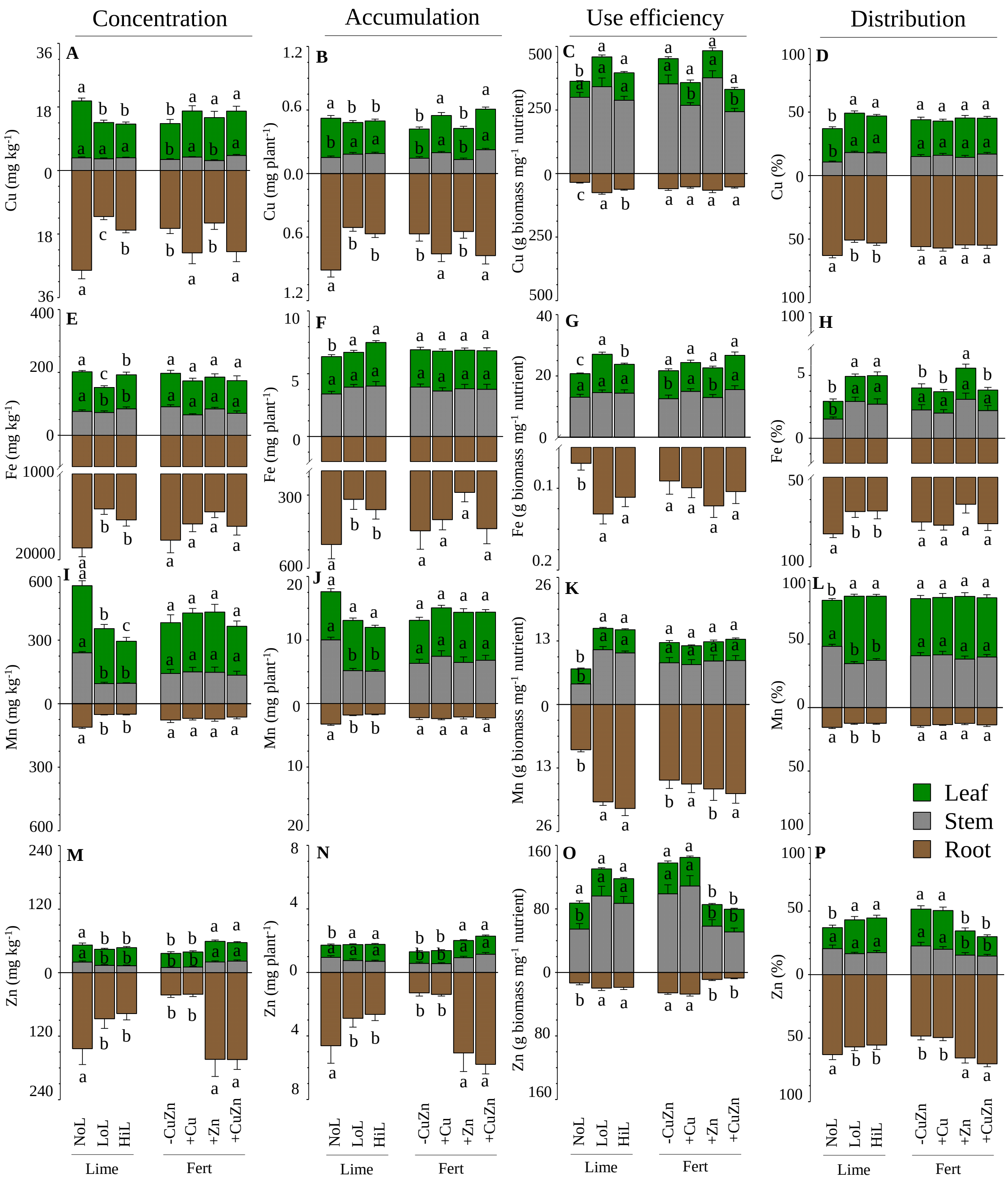



**Fig. 3.** Concentration, accumulation, use efficiency, and distribution of Cu (A-D), Fe (E-H), Mn (I-L), and Zn (M-P) in *Eucalyptus grandis* seedlings, at 80 days grown under lime application (lime) and fertilizers with Cu and Zn (fert). Lime treatments: no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%). Fert treatments: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu kg$^{-1}$ soil (+Cu); 5.16 mg Zn kg$^{-1}$ soil (+Zn); and 1.72 mg Cu kg$^{-1}$ soil plus 5.16 mg Zn kg$^{-1}$ soil (+CuZn). Standard error bars with different lowercase letters in the same condition (lime or fert) indicate differences between treatments by LSD test ($P$ < 0.05) (n = 5).

Lime application (i.e., LoL and HiL treatments) decreased the Cu, Fe, Mn, and Zn concentrations in all plant tissues (i.e., leaf, stem, and root) (Fig. 3A, E, I, M). Copper and Zn applications (i.e., +Cu, +Zn, and +CuZn treatments) increased the concentration of Cu and Zn in all plant compartments (Fig. 3A, M). Overall, higher Mn concentration was observed in the leaves, while higher concentration values of Cu, Fe, and Zn were found in the roots. Among the micronutrients in all plant compartments, higher concentration were found, as follows: leaf = Mn > Fe > Zn > Cu, stem = Mn > Fe > Zn > Cu, and root = Fe > Zn > Mn > Cu.

Plants cultivated under liming presented a decrease in Cu (41%), Fe (33%), Mn (46%), and Zn (40%) accumulation in the roots (Fig. 3B, F, J, N), whereas 18% of Cu accumulation were reduced in leaf (Fig. 3B) and 49% of Mn in the stem (Fig. 3J) relative to plants without lime, respectively. Nevertheless, plants cultivated under lime had increased leaf accumulation of Fe and Zn (Fig. 3J, N), and only Cu in the stem (Fig. 3B). Plants fertilized with Cu plus Zn showed higher accumulation of Cu and Zn in the leaf (39% of Cu and 56% of Zn), stem (55% of Cu and 102% of Zn), and root (36% of Cu and 348% of Zn) relative to plants without Cu and Zn application (Fig. 3B, N), while higher accumulation for Cu and Zn was obtained in the root.

The use efficiency of Cu, Fe, Mn, and Zn in leaves, stems, and roots was increased in the plants grown under lime application (LoL and HiL treatments) (Fig. 3C, G, K, O). When Cu fertilizer was applied (+Cu and +CuZn treatments), the use efficiency of Cu decreased in the *Eucalyptus* stem (Fig. 3C) relative to plants under treatment without Cu plus Zn (-CuZn), and with Zn addition (+Zn and +CuZn treatments), the use efficiency of Zn decreased in all plant compartments as compared with -CuZn treatment (Fig. 3O). Overall, the use efficiency in *Eucalyptus* seedlings among all metallic micronutrients was observed in the following order: Cu > Zn > Fe > Mn. In addition, a higher use efficiency for Cu and Zn was obtained in the stem.

Among *Eucalyptus* compartments, a higher distribution of Cu, Fe, and Zn was obtained in the root (Fig. 3D, H, P), and Mn in the leaf (Fig. 3L). A comparison between root and shoot (leaf + stem) showed a higher distribution of Cu, Fe, and Zn in the root and Mn in the shoot. In addition, the distribution of these micronutrients in root decreased in the treatments under lime as compared with those without lime application. Among treatments under Cu plus Zn fertilization, there was no difference in Cu distribution in the *Eucalyptus* compartments (Fig. 3D). The *Eucalyptus* seedlings that were grown under +Zn and +CuZn treatments showed a higher distribution of Zn in the root relative to treatments under -CuZn and +Cu, which had lower Zn Dist in the leaf and stem (Fig. 3P).

### 4.2.6. Multivariate analysis characterized treatments under liming and Cu plus Zn application

The first component (PC1) explained 56% of the data variance from TB, LA, flavonoid, and chlorophyll content, while the second component (PC2) explained 33% of the variance associated with the same variables (Fig. 4A). The treatments under combined application of lime and Cu plus Zn (i.e., LoL × +CuZn and HiL × +CuZn) were characterized by higher values of TB, LA, chlorophyll, and flavonoid as compared with treatment with no lime and no Cu plus Zn (i.e., NoL × -CuZn). Separate application of lime (LoL and HiL) and Cu plus Zn (+CuZn) were closely associated with biomass production and leaf area.

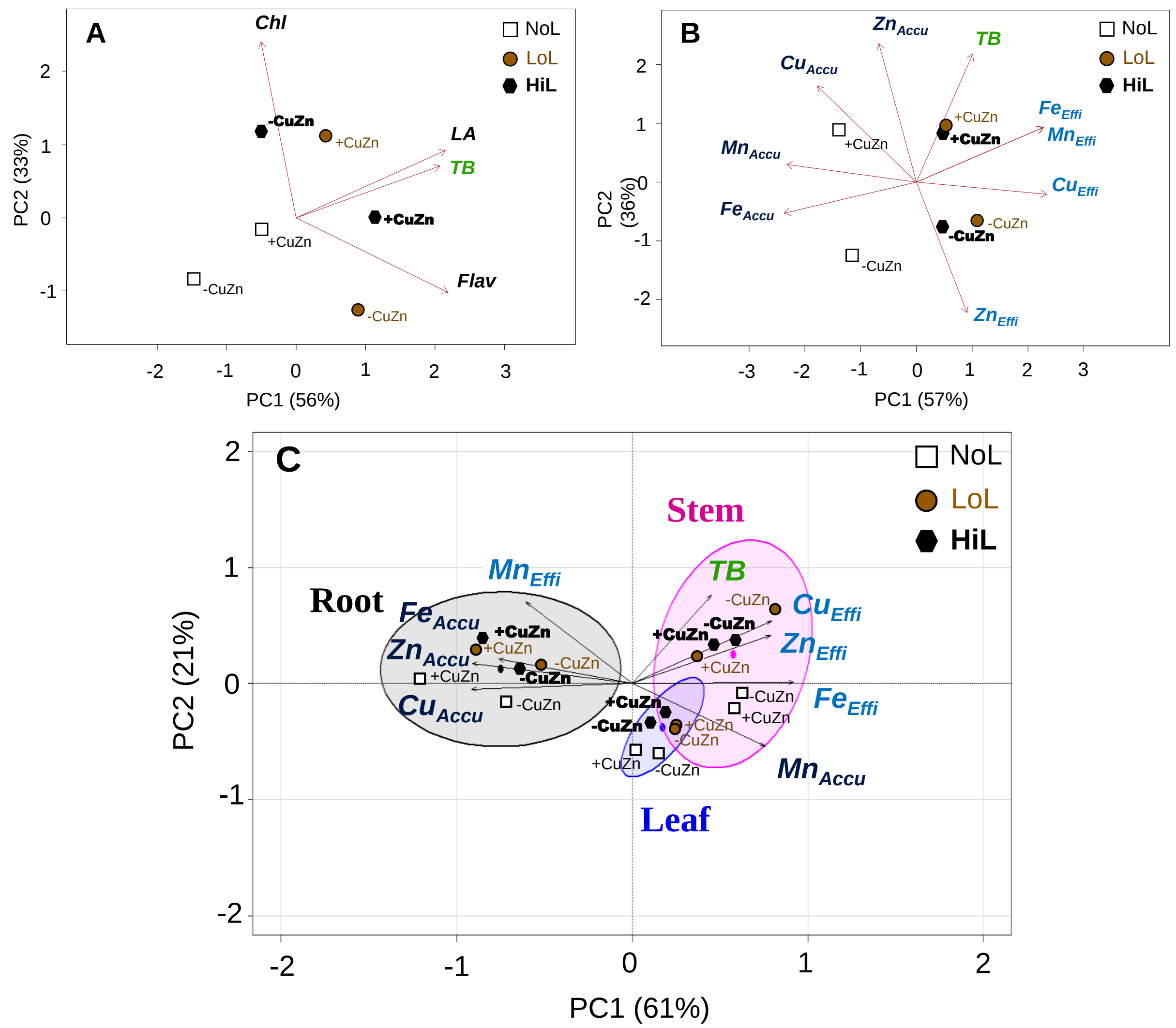



**Fig. 4.** Principal component analysis (PCA) among total biomass (TB), leaf area (LA), chlorophyll (Chl) and flavonoids (Flav) contents (A) and TB, accumulation (Accu), and use efficiency (Effi) of Cu, Fe, Mn, and Zn in *E.grandis* (B) and their tissues (C), at 80 days grown under lime application (lime) and fertilizers with Cu and Zn (fert). Lime treatments: no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%). Fert treatments: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu $kg^{-1}$ soil (+Cu); 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn).

