

UNIVERSIDADE ESTADUAL DE CAMPINAS Instituto de Biologia

CINTHIA APARECIDA DA SILVA

EFFECTS OF LAND USE CHANGE AND SEASONALITY ON FINE ROOTS FROM ATLANTIC FOREST

EFEITOS DA MUDANÇA NO USO DA TERRA E DA SAZONALIDADE NAS RAÍZES FINAS DA FLORESTA ATLÂNTICA

CAMPINAS 2022

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Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa de Pós-graduação em Biologia Vegetal do Instituto de Biologia.

DEDICATION

To my students and trainees, whose enchantment with life reminded me of the choices that have brought me here.

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RESUMO

A compreensão, promoção e manutenção do funcionamento de florestas antigas e florestas secundárias ricas em espécies são cruciais para a conservação da biodiversidade em um mundo em constante mudança. Essa afirmação vale especialmente para a Mata Atlântica, um dos biomas brasileiros mais biodiversos, e cuja área de abrangência tem sido drasticamente afetada pelas mudanças no uso da terra. Esta tese teve como objetivo entender como os usos passados da terra e as mudanças climáticas podem afetar a Mata Atlântica, com foco particular no seu funcionamento abaixo do solo, especificamente aqui representado, pelas raízes finas. Estruturas mais ativas do sistema radicular e que absorvem e transportam a água e os nutrientes necessários para o crescimento e manutenção das plantas no meio. Para isso, foram feitas medições em 11 parcelas permanentes de um hectare para observar as respostas em uma variedade de florestas antigas e modificadas pelo homem. Primeiro, devido à existência de vários métodos para quantificar o estoque e a produção de raízes finas, aprofundamos o método de previsão temporal para reduzir o tempo de coleta em campo sem afetar a precisão da estimativa de biomassa de raízes finas. Então, observamos se o investimento em produtividade de raízes finas variou entre diferentes florestas atlânticas modificadas pelo homem, anos e estações. Examinamos os principais impulsionadores edáficos da produtividade de raízes finas em quatro tipos de cobertura florestal e investigamos se havia um trade-off de alocação de recursos entre produtividade de raízes finas e estoque de madeira e/ou produtividade de madeira em cada um deles. Por fim, exploramos a relação fungoraiz, buscando entender se a produção de raízes finas continuou a diferir entre os tipos de cobertura florestal e as estações do ano após o controle de algumas estruturas e substâncias produzidas por fungos micorrízicos arbusculares (FMA), bem como o mínimo tempo necessário para detectar essas diferenças. Descobrimos que, reduzindo o tempo gasto na remoção de raízes usando o método de previsão temporal, os pesquisadores podem estimar com precisão a massa de raízes finas e, consequentemente, aumentar o número de testemunhos de solo extraídos por local de estudo e melhor caracterizá-lo. Destacamos semelhanças e diferenças nas trajetórias de recuperação da produtividade, e pudemos observar uma tendência da biomassa de raízes finas se tornar menos conservadora à medida que a cobertura florestal muda, embora não tenhamos encontrado o mesmo padrão para quase todas as características de FMAs analisadas.

Palavras-chave: produtividade subterrânea, biomassa, alocação, funcionamento ecossistêmico.

ABSTRACT

The understanding, promotion, and functioning maintenance of old-growth forests and species-rich secondary forests are crucial for biodiversity conservation in a constantly changing world. This statement is especially true for Atlantic Forests, one of the most biodiverse Brazilian biomes, and whose coverage area has been drastically affected due to changes in land use. This work aimed to understand how past land uses and climate changes can affect Atlantic Forests with a particular focus upon their belowground functioning, specifically represented here, by the fine roots. More active structures of the root system, which absorb and transport water and nutrients necessary for the growth and maintenance of plants in the environment. For this, measurements were made at 11 one-hectare permanent plots to observe responses across a range of old-growth and human-modified forests. First, due to the existence of several methods to quantify the stock and production of fine roots, we delved deeper into the temporal prediction method to reduce collection time in the field without affecting the accuracy of fine-root biomass estimation. Then, we observed whether the investment in fine-root productivity varied between different human-modified montane Atlantic Forests, years, and seasons. We examined the main edaphic drivers of fineroot productivity in four types of forest cover and investigated if there was a resource allocation trade-off between fine-root productivity and wood stock and/or wood productivity in each one of them. Finally, we explored the root-fungus relationship, seeking to understand whether the production of fine roots continued to differ between types of forest cover and seasons after controlling for some structures and substances produced by arbuscular mycorrhizal fungi (AMF), as well as the minimum time needed to detect these differences. We found that, by reducing the time spent on removing roots using the temporal prediction method, researchers can accurately estimate fineroot mass, and consequently, increase the number of soil cores extracted per study site and better characterize it. We highlighted similarities and differences in recovery trajectories of productivity, and we could observe a tendency for fine-root biomass to become less conservative as forest cover changes, although we did not find the same pattern for almost all AMF traits analyzed.

Keywords: belowground productivity, biomass, allocation, ecosystem functioning.

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GENERAL INTRODUCTION

Anthropogenic practices are expected to affect successional trajectories in all ecosystems, sometimes through changes in their physical and chemical properties and sometimes through biotic alterations (Mesquita et al. 2015). On a local scale, land use change can bring economic and social benefits, but on regional, global, and temporal scales, it can also promote ecological degradation (Foley et al. 2005). In this context, forest ecosystems have received special attention.

Forests provide food, habitats, and medicines. They maintain biodiversity and regulate hydrological and biogeochemical cycles (Foley et al. 2005; Postel & Thompson 2005; Bonan 2008; Pöschl et al. 2010; Spracklen et al. 2012). Tropical forests, especially, are a critical component of the global carbon cycle, accounting for almost one-third of the global terrestrial net primary productivity (Melillo et al. 1993; Beer et al. 2010). The deforestation, cultivation and land abandonment of these forests have resulted in forest covers with different transition stages (Corlett 1995; Chazdon 2003).

Essentially, plants need solar radiation, water and nutrients (Resende et al. 2002). When the availability of these resources is affected, new habitats are created and occupied by species with different needs (Tabarelli & Mantovani 1999). The fast-growing species tend to outnumber late species in abundance through interspecific competition, while producing an environment that is conducive to their development (Horn 1974), thus giving rise to the secondary forests.

To maintain different colonization and growth strategies, a considerable and variable part of plant productivity is allocated to fine roots (Jobbagy & Jackson 2001; Raich et al. 2014; McCormack et al. 2015). These roots are the most active parts of the root system and act to transport and absorb an adequate supply of water and nutrients for plant growth and maintenance (Børja et al. 2008; McCormack et al. 2015). They are morphologically classified as having a diameter of less than or equal to 2 mm (Jackson et al. 1997), and due to their intimate contact with soil particles, some of the most complex chemical, physical and biological interactions experienced by land plants are mediated by such roots (Bais et al. 2006).

The goal of this study was to understand how past land uses and climate can affect the investment in fine-root biomass in Atlantic Forests. To achieve that goal, in **Chapter 1**, we continued the work initialized in my master's research, when we aimed to understand if it was possible to reduce collection time in the field without affecting the accuracy of fine-root biomass estimation by selecting the best-fit model for fine-root reference samples. This methodological comprehension was important due to the existence of several methods to quantify fine-root stock and production, and the fact that almost all of them are associated with various sources of error (Berhongaray et al. 2013; Addo-Danso et al. 2016; Sochacki et al. 2017).

In the **Chapter 2**, by trusting the accuracy of the sampling method, we explored the root-fungus relationship, seeking to understand whether the production of fine roots continued to differ between types of forest cover and seasons after controlling for some structures and substances produced by arbuscular mycorrhizal fungi (AMF), as well as the minimum time needed to detect these differences. Additionally, we evaluated the opposite relationship, where we determined whether the characteristics related to AMF were affected by different forest covers and seasons after controlling for fine-root production.

After understanding the belowground functioning of the analyzed forests, in **Chapter 3**, we sought to evaluate whether the investment in fine-root productivity varied between different human-modified montane Atlantic Forests, years, and seasons. We examined the main edaphic drivers of fine-root productivity in four types of forest cover and analyzed, in each one of them, if there was a resource allocation trade-off between fine-root productivity and wood stock and/or wood productivity.

Finally, to summarize all our findings, we presented a general conclusion.

CHAPTER 1 – TESTING COLLECTION-TIME REDUCTION IN FINE-ROOT BIOMASS ESTIMATION IN ATLANTIC FORESTS

1.1 Abstract

Fine roots are essential components of the below-ground layer and play an important role in the carbon cycle. Methods for root extraction and biomass estimation have been proposed, including the temporal prediction method. However, there are doubts if the best model to estimate total root mass varies between study sites. Additionally, there are no records regarding the prediction method's efficiency for shorter collection times than 40 min. Here, we aim to clarify these doubts. We extracted 1080 fine-root samples from two contrasting ecosystems at 60-time intervals of 2 min each. We then performed a model selection to identify the best fit model and used it to find the shortest time suitable for collecting fine-root samples (40, 32, 24, 16, or 8 min). Other 448 root samples were collected from seven ecosystems by employing the shortest time tested (8 min). We calculated the percentage of estimated mass at 120 min and tested for differences between ecosystems. We found that Weibull was the best fit model, and it performed well for modelling root extraction at shorter collection times. All collection times tested had excellent goodness of fit, and there was strong evidence that the estimated mass did not differ between them. Moreover, collections at 8 min were enough to make reliable estimates of fine-root mass at 120 min in all ecosystems. Weibull is a flexible model and can accurately estimate fine-root mass at 120 min in different ecosystems. The extraction of fine roots can be reduced to four-time intervals of 2 min each when using the temporal prediction method. By reducing the time spent removing roots from each soil core, researchers can increase the number of soil cores extracted per study site and characterize the environment properly.

Keywords: fine-root biomass; root collection time; root-sampling method; temporal prediction method.

1.2 Introduction

Fine roots comprise an important plant-photosynthate and resource investment (Jackson et al. 1997b; Yuan & Chen 2012; McCormack et al. 2015). They have been identified by different classification systems, but are mainly known for absorbing and transporting water and nutrients from the soil, and for playing a vital role in the ecosystem carbon (C) cycle (Yuan & Chen 2012; McCormack et al. 2015). Fine roots represent the bulk of a root system's annual turnover (Freschet et al. 2013). Their litter production can exceed the amount of litter from leaves (Röderstein et al. 2005), and their mean residence time of C can be more than twofold higher than that of shoots (Rasse et al. 2005). However, due to the difficulties related to root sampling, fine roots have been often ignored in field studies or estimated as a theoretical proportion of aboveground values (Clark et al. 2001a; Trumbore & Gaudinski 2003).

In the last few decades, a set of different methods have been proposed to quantify the fine-root C pool compartment and production, but their accuracy still needs to be improved due to some sources of error. Especially in fine-root biomass estimations, these errors may be related to soil density, soil seasonal fluctuations of resources and conditions, distance from sampled trees, and the use of sieves with non-standard mesh openings or even made of fragile and easily deformable materials (such as plastic sieves) (Livesley et al. 1999; Addo-Danso et al. 2016; Sochacki et al. 2017). Not surprisingly, there is still a lack of agreement in the literature concerning the most appropriate method for sampling fine roots in forest ecosystems (Clark et al. 2001a; Levillain et al. 2011; Addo-Danso et al. 2016; Sochacki et al. 2017).

Similarly to the sample-based interpolation and extrapolation methods for estimating species richness (Colwell et al. 2012), the temporal prediction method has emerged as an alternative for fine-root mass estimation. The temporal prediction method relies on manually extracting roots from soil cores for 40 min (divided into four time intervals of 10 min each and resulting in four sample masses), and then predicting the root extraction usually by fitting the data to a logarithmic model beyond that period (e.g., up to 120 min) (Metcalfe et al. 2007). This approach reduces fieldwork time during root collection and allows increasing the number of sampling points per area (Metcalfe et al. 2007).

The temporal prediction method estimates fine-root production per unit area and time and corrects for underestimating fine-root mass by fitting the data to a model (Girardin, Malhi, Aragão, Mamani, et al. 2010). However, the same model (e.g., logarithmic) may not converge for all reference samples, and other models should be tested (e.g., exponential and Michaelis-Menten) (Marthews et al. 2014). Additionally, it is assumed that the best-fitting curve formula may vary among study sites (Metcalfe et al. 2007). There are also no records in the scientific literature regarding the efficiency of the prediction method in observed collection times shorter or larger than 40 minutes.

In this study, we measured the fine-root mass extracted from soil over time and tested if it could reduce the fine-root collection time observed by the temporal prediction method without affecting biomass estimation accuracy. Specifically, we aimed to answer the following questions: (1) which statistical model best fits the fine-root mass collected for 120 min?; (2) is the selected model in question 1 capable of making good and reliable estimations of the total root mass for collecting reference samples in collection times shorter than the previously tested 40 min (32, 24, 16, or 8 min)?; (3) does a reduction in the observed collection time affect fine-root mass estimation?; (4) is the relative error in the estimated biomass vary between sites when a short fine-root collection time is selected?

Due to the reduced number of reference samples (n=4), we expect the models with few parameters (e.g., logarithmic) to show a best fit, regardless of the collection time. Assuming the reliability of estimates from the temporal prediction method (Metcalfe et al. 2007), we expect to find no differences in biomass estimates at the different collection times observed. Also, because the method underestimates the fine-root biomass sampled in the field (Koteen & Baldocchi 2013), we predict that soil cores with the largest fine-root biomass would have a greater error associated with the estimates. Finally, because ecosystems have intrinsic characteristics (Marthews et al. 2014), we expect to find variations in the relative estimated biomass by the same model in short observed collection times.

1.3 Methods

1.3.1 Study region

To answer the questions in this study, we carried out our fieldwork along an elevational gradient of the Atlantic Forest from 10 m to 1000 m above sea level (asl).

This is the second largest forest complex in the American continent and an important biodiversity hotspot (Colombo and Joly, 2010; Myers and Mittermeier, 2000). Specifically, we worked in seven sites of *Serra do Mar* State Park, São Paulo, south-eastern Brazil (Fig. 1A): an old-growth seasonally flooded forest - *Restinga* (RES, \pm 13 m asl), an old-growth lowland forest (LOW, \pm 70 m asl), a lower submontane forest post-selective logging (LSM(SL), \pm 150 m asl), an old-growth lower submontane forest (LSM, \pm 248 m asl), an old-growth upper sub-montane forest (USM, \pm 370 m asl), a montane forest post-selective logging (MON(SL), \pm 1031 m asl), and finally, an old-growth montane forest (MON, \pm 1,038 m asl) (Fig. 1B). We took advantage of the fact that, in each of these places, there were 1-ha plots (a grid of 100 x 100 m divided into 100 subplots) installed to carry out long-term ecological research (Joly et al. 2012). These sites varied in elevation, soil texture, soil chemistry, and above-ground biomass (Table 1).



Figure 1. The study was carried out in the northern part of *Serra do Mar* State Park (red rectangle), São Paulo, south-eastern Brazil (a). Seven ecosystems along an elevational gradient were selected for collecting root samples (b). Acronyms: RES: old-growth *Restinga*; LOW: old-growth lowland forest; LSM(SL): lower sub-montane forest post-selective logging; LSM: old-growth lower sub-montane forest; USM: old-growth upper sub-montane forest; MON(SL): montane forest post-selective logging; MON: old-growth montane forest. Coordinate System: GCS SIRGAS 2000; Datum: SIRGAS 2000; Author: VL.

Table 1. Characteristics of ecosystems along an elevational gradient in *Serra do Mar* State Park, São Paulo State, south-eastern Brazil. Adapted from Alves et al. (2010) and Martins et al. (2015).

Paramotor -	Atlantic Forest physiognomy			
Falameter -	Restinga	Lowland	Sub-montane	Montane
Plot codes	RES	LOW	LSM(SL), LSM and USM	MON(SL) and MON
Elevation (m)	0–50	50–100	100–500	500–1,200
Rainfall (mm) *	2,146	2,146	2,146	1,975
Temperature (°C) *	22.3	22.3	22.3	16.3
Slope (°)	0–10	10–30	> 30	> 30
Soil type	Entisol (Quartzipsamments)	Inceptisol (Typic Dystrudepts)	Inceptisol (Typic Dystrudepts)	Inceptisol (Typic Dystrudepts)
Soil texture				
Clay (%)	5.6	34.8	20.6	20.8
Silt (%)	4.2	7.8	17.0	24.6
Sand (%)	90.2	57.4	62.5	54.7
Soil chemistry				
Bulk density (g.cm ⁻³)	1.2	1.2	1.2	0.9
C (Mg.ha ⁻¹)	63.4	102.6	126.2	139.5
N (Mg.ha ⁻¹)	3.7	7.6	10.0	10.6
P (mg.kg ⁻¹)	11.5	17.6	12.9	21.2
K (mmolc.kg ⁻¹)	1.0	1.8	2.2	2.1
Mg (mmolc.kg ⁻¹)	1.1	4.6	7.2	5.3
Ca (mmolc.kg ⁻¹)	1.5	5.4	12.0	7.2
AI (mmolc.kg ⁻¹)	15.6	14.3	25.1	26.7
рН	3.6	4.6	3.8	3.7
CEC (mmolc.kg ⁻¹)	85.4	110.9	160.2	121.3
Base saturation (mmolc.kg ⁻¹) <i>Aboveground biomass</i>	3.0	7.2	15.3	8.1
Trees (Mg.ha ⁻¹)	163.5	204.8	247.7	271.3
Palms (Mg.ha ⁻¹)	2.8	3.9	6.0	11.3
Ferns (Mg.ha ⁻¹)	0.0	0.2	0.1	0.8

(*): Annual rainfall and temperature data are from the automatic weather stations installed by the Biota Functional Gradient Project, near the plot in the montane forest, and by the Agrometeorological Information Centre (CIIAGRO), near the plots in the *Restinga*, lowlands and sub-montane forests for the period from March/2013 to February/2014. Acronyms: RES: old-growth *Restinga*; LOW: old-growth lowland forest; LSM(SL): lower sub-montane forest post-selective logging; LSM: old-growth lower sub-montane forest; USM: old-growth upper sub-montane forest; MON(SL): montane forest post-selective logging; MON: old-growth montane forest.

1.3.2 Root sampling

The fine roots (roots ≤ 2 mm) sampled to fit the best model were collected in USM and MON forests. These two sites had been previously studied (Sousa Neto et al. 2011) and were known for containing contrasting fine-root stocks (small in the submontane forest and large in the montane forest). Soil cores with the stocked fine roots were extracted from nine subplots in each study site (sub-montane and montane forests) during fieldwork in March 2013. We used a systematic design to collect the soil cores: three subplots were selected at the bottom of the grid, three in the middle, and three in the upper part. Soil cores (measuring 14 cm in diameter and 10 cm in depth) were extracted at the right upper corner of each subplot using a manual auger. Roots that had not been cut by the auger and remained on the wall of the open soil cavity were cut off using scissors. We maintained a minimum distance of 40 m in the soil core collections. The sampling points were moved up to a maximum of 2 m away if they fell on rocks or trees.

The soil cores extracted from both study sites (n = 18) were placed on previously identified trays, and roots were hand-picked in the field for 120 min, split into 2-min time intervals (60-time intervals of 2 min each, 1080 min per study site). Six people participated in removing the roots from the soil cores, and they were instructed to maintain the same collection pace throughout the sampling. Also, if a person quickly collected the roots from a portion of the soil, (s)he would be instructed to keep searching for roots (at the same pace) until the allotted time elapsed. We did not evaluate the performance of more than one field worker on the same soil sample.

In total, we collected 540 root samples per study site. The roots collected at the end of each 2-min interval were stored in identified paper bags with the site identification, the soil core number, and the collection time interval (e.g., 0–2 min, 2–4 min, 118–120 min). The root samples were taken to the Laboratory of Ecology, Department of Plant Biology at the University of Campinas, where the roots were washed in a particle size sieve (0.50 and 0.25-mm opening) and oven-dried at 60 °C until reaching a constant dry weight. We weighed the 540 root samples from each study site on a precision scale and summed their masses (in g) according to the soil core. Thus, we attained the information concerning the observed root mass at different time intervals over 120 min.

1.3.3 Testing the shortest observed collection time under different conditions

We carried out another fieldwork in July 2014 to collect new fine-root samples (also, roots ≤ 2 mm) after identifying the best fit model and the shortest time for collecting reference samples. These samples were used to test if the percentage of estimated mass differed between ecosystems, soil types, and land uses. We tested for differences in the estimated fine-root mass percentage (and not for the absolute mass) because the absolute mass would vary naturally between ecosystems. Our objective was to verify the model's efficiency.

New fine-root samples were collected in the Serra do Mar State Park covering all seven sites selected for this study (Fig. 1B). Soil cores (diameter, 14 cm; depth, 10 cm) were extracted in the right upper corner of 16 systematically assigned 100-m² subplots. We collected soil cores in four subplots at the bottom of the grid, eight soil cores in the middle, and four in the upper part. The subplots were 30 m away from each other. A manual auger was used to extract the soil cores, and before rotating the auger, the surface roots were cut off to prevent fine-root samples higher than 14 cm from being sampled. Again, the sampling points were moved up to a maximum of 2 m away if they fell on rocks or trees. The soil cores extracted were placed on identified trays, and roots were hand-picked in the field in the four-time intervals of 2 min each (8 min per soil core, 128 min per study site) – the shortest collection time tested, as described in the subsection Statistical analysis. We chose to collect roots in the shortest time to capture the increments of root biomass at the early phases of the root picking (Berhongaray et al., 2013). Thus, with less time spent in the field it would be in that shortest time if the method were unstable. Sixty-four root samples were extracted per study site, and they were placed in identified paper bags. Root samples were taken to the Laboratory of Ecology at the University of Campinas, where the roots were washed, oven-dried, and weighed on a precision scale. The absolute mass was calculated in Mg.ha⁻¹.