PC1 (57%) and PC2 (36%) explained 83% of the data variance for TB, metallic micronutrient (i.e., Cu, Fe, Mn, and Zn) accumulation (Accu), and their use efficiency (Effi) (Fig. 4B). Treatments with the combined application of lime and Cu plus Zn were associated with TB, Accu of Cu and Zn, and Effi of Fe and Mn. In addition, plants grown with lime (regardless of LoL and HiL rate) were associated with TB and the Effi of metallic micronutrients, while plants with no lime treatment (i.e., NoL) were closely related to the Accu of metallic micronutrients. The plants grown under treatments with Cu plus Zn rate (i.e., +CuZn) were characterized as having higher TB and Accu of Cu and Zn, with lower Effi of these same micronutrients relative to plants grown under treatment without Cu plus Zn (i.e., -CuZn). With 82% (PC1 + PC2) explanation of data variance (Fig. 4C), the stem and leaf were closely associated with biomass production, Effi of Cu, Fe, and Zn, and Accu of Mn, while roots were related to the Accu of Cu, Fe, and Zn and Effi of Mn.

## 4.3. Discussion

### 4.3.1. Enhanced soil fertility caused by lime or Cu plus Zn application improved leaf traits and plant growth

The lime application rate increased the soil pH, reduced the H+Al and Fe soil availability, while the Cu, Mn, and Zn soil levels were not affected ($P < 0.05$). The increase in soil pH was directly related to soil nutrient availability (Alleoni et al. 2005; Barman et al. 2014), therefore, there is a rearrangement of Cu, Fe, Mn, and Zn in the soil under lime application (Faria et al. 2020; Holland et al. 2018; Paradelo et al. 2015; Rahman et al. 2018). These changes in soil chemical features under liming have been reported in other studies (Bossolani et al. 2020; Chatzistathis et al. 2015; Gabriel et al. 2018).

Copper and Zn application separately or combined in a single fertilizer increased its concentrations in the soil (Table 1) and, consequently, enhanced *Eucalyptus* seedlings uptake and growth. The metallic micronutrient (i.e., Cu, Fe, Mn, and Zn) contents in tropical soils were primarily associated with recalcitrant soil fractions, mainly Mn oxides and amorphous and crystalline Fe oxides (Silveira et al. 2006). Evidence indicates that plants have optimized uptake of these metals, facilitating their distribution from one compartment to another, depending on the requirement by different physiological processes (Roschzttardtz et al. 2019). *Eucalyptus* responses to Cu and Zn mineral fertilization under oxidic and acidic soils tend to be more common after several rotations or over the long term, mainly in soil that has never been fertilized with metallic micronutrients (Gonçalves et al. 2013). Thus, it is necessary to apply fertilizers to guarantee higher plant development and avoid environmental stress such as that from nutrient deficiency (Smethurst 2010).

The individual effects of lime and Cu plus Zn (i.e., fert application) showed that this nutritional management was essential for enhancing plant growth (Table 2), and that there was a synergy effect between lime and Cu plus Zn application (i.e., +CuZn treatments) (FIG. 1C and Supplementary Fig. S2). Copper and zinc participate in the most abundant superoxide dismutase (SOD) in plant cells (Broadley et al. 2012) and under Zn deficiency reactive oxygen species (ROS) accumulate in plant tissues, increasing membrane lipid peroxidation and membrane permeability (Cakmak and Marschner 1988), which is associated with vegetal tissue senescence (Dhindsa et al. 1981). In this study, plants under fertilization with Cu plus Zn, the contents of Cu and Zn were increased (Fig. 3) and the flavonoid contents were enhanced (Fig. 2), which is directly associated with plant antioxidant functions in response to several antioxidant enzymes, including ROS accumulation (Pollastri and Tattini 2011).

*Eucalyptus* seedlings growth were favored under soil lime application; under such conditions, higher biomass production was related to better soil chemical attributes (Table 1). Moreover, under lime application, *Eucalyptus* seedlings may be favored by better soil nutrient mineralization (Rocha et al. 2019), solubilization (González-Alcaraz et al. 2013), and cycling due to the increase in biological properties (Allen et al. 2020; Caires et al. 2011; Chatzistathis et al. 2015; González-Alcaraz et al. 2013; Wachendorf 2015). In our study, the root:shoot ratio was reduced (Table 2), which indicates higher plant root biomass distribution under liming relative to plants grown without liming. Within the first situation is expected better nutrient and water uptake by plants. Thus, better biomass yield partitioning in plants grown under lime rate (lower root:shoot ratio) is a plant strategy to increase root surface, for example, to improve the uptake and the use efficiency of water and nutrient (Han et al. 2016; Stape et al. 2008, 2010). The nutrients were not homogeneously distributed in the different tissues, as seen in the variation along the root and shoot (Fig. 3), in which nutrient partitioning in plants is closely associated with soil fertility and mineral fertilization, which is responsible for the nutritional status of plants (Valentinuzzi et al. 2020). The improvement in biomass production is related to the effect of lime application rate, which increases the calcium content in *Eucalyptus* plants (Rocha et al. 2019). The increase in the root:shoot ratio can be explained by the supply of Ca content after liming because the formation of new root tissues depends on continuous Ca uptake, which is thought to be involved in the development of lateral and primary root meristems (Ramírez-Builes et al. 2020). *Eucalyptus* plants with higher root biomass distribution can stimulate microbial activity to accelerate organic acid production from root exudates, mainly to solubilize unavailable compounds in the soil (Dakora and Phillips 2002).

Copper or Cu plus Zn applications increased plant growth and thereafter plant compartment biomass [i.e., leaf, stem, and root (Table 2, Fig. 1, and Supplementary Fig. S2)]. Overall, it appears that *Eucalyptus* seedlings dry matter is enhanced after soil Cu and Zn application under low soil-moisture conditions as well as under soil where micronutrients fertilization have never been performed (Gonçalves et al. 2013).

Lime application improved leaf area (Table 3) regardless of the treatments (i.e., LoL and HiL), which determined the adaptability of *Eucalyptus* under these conditions (Table 3). Plants from LoL and HiL treatments showed higher LA (60%) and SLA (11%) than plants grown without liming (i.e., NoL treatment), which may have led to higher biomass production and, therefore, greater plant growth under the former situation. Changes in leaf area traits associated with increased biomass production play a key role in proper plant functioning (Liu et al. 2017), being decisive for greater water- and nutrient-use efficiency (Bieker and Zentgraf 2013) as well as improved light interception (Koester et al. 2014). The increases in LA and SLA of plants grown under lime application may have been indirectly affected by soil-plant-microbiome interaction, which can improve nutrient soil availability for plants and better plant growth (Chaín et al. 2020; Jez et al. 2016; Pereira et al. 2019).

*Eucalyptus* seedlings grown under Cu plus Zn application (+CuZn treatment) increased LA (8%) and SLA (5%) relative to plants without Cu plus Zn (-CuZn treatment) (Table 3), which may have enhanced biomass production (Table 2). In addition to improved biomass production, the lower values of LA and SLA in the plants without Cu and Zn supplies may relate to the lower micronutrient uptake by plants. Leaf area and SLA are closely related to internal factors such as transpiration rates. However, larger leaves are not necessarily associated with higher transpiration rates, because several adaptation strategies are involved in water control (e.g., stomata, pore size, and leaf-shoot ratio) (Bhatla and Lal 2018), and consequently, improved nutritional status and growth performance.

Preferable soil chemical attributes (Table 1), seedling responses [i.e., leaf area traits (Table 3)] and biomass production (Table 2) under lime application led to increased flavonoid contents (Fig. 2B), while Cu and Zn application increased the chlorophyll content (Fig. 2A). In contrast, the plants’ responses for NBI under lime or Cu plus Zn application was not significant ($P < 0.05$) (Fig. 2C). Chlorophyll is an important photosynthetic attribute indicator, as it is capable of capturing light for photosynthesis and other photochemical or non-photochemical reactions (Li et al. 2020). Flavonoids are polyphenolic secondary metabolites that act on the plant's antioxidant metabolism - i.e., in the ascorbate cycle to scavenge the hydrogen peroxide that leaks out of mesophyll cells (Meyer et al. 2006; Pinkard et al. 2006)- and are associated with plant responses to ultraviolet radiation (Verdaguer et al. 2017). Chlorophyll, flavonoid, and NBI are directly related to plant responses to light intensity; consequently, these parameters are dependent on canopy structure (i.e., the plant's ability to intercept light), soil management (with or without nutrient addition), and plant growth (Overbeck et al. 2018). Thus, these variables provide crucial information for interpreting light intensity in *Eucalyptus* and acting as a guide to good plant management. The low soil pH (Table 1) may have favored a reduction in chlorophyll content in leaves, because at low pH (< 5.0), Al availability is high (Panda et al. 2009), and under such conditions, the chlorophyll content can be inhibited by $Al^{+3}$ uptake (Yang et al. 2015). The increase in flavonoid content may be related to high plant development (e.g., leaf biomass in Table 2) and high photosynthetic rate (Verdaguer et al. 2017).

### 4.3.2. *Eucalyptus* nutritional parameters by metallic micronutrients as affected by liming and Cu plus Zn fertilization

The parameters of concentration, accumulation, use efficiency, and distribution of Cu, Fe, Mn, and Zn in *Eucalyptus* seedlings were influenced by lime and Cu plus Zn application (Fig. 3). These micronutrients can uptake by *Eucalyptus* seedling in the form of metal ions via transpiration flow in the xylem (Alejandro et al. 2020); and these ions (e.g., $Cu^{2+}$, $Fe^{3+}$, $Mn^{2+}$, and $Zn^{2+}$) act as cofactors for numerous enzymes and are therefore indispensable for plant growth (Saito and Uozumi 2020). Low Cu, Fe, Mn, and Zn concentration in *Eucalyptus* plant compartments (leaf, stem, and root) under lime application (Fig. 3A, E, I, M) may express the effect of lime to decrease the uptake of these micronutrients by seedlings. In contrast, the increase in Cu and Zn concentration in plants tissues under Cu and Zn fertilization revealed the crucial role of nutrient supply (i.e., Cu plus Zn fertilization) adoption to overcome the reduction in the availability of these metals after liming (Fig. 3A, M), thereby increasing Cu and Zn contents in plant organs. Therefore, liming has widely been related to the reduction of the metallic micronutrient content in plants (Faria et al. 2020; Holland et al. 2018).

Low metallic micronutrient concentration in plants under lime application (Fig. 3A, E, I, M) led to a reduction in the accumulation of these metals in roots, whereas in leaves only the accumulation of Cu decreased, and in the stem only accumulatio of Mn was reduced. Nonetheless, liming increased Fe and Zn accumulation in leaves, but only Cu accumulation was raised in stems (Fig. 3B, F, J, N). These results were associated with the liming effect of increased biomass production in seedlings, which in turn reduced micronutrients accumulation and concentration in *Eucalyptus* seedlings tissues. Under lime application, crops can increase their dry biomass yield, which is related to the uptake of nutrients; however, on the other hand decreasing nutrient concentration in the plants tissues, as known as dilution effect (Holland et al. 2018; Jarrell and Beverly 1981). Seedlings fertilized with Cu and Zn increased the accumulation of these elements in the leaves, stems, and roots relative to plants without Cu and Zn fertilization (Fig. 3B, N). This may be due to greater Cu and Zn uptake under the treatments where the micronutrients were applied. The mechanisms involved in the uptake and accumulation of metallic micronutrients in *Eucalyptus* play a key role in understanding plant homeostasis because plants have developed complex systems to control metal uptake and deliver to the cell tissues (Roschzttardtz et al. 2019).

Lime application increased Cu, Fe, Mn, and Zn use efficiency in leaves, stems, and roots (Fig. 3C, G, K, O), while Cu plus Zn fertilizer reduced Cu use efficiency in the stem, and Zn use efficiency in the *Eucalyptus* leaf, stem, and root (Fig. 3C, O). This pattern of micronutrient use efficiency suggests that special attention should be given to the proper management of lime and fertilizers containing Cu and Zn for suitable *Eucalyptus* plant development. The higher use efficiency of Cu > Zn > Fe > Mn in leaves; Cu > Zn > Fe > Mn in stems; and Cu > Zn > Mn > Fe in the roots (Fig. 3C, G, K, O) indicates that Cu followed by Zn are the metallic micronutrients that have the greatest potential to limit *Eucalyptus* plant growth, since their availabilities in the soil are low. However, this limitation may be avoided if Cu and Zn fertilizers are applied. Nutrients with higher use efficiency in plants should be the key nutrients that limit the improvement of biomass yield (Santana et al. 2002; Smethurst 2010; Srivastava and Malhotra 2017), mainly during plant development and aging (Laclau et al. 2000).

Liming reduced the Cu, Fe, Mn, and Zn distribution in *Eucalyptus* roots, while increasing distribution of these metals in leaves (Fig. 3D, H, L, P). Overall, Cu and Zn or both applications did not significantly change the distribution of Cu, Fe, and Mn in *Eucalyptus* compartments, whereas Zn application increased the distribution of Zn in roots and decreased this element in leaves and stems (Fig. 3D, H, L, P). Increasing evidence emphasizes a co-regulation among the homeostasis of different nutrients in plants (Schjoerring et al. 2020); for example, plants with Fe deficiency showed a greater distribution of Zn in chloroplasts and leaf mitochondria (Vigani et al. 2018). Consequently, the evolution of metallic micronutrient distribution in metabolism could reflect how some metalloenzymes can replace their demands for metal cofactors in plants according to availability (Roschzttardtz et al. 2019).

Among *Eucalyptus* compartments (leaf, stem, and root), the higher concentration, accumulation, and distribution of Cu, Fe, and Zn in the root (Fig. 3) probably reflects the greater contribution to plant uptake of these metals from the soil and export to the xylem, orchestrated by various enzymatic transporters (Dimkpa et al. 2020). Other study suggests that high root concentrations of Fe, Cu and Zn in relation to leaf were adsorbed to root cation exchange capacity, limiting their entry into the symplast (Gomes et al. 2019). The higher concentration, accumulation, and distribution of Mn in leaves (Fig. 3I, J, L) can be associated with the high demand for Mn by the *Eucalyptus* leaf; and *Eucalyptus grandis* probably is closely associated with greater contents to Mn in shoot (i.e., leaf + stem) than to contents of Cu, Fe, and Zn. Manganese plays an important role in the functioning of chloroplasts, and recent studies have identified and characterized Mn transporters in the chloroplast membrane (Alejandro et al. 2020). In addition, when chelated Fe movement to the surface root is reduced, the $Fe^{2+}$ transporter aids the uptake, and in this process, other metallic micronutrients, such as Cu, Mn, and Zn, are absorbed by the plant, since these transporters are not specific (Lambers et al. 2015).

### 4.3.3. The improvement of morpho-physiological traits in *Eucalyptus* seedlings was closely associated with liming and Cu plus Zn fertilization

The PCA analysis indicated that under lime and Cu plus Zn application, total biomass (TB) of *Eucalyptus* seedlings was closely associated with leaf area (LA) and leaf chlorophyll and flavonoid contents (Fig. 4A). Thus, the combination of lime and Cu plus Zn application (i.e., +CuZn) likely improved the anti-stress system of the plant, which enhanced LA and, consequently, increased biomass production. Higher biomass yield results from increased leaf area and specific leaf area (Granda et al. 2014). Copper and Zn are involved in different responses of plant metabolism to control the activity of antioxidant enzymes, such as superoxide dismutase (SOD) (Soares et al. 2019). Therefore, copper plus Zn fertilization can offset the reduction of soil Cu and Zn availability and, thereafter, the content of these micronutrients in *Eucalyptus* seedlings grown under lime application, leading to enhanced biomass yield and other responses for improved plant growth ( Fig. 4, Fig. 1, and Supplementary Fig. S2). The implications of Cu and Zn in the *Eucalyptus* plants to enhance the nutritional status, growth, and reproduce are linked with the biogeochemicas processes and ecosystem functions (Kaspari, 2021).

The high lime application rate (i.e., HiL) in the absence of Cu plus Zn rate (i.e., -CuZn) appears to improve chlorophyll content, whereas flavonoid content was correlated with lime application regardless of lime application rate (Fig. 4A). Chlorophylls (mostly Chl *a* and Chl *b*) are required for the transformation of light energy to chemical bonds, and their content estimation has been used successfully to estimate the stress physiology and abiotic stresses, such as plant nutritional performance (Bang et al. 2021; Kalaji et al. 2017). Changes in canopy traits and increase in biomass yield can be affected by the production of leaf pigments, such as flavonoids, which are associated with plant responses to ultraviolet radiation and antioxidant metabolism (Meyer et al. 2006; Overbeck et al. 2018). Flavonoids are involved in regulating the activities of different protein kinases, which regulate the ROS-induced signaling cascades that are vital to cell development (Brunetti et al. 2013; Pollastri and Tattini 2011). In tropical acidic soils ($pH < 5$), the availability of metallic micronutrients (e.g., Cu and Zn) and Al is high, which can cause nutritional imbalance and consequently lead to low plant growth (Faria et al. 2020; Rahman et al. 2018). Thus, through PCA, there was a synergy between the combined application of lime and Cu plus Zn for beneficial development of *Eucalyptus* seedlings (Fig. 1 and Supplementary Fig. S2), as evidenced by the increase in leaf area, leaf pigments, and biomass production (Fig. 4A, B).

The higher biomass yield and accumulation of Cu and Zn in plants grown under treatments with combined application of lime and Cu plus Zn (Fig. 4B) revealed that *Eucalyptus seedlings* require a greater amount of Cu and Zn to enhance nutritional status. The root system was the plant compartment that accumulated higher Cu, Fe, and Zn, and where Mn use efficiency occurred, while the shoot (i.e., leaf and stem) was closely associated with biomass production (Fig. 4C). Moreover, the stem had better Mn accumulation, as well as higher use efficiency of Cu, Fe, and Zn (Fig. 4C). This pattern of Mn distribution across the two aforementioned plant components may be explained by the high requirement of Mn in leaves to perform a water-splitting reaction during the photosynthesis process (Schmidt et al. 2016). Moreover, between the lime management methods (i.e., the presence and absence of lime application), Mn use efficiency was most closely associated with lime application, which reinforces the effect of lime to precipitate Mn and reduces its availability to the soil for plant uptake (Farid and Khordadpour 2008; Hue and Mai 2002). The high lime application rate reduced the Mn content in the plant, which could cause Mn deficiency (Tanaka et al. 1992). Under liming, the Fe and Mn use efficiency were favored, which was correlated with the lower uptake of these micronutrients by *Eucalyptus* seedlings (Fig. 4B). The reduction of Fe and Mn in plants can be associated with the liming effect that increased the Ca and Mg contents in plants, which can cause an imbalance between Fe and Mn in *Eucalyptus* seedlings. Iron and Mn deficiency is a serious, widespread plant nutritional disorder under high pH (i.e., in calcareous soils or higher lime application rates), which leads to a reduced number of Fe and Mn complexes in photosystem II, thereby lowering the net photosynthesis rate (Alejandro et al. 2020; Gheshlaghi et al. 2020; Schmidt et al. 2016).

Studies have reported that nutrients with higher use efficiency are the first to limit plant nutritional status and therefore plant growth (Santana et al. 2002; Smethurst 2010). Other responses of *Eucalyptus* plants are associated with nutrient availability in the soil and their uptake by plants (Laclau et al. 2000; Turner and Lambert 2014). The high Cu and Zn use efficiency in leaves and stems (Fig. 4C) indicated that lime application can decrease the allocation of Cu and Zn in *Eucalyptus* seedlings. Therefore, when this occurs, the Cu plus Zn application can guarantee the supply of these micronutrients to plants, aiming to improve growth development (Fig. 4). The Cu and Zn fertilization (i.e., +CuZn treatment), regardless of lime rate (i.e., without or with lime), increased the accumulation of these micronutrients and the total biomass production in *Eucalyptus* seedlings (Fig. 4B). This fact can be associated with the contribution of Cu and Zn fertilization that improved the development of *Eucalyptus* (e.g., total biomass production), mainly when grown under lime application (which reduced these metals in plants). Copper and Zn interact with other nutrients (macro and micronutrients), and Zn has higher plant requirements than Cu (López-Hernández 2008).