1.3.4 Statistical analysis

To identify the best-fit model for the fine-root mass accumulation curve (study question 1), we constructed a cumulative curve of fine-root mass for 120 min for each

soil core (the reference samples). Thus, we had 18 cumulative curves in total and fitted the models to each one of them. We evaluated the fine-root mass cumulative curves' shape by assessing ten statistical models' predictive accuracy using the Akaike Information Criterion (AIC) obtained from the *aictab* function of the *AlCcmodavg* package (Table 2). Only models having $\Delta AIC \leq 2$ were considered to be models with substantial best-fit support/evidence (Burnham & Anderson 2004). Next, an independence test with the most appropriate models was performed to quantify the percentage of fit of the mass curves. In situations where there was a tie, i.e., more than one model best fitted the curves, all models were considered. **Table 2**. Models tested for best fit for fine-root mass accumulation. Root samples were collected over 120 minutes in time intervals of 2 min each in Sub-Montane and Montane Forests (540 root samples per area) in *Serra do Mar* State Park, south-eastern Brazil.

Model	Fit formula	Reference
(1) Chapman-Richards	$R_t = a(1 - e^{bt})^c) + \varepsilon$	(Richards 1959; Huang et al. 1992)
(2) Exponential	$R_t = e^{a+b/t+1} + \varepsilon$	(Wykoff et al. 1982; Huang et al. 1992)
(3) Gompertz	$R_t = a e^{-b \exp(-ct)} + \varepsilon$	(Winsor 1932; Huang et al. 1992)
(4) Hyperbolic	$R_t = at/(b+t)$	(Bates & Watts 1980; Ratkowsky & Reedy 1986)
(5) Logarithmic	$R_t = a + b \log(t) + \varepsilon$	(Curtis 1967; Arabatzis & Burkhart 1992)
(6) Logistic	$R_t = a/(1+be^{-ct}) + \varepsilon$	(Pearl & Reed 1920; Huang et al. 1992)
(7) Monomolecular	$R_t = a(1 - c e^{-bt}) + \varepsilon$	(Brody 1945; Draper & Smith 1981)
(8) Power law	$R_t = at^{b} + \varepsilon$	(Stoffels & van Soeset 1953; Stage 1975; Huang et al. 1992)
(9) Second-order polynomial	$R_t = a + bt + ct^2 + \varepsilon$	(Henriksen 1950; Curtis 1967)
(10) Weibull	$R_t = a[1 - \exp(-bt^c)] + \varepsilon$	(Bailey & Dell 1972; Fang & Bailey 1998)

Notes: R_t is the cumulative root mass at time t; a, b, c are parameters estimated by least squares, and ϵ is the statistical error with Gaussian distribution, zero mean and constant variance.

To analyse the performance of the best-fit model at shorter collection times lower than that previously tested (40 min), we kept the original number of reference samples (n = 4) and tested different time interval reductions of 2, 4, 6 and 8 min (study question 2). The reductions resulted in the following collection times: 40 min (four time intervals of 10 min each), 32 min (four time intervals of 8 min each), 24 min (four time intervals of 6 min each), 16 min (four time intervals of 4 min each), and 8 min (four time intervals of 2 min each). To observe the mass per each time interval, we used the mass collected over 120 min (Figure 2, observed mass/reference samples).

We chose not to work with time intervals shorter than 2 min due to the increased chance of collecting large fine root masses at one time interval after another, in which we found little or no mass (where the opposite is expected by the temporal prediction method). This could result in poor model fits and, consequently, errors in fine-root mass estimates, especially in ecosystems where fine-root stock is large. Additionally, these

tests are laborious, especially when cleaning the samples, which made us believe that five different lengths of time intervals would be enough to explore the method's potential.

Estimations of total fine-root mass were noticed to be significantly improved by the parameter controlling the asymptote in one of the ten models tested (parameter alpha (α) of the Weibull model). Thus, we performed a simple linear regression between the observed cumulative mass at 40, 32, 24, 16, and 8 min (the predictive variable) and the α parameter (the response variable). The α parameter calculated for the observed cumulative mass at different observed times was used to construct back-transformed equations (Table S1 – Appendix A). The α parameter is necessary as a starting value during the Weibull model's optimization procedure to estimate the fine-root mass accumulated at the same cut-off point used for the observed data. After this adjustment, the model can be used to predict the fine-root mass at 120 min (Figure 2 - estimated mass/extrapolated time), the period in which we observed total accumulated dry mass in the soil cores. Therefore, it is noteworthy that we collected roots for 120 min (observed mass). Then we used the observed mass at different time intervals (40, 32, 24, 16, and 8 min) to predict the mass at 120 min (estimated mass).

We applied the Relative Root Mean Squared Error (RRMSE) using the *gofRRMSE* function of the *ehaGoF* package (Gulbe & Eyduran 2020), which provides information about a model's performance (goodness of fit) to investigate whether a reduction in fine-root observed collection time affects fine-root mass estimation (study question 3). Model accuracy was considered excellent when RRMSE was < 10%; good when RRMSE was \geq 10% and < 20%; fair when \geq 20% and < 30%; and poor when RRMSE was \geq 30% (Despotovic et al. 2016). Additionally, we performed an analysis of variance (one-way ANOVA) to test if the estimated mass differed between collection times (40, 32, 24, 16, and 8 min) (categorical variable: collection time, response variable: estimated mass for 18 soil cores).

To test if the relative error in the model's estimations was associated with fieldobserved mass (study question 4), we performed simple linear regressions for each observed collection time by summing each soil core's mass values. The model's relative prediction error was calculated as follows: (observed mass - predicted mass)/observed mass x 100. The test has the purpose of evaluating if the model's error is associated with the observed mass, e. g. if large samples have large errors (40 min) and small samples have minor errors (8 min). The observed mass was used to predict the relative error (response variable) in performing the linear regressions.

We fitted the best model selected from previous analyses to the observed fineroot mass and estimated the mass at 120 min (Figure 2 - extrapolated time/estimated mass). Then, we calculated the estimated/total mass ratio (proportion data) and transformed the proportions into logit to meet the assumptions for ANOVA (Warton & Hui 2011). We performed a one-way ANOVA to analyse if the estimated mass differed between ecosystems (*Restinga*, lowland forest, lower sub-montane forest, lower submontane forest post-selective logging, upper sub-montane forest, montane forest postselective logging, and old-growth montane forest), soil types (Entisol versus Inceptisol), and land uses (post-selective logging versus old-growth) (study question 5). Logit data were back-transformed to present the results of the percentage of estimated fine-root mass. We used R 4.0.3 (R Core Team 2020) to perform the analyses.



Figure 2. Hypothetical representation of how the best-fit model was applied to the temporal prediction method. The chequered background represents the observed mass collected in the field. The white background represents the mass estimated by the model. A is the fine-root mass accumulated over the first-time interval; B is the mass accumulated at the end of the first-time interval plus the fine-root mass accumulated in the second-time interval; C is the mass accumulated at the end of the second-time interval plus the fine-root mass accumulated in the third-time interval; D is the mass accumulated at the end of the third-time interval plus the fine-root mass accumulated in the fourth-time interval. Based on these first four reference samples, the model of best fit to the observed data was again fitted and used to estimate the total accumulated fine-root mass for 120 min (E).

1.4 Results

1.4.1 Best fit model and the shortest collection time

We found that Gompertz, logistic, monomolecular, and second-order polynomial models did not converge for accumulated mass curves using the full 120-min dataset (observed mass). Among the remaining models, the best-fit model was Weibull (study question 1); its fit was independent (had no association with the observed fine-root

collection times ($\chi^2 = 10.88$; df = 16; p = 0.82) and had 58.6% of relative frequency (Table 3). Other models with the best goodness of fit (Δ AIC \leq 2), but with a low fit percentage were Chapman-Richards (25.2%), power (8.1%), logarithmic (4.5%), and hyperbolic (3.6%) (Table 3).

Table 3. Absolute (n) and relative (%) frequency for models with the best fit ($\Delta AIC \leq$ 2) for 1,080 cumulative fine-root mass samples collected in different durations of observed time over 120 minutes, and in 18 sampling points.

Number and duration of each time interval	Model				
	Chapman- Richards	Hyperbolic	Logarithmic	Power	Weibull
12 intervals of 10 min	6 (23.1)	2 (7.7)	2 (7.7)	3 (11.5)	13 (50.0)
15 intervals of 8 min	7 (31.8)	1 (4.5)	1 (4.5)	1 (4.5)	12 (54.5)
20 intervals of 6 min	7 (30.4)	1 (4.3)	1 (4.3)	2 (8.7)	12 (52.2)
30 intervals of 4 min	5 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (75.0)
60 intervals of 2 min	3 (15.0)	0 (0.0)	1 (5.0)	3 (15.0)	13 (65.0)
Total (%)	28 (25.2)	4 (3.6)	5 (4.5)	9 (8.1)	65 (58.6)

We found an excellent fit ($R^2 \ge 93\%$) by regressing the mass estimated by the Weibull model at each observed collection time as a function of the observed mass at the same time intervals (Figure 3). This result implies that there is no evidence of accuracy loss when we fitted the same model for all sample roots (study question 2). The goodness of fit of the Weibull predictions was also excellent for all collection times (RRMSE < 10%) (Figure 3). Moreover, we found no significant differences in the relative estimated mass among the different observed time collections ($F_4 = 0.08$; p =0.99). This result indicates that there are no grounds to state that reductions in the observed time interval can affect fine-root estimation (study question 3). We also found no significant relationship between the relative prediction error of the Weibull model and the observed mass for any observed collection times (study question 4) (Figure 4). Thus, the estimated mass in long (40 min) or short (8 min) time intervals was not associated with estimation errors.



Figure 3. Fine-root mass estimated (rarefied) by the Weibull model at 40, 32, 24, 16, and 8 min as a function of the observed root mass for the same periods. Notice that all estimations were significant (*p*-value), had a high coefficient of determination (R^2), and excellent accuracy (RRMSE < 10). The confidence intervals (95%) at each point are displayed in a grey shade. RRMSE = Relative Root Mean Squared Error.



Figure 4. The Weibull model estimation relative error at different rarefaction times (40, 32, 24, 16, and 8 min) as a function of the observed fine-root mass. There was no association between the relative error and observed mass for any collection times (p-value > 0.05).

1.4.2 Reducing the observed collection time does not affect fine-root estimation under different conditions

We observed that collecting fine roots from soil cores in four-time intervals of 2 min each was sufficient to collect approximately 64.5% of the mass in the first 8 min, and 35.5% was predicted by the model (Figure 5). As expected, the ecosystems' absolute mass varied, but there was strong evidence that the percentage of the observed mass collected for 8 min did not differ across sites from the percentage of relative biomass estimated by the model (*F*_{6, 105} = 0.897; *p* = 0.499) (study question 5).

Figure 5. Absolute (Mg ha-1) and relative (%) fine-root mass quantified for 120 min in seven ecosystems along an elevational gradient of Atlantic Forests. The black colour



represents the percentage of the mass collected in the first 8 min (fieldwork with four series of 2 min/each), and the grey colour represents the percentage of mass estimated (uncollected) by the Weibull model at 120 min. The percentage of collected and estimated mass did not differ among ecosystems, soil types (Entisol versus Inceptisol), and land-use histories (old-growth versus selective-logging). RES: old-growth *Restinga*; LOW: old-growth lowland forest; LSM(SL): lower sub-montane forest post-selective logging; LSM: old-growth lower sub-montane forest; USM: old-growth upper sub-montane forest; MON(SL): montane forest post-selective logging; MON: old-growth montane forest.

1.5 Discussion

The results provide insights into the usefulness of predictive models and the appropriate time to extract fine roots from soil cores. We found that the Weibull model best fitted the observed mass for 120 min and predicted the fine-root mass correctly at shorter collection times. Unlike other studies suggesting that the model for predicting fine-root mass can vary among study sites (Metcalfe, Williams, Aragão, Da Costa, De Almeida, Braga, Gonçalves, Silva, et al. 2007; Marthews et al. 2014), we observed that the same model could estimate root mass for different ecosystems and conditions. Our results are partially explained by the fact that we were dealing with mass accumulation curves of similar shapes. The same model could fit almost all of them, partially because

of the model properties. Weibull is a type of distribution that has been known as highly flexible and able to assume virtually all monotonically increasing sigmoid growth shapes, allowing an increase or decrease in the rate over time (Yang et al. 1978).

The Weibull distribution has been commonly used in forest science, particularly to predict patterns of above-ground structures, such as tree diameter distributions (Zhang & Liu 2006; Mcgarrigle et al. 2011) and height-diameter relationships (Huang et al. 1992; Scaranello et al. 2012). Although the Weibull model has been shown to provide the best realistic growth pattern above-ground (Payandeh & Wang 1995), few attempts have been made to test this model for below-ground structures (Schwarz et al. 2013; Guo et al. 2021). We found that Weibull works well for modelling root removal over time and suggest other studies to consider this model when fitting their data collected below-ground. Our results also show that there is no appropriate time for extracting fine roots from the soil as there was no evidence of difference between collection times. This finding is significant, as researchers can spend less time collection time per soil core to 8 min, we spent 88% less time extracting roots per study site - from 1080 min to 128 min.

The temporal prediction method has raised doubts concerning its accuracy (Koteen & Baldocchi 2013), especially due to the small roots remaining in the soil matrix. However, our study shows that, despite not using all the root mass present in the soil sample, it proved to be efficient by comparing samples of different sites even when shorter collection times were used. Since most of the total estimated mass for 120 min was collected in the initial minutes, even at the shortest observed collection time (whose accumulated reference sample masses represented 64.5%, and the remaining mass was estimated by modelling), it is possible to observe that more than half of the root mass could be collected. In a high-density *Populus* plantation in Belgium, for example, 10 min was enough to pick 90% of the fine-root biomass in the summer (Berhongaray et al. 2013). Thereby, based on the collected reference samples, the remaining biomass in the soil is not neglected, but considered by the modelling process, whose final estimate will have the built-in correction.

Thus, this study corroborates the method's estimation efficiency evaluated by Metcalfe et al. (2007), as well as defending the suggestion that assertive reference

sample collections combined with models that best fit them provide two ways to achieve more realistic values even at shorter time intervals than those previously used. The lack of differences in the estimated mass percentage that was observed along the Atlantic Forest's elevational gradient (Figure 5) shows that the method is consistent regardless of the mass variations among the different ecosystems analyzed.

Given the fact that we tested different models on soil samples up to 10 cm in depth only, the soil layer where the greatest fine-root biomass is found in the Atlantic Forest (Rosado et al. 2011a; Sousa Neto et al. 2011; Silva et al. 2020), we are still unable to state whether similar results can be obtained when soil layers above 10 cm are handled during the same collection times as those tested. However, others can apply these procedures in ecosystems with similar conditions to ours. In addition, we still need further clarification concerning the effect of soil texture, organic matter, and soil water content during fine-root removal, since, based on our experiences in the field, it has been noticed that the soil becomes very sticky as the water content increases. This can become a big problem when sampling roots, especially those growing in rainforest oxisols, for example.

Choosing the most appropriate method to answer the questions of below-ground research is crucial for studies to ensure that sufficient and meaningful replication is statistically robust (Freschet et al., 2021). We conclude that the findings obtained here reinforce the usefulness of the temporal prediction method to achieve these goals and have a broader impact in the root ecology field. This impact is significant in a changing environment, where roots and their associated microorganisms can shape how ecosystems respond to climate change (Pennisi & Cornwall 2020), and we still know little about how this happens.

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1.7 Authors' contributions

C.A.S conceived the ideas and designed the methodology; C.A.S. collected the data; C.A.S., A.M.D'A., and M.A.S.S. analyzed the data; B.B. structured the manuscript's draft; V.L. and C.A.S. led the manuscript's writing; C.A.J. and S.A.V. obtained funding and supervised the research group.

1.8 Data availability statement

All data are available on Mendeley Data: http://dx.doi.org/10.17632/744tc82859.17

CHAPTER 2 – FINE ROOT-ARBUSCULAR MYCORRHIZAL FUNGI INTERACTION IN TROPICAL MONTANE FORESTS: EFFECTS OF COVER MODIFICATIONS AND SEASON

2.1 Abstract

Tropical Montane Forests are unique climate-influenced ecosystems with a vital role for some ecosystem services, of which one of the most important is soil carbon storage. Changes in forest cover affect forest structure, composition and functioning, but little is known about how such changes influence the belowground carbon content. In this study, we addressed this issue by evaluating whether fine-root production differed between forest-cover types and contrasting seasons after controlling arbuscular mycorrhizal fungi (AMF) traits, and what the minimum time required to detect such differences is. We also determined whether AMF-related traits were affected by distinct forest cover and seasons after controlling fine-root production. The objects of study were four forests with distinct degradation histories in the Atlantic Forest: old-growth, post-selective logging, post clear-cut and post-pasture forests. Data were collected in four 2500-m² plots installed in each area and analyzed through multivariate statistics. Fine-root production differed significantly between forest covers, and marginally between seasons, as it was greater in the post-pasture area and in the rainy season. Some AMF traits also differed between forest covers and seasons, especially the abundance of viable spores in the dry season. We found that six months was the shortest period necessary to identify differences in the production of fine roots. This study shows that forests with different degradation histories and annual climatic variations significantly affect fine-root production and AMF dynamics in Tropical Montane Forests. In particular, ecosystems that have been more perturbed tend to invest more in fine-root production (post-pasture). Our results help to better understand belowground interactions and biomass investment under different managed ecosystems.

Keywords: Atlantic Forest; belowground functioning; biomass; seasonality.
2.2 Introduction

Tropical Montane Forests are the predominant forest type in zones of maximum cloud condensation in mountain ranges (Boehmer 2011). Although fragmented, reduced and disturbed by human intervention, these forests are considered hotspots of biodiversity (Gradstein et al. 2008) and are strongly influenced by temperature, precipitation and soil fertility gradients, generally associated with elevation. Montane Forests invest substantial amounts of biomass in the production and functioning of belowground structures (Hertel et al. 2009; Moser et al. 2011; Girardin et al. 2013) that are vital for some ecosystem services, such as hydrology, slope stability and, especially, soil carbon (C) storage (Girardin, Malhi, Aragão, Mamani, et al. 2010; Vieira et al. 2011).

Together, natural and man-induced disturbances can modify the intensity, duration and frequency of the time that it takes for systems to restore (Liebsch et al. 2008; Brearley 2011). Such modifications occur because once resource availability is affected (such as changes in water, light and nutrient availabilities), new habitats are created and occupied by species with different needs (Tabarelli & Mantovani 1997; Tabarelli 1999). A considerable and variable part of plant productivity is destined to belowground structures, such as fine roots, in order to maintain diverse growth strategies (Litton et al. 2007; Malhi et al. 2011; Raich et al. 2014). Therefore, it is expected that root biomass will vary depending on the type of disturbance and recovery time, although few studies have addressed these effects on the belowground compartment in wet tropical forests (Hertel et al. 2007).

Fine roots, especially, are key to the root system because they actively function in the transportation and absorption of adequate supplies of water and nutrients for plant growth and maintenance (Gordon & Jackson 2000; McCormack et al. 2015). They are morphologically classified with a diameter less than or equal to 2 mm (Jackson et al. 1997a) and, due to their close contact with soil particles, some of the most complex chemical, physical and biological interactions experienced by terrestrial plants are mediated by these types of roots (Vogt et al. 1993; Bais et al. 2006). Although C inputs to the soil through fine-root turnover are lower as compared to C inputs via aboveground sources, they are the main source for soil organic C formation (Berhongaray et al. 2019). In general, root production in tropical forests occurs continuously throughout the year, but its growth is affected by environmental and plant characteristics (Meier et al. 2013; Mommer et al. 2016) as well as by the microorganisms with which it interacts. Root-fungi associations, in particular, are reported as the most widespread symbiotic associations, and they occur in more than 80% of higher-plant species (Wang & Qiu 2006; Smith & Read 2008). This is particularly important in tropical forest where the C stored in aboveground biomass is mainly derived from plants that are associated with arbuscular mycorrhizal fungi (Soudzilovskaia et al. 2019; Steidinger et al. 2019).

In this association, arbuscular mycorrhizal fungi (AMF), which are necessarily biotrophs, completely depend on their plant hosts for C supply (Bago et al. 2003). AMF, in turn, improve plant fitness and productivity by increasing nutrient uptake capacity, required in photosynthetic and other metabolic processes (van der Heijden et al. 2008; Cavagnaro et al. 2015). Regarding C fluxes, arbuscular mycorrhizal symbiosis significantly contributes to C allocation to both roots and AMF structures in the soil (Drigo et al. 2010). Considering fine-root production as a source of potential entry points of AMF hyphae, understanding how root-microbial interactions respond to seasonal climate variations may shed light on net primary productivity and soil C-storage capacities (Leake et al. 2006; Newbold et al. 2015).

In order to address the consequences of forest-cover changes on the belowground C content, our goals in this study were to analyze the effects of forest cover on fine-root production as well as evaluate the minimum time required to detect these effects by directly measuring fine-root biomass production. Moreover, considering that recently produced fine roots are the structures by which AMF accesses photoassimilated C and that it can significantly influence plant growth and nutrients and water acquisition (Pasqualini et al. 2007), we investigated if by controlling the production of fine roots, AM symbiosis components would be altered by different forest covers and seasonality. We also checked if soil parameters differed between forest-cover types and seasons and if they were correlated with fine-root production and AMF symbiosis components.

Based on previous studies which predict that in Montane Forests higher radiation and vapor pressure deficits lead to higher water loss from trees (Rosado et al. 2016), we expect that different forest-cover types will influence fine-root biomass production as well as its AMF association. Thus, the more managed the ecosystems

are (in this case, a post-pasture area), the more roots will be produced and/or the more permissive these roots will be to AMF associations due to such higher radiation and water loss. In addition, because, in rainy seasons, plant metabolic activities are high in these forests (Marchiori et al. 2019), we also expect to find variations between contrasting seasons.

2.3 Materials and Methods

2.3.1 Study site and sample collection

The study was carried out in the protected area of Serra do Mar State Park in São Paulo state, southeastern Brazil (Fig. 1a). Serra do Mar State Park holds the most well preserved Atlantic Forest remnants (Oliveira-Filho & Fontes 2000; IBGE 2012). Since the 1970s, with the State Park creation, changes in forest use in many areas have ceased or have been reduced (Tabarelli et al. 1993; SIMA 2006).

The regional climate is the Cwa type in Köppen's classification (Alvares et al. 2013). The winter (from June to September) is usually cold and, exceptionally, in June and July, there is scarce humidity and precipitation (Colombo & Joly 2010). In contrast, the summer (from December to March) is hot and humid. Fog is observed sporadically throughout the year, being common during the dry season (Salemi et al. 2013). Here, we consider both mentioned periods and all their intrinsic processes as contrasting seasons and, for comparative purposes, we call winter the dry season and summer the rainy season.

In the study period (from July 2016 to June 2017), the mean annual temperature was approximately 17 °C, with monthly averages ranging from a minimum of 13 °C in July to a maximum of 20 °C in January. Annual precipitation was close to 1500 mm, with high seasonal variation and the monthly amount ranging from a minimum of 2.2 mm in July/2016 to a maximum of 327 mm in January/2017, according to data from meteorological stations close to the plots and provided by the Climate and Biosphere Laboratory - DCA/IAG-USP and by the São Paulo State Forestry Institute.

We selected 16 plots of 50 m x 50 m in size (2500 m² each) with four replicate plots representing forests with past modifications that affected the characteristics of the forest cover, which we considered here as forest-cover types and defined as

follows: (i) Old-growth forest (OG), a forest without evidence of human impact; (ii) Postselective logging forest (SL), a forest characterized by irregular extraction of largediameter stems and considered here as low to medium human impact; (iii) Post clearcut forest (PCC), a forest grown after a total cut of tree individuals, and thus, classified as a tree community after medium to high human impact, and (iv) Post-pasture forest (PP), a forest undergoing a regeneration process after forest-cover conversion by clear-cut followed by pasture, considered a regenerating forest cover after high human impact (Fig. 1b). All these plots are included in an old-growth forest matrix and the maximum distance from each other is approximately 13 km (Fig.1b).



Figure 1. The study areas were in the Serra do Mar State Park, São Paulo State, southeastern Brazil (a). Four forests with distinct levels of human impact in the past were the objects of

study: Old-growth was a mature forest; Post-selective logging had irregular extraction of largediameter stems; Post clear-cut had all trees removed; and Post-pasture was an area used for livestock and then abandoned for natural regeneration (**b**). The level of human impact (hemeroby) can be considered from low to high from Old-growth to post-pasture.

Regional vegetation is typical of Montane Atlantic Forest and the above-ground biomass tends to decrease with increasing intensity of human modifications. The plots are close to 1000 m of altitude (Alves et al., 2010; Marchiori et al., 2016) and according to the testimony of residents next to the forest, the last modifications in the structure of the tree community occurred about 50 years ago, when the Serra do Mar State Park was created. In addition, the most abundant tree families make associations with AMF (Table 1).

The soils are mostly classified as Inceptisols, with medium texture and shallow depth (Martins et al., 2015; SIMA, 2006). The chemical soil analyses of the plots were carried out at the Soil Science Department of Luiz de Queiroz College of Agriculture (ESALQ-USP), in which pH was measured in 0.01 M CaCl₂ with a soil-solution ratio of 1:2.5; exchangeable Al by means of extraction with solution of KCl 1 N and titration with 0.05 mol L–1 NaOH in the presence of bromothymol blue; potential acidity by means of pH; K, Na, Ca and Mg with extraction of elements by ion exchange resins and reading by an atomic absorption spectrophotometer (Ca and Mg) and flame photometer (K and Na); and P by water extraction using anion exchange resins and quantification by colorimetry. N concentrations were determined by a Carlo Erba elemental analyzer (FlashEA1112) at the Center of Nuclear Energy in Agriculture – University of São Paulo CENA/USP. Total organic C was determined at the Center for Research and Development of Soils and Environmental Resources – Agronomic Institute of Campinas (IAC) by dichromate oxidation method.

	Forest cover ¹								
Site parameter	OG	PSL	PCC	PP	F	р			
Tree community characteristics									
Aboveground biomass (Mg ha ⁻¹) ²	303.10	242.90	171.78	126.31	*	*			
	Arecaceae	Chrysobalanaceae	Euphorbiaceae	Euphorbiaceae					
Most abundant families ³	Sapotaceae	Monimiaceae	Arecaceae	Primulaceae	*	*			
	Chrysobalanaceae	Sapotaceae	Cyatheaceae	Lauraceae					
Mycorrhizal status ⁴	AM only	AM only	>90% AM	>90% AM	*	*			
Soil chemical characteristics ⁵									
C organic matter (g kg ⁻¹)	11.39±0.48ª	8.21±0.33 ^b	8.68±0.35 ^{ab}	7.65±0.25 ^b	5.25	0.02			
N (g kg ⁻¹)	6.75±0.53 ^a	4.51±0.39 ^b	5.92±0.43 ^{ab}	7.69±0.52 ^a	8.08	<0.01			
P (mg kg ⁻¹)	19.22±5.05a ^b	13.25 ± 2.15 [♭]	16.13±0.62 ^{ab}	20±1.57 ^a	3.66	0.02			
K (mg kg ⁻¹)	60.85±6.70 ^a	67.20±6.67 ^a	66.23±3.98 ^a	79.67±4.48 ^a	2.02	0.12			
Mg (mg kg⁻¹)	56.25±9.63 ^a	33±5.37 ^b	25.13±3.25 ^b	37.88±3.54 ^{ab}	5.28	<0.01			
S (mg kg ⁻¹)	24.38±5.64ª	22.03±3.45 ^a	25.13±1.58ª	20.25±1.17 ^a	1.20	0.32			
Ca (mg kg ⁻¹)	96.88±28.01 ^{bc}	90 ± 16.38^{ab}	46.25±13.87°	173.75±30.83 ^a	8.11	<0.0001			
AI (mg kg ⁻¹)	245.81±19.67 ^a	204.75±11.73 ^a	193.50±10.29 ^a	192.09±17.72 ^a	2.68	0.05			
H+AI	146.72±15.52 ^a	114.03±6.99 ^{ab}	122.09±9.34 ^{ab}	103.31±10.46 ^b	2.80	0.05			
рН	3.51±0.05 ^b	3.63±0.03 ^{ab}	3.76 ± 0.03^{a}	3.76 ± 0.08^{a}	4.89	<0.01			
SB (mmolc kg ⁻¹)	12.30±2.32ª	9.88±1.19 ^a	4.68±0.66 ^b	13.51±1.79 ^a	11.00	<0.0001			
CTC (mmolc kg ⁻¹)	159.02±16.74 ^a	123.91±7.47 ^a	126.78±9.08 ^a	116.82±9.98 ^a	2.07	0.11			

Table 1 – Tree community and soil chemical characteristics in different forest-cover types in Serra do Mar State Park, Brazil. Values represent the means and standard errors.

¹Forest cover types: OGF = Old-Growth Forest; PSLF = Post Selective Logging Forest; PCCF = Post Clear-Cut Forest; PPF = Post Pasture Forest; ²Data from Ramos et al. (unpublished); ³Data from Padgurschi et al. (2011), Marchiori et al. (2016) and Oliveira et al. (unpublished). ⁴Estimated according to Wang and Qiu (2006) and Steidinger et al. (2015); ⁵Martins et al. (2015) and Silva et al (present study); AM= arbuscular mycorrhizal; SB = Sum of basis; CEC = Cationic Exchange Capacity. Lower case letters indicate differences between forest cover types (p < 0.05).

2.3.2 Fine-root production characterization

We accessed net fine-root biomass production by the ingrowth core method (Steingrobe et al. 2001; Hendricks et al. 2006). To that end, we first opened four cavities per plot (14 cm in diameter by 30 cm in height) in a systematic design of about 30 m equidistant. Then, to avoid the transfer of soil with high nutrient content from the top to the bottom layers (as suggested by Aragão et al., 2009), we held each soil sample on trays referring to 0-10, 10-20 and 20-30 cm soil depths and manually collected stored roots from soil by the temporal prediction method (Metcalfe et al., 2007). We kept the collected roots in paper bags so that we could later (in the laboratory) triage, carefully wash, dry, weigh and, with measured masses, convert them to an Mg ha⁻¹ scale. Live and eventually dead roots were not separated but treated together under the term biomass.

In each open cavity, we inserted a plastic mesh (1 cm-opening) and filled it with root-free soil. At the end of each season, we removed the fine roots produced inside the mesh (according to the same method previously mentioned) until one year of monitoring was completed. Due to variation in the response time between the different types of forest cover, we evaluated the fine-root biomass produced in the winter (June to September/2016) and summer (December to March/2017) as well as in a longer period of time, consisting of the sum of the fine-root biomass produced in the autumn and winter (March to September/2016) as well as in the spring and summer (September/2016 to March/2017). Here, we identified them as samples of three and six months.

To avoid comparisons of different belowground performances, we carried out Kruskal-Wallis tests to evaluate differences between soil depths and observed that biomass root production differed and was higher in the superficial layer (0-10 cm) in all study areas (Old-growth: $X^2 = 11.08$, p = 0.004; Post-pasture: $X^2 = 19.2$, p <0.0001; Selective logging: $X^2 = 16.09$, p <0.001; Post clear-cut: $X^2 = 19.69$, p <0.0001) (Figure S1, Appendix B). Thus, we restricted this study only to the evaluation of processes that occurred at a depth of 0-10 cm, which is in direct contact with decomposing litter, recognized as an important driver of root proliferation (Sayer et al., 2006).

After quantifying the biomass produced from fine roots, we selected three samples of each forest-cover type for ¹³C content analysis. Fine-root carbon

concentrations were determined by Carlo Erba elemental analyzer (FlashEA1112) at the Center of Nuclear Energy in Agriculture – University of São Paulo CENA/USP.

2.3.3 Microbial soil C and AMF traits

Near each ingrowth core, we randomly sampled a volume of soil equivalent to 10 cm in diameter and 10 cm in depth in order to analyze the relation between fine roots and AMF traits. We homogenized the roots and soil from each plot, totalizing four composed samples by forest-cover type. We quantified soil microbial biomass C (μ C g⁻¹ soil) (Jenkinson & Powlson 1976) and soil basal respiration (CO₂ g⁻¹ soil h⁻¹) (Alef & Nannipieri 1995) in order to estimate C stocks and efflux capacity through soil microbiota. The metabolic quotient (qCO₂) was calculated using the ratio between the basal respiration of the soil and soil microbial biomass C (Anderson & Domsch 1993b). We conducted the following procedures to characterize AMF traits: intraradical colonization, extraradical hyphae, viable spores and soil glomalin contents.

We analyzed the percentage of fine-root length colonized by AMF by first clarifying the roots with 10% (w/v) KOH, and in case roots were still dark, they were treated with alkaline peroxide (3% H_2O_2 10v and 20% NH₄OH) (Koske & Gemma 1989). After bleaching, the roots were acidified in 2% HCl and stained in 0.05% (w/v) trypan blue in acidified glycerol (Giovannetti & Mosse 1980). We observed 30 root segments of 1 cm under a light microscope (100-400x magnification) and then calculated the percentage of colonized root length (Trouvelot 1986) for each composed sample in each plot (n = 30).

We estimated the extent of the extraradical mycelium using the flotation and quantification method, after filtration and staining (Boddington et al. 1999; Melloni & Cardoso 2001). To that end, 10 g of soil from each composed sample were used for hyphal extraction. The washing solution was passed through a 0.45-mm mesh and then collected in dark flasks. The mycelium suspension was vacuum filtered using a nitrocellulose membrane with a cross-linked grid and stained with 0.05% trypan blue in acidified glycerol. The total length of extraradical mycelium was estimated under an optical microscope (100x magnification) in 64 fields. Only hyphae with AMF characteristics and morphology were considered (blue stained non-melanized,

aseptate hyphae with characteristic bifurcations angles), and the length of extraradical hyphae was expressed as meters per gram of soil (m g⁻¹ soil).

Viable spores were determined under the light of a stereomicroscope lens by counting those with an intact wall and clear internal content and, expressed by number of spores per g⁻¹ soil (Gerdemann & Nicolson 1963). The spores were extracted from a 20-g soil subsamples of each composite sample by wet sieving through 0.250- and 0.025-mm sieves. The content in the later sieving was transferred to a tube and centrifuged in 70% sucrose solution.

A significant amount of photosynthetically fixed carbon is allocated by AMF to glomalin- related soil-protein (GRSP) production (Rillig et al. 2001; Lovelock et al. 2004). We extracted GRSP from 1 g of soil (three technical replicates) with 8 mL of sodium citrate (50 mmol L⁻¹ pH 8) and autoclaved it at 121 °C for 1 h. The solution was centrifuged at 8000*g* for 15 min and the supernatant separated. This process was repeated until the supernatant solution became translucent (Wright & Upadhyaya 1998). Bradford assay was used to estimate the protein amount in the extracts, using bovine serum albumin as a standard (Bradford 1976) and expressed by mg g⁻¹ soil.

2.3.4 Temperature and soil-water content monitoring

We monthly measured soil temperature and soil-water content, totalizing four measurements per plot (n = 16 by forest-cover type). To that end, we used precision sensors of ± 0.3 °C (STP-2, PP Systems) and 3% (HydroSense II, Campbell Scientific), respectively.

2.3.5 Data analysis

We began by checking the assumptions of multivariate normality and homogeneity of variance-covariance matrices through Mardia and Box's M tests, respectively. The data were then transformed into log+1 to standardize the variables and achieve such assumptions. A two-way multivariate covariance analysis (two-way MANCOVA) was performed to evaluate whether the type of forest cover and season (independent variables) as well as their interaction affected the biomass production of fine roots collected every three and six months (dependent variables). The AMF traits (i.e. root colonization, extraradical mycelium length, number of viable spores and GRSP) were used as covariates. To avoid Type-I error across the pairwise comparisons, we used Bonferroni procedure.

A two-way MANCOVA was also performed to evaluate the opposite direction of interaction, that is, whether the type of forest cover, season and root production affected AMF-related traits. In this case, the type of forest cover and season were used as independent variables and the performance of the AMF traits as dependent variables, after controlling the fine-root production identified in the first MANCOVA. Bonferroni tests were also used for pairwise comparisons.

We performed a two-way multivariate analysis of variance (two-way MANOVA) to examine if soil parameters (microbial biomass C, basal respiration, soil temperature and water content) differed between forest-cover types and seasons. Finally, Pearson's correlations were performed to evaluate the relation between biomass fine-root production and AMF traits with a series of soil variables (these variables are described in Tables 3 and 4). The statistical analyses were carried out by the R i386 3.6.0 free software environment (Wickham et al. 2016; R Core Team 2020) and SPSS 23 software (IBM 2019).

2.4 Results

We found a significant difference between forest-cover types ($F_{6, 38} = 2.58$, P = 0.03, Wilks' $\Lambda = 0.505$) and a marginal significant difference between seasons ($F_{2, 19} = 3.01$, P = 0.07, Wilks' $\Lambda = 0.759$) for the combined dependent variables (biomass fine-root production every three and six months) after controlling the studied AMF traits (mycelium colonization, mycelium length, viable spores and GRSP content), which were not significant in the model (Fig. 2). There was also a significant interaction between forest cover and season in biomass fine-root production after controlling the covariates ($F_{6, 38} = 2.56$, P = 0.04, Wilks' $\Lambda = 0.507$).

Looking specifically at the collection period (three and six months), we observed that fine-root production differed between forest-cover types and between seasons only when roots were collected every six months ($F_3 = 5.28$, P = 0.01, Wilks' $\Lambda = 0.505$ forest cover; $F_1 = 5.39$, P = 0.03, Wilks' $\Lambda = 0.759$ season) (Fig. 2). The average

production of fine roots was greater in the post-pasture forest and in the rainy season (Fig. 2b).



Figure 2 Fine-root biomass production at (**a**) three months and (**b**) six months. Forestcover types: OG - Old-growth, PSL - Post-selective logging, PCC - Post clear-cut and PP - Post-pasture. Bars represent the standard error (n = 16). Upper-case letters indicate significant differences between forest-cover types and lower-case letters indicate significant differences between seasons for the same forest type.

Regarding the influence of forest cover and season on AMF traits, we found a statistically significant difference between forest cover ($F_{12, 50.56} = 2.11$, P = 0.03, Wilks' $\Lambda = 0.341$) and season ($F_{4, 19} = 14.66$, P < 0.001, Wilks' $\Lambda = 0.245$) in the combined AMF traits after controlling the production of fine roots, which were not significant in the model (Fig. 3). There was also a significant interaction of forest cover and season in AMF traits after controlling the production of fine roots ($F_{12, 50.56} = 3.93$, P < 0.0001, Wilks' $\Lambda = 0.175$).

Analyzing each component of the AMF traits separately, we found no significant differences in root colonization between forest-cover types ($F_3 = 1.83$, P = 0.17, Wilks' $\Lambda = 0.341$), but significant differences between seasons ($F_1 = 25$, P < 0.01, Wilks' $\Lambda = 0.245$), with more colonization occurring in the rainy season (Fig. 3a). Extraradical mycelium length and soil GRSP content differed between seasons ($F_1 = 18.50$, P < 0.01, P <

0.001, Wilks' $\Lambda = 0.245$ for mycelium, and $F_1 = 5.00$, P = 0.036, Wilks' $\Lambda = 0.245$ for GRSP), and both components were more produced in the rainy season (Fig. 3b/d). The number of viable soil spores differed significantly between forest-cover types ($F_3 = 7.27$, P = 0.001, Wilks' $\Lambda = 0.341$) (higher in old-growth and post-pasture) and seasons ($F_1 = 16.83$, P < 0.001, Wilks' $\Lambda = 0.245$) (higher in the rainy season) (Fig. 2c).



Figure 3. Arbuscular Mycorrhizal Fungi (AMF) traits based on (**a**) Fine-root colonization, (**b**) Extraradical mycelium length, (**c**) Number of viable spores and (**d**) Glomalin-related soil protein of the forest covers examined in this study. Forest-cover types: OG – Old-growth, PSL – Post-selective logging, PCC – Post clear-cut and PP – Post-pasture. Error bars represent one standard error (n = 4). Upper-case letters indicate significant differences between forest covers and lower-case letters indicate significant differences between seasons for the same forest type.

We found that biotic and abiotic soil parameters significantly differed between forest covers ($F_{3, 15} = 2.28$, P = 0.01, Wilks' $\Lambda = 0.27$), and mainly between seasons ($F_{1, 5} = 13.59$, P < 0.001, Wilks' $\Lambda = 0.23$). There was no interaction between forest cover and season in soil biotic and abiotic parameters ($F_{3, 15} = 1.22$, P = 0.28, Wilks' $\Lambda = 0.46$). In addition, we observed that soil temperature varied between forest covers

($F_3 = 5.30$; P < 0.01); qCO₂ was also the highest ($F_1 = 43.01$; P < 0.001), and soil-water content was the lowest ($F_1 = 24.47$; P < 0.001) in the dry season. Opposite results were found for the rainy season (Table 2).

Table 1 – Average of soil parameters in four forest-cover types analyzed in the dry and in the rainy seasons at Serra do Mar State Park, southeastern Brazil.

			Univariate statistics								
– Soil parameters	00	3	P	SL	PC	C	Р	Ρ			
				Seaso	n						
-	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Cover	Season	Cover*Season
Fine-root biomass C	27.0.4.0	20.2.2.4	22.2.0.2	20.2.2.0	24.2.0.5	20 7.4 5	20.0.1.0		F ₃ =1.35;	F₁=1.54;	F ₃ =1.86;
(%)	37.9±1.0	30.3±3.1	33.2±0.2	36.3±2.9	31.3±0.5	36.7±1.5	30.0±1.2	35.9±0.5	P=0.29	P=0.23	P=0.18
Microbial biomass C	1,358.0±	1,238.1±	1,100.0±	945.2±	909.3±	1,139.0±	1,048.8±	960.0±	<i>F</i> ₃ =1.53;	<i>F</i> ₁ =0.24;	<i>F</i> ₃ =0.93;
(µC g⁻¹ soil)	193.7	241.7	48.1	86.4	49.6	134.2	99.1	127.4	<i>P</i> =0.23	<i>P</i> =0.63	<i>P</i> =0.44
Basal respiration		4 7 0 0							<i>F</i> ₃ =0.90;	<i>F</i> ₁ =4.96;	<i>F</i> ₃ =0.44;
(CO ₂ g ⁻¹ soil h ⁻¹)	8.1±1.2	4.7±0.6	6.2±0.9	3.0±0.2	6.9±1.3	10.7±0.2	5.8±0.5	4.3±0.3	<i>P</i> =0.45	<i>P</i> =0.04	<i>P</i> =0.73
~ CO	60.012	2 0 · 0 2h	E C . O 93	2 1 . 0 2h	77.1 59	2 0 . 0 2h	E C . O Oa	2 2 . 0 2h	<i>F</i> ₃ =1.85;	<i>F</i> ₁ =43.01;	<i>F</i> ₃ =0.31;
$q \cup O_2$	6.0±0.1ª	3.9±0.3 ⁵	5.6±0.8ª	3.1±0.3°	/./±1.5ª	3.9±0.3°	5.0±0.2°	3.3±0.2°	<i>P</i> =0.17	<i>P</i> <0.001	<i>P</i> =0.82
Soil temperature	14 1 . O OAB	10 7 . O 2AF			147.028	10.0.0.04	12 7 0 1B	10 2 0 2B	<i>F</i> ₃ =5.30;	<i>F</i> ₁ =0.01;	<i>F</i> ₃ =3.07;
(°C)	14.1±0.0 ^{,18}	10.7±0.2	° 14.0±0.1∾ 18.8±0.1		² 14.7±0.3 ^A	19.0±0.0	13.7±0.19	19.3±0.2°	<i>P</i> <0.01	<i>P</i> =0.93	<i>P</i> =0.05
Soil water content		00.0.0.53		31.3±2.2ª	00 0 . 0 Ch	00 0 . 0 43	117.0 Oh	00.4 . 4.03	<i>F</i> ₃ =3.83;	<i>F</i> 1=24.47;	<i>F</i> ₃ =1.90;
(%)	ZZ.3±Z.2°	∠9.8±3.5ª	∠0.2±3.1°		22.9±3.6°	28.2±0.4ª	$14.7 \pm 0.3^{\circ}$	28.1±1.0ª	<i>P</i> =0.02	<i>P</i> <0.001	<i>P</i> =0.16
¹ Forest-cover types:	OG – Old-g	rowth, PS	L – Post-s	selective lo	ogging, PC	C – Post	clear-cu	t and PP -	Post-past	ure. Uppe	r-case letters

¹Forest-cover types: OG – Old-growth, PSL – Post-selective logging, PCC – Post clear-cut and PP – Post-pasture. Upper-case letters indicate significant differences between forest-cover types and lower-case letters indicate significant differences between seasons (*P* < 0.01), respectively.