In a comprehensive overview, the balance of metallic micronutrients (e.g., Cu and Zn) with other nutrients in the soil-plant system must be taken into account to more accurately interpret productivity responses (Srivastava and Malhotra 2017), as well as the uptake rate and nutritional status of plants. Although liming is widely adopted for annual crops (e.g., wheat and soybean) to reduce soil acidity [i.e., HiL treatment (Raij et al. 1997)], this is not a practice widely adopted for *Eucalyptus* cultivation (Gonçalves 2010). In *Eucalyptus* plantations, the recommendation (i.e., LoL treatment) is to supply Ca and Mg for plant nutrition and to benefit their development (Gonçalves 2010; Rocha et al. 2019). The present study highlights lime application combined with Cu plus Zn fertilizers as a promising strategy to alleviate abiotic stress for *Eucalyptus grandis* seedling growth, as well as a path to mitigate the inherent consequences of metabolic activity, such as, reactive oxygen species production (Brunetti et al. 2013; Julkunen-Tiitto et al. 2015; Pollastri and Tattini 2011), which is associated with improved seedling biomass partitioning and power total biomass production.

### 4.4. Conclusion

Although lime application is widely adopted for reducing soil acidity for annual crops, lime recommendation for *Eucalyptus* cultivation is a nutritional management strategy to supply calcium and magnesium. The present study highlights lime application as a promising strategy to enhance growth of *Eucalyptus grandis* seedlings as well as a way to mitigate the inherent consequences of nutritional status and metabolic activity, e.g., leaf chlorophyll and flavonoids contents and leaf area. Moreover, application of lime and Cu plus Zn fertilization is associated with better *Eucalyptus* seedling biomass partitioning and higher biomass production. Even under lime application, copper and zinc contents in *Eucalyptus grandis* seedlings are reduced, fertilization with copper plus zinc shows up it is a strategy to overcome undesirable implications of liming to the soils and thereby increase their contents in plant tissue. Therefore, Cu plus Zn fertilization improves the nutritional status of *Eucalyptus grandis* seedlings, leading to enhance accumulation of Cu and Zn, leaf pigments, leaf area and with the addition of lime could prove a crucial strategy for increased plants growth and, consequently, boosting biomass yield of *Eucalyptus* seedlings.

### References

Alejandro, S., Höller, S., Meier, B., Peiter, E., 2020. Manganese in Plants: From Acquisition to Subcellular Allocation. Front. Plant Sci. https://doi.org/10.3389/fpls.2020.00300

Allen, J., Maunoury-Danger, F., Felten, V., Danger, M., Legout, A., Guérold, F., 2020. Liming of acidified forests changes leaf litter traits but does not improve leaf litter decomposability in forest streams. For. Ecol. Manage. 475. https://doi.org/10.1016/j.foreco.2020.118431

Alleoni, L.R.F., Iglesias, C.S.M., Mello, S.D.C., Camargo, O.A. De, Casagrande, J.C., Lavorenti, N.A., 2005. Atributos do solo relacionados à adsorção de cádmio e cobre em solos tropicais. Acta Sci. Agron. 27, 729–737. https://doi.org/10.4025/actasciagron.v27i4.1348

Alloway, B.J., 2008. Micronutrient deficiencies in global crop production, Micronutrient Deficiencies in Global Crop Production. Springer Netherlands. https://doi.org/10.1007/978-1-4020-6860-7

Ameyu, T., 2019. A Review on the Potential Effect of Lime on Soil Properties and Crop Productivity Improvements. J. Environ. Earth Sci. https://doi.org/10.7176/jees/9-2-03

Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New Phytol. 229, 2446–2469. https://doi.org/10.1111/nph.17074

Barman, M., Shukla, L.M., Datta, S.P., Rattan, R.K., 2014. Effect of applied lime and boron on the availability of nutrients in an acid soil. J. Plant Nutr. 37, 357–373. https://doi.org/10.1080/01904167.2013.859698

Barnard, R.O., van der Watt, H. v. H., Dekker, J., Cronje, I., Mentz, W.H., Cillie, G.E.B., Laker, M.C., 1992. Application of Fe and Zn to lime-rich soils in the form of formulated coal products. Sci. Total Environ. 117–118, 569–574. https://doi.org/10.1016/0048-9697(92)90121-8

Battie-Laclau, P., Laclau, J.P., Beri, C., Mietton, L., Muniz, M.R.A., Arenque, B.C., De Cassia Piccolo, M., Jordan-Meille, L., Bouillet, J.P., Nouvellon, Y., 2014a. Photosynthetic and anatomical responses of *Eucalyptus grandis* leaves to potassium and sodium supply in a field experiment. Plant, Cell Environ. 37, 70–81. https://doi.org/10.1111/pce.12131

Battie-Laclau, P., Laclau, J.P., Domec, J.C., Christina, M., Bouillet, J.P., de Cassia Piccolo, M., de Moraes Gonçalves, J.L., Moreira, R.M., Krusche, A.V., Bouvet, J.M., Nouvellon, Y., 2014b. Effects of potassium and sodium supply on drought-adaptive mechanisms in *Eucalyptus grandis* plantations. New Phytol. 203, 401–413. https://doi.org/10.1111/nph.12810

Bhatla, S.C., Lal, M.A., 2018. Plant Physiology, Development and Metabolism, Plant Physiology, Development and Metabolism. https://doi.org/10.1007/978-981-13-2023-1

Bieker, S., Zentgraf, U., 2013. Plant Senescence and Nitrogen Mobilization and Signaling, in: Senescence and Senescence-Related Disorders. InTech. https://doi.org/10.5772/54392

Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375. https://doi.org/10.1016/j.geoderma.2020.114476

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., 2012. Function of Nutrients: Micronutrients, Marschner's Mineral Nutrition of Higher Plants: Third Edition. https://doi.org/10.1016/B978-0-12-384905-2.00007-8

Brunetti, C., Di Ferdinando, M., Fini, A., Pollastri, S., Tattini, M., 2013. Flavonoids as antioxidants and developmental regulators: Relative significance in plants and humans. Int. J. Mol. Sci. https://doi.org/10.3390/ijms14023540

Caires, E.F., Barth, G., Garbuio, F.J., 2006. Lime application in the establishment of a no-till system for grain crop production in Southern Brazil. Soil Tillage Res. 89, 3–12. https://doi.org/10.1016/j.still.2005.06.006

Caires, E.F., Barth, G., Garbuio, F.J., Churka, S., 2008. Soil acidity, liming and soybean performance under no-till. Sci. Agric. 65, 532–540. https://doi.org/10.1590/s0103-90162008000500013

Caires, E.F., Joris, H.A.W., Churka, S., 2011. Long-term effects of lime and gypsum additions on no-till corn and soybean yield and soil chemical properties in southern Brazil. Soil Use Manag. 27, 45–53. https://doi.org/10.1111/j.1475-2743.2010.00310.x

Cakmak, I., Marschner, H., 1988. Increase in Membrane Permeability and Exudation in Roots of Zinc Deficient Plants. J. Plant Physiol. 132, 356–361. https://doi.org/10.1016/S0176-1617(88)80120-2

Camargo, O.A., Moniz, A.C., Jorge, J.A., Valadares, J.M., 1986. Methods of soil chemical, physical, and mineralogical analysis of the Agronomic Institute in Campinas.

Cerovic, Z.G., Cartelat, A., Goulas, Y., Meyer, S., 2005. In-field assessment of wheat-leaf polyphenolics using the new optical leaf-clip Dualex, in: Precision Agriculture 2005, ECPA 2005. pp. 243–250.

Cerovic, Z.G., Masdoumier, G., Ghozlen, N. Ben, Latouche, G., 2012. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiol. Plant. 146, 251–260. https://doi.org/10.1111/j.1399-3054.2012.01639.x

Chaín, J.M., Tubert, E., Graciano, C., Castagno, L.N., Recchi, M., Pieckenstain, F.L., Estrella, M.J., Gudesblat, G., Amodeo, G., Baroli, I., 2020. Growth promotion and protection from drought in *Eucalyptus grandis* seedlings inoculated with beneficial bacteria embedded in a superabsorbent polymer. Sci. Rep. 10, 18221. https://doi.org/10.1038/s41598-020-75212-4

Chatzistathis, T., Alifragis, D., Papaioannou, A., 2015. The influence of liming on soil chemical properties and on the alleviation of manganese and copper toxicity in Juglans regia, Robinia pseudoacacia, *Eucalyptus sp*. and *Populus sp*. Plantations. J. Environ. Manage. 150, 149–156. https://doi.org/10.1016/j.jenvman.2014.11.020

Croft, H., Chen, J.M., 2017. Leaf pigment content, in: Comprehensive Remote Sensing. Elsevier, pp. 117–142. https://doi.org/10.1016/B978-0-12-409548-9.10547-0

Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil. https://doi.org/10.1023/A:1020809400075

Dhindsa, R.S., Plumb-dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32, 93–101. https://doi.org/10.1093/jxb/32.1.93

Dimkpa, C.O., Andrews, J., Fugice, J., Singh, U., Bindraban, P.S., Elmer, W.H., Gardea-Torresdey, J.L., White, J.C., 2020. Facile Coating of Urea With Low-Dose ZnO Nanoparticles Promotes Wheat Performance and Enhances Zn Uptake Under Drought Stress. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00168

Faria, Á.J.G. de, Franciely Machado, Â., Nunes Lucena, G., Vidal de Negreiros Neto, J., Santana da Silva Carneiro, J., da Costa Leite, R., Ribeiro da Silva, R., 2020. Potential of Limestone Tailings for Correction and Fertilization of an Oxisol in the Brazilian Savanna. Commun. Soil Sci. Plant Anal. 51, 1024–1035. https://doi.org/10.1080/00103624.2020.1744629

Farid, R., Khordadpour, G., 2008. The effect of lime on controlling manganese toxicity, tea yield and quality in sprinkler condition.

Gabriel, C.A., Cassol, P.C., Simonete, M.A., Moro, L., Pfleger, P., Mumbach, G.L., 2018. Lime and gypsum applications on soil chemical attributes and initial growth of eucalyptus. Floresta 48, 573–582. https://doi.org/10.5380/rf.v48i4.57455

Gheshlaghi, Z., Luis-Villarroya, A., Álvarez-Fernández, A., Khorassani, R., Abadía, J., 2020. Iron deficient Medicago scutellata grown in nutrient solution at high pH accumulates and secretes large amounts of flavins. Plant Sci. https://doi.org/10.1016/j.plantsci.2020.110664

Gomes, S.D.S., Gonçalves, J.L.D.M., Rocha, J.H.T., Menegale, M.L.D.C., 2019. Tolerance of *Eucalyptus* and *Pinus* seedlings to exchangeable aluminium. Sci. Agric. 76, 494–500. https://doi.org/10.1590/1678-992x-2018-0011

Gonçalves, J.L. de M., 2010. Eucalipto. In: “Boas práticas para o uso eficiente de fertilizantes”. Piracicaba: International Plant Nutrition Institute Brazil, V.3, pag. 309-369.

Gonçalves, J.L. de M., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., Ferraz, S.F. de B., Lima, W. de P., Brancalion, P.H.S., Hubner, A., Bouillet, J.P.D., Laclau, J.P., Nouvellon, Y., Epron, D., 2013. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. For. Ecol. Manage. 301, 6–27. https://doi.org/10.1016/j.foreco.2012.12.030

Gonçalves, J.L.D.M., Valeri, S.V., 2001. Eucalipto e pínus, in: Ferreira, M.E., Cruz, M.C.P., Raij, B., Abreu, C.A. (Eds.), Micronutrientes: Elementos Tóxicos Na Agricultura. CNPq/FAPESP/PATAFOS, Jaboticabal, Pp. 393–423. Jaboticabal (SP), pp. 393–423.