When analyzing the correlations of soil parameters with fine roots and AMF traits in the dry season, we observed strong positive correlations of viable AMF spore numbers with extraradical mycelium length (r = 0.95, P < 0.01), and of viable spores with soil GRSP content (r = 0.91, P < 0.01) (Table 3). However, we also found a strong negative correlation between spore numbers and soil pH in the dry season (r = -0.81, P < 0.01) (Table 3). In the rainy season, the strongest positive correlations of viable spores were found with fine-root yield (r = 0.76, P < 0.01), AM root colonization (r = 0.84, P < 0.01) and extraradical mycelium length (r = 0.75, P < 0.01) (Table 4). During this season, the cation exchange capacity (CEC) of the soil was the variable with the highest negative correlation with viable spores (r = -0.74, P < 0.01) (Table 4).

		,	-	2102	_	-	_												
Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. FRP																			
2. FRC	.06																		
3. EML	.54*	25																	
4. VSN	.42	13	.95**																
5. GRSP	.36	20	.92**	.91**															
6. MB	.34	29	.89**	.88**	.95**														
7. MR	.32	46*	.53*	.40	.66**	.70**													
8. MQ	.04	29	31	48*	19	20	.56**												
9. SWC	30	70**	03	07	09	.00	02	03											
10. TMP	27	30	35	53*	38	32	02	.34	.57**										
11. N	.28	.60**	.12	.20	.12	08	31	33	68**	58**									
12. P	.04	.44	.30	.52*	.41	.27	21	59**	22	56*	.67**								
13. K	20	.51*	49*	30	52*	62**	83**	42	15	29	.60**	.51*							
14. Ca	24	.50*	12	.10	10	21	65**	65**	34	62**	.72**	.67**	.80**						
15. Mg	.28	.01	.78**	.89**	.78**	.70**	.12	64**	06	59**	.46*	.77**	.07	.44					
16. S	.45*	35	.02	13	28	19	19	04	.43	.26	29	44	03	26	18				
17. Al	.64**	36	.82**	.71**	.80**	.76**	.63**	01	.14	09	05	.17	54*	42	.55*	.16			
18. pH	40	.35	83**	79**	83**	86**	65**	.10	25	.05	.26	20	.61**	.46*	55*	.04	87**		
19. SB	10	.33	.22	.42	.23	.12	44	74**	25	71**	.70**	.79**	.62**	.93**	.73**	25	10	.13	
20. CEC	.55*	40	.78**	.65**	.70**	.64**	.44	13	.28	.05	05	.15	46*	37	.53*	.28	.95**	79**	07

Table 3 – Pearson correlation coefficients (r) between soil variables collected during the dry season in four forest-cover types in Serra do Mar State Park, southeastern Brazil.

Acronyms mean: FRP - Fine-root production, FRC - Fine-root colonization, EML - Extraradical mycelium length, VSN - Viable spores number, GRSP - Glomalin-related soil protein, MB - Microbial biomass, BR - Basal respiration, MQ - Metabolic quotient, SWC = Soil-water content, TMP - Soil temperature, SB - Sum of bases and CEC - Cation exchange capacity. (*) P < 0.05 and (**) P < 0.01.

Table 4 – Pearson correlation coefficients (r) between soil variables collected during the rainy season in four forest-cover types in Serra do Mar State Park, southeastern Brazil.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. FRP																			
2. FRC	.57**																		
3. EML	.75**	.73**																	
4. VSN	.74**	.80**	.71**																
5. GRSP	.13	.03	36	.18															
6. MB	70**	48*	66**	32	.29														
7. MR	09	26	12	18	.15	.04													
8. MQ	.11	30	02	37	32	53*	.35												
9. SWC	82**	30	65**	44	.09	.74**	29	51*											
10. TMP	.94**	.57**	.68**	.63**	.22	63**	08	.05	71**										
11. N	.48*	.33	.39	.67**	01	.04	52*	32	16	.37									
12. P	.07	.15	12	.28	.50*	.49*	40	59**	.33	.24	.55*								
13. K	.33	.67**	.61**	.56**	29	15	68**	52*	.07	.35	.62**	.43							
14. Ca	.00	.47*	.18	.56**	.11	.37	58**	86**	.44	01	.63**	.61**	.74**						
15. Mg	43	34	60**	17	.33	.76**	47*	47*	.70**	37	.37	.74**	.14	.51*					
16. S	75**	56**	54*	75**	50*	.28	05	.19	.60**	77**	41	37	23	19	.19				
17. Al	23	72**	75**	56*	.40	.29	.07	.33	.19	17	13	.19	56*	42	.50*	.26			
18. pH	.10	.54*	.59**	.51*	37	08	.02	43	05	04	.17	25	.46*	.48*	39	- .10	90**		
19. SB	20	.19	14	.33	.21	.58**	62**	81**	.62**	19	.59**	.72**	.58**	.93**	.78**	- .03	09	.20	
20. CEC	45*	83**	85**	67**	.27	.48*	.13	.27	.35	41	16	.16	58**	36	.56**	.44	.95**	79**	02

Acronyms mean: FRP - Fine-root production, FRC - Fine-root colonization, EML - Extraradical mycelium length, VSN - Viable spores number, GRSP - Glomalin-related soil protein, MB - Microbial biomass, BR - Basal respiration, MQ - Metabolic quotient, SWC - Soil-water content, TMP - Soil temperature, SB - Sum of bases and CEC - Cation exchange capacity. (*) *P* < 0.05. (**) *P* < 0.01.

2.5 Discussion

Our study showed relevant information about the production of fine roots, its associated AMF and soil microbial C. In general, both fine-root production and AMF traits were influenced by forest-cover types indicating that past disturbance (and the intensity of such disturbance) in an ecosystem can alter the belowground interaction and C dynamics over many years. This can be observed by the increased investment in fine roots in those forests with the highest human impact in the past, i.e. post clear-cut and post-pasture. A study on soil C dynamics in a chronosequence of secondary tropical rainforests showed that pasture soils stored 19% more C than early and late successional sites in the top 10 cm of mineral soil, and successional sites stored 14–18% more C in soil than pastures between 10 and 30 cm in depth (Schedlbauer & Kavanagh 2008).

Contrarily to previous studies in Montane Atlantic forests (Sousa Neto et al. 2011; Rosado et al. 2011b), we found that the most productive period of fine roots was in the rainy season, confirming the hypothesis of higher production with greater precipitation, since temperature varies less than precipitation during a single growing season (Wang et al. 2017). Is important to consider that the inferences of fine-root biomass made in the abovementioned studies were based on the biomass stored in the soil and because such biomass is a cumulative response, as there is no way to ensure that the growing season is the same as that when fine-root sampling was made. For this reason, by removing the roots produced at the end of each season, our study can safely confirm that the rainy season is the most productive in that region.

An important issue in studying fine-root production is the adequate period to collect samples in the field. Here, we found that more than 3 months was needed for fine-root sampling, which coincides with contrasting dry and rainy seasons (6 months). The residence time of fine roots in the soil ranges from a few weeks to 1–2 years for first-order roots, and from years to decades for second- and third-order roots (Vargas & Allen 2008). Thus, the observed biomass found here can be considered an accumulated investment. Studies carried out in tropical forests, and specially in Montane forests, should consider the time necessary for fine roots to grow before collecting them (i.e. six months and at the end of the growing season).

Studies on Montane Atlantic Forests have found that there is not a clear pattern of litterfall inputs, but it is known that stocks are high and decomposition coefficients are low (Sousa-Neto et al, 2011, Marchiori et al, 2019). In the dry season, microbial biomass and respiration positively correlated with AMF traits, as there was abundance of spores in soils and extraradical mycelium, indicating that AM symbiosis contributed to microbial soil C dynamics, with drier periods of the year significantly diminishing the activity of soil AMF communities (measured as GRSP content and ERM length), which could reflect their smaller contribution to soil microbial biomass and activity (measured as basal respiration).

In general, forest soils showed a higher metabolic quotient (qCO₂) in the dry season, which indicates more stressing conditions for the soil microbiota, which loses more C through respiration and is less efficient in incorporating C into the microbial biomass (Anderson and Domsch 1993). On the other hand, lower qCO₂ values in the rainy season may indicate higher stability of soil microbiota under those conditions, and consequently, higher rates of litter decomposition, whose nutrients may be readily transported to fine roots by water through the mass flow.

With regard to the AMF symbiosis-related traits, we found no clear differences between changes in these traits and forest-cover types. Other studies, however, reported higher AM colonization in roots of more disturbed plant communities (Aidar et al. 2004; Zangaro et al. 2008). One possible explanation for these contrasting results may be the time during which forests were left to natural restoration. The investigation areas in the abovementioned studies were younger than our areas, so older areas with distinct histories can recover AM traits and respond very much as old-growth forests do. On the other hand, the high resilience of AMF after several forms of disturbance has been shown (Soteras et al. 2014; Carrillo-Saucedo et al. 2018; de León et al. 2018; Mao et al. 2019). For example, in a study comparing mature tropical forests and a forest after slash-and-burn impact, AMF communities were relatively similar, suggesting the AMF capacity to reestablish in roots of new cover plants (de Leon et al. 2018). Here, we can also hypothesize that AMF communities more easily recovered from land-use perturbations than did belowground fine-root C storage, possibly due to the capacity of AM symbiosis to establish in a wide range of plant species present in forest covers. With this respect, it can be suggested that the recovery of belowground forest functions in C storage is mostly dependent on plant restoration levels.

As for AMF intraradical colonization, we found no significant variation in extraradical mycelium length between forest covers, meaning that this trait can also be restored after distinct disturbance types. In the rainy season, even though the length of AMF extraradical mycelium established an inverse correlation with soil-water content (higher in this season), it correlated positively with soil temperature. Usually, soil-water saturation conditions reduce the initiation of root colonization by AMF either directly or indirectly, but once fungi are established in the roots, they are able to maintain and expand within the growing root (Miller & Sharitz 2000).

In addition, due to the fact that AM extraradical mycelium length is more sensitive to soil warming than the intraradical mycelium directly associated with the host plant (Heinemeyer & Fitter 2004), higher temperatures may lead to higher growth and activity of extraradical hyphae with consequent greater flux of photosynthetic C from the host to the soil via AM symbiosis of fine roots, carbon that would be retained in the form of extraradical mycelium or its secreted compounds to the soil, such as GRSP (Gavito et al. 2005; Heinemeyer et al. 2006). This response of AM mycelium to higher temperatures and the higher photosynthetic activity of this season may explain why more mycelia were produced in the rainy season.

Because AMF spores have characteristics that are relatively easily recognized in the taxonomic identification of AMF biodiversity, this component has been found in some previous studies in other Atlantic Forest physiognomies (Aidar et al. 2004; Stürmer et al. 2006; Zangaro et al. 2008; Bonfim et al. 2013). Such studies report an increase in the number of spores in the rainy season the younger the forest is. On the other hand, we found higher viable spore numbers in the dry season, corroborating the common tendency for AMF species to sporulate during the period of lower plant productivity (Bonfim et al. 2013). In addition, plants also have the possibility to sense the efficiency of the symbiosis and to integrate this information with their nutritional status (Gutjahr & Parniske 2017). Thus, under stressful conditions, as in cold and drier seasons in the tropics, the maintenance of active AM symbiosis in plant roots may depend on the amount of nutrients that the fungi deliver to the plants.

Considering that the number of produced spores greatly varies among the AMF species that are present in the community as well as according to their adaptation to environmental and host characteristics (Bonfim et al. 2013), there may be a genetic tendency for species from a population to sporulate more than others in a given period

of the year (Moreira et al. 2009; Oehl et al. 2009). Therefore, we did not observe a clear pattern in the abundance of soil AM spores in the studied forest-cover types. Yet, another possibility is the functioning resulting from the symbiotic partner composition, host plant and fungal species in these forest covers. In the post-pasture forest cover, for example, it was still possible to observe patches of Gramineae among the trees.

GRSP content differed between seasons in almost all forest-cover types, and it was higher in the rainy season, as well as was slightly higher than that observed in other studies in soils from the Atlantic Forest (Bonfim et al. 2013; da Silva et al. 2016). However, there was no significant difference in GRSP content between forest-cover types. Because GRSP is ubiquitous in terrestrial ecosystems with AMF mycorrhizal plants and also a very recalcitrant compound, with a long permanence in the system after its production (Treseder & Turner 2007; Singh et al. 2013), perhaps the types of disturbances (i.e. selective logging, clear-cutting and pasture) that resulted in the current forest cover were not severe enough to affect the soil communities or, if they did, such communities have already recovered.

We identified a high degree of correlation between fine-root biomass and AMF intraradical colonization, extraradical mycelium and abundance of viable spores in the rainy season; however, these AMF traits were not important covariates in fine-root biomass. Some reasons are possible to explain these findings. Firstly, stored nonstructural carbohydrates may be mobilized to support mycorrhizae (Kobe et al. 2010), with no direct effect of fine-root biomass on AMF traits. Secondly, fine-root morphological traits, such as specific root length, specific root area and root-tissue density, may be also more variable than root biomass within seasonal periods and better predictors in relation to fine-root biomass (Rosado et al. 2011b; Chen et al. 2016; Addo-Danso et al. 2020).

AMF traits were closely correlated with climate seasonality as also observed in other Atlantic Forest physiognomies (Aidar et al. 2004; Zangaro et al. 2008; Bonfim et al. 2013). However, the way this correlation happened was not the same for all. For example, we observed a tendency to have greater abundance of spores in the dry season than in the rainy season, while we found the opposite trend in root AM colonization, extraradical mycelium length and GRSP contents. Because AMF spores are resting structures that can survive under adverse environmental conditions, sporulation can be considered a part of the life cycle of the fungi and a strategy of AMF survival until there are favorable conditions to germinate and colonize susceptible roots in these forests (Lenoir et al. 2016).

Overall, the results show a tendency for fine-root biomass to become less conservative as more forest cover changes. Despite controlling fine-root biomass, we did not find the same pattern for almost all AMF traits analyzed. One possibility is that AM communities are more resilient than plant communities and have already recovered from the damage that occurred 50 years ago in these Tropical Montane Forests. In addition, we found a clear effect of climatic variations on both fine roots and AMF traits. Further studies should be conducted in areas with distinct historical uses, so that we can fully understand how land change impacts belowground interactions and how much time belowground components need to restore in Tropical Montane Forests.

2.6 CRediT author statement

Cinthia Silva: Conceptualization, Investigation, Data Curation and Writing - Original draft preparation.: Vinicius Londe: Formal analysis, Writing - Reviewing and Editing.: Sara Andrade: Methodology, Resources, Writing - Reviewing and Editing, Supervision. Carlos Joly.: Resources, Funding acquisition; Simone Vieira.: Conceptualization, Validation, Supervision.

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CHAPTER 3 - FINE ROOT PRODUCTIVITY IN HUMAN-MODIFIED TROPICAL MONTANE FORESTS

3.1 Abstract

Understanding how forests recover and continue providing vital ecosystem services, such as carbon (C) storage, is crucial for conservation actions in an environmentally changing world. Here, we monitored the above- and belowground pools in 50-year-old naturally regenerating and old-growth forests to analyze if the investment in fine roots varied between forest covers, years, and seasons; to identity which soil properties predicted the investment in fine-root biomass; to examine if forests with different anthropogenic impacts prioritized the resource allocation trade-off to wood or fine roots; to analyze whether and how the wood pool affected the fine-root pool. Six areas in the Tropical Montane Atlantic Forest (TMAF) were selected to represent different land uses: three old-growth forests (OG1, OG2 and OG3), one post-selective-logging forest (PSL), one post-clear-cut forest (PCC), and one post-pasture forest (PP). Fine roots were collected over two years (eight seasons) to calculate fine-root biomass (FRB), primary productivity (NPPFR), C stock (CstockFR), and residence time (RTFR). Plants with diameter at breast height \geq 4.8-cm were measured to compute wood primary productivity (NPP_{wood}) and C stock (Cstock_{wood}). We found strong evidence that FRB differs between forest covers and seasons, but not between years. PSL and OG3 produced more FRB, and production was higher in the summer and spring. PSL and PP stocked lower C in fine roots than other forests. Soil moisture and S content better explained NPP_{FR} in the OG forest, while AI, soil moisture, and Mg were the main predictors in PSL. NPP_{FR} was related with decreasing S in PCC and increasing AI and soil temperature in PP. There was a greater investment in NPPFR than in NPPwood in PP and OG3, and a positive relationship between Cstockwood and NPPwood with NPPFR in PSL and PP forests. NPP_{FR} and NPP_{Wood} can be recovered in the long term in degraded areas within forested matrixes, but more time is required to restore C stocked in fine roots. As in temperate forests, FRB varies between seasons, which should be considered when estimating fine-root production in TMAF. Our study contributes to

better understanding the biomass allocation patterns in TMAF, and which soil variables predict FRB production in mature and secondary forests.

Keywords: montane tropical forests, belowground functioning, productivity, allocation

3.2 Introduction

The patterns observed in a current ecosystem usually reflect the diversity of past acclimations and adaptations accumulated by it. Both phenomena, often combined, make it difficult to explore how organisms respond physiologically to a detected change in the environment (Whitehead, 2012). An example is the uptake of resources by vegetation, which influences and is influenced by spatial-temporal conditions (Gea-Izquierdo et al., 2009; Guo et al., 2004). Nutrients, water and light are the three main resources that limit plant growth and promote competition (Craine and Dybzinski, 2013). Plants usually allocate their net primary productivity (NPP) to pools that potentially increase their resource acquisition (Dybzinski et al., 2011). Leaves, stems, branches, fine roots, coarse roots, reproductive organs, root exudates, and root symbionts are examples of such pools (Clark et al., 2001; Malhi et al., 2011). However, canopy (NPP_{Canopy}), wood (NPP_{Wood}), and fine-root productivity (NPP_{FR}) are the main descriptors of forest ecosystems' functioning (Malhi et al., 2011).

NPP_{canopy} estimation usually differs from other components of NPP, since it measures outputs from canopy biomass (e.g., litterfall) rather than direct inputs (Malhi et al., 2011). NPP_{wood} is estimated from stem diameter, and it is generally measured annually at a height of 1.3 m (Malhi et al., 2011). The largest unknown NPP term is the transference of material out of fine roots, the connecting link between the soil and aboveground pools, either through the production of root exudates directly into the soil or as a carbon supply for mycorrhizae (Högberg and Read, 2006).

In the tropics, the variation in carbon allocation to wood and fine roots seems to be the dominant cause of variation in NPP allocation between above- and belowground pools (Malhi et al., 2011). The enhanced allocation of carbon to root systems can be an important mechanism in promoting biomass accumulation during forest growth (Dybzinski et al., 2011; Raich et al., 2014) as well as in plant survival in adverse periods, such as droughts (Metcalfe et al., 2008). If fine roots are prioritized and less involved in trade-offs, environmental conditions and their variations would predict root productivity better than allocation to roots (Malhi et al., 2011), especially in tropical montane forests (TMF), where there is highly variable resource availability, and structural demands imposed by slope, aspect and soil landslide disturbance determine the variation in allometric partitioning without consistent patterns across sites (Huaraca Huasco et al., 2021; Malhi et al., 2011).

In studies in large-elevation ranges of TMF (e.g., \geq 2000 m), NPP_{FR} is usually reported to increase as NPP_{Wood} decreases (Girardin et al., 2010; Leuschner et al., 2007). But it is still unclear whether TMF at lower-altitude ranges (such as Tropical Montane Atlantic Forests - TMAF) respond in the same way (Alves et al., 2010; Vieira et al., 2011). In addition, there is less agreement about the seasonality, location, and duration of enhanced allocation of carbon to root systems in secondary forests (Raich et al., 2014). Hence, the effect of local-species composition on forest carbon balances may entail different growth rates, partitioning of carbon among biomass pools, tissue turnover rates, and tissue chemistry (Russell et al., 2010; Russell and Raich, 2012).

Considering that the allocation of total NPP_{FR} is crucial for understanding how ecosystems will respond to soil disturbance and climatic variables (Green et al., 2005; Huaraca Huasco et al., 2021; Yuan and Chen, 2010), we questioned in this study: which properties drive NPP_{FR} production in TMAF with different past human-modifications and, possibly, different trade-offs? We expected that secondary TMAF established after different land uses may have different environmental conditions driving their NPP_{FR} and resource allocation trade-offs from those in old-growth forests (Raich et al., 2014). The objectives assessed were: (1) to analyze if fine-root dynamics varied among forest cover types of TMAF, years, and seasons; (2) to identify which soil properties (e.g., nutrient availability, temperature, and moisture) drove carbon allocation to NPP_{FR} in the different forest covers; (3) to examine if forests with different past human-modifications prioritized resource allocation trade-offs to wood or fine roots; (4) to analyze whether and how Cstockwood and NPPwood pools affected the NPP_{FR} pool in different forest covers.

3.3 Materials and methods

3.3.1 Study sites

The study was carried out in the protected forest matrix of Serra do Mar State Park in São Paulo state, southeastern Brazil (Figure 1A). The Serra do Mar State Park holds the most well preserved Atlantic Forest remnants along its 332,000 hectares (IBGE, 2012; Oliveira-Filho and Fontes, 2000). Since the protected area's establishment in 1977, sources of land degradation, such as selective logging and pastures, have ceased or reduced substantially (SIMA, 2006; Tabarelli et al., 1993). The disturbance cessation created a mosaic of secondary forests with different land uses in the past: an ideal environment for investigating ecological processes and succession.