González-Alcaraz, M.N., Conesa, M., Álvarez-Rogel, J., 2013. When liming and revegetation contribute to the mobilisation of metals: Learning lessons for the phytomanagement of metal-polluted wetlands. J. Environ. Manage. 116, 72–80. https://doi.org/10.1016/j.jenvman.2012.11.044

Granda, V., Delatorre, C., Cuesta, C., Centeno, M.L., Fernández, B., Rodríguez, A., Feito, I., 2014. Physiological and biochemical responses to severe drought stress of nine *Eucalyptus globulus* clones: A multivariate approach. Tree Physiol. 34, 778–786. https://doi.org/10.1093/treephys/tpu052

Han, J., Chen, J., Miao, Y., Wan, S., 2016. Multiple Resource Use Efficiency (mRUE): A New Concept for Ecosystem Production. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep37453

Holger, K., Eskilsson, J., 2010. Low manganese (Mn) and copper (Cu) concentrations in cereals explained yield losses after lime application to soil. Acta Agric. Scand. Sect. B Soil Plant Sci. 60, 569–572. https://doi.org/10.1080/09064710903233847

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.08.020

Hue, N. V., Mai, Y., 2002. Manganese toxicity in watermelon as affected by lime and compost amended to a Hawaiian acid Oxisol. HortScience 37, 656–661. https://doi.org/10.21273/hortsci.37.4.656

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106.

Jarrell, W.M., Beverly, R.B., 1981. The dilution effect in plant nutrition studies. Adv. Agron. 34, 197–224. https://doi.org/10.1016/S0065-2113(08)60887-1

Jez, J.M., Lee, S.G., Sherp, A.M., 2016. The next green movement: Plant biology for the environment and sustainability. Science (80-. ). https://doi.org/10.1126/science.aag1698

Joris, H.H.A.W., Fonseca, A. da, Da Fonseca, A.F., Asami, V.Y., Briedis, C., Borszowskei, P.R., Garbuio, F.J., 2012. Adsorção de metais pesados após calagem superficial em um Latossolo Vermelho sob sistema de plantio direto, Revista Ciencia Agronomica. https://doi.org/10.1590/S1806-66902012000100001

Julkunen-Tiitto, R., Nenadis, N., Neugart, S., Robson, M., Agati, G., Vepsäläinen, J., Zipoli, G., Nybakken, L., Winkler, B., Jansen, M.A.K., 2015. Assessing the response of plant flavonoids to UV radiation: an overview of appropriate techniques. Phytochem. Rev. https://doi.org/10.1007/s11101-014-9362-4

Kalaji, H.M., Dąbrowski, P., Cetner, M.D., Samborska, I.A., Łukasik, I., Brestic, M., Zivcak, M., Tomasz, H., Mojski, J., Kociel, H., Panchal, B.M., 2017. A comparison between different chlorophyll content meters under nutrient deficiency conditions. J. Plant Nutr. 40, 1024–1034. https://doi.org/10.1080/01904167.2016.1263323

Kaspari M (2021) The Invisible Hand of the Periodic Table: How Micronutrients Shape Ecology. Annu Rev Ecol Evol Syst 52:199–219. https://doi.org/10.1146/annurev-ecolsys-012021-090118

Kirkham, M.B., 2006. Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. Geoderma. https://doi.org/10.1016/j.geoderma.2006.08.024

Koester, R.P., Skoneczka, J.A., Cary, T.R., Diers, B.W., Ainsworth, E.A., 2014. Historical gains in soybean (Glycine max Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. J. Exp. Bot. 65, 3311–3321. https://doi.org/10.1093/jxb/eru187

Kreslavski, V.D., Schmitt, F.J., Keuer, C., Friedrich, T., Shirshikova, G.N., Zharmukhamedov, S.K., Kosobryukhov, A.A., Allakhverdiev, S.I., 2016. Response of the photosynthetic apparatus to UV-A and red light in the phytochrome B-deficient Arabidopsis thaliana L. hy3 mutant. Photosynthetica 54, 321–330. https://doi.org/10.1007/s11099-016-0212-z

Laclau, J.-P.P., Bouillet, J.-P.P., Ranger, J., 2000. Dynamics of biomass and nutrient accumulation in a clonal plantation of *Eucalyptus* in Congo. For. Ecol. Manage. 128, 181–196. https://doi.org/10.1016/S0378-1127(99)00146-2

Lambers, H., Hayes, P.E., Laliberté, E., Oliveira, R.S., Turner, B.L., 2015. Leaf manganese accumulation and phosphorus-acquisition efficiency. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2014.10.007

Lavres, J., Cabral, C.P., Rossi, M.L., Nogueira, T.A.R., Nogueira, N. de L., Malavolta, E., 2012. Deficiency symptoms and uptake of micronutrients by castor bean grown in nutrient solution. Rev. Bras. Ciência do Solo 36, 233–242. https://doi.org/10.1590/s0100-06832012000100024

Li, D., Chen, J.M., Zhang, X., Yan, Y., Zhu, J., Zheng, H., Zhou, K., Yao, X., Tian, Y., Zhu, Y., Cheng, T., Cao, W., 2020. Improved estimation of leaf chlorophyll content of row crops from canopy reflectance spectra through minimizing canopy structural effects and optimizing off-noon observation time. Remote Sens. Environ. 248. https://doi.org/10.1016/j.rse.2020.111985

Li, Y., Cui, S., Chang, S.X., Zhang, Q., 2019. Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. J. Soils Sediments 19, 1393–1406. https://doi.org/10.1007/s11368-018-2120-2

Liu, M., Wang, Z., Li, S., Lü, X., Wang, X., Han, X., 2017. Changes in specific leaf area of dominant plants in temperate grasslands along a 2500-km transect in northern China. Sci. Rep. 7, 1–9. https://doi.org/10.1038/s41598-017-11133-z

López-Hernández, D., 2008. Biogeochemistry and cycling of zinc and copper in a dyked seasonally flooded savanna. Chem. Ecol. 24, 387–399. https://doi.org/10.1080/02757540802491320

Lukin, V. V., Epplin, F.M., 2003. Optimal frequency and quantity of agricultural lime applications. Agric. Syst. 76, 949–967. https://doi.org/10.1016/S0308-521X(02)00016-1

Malavolta, E., Vitti, G., Oliveira, S., 1997. Avaliação do estado nutricional das plantas: princípios e aplicações.

Mallarino, A.P., Pagani, A., Sawyer, J.E., 2018. Corn and soybean response to soil pH level and liming. https://doi.org/10.31274/icm-180809-74

Masullo, L.S., Derisso, V.D., Manarim, G.R., de Vicente Ferraz, A., Rocha, J.H.T., de Ávila, P.A., Florentino, A.L., de Aguiar, C.L., Lavres, J., de Moraes Gonçalves, J.L., 2021. Modulation of structural carbohydrates, phenol compounds and lignin content in *Eucalyptus urophylla* cuttings grown under boron, copper and zinc induced-deficiency. New For. 1–16. https://doi.org/10.1007/s11056-021-09859-w

Mateus, N. de S., Florentino, A.L., Santos, E.F., Ferraz, A. de V., Goncalves, J.L. de M., Lavres, J., 2021. Partial Substitution of K by Na Alleviates Drought Stress and Increases Water Use Efficiency in *Eucalyptus* Species Seedlings. Front. Plant Sci. 12, 219. https://doi.org/10.3389/fpls.2021.632342

Meyer, S., Cerovic, Z.G., Goulas, Y., Montpied, P., Demotes-Mainard, S., Bidel, L.P.R.R., Moya, I., Dreyer, E., 2006. Relationships between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: A signature of the carbon-nitrogen balance within leaves? Plant, Cell Environ. 29, 1338–1348. https://doi.org/10.1111/j.1365-3040.2006.01514.x

Moreno-Lora, A., Delgado, A., 2020. Factors determining Zn availability and uptake by plants in soils developed under Mediterranean climate. Geoderma 376, 114509. https://doi.org/10.1016/j.geoderma.2020.114509

Morton, J.D., 2020. A review of research on the effect of lime on New Zealand soils and pastures. New Zeal. J. Agric. Res. 63, 189–201. https://doi.org/10.1080/00288233.2018.1537293

Novaes, R.F. de, Neves, J.C.L., Barros, N.F. de, 1991. Ensaio em ambiente controlado, in: Métodos de Pesquisa Em Fertilidade Do Solo. pp. 189–253.

Osman, K.T., 2013. Soils: Principles, properties and management, Soils: Principles, Properties and Management. Springer Netherlands. https://doi.org/10.1007/978-94-007-5663-2

Overbeck, V., Schmitz, M., Tartachnyk, I., Blanke, M., 2018. Identification of light availability in different sweet cherry orchards under cover by using non-destructive measurements with a Dualex$^{TM}$. Eur. J. Agron. 93, 50–56. https://doi.org/10.1016/j.eja.2017.11.006

Panda, S.K., Baluška, F., Matsumoto, H., 2009. Aluminum stress signaling in plants. Plant Signal. Behav. 4, 592–597. https://doi.org/10.4161/psb.4.7.8903

Paradelo, R., Virto, I., Chenu, C., 2015. Net effect of liming on soil organic carbon stocks: A review. Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2015.01.005

Pereira, A.P.A., Durrer, A., Gumiere, T., Gonçalves, J.L.M., Robin, A., Bouillet, J.P., Wang, J., Verma, J.P., Singh, B.K., Cardoso, E.J.B.N., 2019. Mixed *Eucalyptus* plantations induce changes in microbial communities and increase biological functions in the soil and litter layers. For. Ecol. Manage. 433, 332–342. https://doi.org/10.1016/j.foreco.2018.11.018

Pinkard, E.A., Patel, V., Mohammed, C., 2006. Chlorophyll and nitrogen determination for plantation-grown *Eucalyptus nitens* and *E. globulus* using a non-destructive meter. For. Ecol. Manage. 223, 211–217. https://doi.org/10.1016/j.foreco.2005.11.003

Pollastri, S., Tattini, M., 2011. Flavonols: Old compounds for old roles. Ann. Bot. https://doi.org/10.1093/aob/mcr234

R Core Team, 2016. R Development Core Team, R: A language and environment for statistical computing, R Foundation for Statistical Computing. R A Lang. Environ. Stat. Comput.

Rahman, M.A., Lee, S.H., Ji, H.C., Kabir, A.H., Jones, C.S., Lee, K.W., 2018. Importance of mineral nutrition for mitigating aluminum toxicity in plants on acidic soils: Current status and opportunities. Int. J. Mol. Sci. https://doi.org/10.3390/ijms19103073

Raij, B. van, Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Análise química para avaliação da fertilidade de solos tropicais, Campinas: Instituto Agronômico.

Raij, B. van, Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1997. Recomendacoes da adubacao e calagem para o Estado de Sao Paulo 1–88.

Ramírez-Builes, V.H., Küsters, J., de Souza, T.R., Simmes, C., 2020. Calcium Nutrition in Coffee and Its Influence on Growth, Stress Tolerance, Cations Uptake, and Productivity. Front. Agron. 2, 590892. https://doi.org/10.3389/fagro.2020.590892

Resquin, F., Navarro-Cerrillo, R.M., Carrasco-Letelier, L., Casnati, C.R., Bentancor, L., 2020. Evaluation of the nutrient content in biomass of *Eucalyptus* species from short rotation plantations in Uruguay. Biomass and Bioenergy 134. https://doi.org/10.1016/j.biombioe.2020.105502

Rocha, J.H.T., du Toit, B., Gonçalves, J.L. de M., 2019. Ca and Mg nutrition and its application in *Eucalyptus* and *Pinus* plantations. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2019.03.062

Rogers, C., Dell, B., Xu, D., 2001. Micronutrient Disorders.

Roschzttardtz, H., González-Guerrero, M., Gomez-Casati, D.F., 2019. journal of. Front. Plant Sci. 10, 927. https://doi.org/10.3389/fpls.2019.00927

Saito, S., Uozumi, N., 2020. Calcium-Regulated Phosphorylation Systems Controlling Uptake and Balance of Plant Nutrients. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00044

Santana, R.C., Barros, N.F. de, Neves, J.C.L., 2002. Eficiência de utilização de nutrientes e sustentabilidade da produção em procedências de *Eucalyptus grandis* e *Eucalyptus saligna* em sítios florestais do estado de São Paulo. Rev. Árvore 26, 447–457. https://doi.org/10.1590/s0100-67622002000400007

Santos, E.F., Mateus, N.S., Rabêlo, F.H.S., Macedo, F.G., Lavres, J., 2020. Diagnosing early disorders in *Jatropha curcas* to calcium, magnesium and sulfur deficiency. J. Plant Nutr. 43, 1604–1616. https://doi.org/10.1080/01904167.2020.1730899

Santos, E.F., Pongrac, P., Reis, A.R., Rabêlo, F.H.S., Azevedo, R.A., White, P.J., Lavres, J., 2021. Unravelling homeostasis effects of phosphorus and zinc nutrition by leaf photochemistry and metabolic adjustment in cotton plants. Sci. Reports 2021 111 11, 1–14. https://doi.org/10.1038/s41598-021-93396-1

SAS Institute Inc, 2016. SAS Institute Inc., SAS Institute Inc.

Schjoerring, J.K., Bienert, G.P., Santa-María, G.E., Nieves-Cordones, M., Rubio, F., 2020. Editorial: Nutrient Use-Efficiency in Plants: An Integrative Approach. Front. Plant Sci. 11, 623976. https://doi.org/10.3389/fpls.2020.623976

Schmidt, S.B., Jensen, P.E., Husted, S., 2016. Manganese Deficiency in Plants: The Impact on Photosystem II. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2016.03.001

Silveira, M.L., Alleoni, L.R.F., O'Connor, G.A., Chang, A.C., 2006. Heavy metal sequential extraction methods-A modification for tropical soils. Chemosphere 64, 1929–1938. https://doi.org/10.1016/j.chemosphere.2006.01.018

Smethurst, P.J., 2010. Forest fertilization: Trends in knowledge and practice compared to agriculture. Plant Soil 335, 83–100. https://doi.org/10.1007/s11104-010-0316-3

Soares, C., Carvalho, M.E.A., Azevedo, R.A., Fidalgo, F., 2019. Plants facing oxidative challenges—A little help from the antioxidant networks. Environ. Exp. Bot. 161, 4–25. https://doi.org/10.1016/j.envexpbot.2018.12.009

Sperotto, R.A., Ricachenevsky, F.K., Williams, L.E., Vasconcelos, M.W., Menguer, P.K., 2014. From soil to seed: micronutrient movement into and within the plant. Front. Plant Sci. 5, 438. https://doi.org/10.3389/fpls.2014.00438

Srivastava, A.K., Malhotra, S.K., 2017. Nutrient use efficiency in perennial fruit crops-A review. J. Plant Nutr. 40. https://doi.org/10.1080/01904167.2016.1249798

Stape, J.L., Binkley, D., Ryan, M.G., 2008. Production and carbon allocation in a clonal Eucalyptus plantation with water and nutrient manipulations. For. Ecol. Manage. 255, 920–930. https://doi.org/10.1016/j.foreco.2007.09.085

Stape, J.L., Binkley, D., Ryan, M.G., Fonseca, S., Loos, R.A., Takahashi, E.N., Silva, C.R., Silva, S.R., Hakamada, R.E., Ferreira, J.M. de A., Lima, A.M.N.N., Gava, J.L., Leite, F.P., Andrade, H.B., Alves, J.M., Silva, G.G.C.C., Azevedo, M.R., 2010. The Brazil Eucalyptus Potential Productivity Project: Influence of water, nutrients and stand uniformity on wood production. For. Ecol. Manage. 259, 1684–1694. https://doi.org/10.1016/j.foreco.2010.01.012

Tanaka, R.T., Mascarenhas, H.A.A., Bulisani, E.A., 1992. Deficiência de manganês em soja induzida por excesso de calcário. Pesqui. Agropecuária Bras. 27, 247–250.

Turner, J., Lambert, M.J., 2014. Analysis of nutrient use efficiency (NUE) in Eucalyptus pilularis forests. Aust. J. Bot. 62, 558–569. https://doi.org/10.1071/BT14162

Valentinuzzi, F., Mimmo, T., Cesco, S., Al Mamun, S., Santner, J., Hoefer, C., Oburger, E., Robinson, B., Lehto, N., 2015. The effect of lime on the rhizosphere processes and elemental uptake of white lupin. Environ. Exp. Bot. 118, 85–94. https://doi.org/10.1016/j.envexpbot.2015.06.010

Valentinuzzi, F., Pii, Y., Carlo, P., Roberto, T., Fontanella, M.C., Beone, G.M., Astolfi, S., Mimmo, T., Cesco, S., 2020. Root-shoot-root Fe translocation in cucumber plants grown in a heterogeneous Fe provision. Plant Sci. 293, 110431. https://doi.org/10.1016/j.plantsci.2020.110431

Verdaguer, D., Jansen, M.A.K., Llorens, L., Morales, L.O., Neugart, S., 2017. UV-A radiation effects on higher plants: Exploring the known unknown. Plant Sci. 255, 72–81. https://doi.org/10.1016/j.plantsci.2016.11.014

Vigani, G., Bohic, S., Faoro, F., Vekemans, B., Vincze, L., Terzano, R., 2018. Cellular fractionation and nanoscopic X-ray fluorescence imaging analyses reveal changes of zinc distribution in leaf cells of iron-deficient plants. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.01112

Wachendorf, C., 2015. Effects of liming and mineral N on initial decomposition of soil organic matter and post harvest root residues of poplar. Geoderma 259–260, 243–250. https://doi.org/10.1016/j.geoderma.2015.06.013

Wisawapipat, W., Janlaksana, Y., Christl, I., 2017. Zinc solubility in tropical paddy soils: A multi-chemical extraction technique study. Geoderma 301, 1–10. https://doi.org/10.1016/j.geoderma.2017.04.002

Yang, M., Tan, L., Xu, Y., Zhao, Y., Cheng, F., Ye, S., Jiang, W., 2015. Effect of Low pH and Aluminum Toxicity on the Photosynthetic Characteristics of Different Fast-Growing Eucalyptus Vegetatively Propagated Clones OPEN ACCESS. Propagated Clones. PLoS ONE 10, 130963. https://doi.org/10.1371/journal.pone.0130963

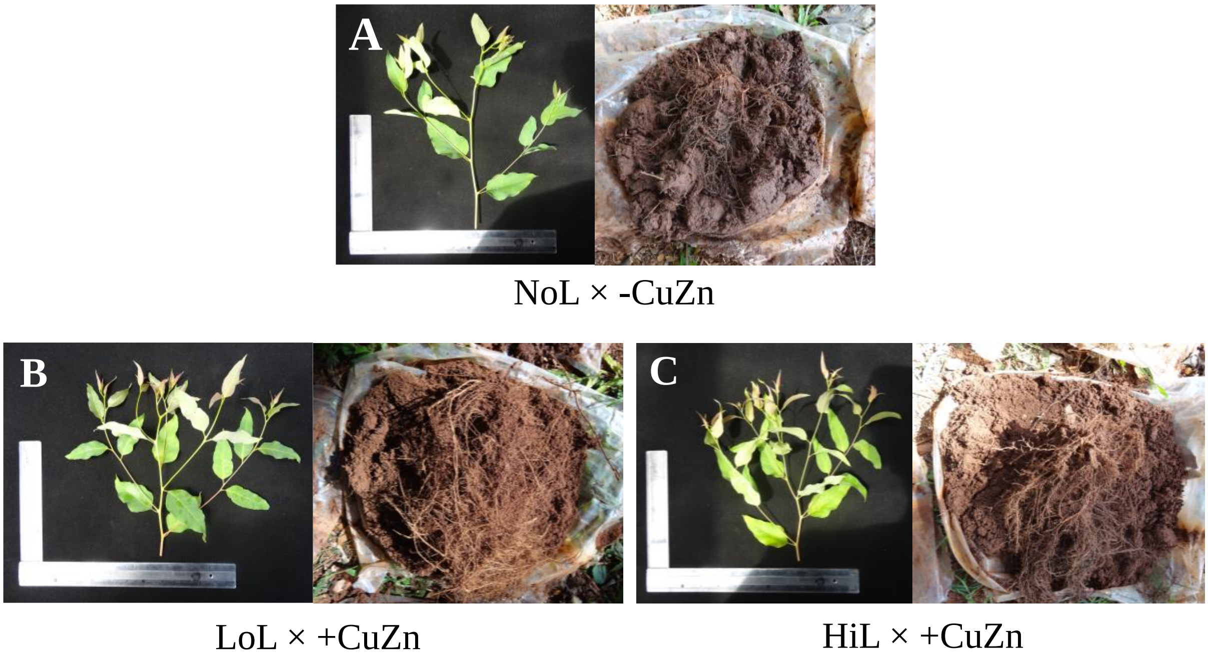
## Supplementary materials

**Supplementary Table S1**
Recommended lime rates (lime) and fertilizer rates (fert) for *Eucalyptus grandis* seedlings grown out in a greenhouse.

| Treat | Lime | Fert | $CaCO_3$ | $MgCO_3$ | $CuSO_4.5H_2O$ | $ZnSO_4.7H_2O$ |
|---|---|---|---|---|---|---|
| | | | ------ g/kg soil ------ | | ------ mg/kg soil ------ | |
| 1 | NoL | -CuZn | 0.00 | 0.00 | 0.0 | 0.0 |
| 2 | NoL | +Cu | 0.00 | 0.00 | 6.8 | 0.0 |
| 3 | NoL | +Zn | 0.00 | 0.00 | 0.0 | 22.8 |
| 4 | NoL | +CuZn | 0.00 | 0.00 | 6.8 | 22.8 |
| 5 | LoL | -CuZn | 0.77 | 0.17 | 0.0 | 0.0 |
| 6 | LoL | +Cu | 0.77 | 0.17 | 6.8 | 0.0 |
| 7 | LoL | +Zn | 0.77 | 0.17 | 0.0 | 22.8 |
| 8 | LoL | +CuZn | 0.77 | 0.17 | 6.8 | 22.8 |
| 9 | HiL | -CuZn | 1.42 | 0.30 | 0.0 | 0.0 |
| 10 | HiL | +Cu | 1.42 | 0.30 | 6.8 | 0.0 |
| 11 | HiL | +Zn | 1.42 | 0.30 | 0.0 | 22.8 |
| 12 | HiL | +CuZn | 1.42 | 0.30 | 6.8 | 22.8 |

Lime treatments: no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%). Fert treatments: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu $kg^{-1}$ soil (+Cu); 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn), represented by 6.8 mg copper sulfate $kg^{-1}$ soil ($CuSO_4.5H_2O$) and 22.8 mg zinc sulfate $kg^{-1}$ soil ($ZnSO_4.7H_2O$). The sources of nutrients were used in the degree of impurity PA.