The predominant vegetation type at Serra do Mar State Park is Dense Montane Ombrophilous Forest (IBGE, 2012), hereafter Tropical Montane Atlantic Forest (TMAF). However, there is also the presence of taxonomic groups from temperate Austral/Antarctic lineages, such as *Myrceugenia*, *Drimys* and *Weinmannia* (Meireles and Shepherd, 2015; Padgurschi et al., 2011). These plant groups are associated with areas of higher precipitation and lower temperatures rather than tropical evergreen regions and deciduous species (Barros et al., 2022). TMAF holds the highest aboveground biomass along the elevation gradient where it occurs (Alves et al., 2010). Carbon stocked in TMAF conserved soils may surpass that of Amazonian forests, and little carbon is lost to water leach (Andrade et al., 2011; Vieira et al., 2011).

We selected six sites to investigate above- and belowground biomass, but the data used varied according to the study's objective. These study sites have a permanent 1-hectare plot installed (a grid of 100 m x 100 m), where long-term ecological research has been carried out (Joly et al., 2012; Marchiori et al., 2016). Each 1-ha plot is divided into 100 subplots of 100 m² each (10 m x 10 m). In general, we were interested in representing four forest cover types (our sample unit) with no or different land uses in the past. The land uses affected the characteristics of the original forest cover, which we consider here as forest cover types defined as follows: (1) old-growth forests [site 1 (OG1; 1066 m a.s.l.; n = 1), site 2 (OG2; 1051 m a.s.l.; n = 1), and site 3 (OG3; 1045 m a.s.l.; n = 1)], forests without evidence of human impacts in

the last 50 years; (2) post-selective-logging forest (PSL; 1025 m a.s.l; n = 1), characterized by the irregular extraction of large-diameter stems (DBH > 50 cm) in the 1970s, and from low to medium human impact; (3) post-clear-cut forest (PCC; 1020 m a.s.l.; n = 1), a secondary forest growing naturally in the last 50 years after a total cut of tree individuals, and thus, classified as a tree community after medium to high human impact; (4) post-pasture forest (PP; 1148 m a.s.l.; n = 1), a secondary forest under natural regeneration for 50 years after forest cover conversion by clear-cut followed by pasture implementation (high human impact). All forest cover types are included in an old-growth forest matrix, and the maximum distance between them was approximately 13 km (Figure 1B).



Figure 1. The study sites are in the Serra do Mar State Park, São Paulo State, southeastern Brazil (**A**). Six forest stands with distinct levels of human impact in the past were the objects of study: Old-growth are mature forests [sites 1 (OG1), 2 (OG2), and 3 (OG3)]; Post-selective logging (PSL) had irregular extraction of large-diameter stems; Post clear-cut (PCC) had all trees removed; Post-pasture (PP) was an area used for livestock and then abandoned for natural regeneration (**B**). The level of human impact can be considered from low (OG) to high (PP). Map information: Coordinate System: GCS SIRGAS 2000; DATUM: SIRGAS 2000; Units: Degree; Satellite imagery (B): Google Earth®; Imagery date: 7/21/2020; Author: VLonde.

Soils in the study areas are classified as Inceptisols (Typic Dystrudepts), with medium texture and shallow depth (Martins et al., 2015; SIMA, 2006). The regional

climate is Cwa (Humid subtropical zone with dry winters and hot summers), according to Köppen's classification (Alvares et al., 2013). The average annual temperature was around 17 °C in the study period (from July 2015 to June 2017): monthly averages ranged from a minimum of 13 °C in July to a maximum of 20 °C in January. The annual precipitation was close to 1,600 mm year⁻¹, a low record in relation to that in previous studies in the same areas (Bonfim et al., 2013; Sousa Neto et al., 2011). Specifically, the precipitation was around 310 mm in January 2016 and 2.2 mm in July 2016. Climate data were obtained from weather stations installed close to the study region (as provided by the Climate and Biosphere Laboratory, DCA/IAG, University of São Paulo, and São Paulo State Forestry Institute).

3.3.2 Fine-root productivity

Fine-root biomass productivity was estimated through the ingrowth core method (Hendricks et al., 2006; Steingrobe et al., 2001), following the Global Ecosystem Monitoring (GEM) protocol (Marthews et al., 2014). Sixteen holes, measuring approximately 14 cm in diameter and 30 cm in depth (96 in total), were dug in each site using a post hole digger. Such holes were opened 30 m away from each other and were distributed in a systematic design into the 1-hectare plot. To avoid the transference of soil with high nutrient content from the top to the bottom layers (Aragão et al., 2009), we kept each soil sample on separated trays and identified them as 0-10 cm, 10-20 cm, and 20-30 cm soil depths.

In order to quantify the carbon present in the fine-root biomass stock (Cstock_{FR}, Mg C ha⁻¹), we manually collected fine roots (< 2 mm) from each tray according to the temporal prediction method (Metcalfe et al., 2007; Silva et al., 2022). We stored the roots in identified paper bags and sent them to the Laboratory of Ecophysiology, Department of Plant Biology at the University of Campinas. Live and (occasionally) dead roots were carefully washed, oven-dried until reaching constant weight, and weighed on a precision scale. Then, we converted the dry mass into carbon units by their specific carbon content (Silva et al., 2020).

To monitor the fine-root net primary productivity (NPP_{FR}) over time, we inserted 1-cm mesh plastic netting into each open hole and filled it with its own root-free soil. At the end of each season (i.e., September, December, March, and June), we collected the fine roots that had grown inside the plastic mesh repeating the same fine root removal method used during the quantification of Cstock_{FR}. We evaluated the fine-root biomass production for two consecutive years (from July 2015 to June 2017) comprising all four seasons twice, since response time varies between forest cover types. To assess the carbon content (Mg C ha⁻¹ year⁻¹), we also converted the dry mass into carbon units by their specific carbon content (Silva et al., 2020). For the purpose of forest cover characterization, we estimated fine-root carbon residence time (RT_{FR}) by dividing Cstock_{FR} by the annual NPP_{FR} (Girardin et al., 2010).

3.3.3 Soil properties

Soil chemistry for the first 30-cm depth in OG1 and PSL forests were obtained from a previous study (Martins et al., 2015). To characterize the soil chemistry in the PCC and PP areas, 32 soil samples (16 per site) were removed in a systematic design following the same sampling procedures and soil depth used by Martins et al. (2015). As the sampling design was the same for fine roots and soil chemistry, the soil samples were collected within a maximum radius of 10 m from each ingrowth core. Soil samples were sent for chemical analyses at the Department of Soil Science, School of Agriculture "Luiz de Queiroz", University of São Paulo (ESALQ/USP). There, the potential of hydrogen (pH) was measured in 0.01 M CaCl₂ with a soil-solution ratio of 1:2.5 (m/v); total aluminum (AI, mg kg⁻¹) by means of extraction using a KCI 1 N solution and titration with 0.05 mol L⁻¹ NaOH in the presence of bromothymol blue; potential acidity (PA, H+AI), by means of pH; total potassium (K, mg/kg⁻¹), total sodium (Na, mg/ kg⁻¹), total calcium (Ca, mg kg⁻¹) and total magnesium (Mg, mg kg⁻¹) with extraction of elements by ion-exchange resins and determined by absorption spectrophotometry (Ca and Mg) and flame photometry (K and Na); and total phosphorus (P-resin, mg kg⁻ ¹) by water extraction using anion-exchange resins and quantification by colorimetry. Total sulphur (S, mg kg⁻¹) was determined by turbidimetry and extraction with 0.01 mol L⁻¹ calcium phosphate.

The sum-of-bases (SB, mmolc kg⁻¹) was obtained by the sum of exchangeable cations (Ca²⁺ + Mg²⁺ + K⁺ + Na⁺). The cation exchange capacity (CEC, mmol_c kg⁻¹) was determined by the compulsive exchange method using a BaCl₂ solution (Gillman, 1979). The soil base saturation (BS, %), which represents the percentage of CEC

occupied by bases (Ca²⁺, Mg²⁺, K⁺, and Na⁺), was calculated as the ratio between 100×SB and CEC. Aluminum saturation (AS, %) represents the percentage of effective CEC that is occupied by AI. Total nitrogen (N, %) concentrations were determined by a Carlo Erba elemental analyzer (FlashEA1112) at the Center of Nuclear Energy in Agriculture, University of São Paulo (CENA/USP). The content of soil organic matter (OM, g kg⁻¹) was determined by the Walkley–Black wet combustion method (Raij et al., 2001).

To complement the database, we monitored the soil temperature and soil water content monthly, from July 2015 to June 2017, to estimate the mean annual soil temperature (MAST, °C) and the mean annual soil moisture (MASM, %) next to the ingrowth cores, totalizing twenty-four records per forest. The measurements were taken using a soil temperature probe (STP-2, PP Systems) and a soil moisture monitor (HydroSense II, Campbell Scientific).

3.3.4 Woody biomass production

The wood net primary productivity (NPP_{wood}) was estimated considering the wood biomass production quantified through repeated censuses from November 2015 to March 2016 and from November 2016 to March 2017. Tree, palm and tree-fern diameters, and wood newly recruited in 96 plots (20 x 20 m; 400 m² each) were used to monitor the forest growth in the vicinity of the ingrowth cores installed in the soil. The diameter of all individuals at 1.3-m breast height \geq 4.8 cm were measured, including trees, tree palms, and ferns. Tree height was estimated using a specific allometric equation adjusted to the Montane Atlantic Forest (Scaranello et al., 2012).

Wood density for species was consulted on the Global Wood Density Database (Chave et al., 2009). We used the genus or family wood density when the information for a species was not found. If no information was available, the average wood density of the 400-m² plot was used. We estimated the wood stock (Mg ha⁻¹) for each plot through allometric equations for trees, palm trees, and ferns (Chave et al., 2014; Hughes et al., 1999). To assess the biomass-stocked C (Cstock_{Wood}; Mg C ha⁻¹), we multiplied the stocked biomass by 45.2%, which is the specific C concentration for TMAF (Vieira et al., 2011). Wood productivity (NPP_{Wood}; Mg C ha⁻¹ year⁻¹) was

estimated by subtracting the Cstock_{Wood} of the surviving trees in the second census from that in the first census, and then, dividing by the time interval.

3.3.5 Data analysis

We submitted the data collected over two years (from September 2015 to June 2017) to a three-way repeated measures ANOVA to check if the fine-root biomass differed among forest cover types (OG1, OG2, OG3, PSL, PCC and PP), between years (1 and 2), and among seasons (winter, spring, summer, and autumn) (objective 1). We transformed the Cstock_{FR}, NPP_{FR}, and RT_{FR} data into Log₁₀+1 to reach the assumptions of normality, sphericity, and no significant outliers. Post-hoc tests included a one-way model at each factor plus multiple pairwise comparisons (Tukey HSD; $\alpha = 0.05$) to identify which pairs of variables differed. All computations were performed in the R package *rstatix* (Kassambara, 2021).

Previously to the identification of the possible causal relations among soil drivers and NPP_{FR} in different forest covers, we performed one-way ANOVAs followed up by Tukey post-hoc tests to understand the nutritional status of the soil forests. To identify which soil properties affected NPP_{FR} (objective 2), we performed multiple linear regressions combined with the backward elimination process (Lilja, 2016) for each forest cover type. For this propose, we checked the assumption of linearity through a scatterplot matrix, homoscedasticity by plotting the predicted values and residuals on a scatterplot, and normality through Shapiro-Wilk tests and normal Q-Q plots. Moreover, significant outliers were checked through Cook's Distance, independence of residuals, and multicollinearity through the variance inflation factor (VIF < 2.5) (Johnston et al., 2018). Due to the dependence between pH, SB, CEC, BS, AS, PA, OM, and soil nutrients, we decided not to add them into the modelling. MAST, MASM, N, P, K, Ca, Mg, AI, and S were used as NPP_{FR} predictors.

We used the NPP_{FR} data transformed in Log₁₀+1 to reach the assumptions of multivariate normality, homoscedasticity, linearity, independence, and randomness. To examine if forests with different land uses in the past prioritized the investment aboveor belowground (objective 3), we performed a multivariate analysis of variance (oneway MANOVA) using the data collected in year 2. Data of only one year was used because biomass production was similar between years. The normality assumption was checked through the Shapiro-Wilk test and normal Q-Q plots (for each dependent variable), and homogeneity of variance-covariance matrices through the Box's M test. We used Tukey HSD for post-hoc multiple comparisons ($\alpha = 0.05$). The relationships between above- (Cstock_{Wood} and NPP_{Wood}) and belowground investment (NPP_{FR}) (objective 4) was examined through simple linear regressions for each forest cover. Results were graphically represented using the *ggplot2* package, and all analyses were conducted in the R environment (R Core Team, 2020; Wickham et al., 2016).

3.4 Results

We found high variability in Cstock_{FR}, NPP_{FR}, and RT_{FR} across the four forest cover types analyzed. PSL and PP had the lowest mean Cstock_{FR} and were similar to each other, while OG and PCC had the highest means ($F_{(5, 90)} = 6.78$; P < 0.001) (Fig. 2A). When we analyzed the annual NPP_{FR} variation, we found similar productivity between almost all forest covers (1.54 ± 0.19 to 2.09 ± 0.16 Mg C ha⁻¹ year⁻¹), except for the high productivity in the PP forest (3.17 ± 0.31 Mg C ha⁻¹ year⁻¹) ($F_{(5, 90)} = 6.56$; P < 0.001) (Fig. 2B), which implied a lower RT_{FR} (0.24±0.03 year) than that in other forest covers (0.40 ± 0.03 to 0.84 ± 0.18 year) ($F_{(5, 90)} = 7.92$; P < 0.0001) (Fig. 2C).



Figure 2. Variation of (**A**) fine-root C stock (Cstock_{FR}), (**B**) fine-root net primary productivity (NPP_{FR}), and (**C**) fine-root residence time (RT_{FR}) in six Tropical Montane Atlantic Forests with four cover types (n = 16). Lower-case letters indicate significant differences between sites. Forest cover types: OG = old-growth [sites 1 (OG1), 2 (OG2)]

and 3 (OG3)], PSL = post-selective logging, PCC = post-clear-cut, and PP = postpasture.

We found differences in fine-root biomass between forest cover types ($F_{(5, 15)} =$ 18.97; P < 0.0001) and seasons ($F_{(3, 15)} = 37.46$; P < 0.0001), but not between the monitoring years ($F_{(1, 15)} = 0.12$; P = 0.73). In general, FRB was mostly produced in spring and summer seasons when compared to autumn and winter ones (Fig. 3). Also, we found significant interactions between forest cover types and the monitoring year ($F_{(5, 15)} = 6.06$; P < 0.0001), the monitoring year and seasons ($F_{(3, 15)} = 23.61$; P < 0.0001), and seasons and forest cover types ($F_{(15, 15)} = 3.44$; P < 0.0001). There was no interaction between the three factors together, i.e., forest cover types, monitoring year and season ($F_{(15, 15)} = 0.76$, P = 0.72).



Monitoring 🚍 Year 1 🛱 Year 2

Figure 3. Fine-root biomass production (FRB) over seasons in four forest cover types of Tropical Montane Atlantic Forest monitored for two years (n = 128). Uppercase letters indicate significant differences between sites, lowercase Greek and Roman letters indicate, respectively, significant differences between seasons in the first and in the second monitoring years, and an asterisk (*) indicates differences within seasons. Forest cover types: OG = old-

growth [sites 1 (OG1), 2 (OG2) and 3 (OG3)], PSL = post-selective logging, PCC = post-clearcut and PP = post-pasture.

In general, no soil chemistry patterns were found for the forest cover types studied (Table 1). In the OG1 forest, we observed the highest contents of N, Mg, and AI. Consequently, OM, CEC, and PA were also higher in OG1 than in the other forest types. In the PSL forest, we found one of the most S soil contents, and this may have contributed to the SB observed in this site (such as from PP and OG). AS was higher in the PCC forest than in others but had similar PA and pH. Also, PCC and PP had the highest K content. Still, we observed the highest MAST, Ca, and BS content in the PP forest.

MASM and S were the soil predictors that contributed most to NPP_{FR} in the OG1 forest ($F_{(2, 13)} = 4.04$; P = 0.04; $R^2_{adj} = 0.29$) (Fig. 4A and 4B). In the PSL forest, Al, MASM, and Mg together better explained changes in NPP_{FR} ($F_{(3, 12)} = 22.38$; P < 0.0001; $R^2_{adj} = 0.81$). While an increase in Al content affected NPP_{FR} positively, an increase in MASM and Mg affected it negatively (Fig. 4C-E). In PCC, S was the only variable affecting (negatively) NPP_{FR} ($F_{(1, 14)} = 4.69$; P = 0.05; $R^2_{adj} = 0.20$) (Fig. 4F). In the PP forest, an increase in NPP_{FR} was positively associated with Al and MAST ($F_{(2, 13)} = 11.49$; p = 0.001; $R^2_{adj} = 0.58$) (Fig. 4G and 4H).

Variables		F _{3,60}	Р			
	OG1	PSL	PCC	PP		
MAST (°C)	15.87±0.09 ^b	15.84±0.06 ^b	16.06±0.07 ^b	16.67±0.16ª	14.19	<0.0001
MASM (%)	31.43±2.41	30.80±1.46	26.66±1.20	29.29±0.73	1.55	0.21
рН	3.64 ± 0.04^{b}	3.76 ± 0.03^{ab}	3.88 ± 0.02^{a}	3.86 ± 0.06^{ab}	6.64	0.001
P (mg kg ⁻¹)	13.17±2.93 ^{ab}	9.11±1.30 ^b	13.95±0.57ª	14.70±1.18ª	5.59	0.002
K (mg kg ⁻¹)	49.00±4.60 ^b	49.80±3.99 ^b	53.08±2.33 ^{ab}	71.21±8.31ª	3.91	0.01
Ca (mg kg ⁻¹)	65.16±15.76 ^{bc}	64.54±8.42 ^b	28.51±4.73°	140.04± 26.22 ^a	8.55	0.0001
Mg (mg kg ⁻¹)	40.25±5.83 ^a	24.30±3.33 ^b	19.78±2.53 [♭]	28.44±2.45 ^{ab}	5.36	<0.01
AI (mg kg ⁻¹)	229.50± 5.21ª	176.34±10.25 ^b	175.64±0.96 ^b	172.22±8.62 ^b	4.31	0.01
S (mg kg ⁻¹)	33.63±6.64 ^{ab}	31.03±3.78 ^a	23.63±1.17 ^{ab}	16.60±1.59 ^b	3.08	0.03
SB (mmol _c kg ⁻¹)	8.90±1.35ª	7.26±0.61 ^a	3.39±0.47 ^b	11.05±1.34 ^a	17.61	<0.0001
CEC (mmol _c kg ⁻¹)	139.66±11.33ª	103.01±6.13 ^b	109.04±6.46 ^{ab}	97.28±6.31 ^b	5.31	<0.01
BS (%)	6.08±0.77 ^b	6.80±0.53 ^b	3.52±0.56°	12.77±1.77ª	18.5	<0.0001
AS (%)	76.68±1.85 ^b	74.69±1.17 ^{bc}	85.73±1.97ª	64.30±4.06°	15.12	<0.0001
N (%)	0.55 ± 0.04^{a}	0.35±0.02 ^b	0.22±0.02 ^c	0.23±0.01°	35.95	<0.0001
PA (mmol₀ kg⁻¹)	130.77±10.42 ^a	95.75±6.03 ^b	105.66±6.61 ^{ab}	86.23±6.78 ^b	6.33	0.001
OM (g kg ⁻¹)	127.75±9.71ª	78.43±5.78 ^b	49.03±2.01°	58.35±3.86°	40.95	<0.0001

Table 1. Soil properties analyzed in the 0-30 cm layer in an old-growth forest (OG1), post-selective-logging forest (PSL), post-clearcut forest (PCC) and post-pasture forest (PP) of the montane Atlantic Forest. Different letters indicate differences between areas.

(1) mean of 16 replications ± SE per site; MAST (mean annual soil temperature); MASM (mean annual soil moisture); SB (sum of bases); BS (base saturation); AS (aluminum saturation); PA (potential acidity); OM (organic matter); mmol_c kg⁻¹: millimoles of charge per kg of dry soil.


Figure 4. Fine-root productivity (NPP_{FR}) as a function of soil properties in different forest cover types of Montane Atlantic Forest. Mean annual soil moisture (MASM) and Sulphur (S) were the main soil drivers in the old-growth forest (**A**, **B**). Aluminum (AI), MASM, and Magnesium (Mg) affected NPP_{FR} in the post-selective-logging forest (PSL) (**C–E**). Sulphur negatively affected NPP_{FR} in the post-clear-cut forest (PCC) (**F**) and AI and the mean annual soil temperature were the main soil properties found in the post-pasture forest (PP) (**G**, **H**). Dashed lines show linear trends for significant relationships. The shaded area represents the 95% confidence interval (n = 16).

Both, NPP_{wood} and NPP_{FR}, differed between forest cover types ($F_{(10, 180)} = 4.78$; P < 0.0005), and except for OG3 and PP, C allocation to wood and fine roots was similar within areas (Fig. 5). Moreover, we found that Cstock_{wood} and NPP_{wood} were positively associated with NPP_{FR} only in the PSL and PP forests, respectively (Fig. 6 and Table S1 — Appendix C).



Figure 5. Above- (wood) and belowground (fine-root) investment of forests with different land uses in the past: old-growth (OG), post-selective-logging (PSL), post-clear-cut (PCC), and post-pasture (PP). Uppercase letters indicate significant differences between sites and lowercase letters indicate significant differences within sites (pools). NPP = net primary productivity.



Figure 6. Fine-root net primary productivity (NPP_{FR}) as a function of wood carbon stock (Cstock_{Wood}) and wood net primary productivity (NPP_{Wood}) in a post-selective-logging (**A**) and post-pasture forest (**B**) in the Montane Atlantic Forest at Serra do Mar State Park, Brazil. Lines show linear trends for significant relations. The shaded area represents the 95% confidence interval, n = 16.

3.5 Discussion

3.5.1 NPPFR over seasons and four forest cover types

Studies of the belowground dynamics in tropical forests have gained much attention in the last few decades (Girardin et al., 2013; Huaraca Huasco et al., 2021; Leuschner et al., 2009), and our work sheds light on how NPP_{FR} may vary in different land uses, seasons and edaphic conditions in montane forests. Contrarily to what we expected, the carbon stocked in fine roots did not follow a degradation continuum depending on the intensity of the anthropic impact suffered in the past (i.e., from none (OG1) to high (PP)). The PSL and PP forests had similar Cstock_{FR}, and PCC was similar to the OG forests, thus indicating that sites with different uses in the past could

stock as much C as mature forests after 50 years of natural regeneration. As many sites in our study, NPP_{FR} was similar between forest cover types in a disturbance gradient in Malaysia (Riutta et al., 2018). The authors speculate that a growth surge may have caused increased demand for nutrients, which can be a plausible explanation for the NPP_{FR} investment in OG3, PCC, and PP forests.