**Supplementary Fig. S1.** Visual aspect of *Eucalyptus grandis* seedlings grown out in a greenhouse, in individual plastic pots (5 L) under the condition of lime application (lime) and fertilizers with Cu and Zn (fert). Description of lime and fert treatments see information in Supplementary Table S1.




Supplementary Fig. S2 Visual aspect of branches and roots from *Eucalyptus grandis* seedlings at 80 days grown under treatment without lime × without fert (i.e., NoL × -CuZn) (A) and under lime × fert [i.e., LoL × +CuZn (B) and HiL× +CuZn (c)]. Lime treatments: no lime rate (NoL), low lime rate (LoL), calculated by 20-[Ca+Mg]/10) and high lime rate (HiL), calculated by base saturation up to 60%. Fert treatments: no Cu and Zn fertilizer (-CuZn); 1.72 mg Cu $kg^{-1}$ soil (+Cu); 5.16 mg Zn $kg^{-1}$ soil (+Zn); and 1.72 mg Cu $kg^{-1}$ soil plus 5.16 mg Zn $kg^{-1}$ soil (+CuZn).

## 5. PRACTICAL APPLICATIONS AND FINAL REMARKS

Through technological advances in Brazilian silviculture, the usage of genetic materials more responsive to mineral fertilizer and with higher potential for wood production has been priority of companies and farmers (Gonçalves et al., 2008, 2020). However, it is not unusual macro and micronutrient deficiency in areas under *Eucalyptus* species cultivation (Battie-Laclau et al., 2016; Masullo et al., 2020; Pulito et al., 2015; Rocha et al., 2019; Rodrigues et al., 2012, 2010). To avoid this implication, forestry companies have applied lime (mainly to increase Ca and Mg availability) and mineral fertilizer [mainly with sources of nitrogen, phosphorus, and potassium (NPK)] to better nutrition and stemwood yields of *Eucalyptus* plantation. The lime application can reduce metallic micronutrients (e.g., Cu and Zn) bioavailabilities for plants, and the Cu and Zn recommendation rate for *Eucalyptus* plantation is not a consensus in Brazilian silviculture (Gonçalves and Valeri, 2001).

Our data showed that lime increased soil pH in the topsoil at the first months after application. This effect can reduce the Cu and Zn soil-availabilities (Aubert et al., 2010; Holland et al., 2018; Kabata-Pendias, 2011). In this sense, agronomic strategy to applied Cu and Zn fertilizers to enhance *Eucalyptus* nutrition should be recommended, mainly with a delay between liming and fertilization with these metals, considering the greater Cu and Zn availability due to the buffer effect within soil and then, when soil pH decreases next to the original levels. In our study, the incorporated soil-lime in planting range (approximately 1.0 m in width) aimed to accelerate soil reaction and to increase soil pH, thus, potentialize implication in the reduction of Cu and Zn availabilities to *Eucalyptus* plant. However, Brazilian forestry companies usually apply lime in total area without incorporation. Usually, this practice is done about three months before planting in area under new rotation. Lime incorporation increases the surface area between soil and lime, which speeds up reaction between lime and the soil particles (Caires et al., 2006; Joris et al., 2016).

The liming decreased the Cu and Zn contents in *Eucalyptus* plants, in trials setup either on the field (**chapter 2 and 3**) or greenhouse (**chapter 4**). This effect was observed in plants grown in treatments with low lime rate, calculated by 20-[Ca+Mg]/10, following recommendations for *Eucalyptus* (Gonçalves, 2010), and in high lime rate, calculated by base saturation up to 60% as a recommendation for annual crops (e.g., *Glycine max* L.) according to Raij et al. (1996). However, between these recommendations, no difference was observed for stem volume and leaf area index (**chapter 2**), biomass production of leaf litter (**chapter 3**), and biomass production in *Eucalyptus* seedlings (**chapter 4**). Such findings highlight that lime recommendation rate for this species is enough to supply Ca and Mg levels for better plant growth according to Gonçalves (2010). This low lime rate guarantees the expected growth of *Eucalyptus* to improve wood yields. Thus, it does not justify to applying high lime rate to supply Ca and Mg contents for *Eucalyptus*, such as those applied for annual crops.

Therefore, the reduction of Cu and Zn contents in *Eucalyptus* plant after lime application can offset by Cu plus Zn fertilization. In this sense, our study showed that Cu plus Zn fertilization is closely associated with the nutritional status and growth of *Eucalyptus* plants, as seen by the improvement of wood production, which was characterized in detail through principal component analyses (**chapter 2, 3 and 4**) and hierarchical cluster analysis (**chapter 4**). Based on these results, some responses are also seen by graphical abstract to field experiment (Fig. 1) and greenhouse experiment (Fig. 2). For example, individual application of limestone decreased Cu and Zn contents in soil and plants, regardless of experiment. While this management increased leaf area index and Ca and Mg contents within soil and *Eucalyptus grandis* plantation (Fig. 1); and the integrative effect of lime and Cu plus Zn improved *Eucalyptus* stemwood yields or biomass production, leaf chlorophyll and flavonoid contents in plants grown in the field grown or greenhouse conditions (Fig. 1 and Fig. 2); and in trees increased the activity of antioxidant enzymes (Fig. 1).

The high levels of Cu and Zn in soil linked to organic matter fraction (**chapter 2**) is closely associated with the presence of forest residue (e.g., leaves, branches, leaf litter and other *Eucalyptus* residues). Another effect of forest residue retention can be related to owed with the history of the area that has been planted with *Eucalyptus* for many years, and the harvest part of the forest residues has been kept over the soil. The microbial communities are able to mineralization forest residue and organic matter compounds, enhancing Cu and Zn contents in soil-eucalyptus system (Pereira et al., 2019). In addition, the forest residue maintenance carried out management of the minimum cultivation practices (Gonçalves et al., 2007) has a large implication to increase soil fertility and nutrient store in the ecosystem. Thus, forest residue can mitigate soil organic matter reduction  as well as to protect the soil against erosion, which is a key player to guarantee the sustainability of forest productivity (Gonçalves et al., 2013; Masullo et al., 2020; Rocha et al., 2016).

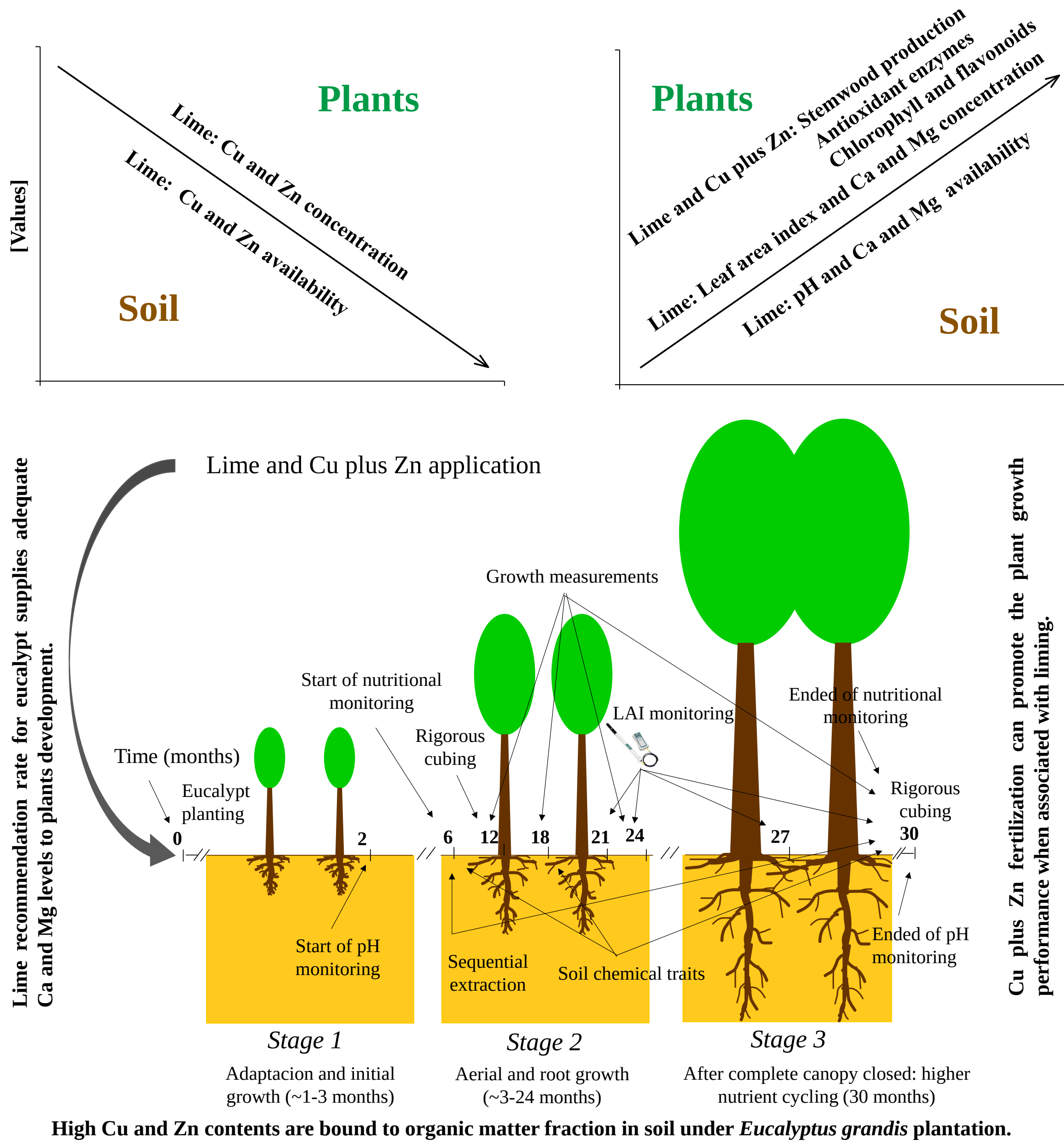



**Fig. 1.** General view of some results in the field experiment.

Overall, our study showed that *Eucalyptus* plants grown under combined application of lime and Cu plus Zn fertilizers leads to better development of plant performance, which was seen by the improvement of biomass yield. In these circumstances, *Eucalyptus* plants potentialize have a strategy to modulate the improved values of chlorophylls, flavonoids, activity of antioxidant enzymes, Ca and Mg accumulations, and Cu and Zn use efficiencies, consequently, is closely associated with higher developmental of the plant.