The RT_{FR} variation found in TMAF is below those found at the same elevation range in Ecuadorian and Andean TMF, respectively (Girardin et al., 2013; Graefe et al., 2008). Considering that soil temperature has been associated as one of the most important variables that explain root turnover patterns (Cusack et al., 2010; McCormack and Guo, 2014), it may be useful to understand RT_{FR}. For example, an increase in temperature is related with an increase in maintenance respiration, higher rates of nutrient mineralization and increased pathogen and herbivore activity, which should decrease root lifespan (Gill and Jackson, 2000). Thus, even in forests belonging to the same altitudinal range, as the soil temperature increases (e.g., due to past human modifications), RT_{FR} also decreases, requiring constant NPP_{FR} so that the plant's soil resource acquisition can be maintained. Indeed, NPP_{FR} was positively associated with soil temperature in PP, the forest cover that invested most in FRB production.

Although seasonal FRB production may be species-specific (Förster et al., 2021; Meinen et al., 2009), we observed that, in the winter and summer, there was similar fine-root production in all forest cover types. Evidence of differences in FRB production was found, especially, in the autumn and spring. A previous study in TMAF found that, during the rainy season (spring and summer), fine roots had larger live mass than dead mass; contrarily, in the dry season (autumn and winter), there was no significant difference between live and dead mass (Sousa Neto et al., 2010). Together, this result and ours, may indicate that, TMAF, similarly to temperate or boreal forests, are "summer-active" ecosystems where, in "regular" years, physiological activity ramps up in spring, levels off in early summer, and begins to taper off by mid-summer before rapidly declining with autumn senescence (Richardson et al., 2010).

It is also important to highlight the greater susceptibility found in this study of modified forests to seasonal variations between years. While in unmodified or little modified forests a season of greater variability in FRB production is followed by the reestablishment of such production in the next (and favorable) season, in modified forests the reestablishment of FRB production seems to take longer. In these secondary forests, fast-growing species have probably been favored, as they are more efficient in absorbing water and nutrients (Rozendaal et al., 2019; Siminski et al., 2021). They have a high return in terms of carbon gain, but at the cost of a riskier behavior (a lower hydraulic safety margin) compared to slow-growing species (Oliveira et al., 2021). Thus, unless these species can make ecophysiological adjustments for more conservative water use – as previous studies have identified in TMAF (Eller et al., 2013; Fauset et al., 2018; Rosado et al., 2016) – they are likely to be more exposed to water deficits in hot and/or dry weather.

3.5.2 NPPFR as a function of soil properties

When we specifically consider the soils on which tropical forests have grown, they are predominantly acidic, due to abundant precipitation, leaching of bases or biological processes driven by nitrification, OM decomposition, dissociation of organic acids, and the excessive uptake of cations over anions by plants (Binkley and Richter, 1987). Here, we could observe high Al content and low pH in the OG1 forest, but concomitantly, it also had the highest soil OM. The main variables affecting NPP_{FR} in this site – MASM (negatively) and S (marginally positive) – are probably the result of OM influences. Because soil OM is essential for retaining nutrients and water in sandy soils as well as negatively related to their degree of compaction (Grigal and Vance, 2000), it could mitigate the soil's adverse conditions to NPP_{FR}.

Globally, it is established that forest productivity tends to increase with increased temperature and precipitation (Grigal and Vance, 2000). Especially in cloud accumulation stands, soil moisture is typically high, and water logging may persist for extended periods (Eller et al., 2016; Graefe et al., 2008). Although the forest community may increase its NPP_{FR} in the rainy season, excessive soil moisture can negatively affect the root system, as found in the OG1 and PSL sites. Water saturates the soil pores and hinders gas exchange, root respiration and nutrient uptake (Pan et al., 2021), thus increasing stress and pressures from pathogens (Jackson and Colmer, 2005; Leppälammi-Kujansuu et al., 2014; McCormack and Guo, 2014).

Considering the context of forests, because a major part of the macronutrient in soils is in organic combinations (Binkley and Fisher, 2012; Schoenau and Malhi, 2015),

in order to be useful to trees, they must be converted to inorganic forms by biochemical or microbiological mineralization (Castellano and Dick, 1991). Due to the negative charge of those mineralized compounds, forms such as sulfates (SO_4^{2-}) could remain in the soil solution. Thus, they could probably be readily leached from upper (and drier) to lower (and moister) slope positions in OG1 and PCC forests. As the variable analyzed in this study considers the total S in the soil, we presume that the marginally inverse effect of that nutrient on NPP_{FR} in the two mentioned forests is related to possible differences in the mineralization rates of OM and, consequently, to the availability of plant-assimilable forms to satisfy the vegetation needs, as found in a TMF in Ecuador (Wilcke et al., 2002).

Acidic and weathered soils also tend to be Mg deficient (Gransee and Führs, 2013; Guo et al., 2016). This nutrient competes with Al for binding sites from the root's membrane, increasing Al-toxicity tolerance and promoting root growth (Li et al., 2020). In the PSL forest, we suspect that the negative relation observed between total soil Mg content and NPP_{FR} is not causal, probably because Al is binding more sites from the root's membrane than Mg. Thus, even if the amount of Mg increases in some regions, the effect of Al on the roots may be predominating, thus decreasing RT_{FR} in this forest. Once an Mg-deficient condition is established, the plant's oxidative stress increases, thus impairing carbon gain and decreasing biomass, due to its effects on night-time transpiration, respiration, or root exudation (Tränkner et al., 2016). If we also consider the negative effect of MASM, the result is a lower NPP_{FR} as compared to other forests.

Not even at present is there consensus on how Al influences root development in acidic soils (Bojórquez-Quintal et al., 2017; Osaki et al., 1997; Rehmus et al., 2014; Wu et al., 2018). Here, we have seen that in forests where there is lower RT_{FR}, such as in PSL and PP, NPP_{FR} is positively associated with Al content. In temperate forests, some studies have observed that low pH associated with high concentrations of free Al may reduce root longevity (Godbold et al., 2003; Raynal et al., 1990). While in the PSL forest we observed that MASM and Mg negatively affect NPP_{FR}, in the PP forest, MAST contributes positively to NPP_{FR}, and this may justify the contrasting productivity found in these forests.

3.5.3 Wood and fine-root investment

In general, the soil environment is extremely complex, and a variety of external factors may be modifying the roots' dynamics and how the shoot-to-root growth ratio varies among plant species during plant ontogenesis (Lynch et al., 2011). In the last few decades, several studies have sought to understand whether there is an internal pattern of below- and aboveground carbon investment in forest ecosystems (Aragão et al., 2009; Girardin et al., 2010; Malhi et al., 2011; Raich et al., 2014).

Here, we have observed that some TMAF seems to follow the resource allocation trade-offs of some montane Andean and old Hawaiian tropical forests (Malhi et al., 2011), where there is higher investment in NPP_{FR} than in NPP_{Wood}. In a disturbance gradient of Indonesian TMF, it was observed that the fractional canopy cover was the most important factor influencing the FRB and necromass of fine roots (Leuschner et al., 2006). In accordance with that study, a reduction in canopy cover from 90% to 75% was associated with a reduction in FRB by approximately 45%. Thus, although the OG3 and PP forests have had different disturbance histories, the canopy of both may have some similarities, contributing to greater C investment in below- than aboveground.

As a positive feedback, it is known that as litter quality declines (due to decreased soil fertility), SOM accumulates, nutrients are immobilized, and biomass allocation may shift from aboveground tissues to fine roots and mycorrhizas (Grigal and Vance, 2000). In this reasoning logic, the C allocation pattern found in OG3 and PP forests would be consistent with flexible and not fixed C allocation patterns (Raich et al., 2014). We agree that further studies considering canopy production (and its nutrient composition), canopy openings, as well as individuals' access to light can contribute to a better elucidation of how C allocation works in these forests.

3.5.4 NPPFR as a function of Cstockwood and NPPwood

We observed a positive effect of $Cstock_{Wood}$ on NPP_{FR} in the PSL forest while, in the PP forest, we found a positive effect of NPP_{Wood}. These positive trends are in agreement with those reported by a study on mature forests and native tree plantations performed in Costa Rica (Raich et al., 2014), in which a significantly positive relationship between below-ground C allocation and aboveground tree growth was found. In the PSL forest, there are individuals with DBH > 50 cm and slow growth, and this characteristic may be indicative that C allocation is determined by changes occurring over a long time (decades or more). In PP forests, due to competition with grasses, the establishment of tree seedlings may be hindered (Zanini et al., 2021). Thus, as in diversity-disturbance relationships (Bohn et al., 2014; Oliveira et al., 2021), the r-selection may favor fast-growth and generalist colonizing species (in less populated areas), while the k-selection, may foster slow-growth and specific-competitor species (in the most populated areas). In this site, carbon allocation would be determined by changes in a short time (years).

Recent studies estimate that there is about 28% of the native vegetation cover for the Atlantic Forest, of which 26% is exclusive to forest formations (Rezende et al, 2018). These forests have been facing non-linear changes over time, either in terms of forest loss and/or gain, as well as in the landscape structure (Costa et al, 2017). In light of this study, we can conclude that even disturbances considered of low intensity (such as selective cutting, for example) affect the interaction between plants and soil. In addition, we observed that old-growth forests tend to function more conservatively, while secondary forests tend to be more sensitive to changes in resource availability and soil conditions, presenting a great variability in their responses. We could observe the same trade-offs in vegetations with different histories (e.g. old-growth and postpasture forests), as well as the significant effect of stock and aboveground productivity on fine root productivity only in a few specific situations. In this context, in addition to quantification, understanding, maintaining, and promoting the proper functioning of vegetation become important ways to ensure the quality of ecosystem services that we want these forests to provide us.

3.6 Acknowledgements

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GENERAL CONCLUSION

In **Chapter 1**, we advanced in some points of knowledge. Hence, we believe that we have contributed to the applicability of the temporal prediction method in the Tropical Montane Atlantic Forest. First, we found that Weibull is a flexible model and can accurately estimate fine-root mass at 120 min in different ecosystems. This discovery was important because a reduction in collection time does not compromise fine-root biomass estimation using the temporal prediction method. These results are encouraging because by reducing the time spent to remove roots from each soil core, researchers can increase the number of soil cores extracted per study site and better characterize it.

Overall, in **Chapter 2**, we found a tendency for fine-root biomass to become less conservative as forest cover changes from old-growth to disturbed status. Despite controlling fine-root biomass, we did not find the same pattern for almost all arbuscular mycorrhizal (AM) traits analyzed. One possibility is that AM communities are more resilient than plant communities and have already recovered from the damage that occurred 50 years ago in these Tropical Montane Forests. In addition, we found a clear effect of climatic variations on both fine roots and AM traits. Further studies should be conducted in areas with distinct historical uses, so that we can fully understand how land use change affects belowground interactions and how much time belowground components need to be restored in Tropical Montane Forests.

When we considered the natural restoration of Tropical Montane Atlantic Forests in the **Chapter 3**, we highlighted similarities and differences in productivity recovery trajectories. We found different fine-root productivity between forest cover types and seasons, but not between years. We also observed that more fine roots are produced in the summer and spring than in the autumn and winter. Furthermore, we noted that soil moisture and S content, together, explain the fine-root production in the old-growth forest (OG). In the post-selective-logging forest (PSL), the main predictors were soil moisture, Mg and Al. The post-clear-cut forest (PCC) was predicted only by S, and in the post-pasture forest (PP), soil temperature and Al were positively associated with fine-root productivity. In general, the investment in biomass was higher

in below- than in aboveground pool in OG3 and PP forests, being that aboveground components (stock and wood productivity) had a positive effect on belowground productivity in the PSL and PP forests, consecutively. We think that such findings concerning how these productivity allocation patterns happen in the Tropical Montane Atlantic Forest as well as the main edaphic factors that regulate it may be significant for the functionality conservation and restoration strategies of those ecosystems, especially those affected by land use changes.

REFERENCES

- Addo-Danso, S.D., Defrenne, C.E., McCormack, M.L., Ostonen, I., Addo-Danso, A.,
 Foli, E.G., Borden, K.A., Isaac, M.E., & Prescott, C.E. 2020. Fine-root
 morphological trait variation in tropical forest ecosystems: an evidence synthesis.
 Plant Ecology 221:.
- Addo-Danso, S.D., Prescott, C.E., & Smith, A.R. 2016. Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: A review. Forest Ecology and Management 359: 332–351.
- Aidar, M.P.M., Carrenho, R., & Joly, C.A. 2004. Aspects of arbuscular mycorrhizal fungi in an atlantic forest chronosequence parque estadual turístico do Alto Ribeira (petar), SP. *Biota Neotropica* 4: 1–13.
- Alef, K., & Nannipieri, P. 1995. *Methods in applied soil microbiology and biochemistry*. London.
- Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L., & Sparovek,
 G. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711–728.
- Alves, L.F., Vieira, S. a., Scaranello, M. a., Camargo, P.B., Santos, F.A.M., Joly, C. a., & Martinelli, L. a. 2010. Forest structure and live aboveground biomass variation along an elevational gradient of tropical Atlantic moist forest (Brazil). *Forest Ecology and Management* 260: 679–691.
- Anderson, T.H., & Domsch, K.H. 1993. he metabolic quotient for CO2 (qCO2) as a specific activity parameter to assess the effects of environmental conditions, such as ph, on the microbial biomass of forest soils. *Soil Biology & Biochemistry* 25: 393–395.
- Andrade, T.M.B., Camargo, P.B., Silva, D.M.L., Piccolo, M.C., Vieira, S.A., Alves,
 L.F., Joly, C.A., & Martinelli, L.A. 2011. Dynamics of dissolved forms of carbon and inorganic nitrogen in small watersheds of the coastal atlantic forest in southeast Brazil. *Water, Air, and Soil Pollution* 214: 393–408.
- Arabatzis, A.A., & Burkhart, H.E. 1992. An Evaluation of Sampling Methods and Model Forms for Estimating Height-Diameter Relationships in Loblolly Pine Plantations. *Forest Science* 38: 192–198.

- Aragão, L.E.O.C., Malhi, Y., Metcalfe, D.B., Silva-Espejo, J.E., Jiménez, E., Navarrete, D., Almeida, S., Costa, A.C.L., Salinas, N., Phillips, O.L., Anderson, L.O., Baker, T.R., Goncalvez, P.H., Huamán-Ovalle, J., Mamani-Solórzano, M., Meir, P., Monteagudo, A., Peñuela, M.C., Prieto, A., Quesada, C.A., Rozas-Dávila, A., Rudas, A., Silva Junior, J.A., & Vásquez, R. 2009. Above- and belowground net primary productivity across ten Amazonian forests on contrasting soils. *Biogeosciences* 6: 2441–2488.
- Bago, B., Pfeffer, P.E., Abubaker, J., Jun, J., Allen, J.W., Brouillette, J., Douds, D.D.,
 Lammers, P.J., & Shachar-Hill, Y. 2003. Carbon export from arbuscular
 mycorrhizal roots involves the translocation of carbohydrate as well as lipid. *Plant Physiology* 131: 1496–1507.
- Bailey, R.L., & Dell, R. 1972. Quantifying Diameter Distributions with the Weibull Function. *Forest Sciences* 19: 97–104.
- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., & Vivanco, J.M. 2006. the Role of Root Exudates in Rhizosphere Interactions With Plants and Other Organisms. *Annual Review of Plant Biology* 57: 233–266.
- Barros, F. de V., Bittencourt, P.L., Eller, C.B., Signori-Müller, C., Meireles, L.D., &
 Oliveira, R.S. 2022. Phytogeographical origin determines Tropical Montane
 Cloud Forest hydraulic trait composition. *Functional Ecology* 36: 607–621.
- Bates, D.M., & Watts, D.G. 1980. Relative Curvature Measures of Nonlinearity. *Royal* Statistical Society 42: 1–25.
- Beer, C., Reichstein, M., Tomelleri, E., Ciais, P., Jung, M., Carvalhais, N.,
 Rödenbeck, C., Arain, M.A., Baldocchi, D., Bonan, G.B., Bondeau, A., Cescatti,
 A., Lasslop, G., Lindroth, A., Lomas, M., Luyssaert, S., Margolis, H., Oleson,
 K.W., Roupsard, O., Veenendaal, E., Viovy, N., Williams, C., Woodward, F.I., &
 Papale, D. 2010. Terrestrial Gross Carbon Dioxide Uptake: Global Distribution
 and Covariation with Climate. *Science* 329: 834–839.
- Berhongaray, G., Cotrufo, F.M., Janssens, I.A., & Ceulemans, R. 2019. Belowground carbon inputs contribute more than above-ground inputs to soil carbon accrual in a bioenergy poplar plantation. *Plant and Soil* 434: 363–378.
- Berhongaray, G., King, J.S., Janssens, I.A., & Ceulemans, R. 2013. An optimized fine root sampling methodology balancing accuracy and time investment. *Plant and Soil* 366: 351–361.

- Binkley, D., & Fisher, R.F. 2012. Ecology and Management of Forest Soils (R. F. Fisher & D. Binkley, Eds.). John Wiley & Sons, Ltd, Chichester, UK.
- Binkley, D., & Richter, D. 1987. Nutrient Cycles and H+ Budgets of Forest Ecosystems. *Advances in Ecological Research* 16: 1–51.
- Boddington, C.L., Bassett, E.E., Jakobsen, I., & Dodd, J.C. 1999. Comparison of techniques for the extraction and quantification of extra-radical mycelium of arbuscular mycorrhizal fungi in soils. *Soil Biology and Biochemistry* 31: 479–482.
- Boehmer, H.J. 2011. Vulnerability of Tropical Montane Rain Forest Ecosystems due to Climate Change. In Brauch, H.G., Oswald Spring, Ú., Mesjasz, C., Grin, J., Kameri-Mbote, P., Chourou, B., Dunay, P., & Birkmann, J. (eds.), *Coping with Global Environmental Change, Disasters and Security: Threats, Challenges, Vulnerabilities and Risks*, pp. 789–802. Hexagon Series on Human and Environmental Security and Peace. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Bohn, K., Pavlick, R., Reu, B., & Kleidon, A. 2014. The strengths of r- And Kselection shape diversity-disturbance relationships. *PLoS ONE* 9:.
- Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I., & Martínez-Estévez, M. 2017. Aluminum, a friend or foe of higher plants in acid soils. *Frontiers in Plant Science* 8: 1–18.
- Bonan, G.B. 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science (New York, N.Y.)* 320: 1444–9.
- Bonfim, J.A., Vasconcellos, R.L.F., Stürmer, S.L., & Cardoso, E.J.B.N. 2013. Arbuscular mycorrhizal fungi in the Brazilian Atlantic forest: A gradient of environmental restoration. *Applied Soil Ecology* 71: 7–14.
- Børja, I., De Wit, H.A., Steffenrem, A., & Majdi, H. 2008. Stand age and fine root biomass, distribution and morphology in a Norway spruce chronosequence in southeast Norway. *Tree Physiology* 28: 773–784.
- Bradford, M.M. 1976. Sistema séptico domiciliario | Rotomoldeo en Colombia Tanques Plasticos En Colombia Rotoplast. *Analytical Biochemistry* 72: 248–254.
- Brearley, F.Q. 2011. Below-ground secondary succession in tropical forests of Borneo. *Journal of Tropical Ecology* 27: 413–420.
- Brody, S. 1945. *Bioenergetics and growth: with special reference to the efficiency complex in domestic animals*. Hafner Publishing Company, Inc., New York.

- Brunner, I., & Godbold, D.L. 2007. Tree roots in a changing world. *Journal of Forest Research* 12: 78–82.
- Burnham, K.P., & Anderson, D.R. 2004. Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods and Research* 33: 261–304.
- Carmo, J.B. Do, de Sousa Neto, E.R., Duarte-Neto, P.J., Ometto, J.P.H.B., & Martinelli, L.A. 2012. Conversion of the coastal Atlantic forest to pasture: Consequences for the nitrogen cycle and soil greenhouse gas emissions. *Agriculture, Ecosystems & Environment* 148: 37–43.
- Carrillo-Saucedo, S.M., Gavito, M.E., & Siddique, I. 2018. Arbuscular mycorrhizal fungal spore communities of a tropical dry forest ecosystem show resilience to land-use change. *Fungal Ecology* 32: 29–39.
- Castellano, S.D., & Dick, R.P. 1991. Cropping and Sulfur Fertilization Influence on Sulfur Transformations in Soil. *Soil Science Society of America Journal* 55: 114– 121.
- Cavagnaro, T.R., Bender, S.F., Asghari, H.R., & van der Heijden, M.G.A. 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science* 20: 283–290.
- Chave, J., Coomes, D., Jansen, S., Lewis, S.L., Swenson, N.G., & Zanne, A.E. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12: 351–366.
- Chave, J., Réjou-Méchain, M., Búrquez, A., Chidumayo, E., Colgan, M.S., Delitti,
 W.B.C., Duque, A., Eid, T., Fearnside, P.M., Goodman, R.C., Henry, M.,
 Martínez-Yrízar, A., Mugasha, W.A., Muller-Landau, H.C., Mencuccini, M.,
 Nelson, B.W., Ngomanda, A., Nogueira, E.M., Ortiz-Malavassi, E., Pélissier, R.,
 Ploton, P., Ryan, C.M., Saldarriaga, J.G., & Vieilledent, G. 2014. Improved
 allometric models to estimate the aboveground biomass of tropical trees. *Global Change Biology* 20: 3177–3190.
- Chazdon, R.L. 2003. Tropcial Forest Recovery: Legaies of Human Impact and Natural Disturbances. 6: 51–71.
- Chen, W., Koide, R.T., Adams, T.S., DeForest, J.L., Cheng, L., & Eissenstat, D.M.
 2016. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proceedings of the National Academy of Sciences of the United States of America* 113: 8741–8746.

Clark, D.A., Brown, S., Kicklighter, D.W., Chambers, J.Q., Thomlinson, J.R., & Ni, J.

2001. Measuring net primary production in forests: Concepts and field methods. *Ecological Applications* 11: 356–370.

- Colombo, A.F., & Joly, C.A. 2010. Brazilian Atlantic Forest lato sensu : the most ancient Brazilian forest, and a biodiversity hotspot, is highly threatened by climate change. 70: 697–708.
- Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.Y., Mao, C.X., Chazdon, R.L., & Longino, J.T. 2012. Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *Journal of Plant Ecology* 5: 3–21.
- Corlett, R.T. 1995. Tropical secondary forests. *Progress in Physical Geography* 19: 159–172.
- Craine, J.M., & Dybzinski, R. 2013. Mechanisms of plant competition for nutrients, water and light. *Functional Ecology* 27: 833–840.
- Curtis, R.O. 1967. Height-Diameter and Height-Diameter-Age Equations For Second-Growth Douglas-Fir. *Forest Science* 13: 365–375.
- Cusack, D.F., Chou, W.W., Yang, W.H., Harmon, M.E., & Silver, W.L. 2009. Controls on long-term root and leaf litter decomposition in neotropical forests. *Global Change Biology* 15: 1339–1355.
- Despotovic, M., Nedic, V., Despotovic, D., & Cvetanovic, S. 2016. Evaluation of empirical models for predicting monthly mean horizontal diffuse solar radiation. *Renewable and Sustainable Energy Reviews* 56: 246–260.
- Draper, N.R., & Smith, H. 1981. *Applied Regression Analysis*. John Wiley & Sons, Ltd.
- Drigo, B., Pijl, A.S., Duyts, H., Kielak, A.M., Gamper, H.A., Houtekamer, M.J.,
 Boschker, H.T.S., Bodelier, P.L.E., Whiteley, A.S., Van Veen, J.A., & Kowalchuk,
 G.A. 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO2. *Proceedings of the National Academy of Sciences of the United States of America* 107: 10938–10942.
- Dybzinski, R., Farrior, C., Wolf, A., Reich, P.B., & Pacala, S.W. 2011. Evolutionarily stable strategy carbon allocation to foliage, wood, and fine roots in trees competing for light and nitrogen: an analytically tractable, individual-based model and quantitative comparisons to data. *The American naturalist* 177: 153–66.

Eller, C.B., Lima, A.L., & Oliveira, R.S. 2016. Cloud forest trees with higher foliar

water uptake capacity and anisohydric behavior are more vulnerable to drought and climate change. *The New phytologist* 211: 489–501.

- Eller, C.B., Lima, A.L., & Oliveira, R.S. 2013. Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, Drimys brasiliensis (Winteraceae). *New Phytologist* 199: 151–162.
- Fang, Z., & Bailey, R.L. 1998. Height-diameter models for tropical forests on Hainan Island in southern China. *Forest Ecology and Management* 110: 315–327.
- Fauset, S., Freitas, H.C., Galbraith, D.R., Sullivan, M.J.P., Aidar, M.P.M., Joly, C.A., Phillips, O.L., Vieira, S.A., & Gloor, M.U. 2018. Differences in leaf thermoregulation and water use strategies between three co-occurring Atlantic forest tree species. *Plant Cell and Environment* 41: 1618–1631.
- Foley, J.A., Barford, C., Coe, M.T., Gibbs, H.K., Helkowski, J.H., Holloway, T.,
 Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Ramankutty, N., DeFries,
 R., Asner, G.P., Bonan, G., Carpenter, S.R., Chapin, F.S., Daily, G.C., Prentice,
 I.C., & Snyder, P.K. 2005. Global consequences of land use. *Science* 309: 570–574.
- Förster, A., Hertel, D., Werner, R., & Leuschner, C. 2021. Belowground consequences of converting broadleaf to conifer forest: Comparing the fine root systems of European beech and Scots pine. *Forest Ecology and Management* 496:.
- Freschet, G.T., Cornwell, W.K., Wardle, D.A., Elumeeva, T.G., Liu, W., Jackson,
 B.G., Onipchenko, V.G., Soudzilovskaia, N.A., Tao, J., & Cornelissen, J.H.C.
 2013. Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. *Journal of Ecology* 101: 943–952.
- Gavito, M.E., Olsson, P.A., Rouhier, H., Medina-Peñafiel, A., Jakobsen, I., Bago, A., & Azcón-Aguilar, C. 2005. Temperature constraints on the growth and functioning of root organ cultures with arbuscular mycorrhizal fungi. *New Phytologist* 168: 179–188.
- Gea-Izquierdo, G., Montero, G., & Cañellas, I. 2009. Changes in limiting resources determine spatio-temporal variability in tree-grass interactions. *Agroforestry Systems* 76: 375–387.
- Gerdemann, J.W., & Nicolson, T.H. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British*

Mycological Society 46: 235–244.

- Giardina, C.P., Ryan, M.G., Binkley, D., & Fownes, J.H. 2003. Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. *Global Change Biology* 9: 1438–1450.
- Gill, R.A., & Jackson, R.B. 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* 147: 13–31.
- Gillman, G.P. 1979. A proposed method for the measurement of exchange properties of highly weathered soils. *Australian Journal of Soil Research* 17: 129–139.
- Giovannetti, M., & Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 489–500.
- Girardin, C.A.J., Aragão, L.E.O.C., Malhi, Y., Huaraca Huasco, W., Metcalfe, D.B., Durand, L., Mamani, M., Silva-Espejo, J.E., & Whittaker, R.J. 2013. Fine root dynamics along an elevational gradient in tropical Amazonian and Andean forests. *Global Biogeochemical Cycles* 27: 252–264.
- Girardin, C.A.J., Malhi, Y., Aragão, L.E.O.C., Mamami, M., Huaraca Huasco, W.,
 Durand, L., Feeley, K.J., Rapp, J., Silva-Espejo, J.E., Silman, M., Salinas, N., &
 Whittaker, R.J. 2010. Net primary productivity allocation and cycling of carbon
 along a tropical forest elevational transect in the Peruvian Andes. *Global Change Biology* 16: 3176–3192.
- Godbold, D.L., Fritz, H.-W., Jentschke, G., Meesenburg, H., & Rademacher, P. 2003. Root turnover and root necromass accumulation of Norway spruce (Picea abies) are affected by soil acidity. *Tree Physiology* 23: 915–921.
- Gordon, W.S., & Jackson, R.B. 2000. Nutrient concentrations in fine roots. 81: 275– 280.
- Gradstein, S.R., Homeier, J., & Gansert, D. 2008. The Tropical Mountain Forest Patterns and Processes in a Biodiversity Hotspot.
- Graefe, S., Hertel, D., & Leuschner, C. 2008. Fine root dynamics along a 2,000-m elevation transect in South Ecuadorian mountain rainforests. *Plant and Soil* 313: 155–166.
- Gransee, A., & Führs, H. 2013. Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. *Plant and Soil* 368: 5–21.
- Green, J.J., Dawson, L. a., Proctor, J., Duff, E.I., & Elston, D. a. 2005. Fine Root

Dynamics in a Tropical Rain Forest is Influenced by Rainfall. *Plant and Soil* 276: 23–32.

- Grigal, D.F., & Vance, E.D. 2000. Influence of soil organic matter on forest productivity. *New Zealand Journal of Forestry Science* 30: 169–205.
- Gulbe, A., & Eyduran, E. 2020. Package 'ehaGoF.' 28.
- Guo, Z., Chang, C., Zou, X., Wang, R., Li, J., & Li, Q. 2021. A model for characterizing dry soil aggregate size distribution. *Catena* 198: 105018.
- Guo, D., Mou, P., Jones, R.H., & Mitchell, R.J. 2004. Spatio-temporal patterns of soil available nutrients following experimental disturbance in a pine forest. *Oecologia* 138: 613–621.
- Guo, W., Nazim, H., Liang, Z., & Yang, D. 2016. Magnesium deficiency in plants: An urgent problem. *Crop Journal* 4: 83–91.
- Gutjahr, C., & Parniske, M. 2017. Cell Biology: Control of Partner Lifetime in a Plant– Fungus Relationship. *Current Biology* 27: R420–R423.
- van der Heijden, M.G.A., Bardgett, R.D., & van Straalen, N.M. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11: 296–310.
- Heinemeyer, A., & Fitter, A.H. 2004. Impact of temperature on the arbuscular mycorrhizal (AM) symbiosis: Growth responses of the host plant and its AM fungal partner. *Journal of Experimental Botany* 55: 525–534.
- Heinemeyer, A., Ineson, P., Ostle, N., & Fitter, A.H. 2006. Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytologist* 171: 159–170.
- Hendricks, J.J., Hendrick, R.L., Wilson, C. a., Mitchell, R.J., Pecot, S.D., & Guo, D.
 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Journal of Ecology* 94: 40–57.
- Henriksen, H.A. 1950. Height/diameter curve with logarithmic diameter: brief report on a more reliable method of height determination from height curves, introduced by the State Forest Research Branch. *Dansk Skovforeningens Tidsskrift* 35: 193–202.
- Hertel, D., Leuschner, C., Harteveld, M., & Wiens, M. 2007. Fine root mass, distribution and regeneration in disturbed primary forests and secondary forests

of the moist tropics. Stability of Tropical Rainforest Margins: Linking Ecological, Economic and Social Constraints of Land Use and Conservation

- Hertel, D., Moser, G., Culmsee, H., Erasmi, S., Horna, V., Schuldt, B., & Leuschner, C. 2009. Below- and above-ground biomass and net primary production in a paleotropical natural forest (Sulawesi, Indonesia) as compared to neotropical forests. *Forest Ecology and Management* 258: 1904–1912.
- Högberg, P., & Read, D.J. 2006. Towards a more plant physiological perspective on soil ecology. *Trends in Ecology and Evolution* 21: 548–554.
- Horn, H.S. 1974. The Ecology of Secondary Succession. *Annual Review of Ecology* and Systematics 5: 25–37.
- Huang, S., Titus, S.J., & Wiens, D.P. 1992. Comparison of nonlinear height-diameter functions for major Alberta tree species. *Canadian Journal of Forest Research* 22: 1297–1304.
- Huaraca Huasco, W., Riutta, T., Girardin, C.A.J., Hancco Pacha, F., Puma Vilca,
 B.L., Moore, S., Rifai, S.W., del Aguila-Pasquel, J., Araujo Murakami, A., Freitag,
 R., Morel, A.C., Demissie, S., Doughty, C.E., Oliveras, I., Galiano Cabrera, D.F.,
 Durand Baca, L., Farfán Amézquita, F., Silva Espejo, J.E., da Costa, A.C.L.,
 Oblitas Mendoza, E., Quesada, C.A., Evouna Ondo, F., Edzang Ndong, J.,
 Jeffery, K.J., Mihindou, V., White, L.J.T., N'ssi Bengone, N., Ibrahim, F., AddoDanso, S.D., Duah-Gyamfi, A., Djaney Djagbletey, G., Owusu-Afriyie, K.,
 Amissah, L., Mbou, A.T., Marthews, T.R., Metcalfe, D.B., Aragão, L.E.O.,
 Marimon-Junior, B.H., Marimon, B.S., Majalap, N., Adu-Bredu, S., Abernethy,
 K.A., Silman, M., Ewers, R.M., Meir, P., & Malhi, Y. 2021. Fine root dynamics
 across pantropical rainforest ecosystems. *Global Change Biology* 27: 3657–3680.
- Hughes, R.F., Kauffman, J.B., & Jaramillo, V.J. 1999. Biomass, carbon, and nutrient dynamics of secondary forests in a humid tropical Region of Mexico. *Ecology* 80: 1892–1907.
- IBGE, I.B. de G. e E. 2012. Manual técnico da vegetação brasileira: sistema fitogeográfico, inventário das formações florestais e campestres, técnicas e manejo de coleções botânicas, procedimentos para mapeamentos. Rio de janeiro - RJ.

- Jackson, M.B., & Colmer, T.D. 2005. Response and adaptation by plants to flooding stress. *Annals of Botany* 96: 501–505.
- Jackson, R.B., Mooney, H.A., & Schulze, E.D. 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences of the United States of America* 94: 7362–7366.
- Jenkinson, D.S., & Powlson, D.S. 1976. The effects of biocidal treatments on metabolism in soil - V: A method for measuring soil biomass. Soil Biology and Biochemistry 8: 209–213.
- Jobbagy, E.G., & Jackson, R.B. 2001. The distribution of soil nutriments with depth : Global patterns of the imprint of plants. *Biogeochemistry* 53: 51–77.
- Johnston, R., Jones, K., & Manley, D. 2018. Confounding and collinearity in regression analysis: a cautionary tale and an alternative procedure, illustrated by studies of British voting behaviour. *Quality and Quantity* 52: 1957–1976.
- Joly, C.A., Assis, M.A., Bernacci, L.C., Tamashiro, J.Y., de Campos, M.C.R., Gomes, J.A.M.A., Lacerda, M.S., dos Santos, F.A.M., Pedroni, F., Pereira, L. de S., Padgurschi, M. de C.G., Prata, E.M.B., Ramos, E., Torres, R.B., Rochelle, A., Martins, F.R., Alves, L.F., Vieira, S.A., Martinelli, L.A., de Camargo, P.B., Aidar, M.P.M., Eisenlohr, P.V., Simões, E., Villani, J.P., & Belinello, R. 2012. Floristic and phytosociology in permanent plots of the Atlantic Rainforest along an altitudinal gradient in southeastern Brazil. *Biota Neotropica* 12: 123–145.
- Kassambara, A. 2021. Package ' rstatix ' : Pipe-Friendly Framework for Basic Statistical Tests. 105.
- Kobe, R.K., Iyer, M., & Walters, M.B. 2010. Optimal partitioning theory revisited: Nonstructural carbohydrates dominate root mass responses to nitrogen. *Ecology* 91: 166–179.
- Koske, R.E., & Gemma, J.N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* 92: 486–488.
- Koteen, L.E., & Baldocchi, D.D. 2013. A randomization method for efficiently and accurately processing fine roots, and separating them from debris, in the laboratory. *Plant and Soil* 363: 383–398.
- Leake, J.R., Ostle, N.J., Rangel-Castro, J.I., & Johnson, D. 2006. Carbon fluxes from plants through soil organisms determined by field13CO2pulse-labelling in an upland grassland. *Applied Soil Ecology* 33: 152–175.

- Lenoir, I., Fontaine, J., & Lounès-Hadj Sahraoui, A. 2016. Arbuscular mycorrhizal fungal responses to abiotic stresses: A review. *Phytochemistry* 123: 4–15.
- de León, D.G., Neuenkamp, L., Moora, M., Öpik, M., Davison, J., Peña-Venegas, C.P., Vasar, M., Jairus, T., & Zobel, M. 2018. Arbuscular mycorrhizal fungal communities in tropical rain forest are resilient to slash-and-burn agriculture. *Journal of Tropical Ecology* 34: 186–199.
- Leppälammi-Kujansuu, J., Salemaa, M., Kleja, D.B., Linder, S., & Helmisaari, H.S. 2014. Fine root turnover and litter production of Norway spruce in a long-term temperature and nutrient manipulation experiment. *Plant and Soil* 374: 73–88.
- Leuschner, C., Harteveld, M., & Hertel, D. 2009. Consequences of increasing forest use intensity for biomass, morphology and growth of fine roots in a tropical moist forest on Sulawesi, Indonesia. *Agriculture, Ecosystems and Environment* 129: 474–481.
- Leuschner, C., Moser, G., Bertsch, C., Röderstein, M., & Hertel, D. 2007. Large altitudinal increase in tree root/shoot ratio in tropical mountain forests of Ecuador. *Basic and Applied Ecology* 8: 219–230.
- Leuschner, C., Wiens, M., Harteveld, M., Hertel, D., & Tjitrosemito, S. 2006. Patterns of fine root mass and distribution along a disturbance gradient in a tropical montane forest, Central Sulawesi (Indonesia). *Plant and Soil* 283: 163–174.
- Levillain, J., Thongo M'Bou, A., Deleporte, P., Saint-André, L., & Jourdan, C. 2011. Is the simple auger coring method reliable for below-ground standing biomass estimation in Eucalyptus forest plantations? *Annals of Botany* 108: 221–230.
- Li, D., Ma, W., Wei, J., Mao, Y., Peng, Z., Zhang, J., Kong, X., Han, Q., Fan, W., Yang, Y., Chen, J., Wu, L., Rengel, Z., Cui, X., & Chen, Q. 2020. Magnesium promotes root growth and increases aluminum tolerance via modulation of nitric oxide production in Arabidopsis. *Plant and Soil* 457: 83–95.
- Liebsch, D., Marques, M.C.M., & Goldenberg, R. 2008. How long does the Atlantic Rain Forest take to recover after a disturbance? Changes in species composition and ecological features during secondary succession. *Biological Conservation* 141: 1717–1725.

Lilja, D. 2016. Linear Regression Using R: An Introduction to Data Modeling.

Litton, C.M., Raich, J.W., & Ryan, M.G. 2007. Carbon allocation in forest ecosystems. *Global Change Biology* 13: 2089–2109.

- Livesley, S.J., Stacey, C.L., Gregory, P.J., & Buresh, R.J. 1999. Sieve size effects on root length and biomass measurements of maize (Zea mays) and Grevillea robusta. *Plant and Soil* 207: 183–193.
- Lovelock, C.E., Wright, S.F., Clark, D.A., & Ruess, R.W. 2004. Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. *Journal of Ecology* 92: 278–287.
- Lynch, J., Marschner, P., & Rengel, Z. 2011. *Effect of Internal and External Factors* on Root Growth and Development. Elsevier Ltd.
- Malhi, Y., Doughty, C., & Galbraith, D. 2011. The allocation of ecosystem net primary productivity in tropical forests. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 366: 3225–45.
- Mao, L., Pan, J., Jiang, S., Shi, G., Qin, M., Zhao, Z., Zhang, Q., An, L., Feng, H., & Liu, Y. 2019. Arbuscular mycorrhizal fungal community recovers faster than plant community in historically disturbed Tibetan grasslands. *Soil Biology and Biochemistry* 134: 131–141.
- Marchiori, N.M., Rocha, H.R. da, Tamashiro, J.Y., & Aidar, M.P.M. 2016. Tree Community Composition and Aboveground Biomass in a Secondary Atlantic Forest, Serra Do Mar State Park, São Paulo, Brazil. *Cerne* 22: 501–514.
- Marchiori, N.M., da Silva, J.G., Dos Santos, F.C., Domingues, L.M., de Freitas, H.C., da Rocha, H.R., de Camargo, P.B., & Aidar, M.P.M. 2019. Effects of seasonal air temperatures and light on leaf traits at atlantic montane forest, Brazilian southeast. *Cerne* 25: 263–272.
- Marthews, T.R., Riutta, T., Oliveras Menor, I., Urrutia, R., Moore, S., Metcalfe, D., Malhi, Y., Phillips, O., Huaraca Huasco, W., Ruiz Jaén, M., Girardin, C., Butt, N., & Cain, R. 2014. Measuring Tropical Forest Carbon Allocation and Cycling: A RAINFOR-GEM Field Manual for Intensive Census Plots (v3.0). *Manual*. doi: doi: 10.5287/bodleian:xp68kh42k
- Martins, S.C., Sousa Neto, E., Piccolo, M.D.C., Almeida, D.Q.A., Camargo, P.B. De, Do Carmo, J.B., Porder, S., Lins, S.R.M., & Martinelli, L.A. 2015. Soil texture and chemical characteristics along an elevation range in the coastal Atlantic Forest of Southeast Brazil. *Geoderma Regional* 5: 106–116.
- McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Helmisaari, H.S., Hobbie, E.A., Iversen, C.M., Jackson, R.B., Leppälammi-

Kujansuu, J., Norby, R.J., Phillips, R.P., Pregitzer, K.S., Pritchard, S.G., Rewald, B., & Zadworny, M. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* 207: 505–518.

- McCormack, M.L., & Guo, D. 2014. Impacts of environmental factors on fine root lifespan. *Frontiers in plant science* 5: 205.
- Mcgarrigle, E., Kershaw, J.A., Lavigne, M.B., Weiskittel, A.R., & Ducey, M. 2011. Predicting the number of trees in small diameter classes using predictions from a two-parameter Weibull distribution. 84: 431–439.
- McGrath, G.S., Paik, K., & Hinz, C. 2012. Microtopography alters self-organized vegetation patterns in water-limited ecosystems. *Journal of Geophysical Research* 117: G03021.
- McNear, D.H. 2013. The Rhizosphere Roots, Soil and Everything In Between. *Nature Education Knowledge* 4: 1.
- Meier, I.C., Avis, P.G., & Phillips, R.P. 2013. Fungal communities influence root exudation rates in pine seedlings. *FEMS Microbiology Ecology* 83: 585–595.
- Meinen, C., Hertel, D., & Leuschner, C. 2009. Root growth and recovery in temperate broad-leaved forest stands differing in tree species diversity. *Ecosystems* 12: 1103–1116.
- Meireles, L.D., & Shepherd, G.J. 2015. Structure and floristic similarities of upper montane forests in Serra Fina mountain range, southeastern Brazil. Acta Botanica Brasilica 29: 58–72.
- Melillo, J.M., McGuire, A.D., Kicklighter, D.W., Moore, B., Vorosmarty, C.J., & Schloss, A.L. 1993. Global climate change and terrestrial net primary production. *Nature* 363: 234–240.
- Melloni, R., & Cardoso, E.J.B.N. 2001. Quantificação de mincélio extrarradicular de fungos micorrízicos arbusculares em plantas cítricas e endófitos. *Revista Brasileira de Ciência do Solo* 53–58.
- Mesquita, R.D.C.G., Massoca, P.E.D.S., Jakovac, C.C., Bentos, T.V., & Williamson,
 G.B. 2015. Amazon Rain Forest Succession: Stochasticity or Land-Use Legacy?
 BioScience 65: 849–861.
- Metcalfe, D.B., Williams, M., Aragão, L.E.O.C., Da Costa, A.C.L., De Almeida, S.S., Braga, A.P., Gonçalves, P.H.L., Silva Junior, J.A., Malhi, Y., & Meir, P. 2007. A

method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy. *New Phytologist* 174: 697–703.