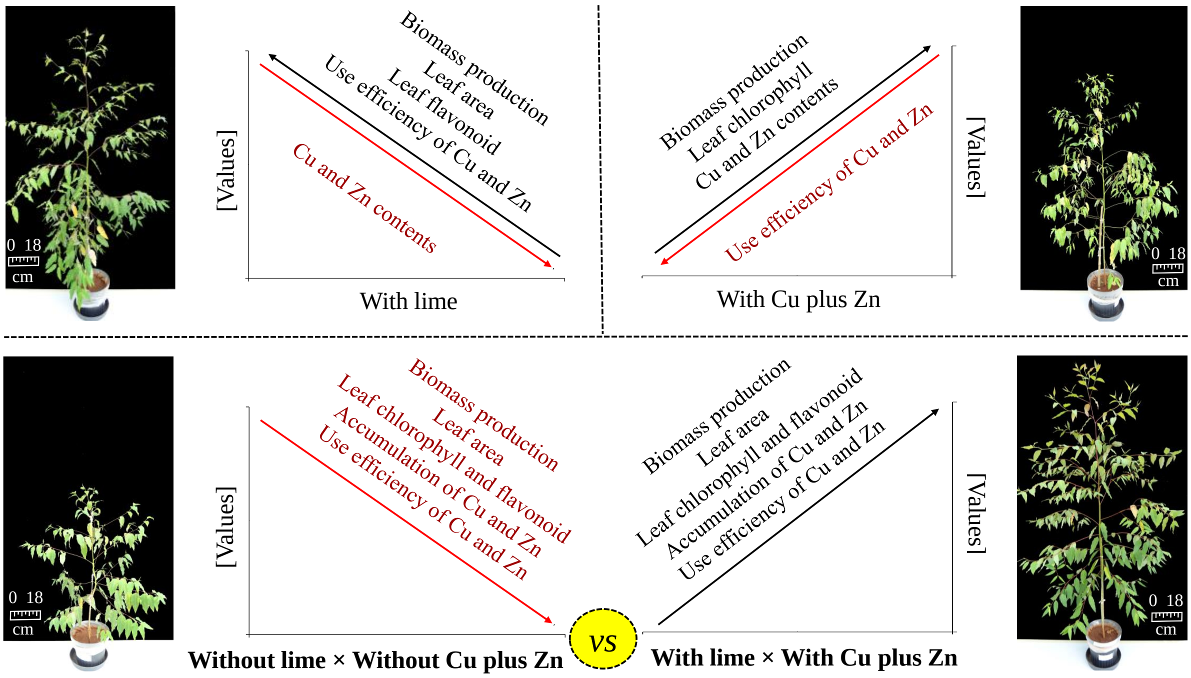



**Fig. 2.** General view of some results in the greenhouse experiment.

Several silvicultural practices can be adopted to improve productivity, including lime application and mineral fertilization. The comprehensive use of lime and mineral Cu and Zn fertilizers in modern agriculture has been key in achieving the best yield by agricultural production (FAO, 2015; Pathak and Nedwell, 2001; Xu et al., 2020). Also, the effects of these factors are associated with mechanisms that can explain stress or nutrient depletion and ultimately lead to resistance against unfavorable conditions (Saito and Uozumi, 2020). Therefore, our study brought new insights into the soil fertility, wood production, leaf area and leaf area index, biochemical traits, antioxidant metabolism, and nutritional status in *Eucalyptus* under lime and Cu plus Zn application rates.

## References

Aubert, H., Pinta, M., Hooda, P., 2010. Trace elements in soils. Trace Elem. Soils 199–226. https://doi.org/10.1002/9781444319477.ch10

Battie-Laclau, P., Delgado-Rojas, J.S., Christina, M., Nouvellon, Y., Bouillet, J.P., Piccolo, M. de C., Moreira, M.Z., Gonçalves, J.L. de M., Roupsard, O., Laclau, J.P., 2016. Potassium fertilization increases water-use efficiency for stem biomass production without affecting intrinsic water-use efficiency in *Eucalyptus grandis* plantations. For. Ecol. Manage. 364, 77–89. https://doi.org/10.1016/j.foreco.2016.01.004

Caires, E.F., Barth, G., Garbuio, F.J., 2006. Lime application in the establishment of a no-till system for grain crop production in Southern Brazil. Soil Tillage Res. 89, 3–12. https://doi.org/10.1016/j.still.2005.06.006

FAO, 2015. Intergovernmental Technical Panel on Soils. Status of the World’s Soil Resources., Intergovernmental Technical Panel on Soils.

Gonçalves, J., Stape, J., Laclau, J., Bouillet, J., Ranger, J., 2008. Assessing the effects of early silvicultural management on long-term site productivity of fast-growing eucalypt plantations: the Brazilian experience Assessing the effects of early silvicultural management on long-term site productivity of fast-growing eu. J. For. Sci. 70, 105–118. https://doi.org/10.2989/SOUTH.FOR.2008.70.2.6.534

Gonçalves, J., Wichert, M., Gava, J., Masetto, A., Junior, A.J.C., Serrano, M., Mello, S., 2007. Soil fertility and growth of *Eucalyptus grandis* in Brazil under different residue management practices. South. Hemisph. For. J. 69, 95–102. https://doi.org/10.2989/SHFJ.2007.69.2.4.289

Gonçalves, J.L. de M., 2010. Capítulo 8 - Livro do IPNI - Adubação do eucalipto.

Gonçalves, J.L. de M., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., Ferraz, S.F. de B., Lima, W. de P., Brancalion, P.H.S., Hubner, A., Bouillet, J.P.D., Laclau, J.P., Nouvellon, Y., Epron, D., 2013. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. For. Ecol. Manage. 301, 6–27. https://doi.org/10.1016/j.foreco.2012.12.030

Gonçalves, J.L. de M., de Vicente Ferraz, A., Tertulino Rocha, J.H., Peressin, M., Alcarde Alvares, C., 2020. Forest outgrower schemes in small and medium-sized farmers in Brazil. For. Ecol. Manage. 456. https://doi.org/10.1016/j.foreco.2019.117654

Gonçalves, J.L.D.M., Valeri, S.V., 2001. Eucalipto e pínus, in: Ferreira, M.E., Cruz, M.C.P., Raij, B., Abreu, C.A. (Eds.), Micronutrientes: Elementos Tóxicos Na Agricultura. CNPq/FAPESP/PATAFOS, Jaboticabal, Pp. 393–423. Jaboticabal (SP), pp. 393–423.

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.08.020

Joris, H.A.W., Caires, E.F., Scharr, D.A., Bini, Â.R., Haliski, A., 2016. Liming in the conversion from degraded pastureland to a no-till cropping system in Southern Brazil. Soil Tillage Res. 162, 68–77. https://doi.org/10.1016/j.still.2016.04.009

Kabata-Pendias, A., 2011. References, in: Trace Elements in Soils and Plants, Fourth Edition. CRC Press, pp. 407–505. https://doi.org/10.1201/b10158-25

Masullo, L.S., Ferraz, A. de V., Gonçalves, J.L. de M., Camargo, L.E.A., de Ávila, P.A., Drago, J.R., Esteves, O., dos Santos Dias, C.T., 2020. Relationship between forest residue management and micronutrient fertilization with eucalyptus rust severity in *Eucalyptus grandis* plantations. For. Ecol. Manage. 475, 118443. https://doi.org/10.1016/j.foreco.2020.118443

Pathak, H., Nedwell, D.B., 2001. Nitrous oxide emission from soil with different fertilizers, water levels and nitrification inhibitors. Water. Air. Soil Pollut. 129, 217–228. https://doi.org/10.1023/A:1010316215596

Pereira, A.P.A., Durrer, A., Gumiere, T., Gonçalves, J.L.M., Robin, A., Bouillet, J.P., Wang, J., Verma, J.P., Singh, B.K., Cardoso, E.J.B.N., 2019. Mixed *Eucalyptus* plantations induce changes in microbial communities and increase biological functions in the soil and litter layers. For. Ecol. Manage. 433, 332–342. https://doi.org/10.1016/j.foreco.2018.11.018

Pulito, A.P., Gonçalves, J.L. de M., Smethurst, P.J., Junior, J.C.A., Alvares, C.A., Rocha, J.H.T., Hübner, A., de Moraes, L.F., Miranda, A.C., Kamogawa, M.Y., Gava, J.L., Chaves, R., Silva, C.R., 2015. Available nitrogen and responses to nitrogen fertilizer in brazilian eucalypt plantations on soils of contrasting texture. Forests 6, 973–991. https://doi.org/10.3390/f6040973

Raij, B. van, Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1996. Recomendações de adubação e calagem para o Estado de São Paulo. Campinas: IAC, 1996. (IAC. Boletim 100). Banana.

Rocha, J.H.T., Gonçalves, J.L. de M., Ferraz, A. de V., Poiati, D.A., Arthur Junior, J.C., Hubner, A., 2019. Growth dynamics and productivity of an *Eucalyptus grandis* plantation under omission of N, P, K Ca and Mg over two crop rotation. For. Ecol. Manage. 447, 158–168. https://doi.org/10.1016/j.foreco.2019.05.060

Rocha, J.H.T., Gonçalves, J.L. de M., Gava, J.L., Godinho, T. de O., Melo, E.A.S.C., Bazani, J.H., Hubner, A., Arthur Junior, J.C., Wichert, M.P., 2016. Forest residue maintenance increased the wood productivity of a *Eucalyptus* plantation over two short rotations. For. Ecol. Manage. 379, 1–10. https://doi.org/10.1016/j.foreco.2016.07.042

Rodrigues, F.A.V., de Barros, N.F., Neves, J.C.L., Alvarez, V.H., Novais, R.F., 2010. Copper availabity for eucalyptus seedlings in Cerrado soils. Rev. Bras. Cienc. do Solo 34, 1923–1932. https://doi.org/10.1590/s0100-06832010000600017

Rodrigues, F.A.V., de Barros, N.F., Neves, J.C.L., Alvarez, V.H. V, Novais, R.F., 2012. Zinc availabity for eucalypt seedlings in cerrado soils. Rev. Bras. Cienc. do Solo. https://doi.org/10.1590/S0100-06832012000400019

Saito, S., Uozumi, N., 2020. Calcium-Regulated Phosphorylation Systems Controlling Uptake and Balance of Plant Nutrients. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00044

Xu, D., Carswell, A., Zhu, Q., Zhang, F., de Vries, W., 2020. Modelling long-term impacts of fertilization and liming on soil acidification at Rothamsted experimental station. Sci. Total Environ. 713. https://doi.org/10.1016/j.scitotenv.2019.136249