- Miller, S.P., & Sharitz, R.R. 2000. Manipulation of flooding and arbuscular mycorrhiza formation influences growth and nutrition of two semiaquatic grass species. *Functional Ecology* 14: 738–748.
- Mommer, L., Kirkegaard, J., & van Ruijven, J. 2016. Root–Root Interactions: Towards A Rhizosphere Framework. *Trends in Plant Science* 21: 209–217.
- Moreira, M., Baretta, D., Tsai, S.M., & Cardoso, E.J.B.N. 2009. Arbuscular mycorrhizal fungal communities in native and in replanted Araucaria forest. *Scientia Agricola* 66: 677–684.
- Moser, G., Leuschner, C., Hertel, D., Graefe, S., Soethe, N., & lost, S. 2011.
 Elevation effects on the carbon budget of tropical mountain forests (S Ecuador): the role of the belowground compartment. *Global Change Biology* 17: 2211–2226.
- Moser, G., Leuschner, C., Röderstein, M., Graefe, S., Soethe, N., & Hertel, D. 2010.
 Biomass and productivity of fine and coarse roots in five tropical mountain forests stands along an altitudinal transect in southern Ecuador. *Plant Ecology & Diversity* 3: 151–164.
- Newbold, T., Hudson, L.N., Hill, S.L.L., & Al., E. 2015. Global effects of land use on local terrestrial biodiversity. *Nature* 520: 45–50.
- Oehl, F., Sieverding, E., Ineichen, K., M\u00e4der, P., Wiemken, A., & Boller, T. 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agriculture, Ecosystems and Environment* 134: 257–268.
- Oliveira-Filho, A.T., & Fontes, M.A.L. 2000. Patterns of Floristic Differentiation among Atlantic Forests in Southeastern Brazil and the Influence of Climate1. *Biotropica* 32: 793–810.
- Oliveira, R.S., Eller, C.B., Barros, F. de V., Hirota, M., Brum, M., & Bittencourt, P.
 2021. Linking plant hydraulics and the fast–slow continuum to understand resilience to drought in tropical ecosystems. *New Phytologist* 230: 904–923.
- Osaki, M., Watanabe, T., & Tadano, T. 1997. Beneficial effect of aluminum on growth of plants adapted to low ph soils. *Soil Science and Plant Nutrition* 43: 551–563.

- Padgurschi, M. de C.G., Pereira, L. de S., Tamashiro, J.Y., & Joly, C.A. 2011. Composição e similaridade florística entre duas áreas de Floresta Atlântica Montana, São Paulo, Brasil. *Biota Neotropica* 11: 139–152.
- Pan, J., Sharif, R., Xu, X., & Chen, X. 2021. Mechanisms of Waterlogging Tolerance in Plants: Research Progress and Prospects. *Frontiers in Plant Science* 11:.
- Pasqualini, D., Uhlmann, A., & Stürmer, S.L. 2007. Arbuscular mycorrhizal fungal communities influence growth and phosphorus concentration of woody plants species from the Atlantic rain forest in South Brazil. *Forest Ecology and Management* 245: 148–155.
- Payandeh, B., & Wang, Y. 1995. Comparison of the modified Weibull and Richards growth function for developing site index equations. *New Forests* 9: 147–155.
- Pearl, R., & Reed, L.J. 1920. On the Rate of Growth of the Population of the United States since 1790 and Its Mathematical Representation. *Proceedings of the National Academy of Sciences of the United States of America* 6: 275–88.
- Pennisi, E., & Cornwall, W. 2020. Hidden web of fungi could shape the future of forests. *Science* 369: 1042–1043.
- Pierick, K., Leuschner, C., & Homeier, J. 2021. Topography as a factor driving smallscale variation in tree fine root traits and root functional diversity in a species-rich tropical montane forest. *New Phytologist* 230: 129–138.
- Pöschl, U., Martin, S.T., Sinha, B., Chen, Q., Gunthe, S.S., Huffman, J.A.,
 Borrmann, S., Farmer, D.K., Garland, R.M., Helas, G., Jimenez, J.L., King,
 S.M., Manzi, A., Mikhailov, E., Pauliquevis, T., Petters, M.D., Prenni, A.J.,
 Roldin, P., Rose, S.R., Artaxo, P., & Andreae, M.O. 2010. Rainforest aerosols
 as biogenic nuclei of clouds and precipitation in the Amazon. *Science* 329:
 1513–1516.
- Postel, S.L., & Thompson, B.H. 2005. Watershed protection: Capturing the benefits of nature's water supply services. *Natural Resources Forum* 29: 98–108.
- R Core Team. 2020. R: A Language and Environment for Statistical Computing (R.D. C. Team, Ed.). *R Foundation for Statistical Computing*.
- Raich, J.W., Clark, D.A., Schwendenmann, L., & Wood, T.E. 2014. Aboveground
 Tree Growth Varies with Belowground Carbon Allocation in a Tropical Rainforest
 Environment (D. Hui, Ed.). *PLoS ONE* 9: e100275.
- Raij, B. van, Andrade, J.C., Cantarella, H., & Quaggio, J.A. 2001. Análise química

para avaliação da fertilidade de solos tropicais.

- Rasse, D.P., Rumpel, C., & Dignac, M.F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil* 269: 341–356.
- Ratkowsky, D.A., & Reedy, T.J. 1986. Choosing Near-Linear Parameters in the Four-Parameter Logistic Model for Radioligand and Related Assays. *Biometrics* 42: 575–582.
- Raynal, D.J., Joslin, J.D., Thornton, F.C., Schaedle, M., & Henderson, G.S. 1990.
 Sensitivity of Tree Seedlings to Aluminum: III. Red Spruce and Loblolly Pine.
 Journal of Environmental Quality 19: 180–187.
- Rehmus, A., Bigalke, M., Valarezo, C., Castillo, J.M., & Wilcke, W. 2014. Aluminum toxicity to tropical montane forest tree seedlings in southern Ecuador: response of biomass and plant morphology to elevated AI concentrations. *Plant and Soil* 382: 301–315.
- Reiners, W.A., Bouwman, A.F., Parsons, W.F.J., & Keller, M. 1994. Tropical Rain Forest Conversion to Pasture: Changes in Vegetation and Soil Properties. *Ecological Applications* 4: 363–377.
- Resende, M., Lani, J.L., & Rezende, S.B. de. 2002. Pedossistemas da Mata Atlântica: considerações pertinentes sobre a sustentabilidade. *Revista Árvore* 26: 261–269.

- Richards, F.J. 1959. A flexible growth model for empirical use. *Journal of Experimental Botany* 10(2): 290–301.
- Richardson, A.D., Black, T.A., Ciais, P., Delbart, N., Friedl, M.A., Gobron, N.,
 Hollinger, D.Y., Kutsch, W.L., Longdoz, B., Luyssaert, S., Migliavacca, M.,
 Montagnani, L., Munger, J.W., Moors, E., Piao, S., Rebmann, C., Reichstein, M.,
 Saigusa, N., Tomelleri, E., Vargas, R., & Varlagin, A. 2010. Influence of spring
 and autumn phenological transitions on forest ecosystem productivity. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365:
 3227–3246.
- Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F., & Torn, M.S. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant and Soil* 233: 167–177.
- Riutta, T., Malhi, Y., Kho, L.K., Marthews, T.R., Huaraca Huasco, W., Khoo, M., Tan, S., Turner, E., Reynolds, G., Both, S., Burslem, D.F.R.P., Teh, Y.A., Vairappan,

C.S., Majalap, N., & Ewers, R.M. 2018. Logging disturbance shifts net primary productivity and its allocation in Bornean tropical forests. *Global Change Biology* 24: 2913–2928.

- Röderstein, M., Hertel, D., & Leuschner, C. 2005. Above- and below-ground litter production in three tropical montane forests in southern Ecuador. *Journal of Tropical Ecology* 21: 483–492.
- Rosado, B.H.P., Joly, C.A., Burgess, S.S.O., Oliveira, R.S., & Aidar, M.P.M. 2016.
 Changes in plant functional traits and water use in Atlantic rainforest: evidence of conservative water use in spatio-temporal scales. *Trees Structure and Function* 30: 47–61.
- Rosado, B.H.P., Martins, A.C., Colomeu, T.C., Oliveira, R.S., Joly, C.A., & Aidar,
 M.P.M. 2011a. Fine root biomass and root length density in a lowland and a montane tropical rain forest, SP, Brazil. *Biota Neotropica* 11: 203–209.
- Rosado, B.H.P., Martins, A.C., Colomeu, T.C., Oliveira, R.S., Joly, C.A., & Aidar,
 M.P.M. 2011b. Fine root biomass and root length density in a lowland and a montane tropical rain forest, SP, Brazil. *Biota Neotropica* 11: 203–209.
- Rozendaal, D.M.A., Bongers, F., Aide, T.M., Alvarez-Dávila, E., Ascarrunz, N., Balvanera, P., Becknell, J.M., Bentos, T. V., Brancalion, P.H.S., Cabral, G.A.L., Calvo-Rodriguez, S., Chave, J., César, R.G., Chazdon, R.L., Condit, R., Dallinga, J.S., De Almeida-Cortez, J.S., De Jong, B., De Oliveira, A., Denslow, J.S., Dent, D.H., DeWalt, S.J., Dupuy, J.M., Durán, S.M., Dutrieux, L.P., Espírito-Santo, M.M., Fandino, M.C., Fernandes, G.W., Finegan, B., García, H., Gonzalez, N., Moser, V.G., Hall, J.S., Hernández-Stefanoni, J.L., Hubbell, S., Jakovac, C.C., Hernández, A.J., Junqueira, A.B., Kennard, D., Larpin, D., Letcher, S.G., Licona, J.C., Lebrija-Trejos, E., Marín-Spiotta, E., Martínez-Ramos, M., Massoca, P.E.S., Meave, J.A., Mesquita, R.C.G., Mora, F., Müller, S.C., Muñoz, R., De Oliveira Neto, S.N., Norden, N., Nunes, Y.R.F., Ochoa-Gaona, S., Ortiz-Malavassi, E., Ostertag, R., Peña-Claros, M., Pérez-García, E.A., Piotto, D., Powers, J.S., Aguilar-Cano, J., Rodriguez-Buritica, S., Rodríguez-Velázquez, J., Romero-Romero, M.A., Ruíz, J., Sanchez-Azofeifa, A., De Almeida, A.S., Silver, W.L., Schwartz, N.B., Thomas, W.W., Toledo, M., Uriarte, M., De Sá Sampaio, E.V., Van Breugel, M., Van Der Wal, H., Martins, S.V., Veloso, M.D.M., Vester, H.F.M., Vicentini, A., Vieira, I.C.G., Villa, P.,

Williamson, G.B., Zanini, K.J., Zimmerman, J., & Poorter, L. 2019. Biodiversity recovery of Neotropical secondary forests. *Science Advances* 5:.

- Russell, A.E., & Raich, J.W. 2012. Rapidly growing tropical trees mobilize remarkable amounts of nitrogen, in ways that differ surprisingly among species. *Proceedings* of the National Academy of Sciences of the United States of America 109: 10398–10402.
- Russell, A.E., Raich, J.W., Arrieta, R.B., Valverde-Barrantes, O., & González, E. 2010. Impacts of individual tree species on carbon dynamics in a moist tropical forest environment. *Ecological Applications* 20: 1087–1100.
- Salemi, L.F., Groppo, J.D., Trevisan, R., de Moraes, J.M., de Barros Ferraz, S.F., Villani, J.P., Duarte-Neto, P.J., & Martinelli, L.A. 2013. Land-use change in the Atlantic rainforest region: Consequences for the hydrology of small catchments. *Journal of Hydrology* 499: 100–109.
- Scaranello, M.A. da S., Alves, L.F., Vieira, S.A., Camargo, P.B. de, Joly, C.A., & Martinelli, L.A. 2012. Height-diameter relationships of tropical Atlantic moist forest trees in southeastern Brazil. *Scientia Agricola*. doi: 10.1590/S0103-90162012000100005
- Schedlbauer, J.L., & Kavanagh, K.L. 2008. Soil carbon dynamics in a chronosequence of secondary forests in northeastern Costa Rica. *Forest Ecology and Management* 255: 1326–1335.
- Schoenau, J.J., & Malhi, S.S. 2015. Sulfur forms and cycling processes in soil and their relationship to sulfur fertility. Sulfur: A Missing Link between Soils, Crops, and Nutrition. doi: 10.2134/agronmonogr50.c1
- Schwarz, M., Giadrossich, F., & Cohen, D. 2013. Modeling root reinforcement using a root-failure Weibull survival function. *Hydrology and Earth System Sciences* 17: 4367–4377.
- da Silva, C.F., Pereira, M.G., dos Santos, V.L., Miguel, D.L., & da Silva, E.M.R. 2016.
 Fungos micorrízicos arbusculares: Composição, comprimento de micélio
 extrarradicular e glomalina em áreas de mata Atlântica, Rio De Janeiro. *Ciencia Florestal* 26: 419–433.
- Silva, C.A. da, Londe, V., Andrade, S.A.L., Joly, C.A., & Vieira, S.A. 2020. Fine rootarbuscular mycorrhizal fungi interaction in Tropical Montane Forests: Effects of cover modifications and season. *Forest Ecology and Management* 476:.

- Silva, C.A., Londe, V., D'Angioli, A.M., Scaranello, M.A.S., Bordron, B., Joly, C.A., & Vieira, S.A. 2022. Testing collection-time reduction in fine-root biomass estimation in Atlantic Forests (M. Bernhardt-Römermann, Ed.). *Applied Vegetation Science* 25:.
- SIMA, S. de I. e M.A. do E. de S.P. 2006. Plano de Manejo do Parque Estadual da Serra do Mar. 679.
- Siminski, A., Zambiazi, D.C., dos Santos, K.L., & Fantini, A.C. 2021. Dynamics of Natural Regeneration: Implications for Landscape Restoration in the Atlantic Forest, Brazil. *Frontiers in Forests and Global Change* 4: 1–15.
- Singh, P.K., Singh, M., & Tripathi, B.N. 2013. Glomalin: An arbuscular mycorrhizal fungal soil protein. *Protoplasma* 250: 663–669.
- Smith, S.E., & Read, D. 2008. Mycorrhizal Symbiosis. Elsevier Ltd.
- Sochacki, S.J., Ritson, P., Brand, B., Harper, R.J., & Dell, B. 2017. Accuracy of tree root biomass sampling methodologies for carbon mitigation projects. *Ecological Engineering* 98: 264–274.
- Soteras, F., Grilli, G., Cofré, M.N., Marro, N., & Becerra, A. 2014. Arbuscular mycorrhizal fungal composition in high montane forests with different disturbance histories in central Argentina. *Applied Soil Ecology* 85: 30–37.
- Soudzilovskaia, N.A., Bodegom, P.M. Van, Terrer, C., & Zelfde, M. Van. 2019. to terrestrial carbon stocks. *Nature Communications*. doi: 10.1038/s41467-019-13019-2
- Sousa Neto, E., Carmo, J.B., Keller, M., Martins, S.C., Alves, L.F., Vieira, S. a.,
 Piccolo, M.C., Camargo, P., Couto, H.T.Z., Joly, C. a., & Martinelli, L. a. 2011.
 Soil-atmosphere exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest. *Biogeosciences* 8: 733–742.
- Spracklen, D.V., Arnold, S.R., & Taylor, C.M. 2012. Observations of increased tropical rainfall preceded by air passage over forests. *Nature* 489: 282–285.
- Stage, A.R. 1975. Prediction of height increment for models of forest growth /. USDA Forest Service, Ogden, Utah.
- Steidinger, B.S., Crowther, T.W., Liang, J., & Al., E. 2019. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* 569: 404–408.

- Steidinger, B.S., Turner, B.L., Corrales, A., & Dalling, J.W. 2015. Variability in potential to exploit different soil organic phosphorus compounds among tropical montane tree species. *Functional Ecology* 29: 121–130.
- Steingrobe, B., Schmid, H., & Claassen, N. 2001. The use of the ingrowth core method for measuring root production of arable crops - Influence of soil and root disturbance during installation of the bags on root ingrowth into the cores. *European Journal of Agronomy* 15: 143–151.
- Stoffels, A., & van Soeset, J. 1953. The main problems in sample plots. *Nederlands Bosbouw Tijdschrift* 25: 190–199.
- Strawn, D.G., Bohn, H.L., & O'Connor, G.A. 2020. Soil Chemistry. John Wiley & Sons Ltd, Hoboken, NJ.
- Stürmer, S.L., Klauberg Filho, O., De Queiroz, M.H., & De Mendonça, M.M. 2006. Occurrence of arbuscular mycorrhizal fungi in soils of early stages of a secondary succession of Atlantic Forest in South Brazil. *Acta Botanica Brasilica* 20: 513–521.
- Tabarelli, M. 1999. A riqueza de espécies arbóreas na floresta atlântica de encosta no estado de São Paulo (Brasil) mações sobre as restingas e a floresta atlântica, repre- senta uma região com índice elevado de endemis- tica na costa brasileira. Nas regiões Sul e Sudest. 2: 217–223.
- Tabarelli, M., & Mantovani, W. 1997. Colonização de clareiras naturais na floresta atlântica no sudeste do Brasil. *Revista Brasileira de Botânica* 20: 57–66.
- Tabarelli, M., & Mantovani, W. 1999. Regeneration of a neotropical montane forest following slash-and-burn (São Paulo-Brazil). *Revista Brasileira de Biologia* 59: 239–250.
- Tabarelli, M., Villani, J.P., & Mantovani, W. 1993. Aspectos da sucessão secundária em trecho da Floresta Atlântica no Parque Estadual da Serra do Mar, SP. *Revista do Instituto Florestal, São Paulo* 5: 95–112.
- Tauc, F., Houle, D., Dupuch, A., Doyon, F., & Maheu, A. 2020. Microtopographic refugia against drought in temperate forests: Lower water availability but more extensive fine root system in mounds than in pits. *Forest Ecology and Management* 476: 118439.
- Tränkner, M., Jákli, B., Tavakol, E., Geilfus, C.M., Cakmak, I., Dittert, K., & Senbayram, M. 2016. Magnesium deficiency decreases biomass water-use

efficiency and increases leaf water-use efficiency and oxidative stress in barley plants. *Plant and Soil* 406: 409–423.

- Treseder, K.K., & Turner, K.M. 2007. Glomalin in Ecosystems. Soil Science Society of America Journal 71: 1257.
- Trouvelot, A. 1986. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. *Physiological and genetical aspects of mycorrhizae*
- Trumbore, S.E., & Gaudinski, J.B. 2003. The Secret Lives of Roots. *Science* 302: 1344–1345.
- Vargas, R., & Allen, M.F. 2008. Dynamics of Fine Root, Fungal Rhizomorphs, and Soil Respiration in a Mixed Temperate Forest: Integrating Sensors and Observations. *Vadose Zone Journal* 7: 1055.
- Vieira, S. a, Alves, L.F., Duarte-Neto, P.J., Martins, S.C., Veiga, L.G., Scaranello, M. a, Picollo, M.C., Camargo, P.B., do Carmo, J.B., Neto, E.S., Santos, F. a M., Joly, C. a, & Martinelli, L. a. 2011. Stocks of carbon and nitrogen and partitioning between above- and belowground pools in the Brazilian coastal Atlantic Forest elevation range. *Ecology and evolution* 1: 421–34.
- Vogt, K.A., Publicover, D.A., Bloomfield, J., Perez, J.M., Vogt, D.J., & Silver, W.L. 1993. Belowground responses as indicators of environmental change. 33:.
- Wang, B., & Qiu, Y.L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16: 299–363.
- Wang, S., Wang, Z., & Gu, J. 2017. Variation patterns of fine root biomass, production and turnover in Chinese forests. *Journal of Forestry Research* 28: 1185–1194.
- Warton, D.I., & Hui, F.K. 2011. The arcsine is asinine: the analysis of proportions in ecology. *Ecology* 92: 3–10.
- Werner, F.A., & Homeier, J. 2015. Is tropical montane forest heterogeneity promoted by a resource-driven feedback cycle? Evidence from nutrient relations, herbivory and litter decomposition along a topographical gradient. *Functional Ecology* 29: 430–440.
- Whitehead, A. 2012. Comparative genomics in ecological physiology: Toward a more nuanced understanding of acclimation and adaptation. *Journal of Experimental Biology* 215: 884–891.

- Wickham, H., Chang, W., & Henry, H. 2016. ggplot2: Elegant Graphics for Data Analysis.
- Wilcke, W., Yasin, S., Abramowski, U., Valarezo, C., & Zech, W. 2002. Nutrient storage and turnover in organic layers under tropical montane rain forest in Ecuador. *European Journal of Soil Science* 53: 15–27.
- Winsor, C.P. 1932. The Gompertz Curve as a Growth Curve. *Proceedings of the National Academy of Sciences* 18: 1–8.
- Woodward, R.A., Harper, K.T., & Tiedemann, A.R. 1984. An ecological consideration of the significance of cation-exchange capacity of roots of some Utah range plants. *Plant and Soil* 79: 169–180.
- Wright, S.F., & Upadhyaya, A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil* 198: 97–107.
- Wu, L., Kobayashi, Y., Wasaki, J., & Koyama, H. 2018. Organic acid excretion from roots: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. *Soil Science and Plant Nutrition* 64: 697–704.
- Wykoff, W., Crookston, N.L., & Stage, A.R. 1982. User's guide to the stand prognosis model. *United States Department of Agriculture*.
- Yang, R.C., Kozak, A., & Smith, J.H.G. 1978. The potential of Weibull-type functions as flexible growth curves. *Canadian Journal of Forest Research* 8: 424–431.
- Yuan, Z.Y., & Chen, H. 2010. Fine Root Biomass, Production, Turnover Rates, and Nutrient Contents in Boreal Forest Ecosystems in Relation to Species, Climate, Fertility, and Stand Age: Literature Review and Meta-Analyses. *Critical Reviews in Plant Sciences* 29: 204–221.
- Yuan, Z.Y., & Chen, H.Y.H. 2012. Indirect methods produce higher estimates of fine root production and turnover rates than direct methods. *PloS one* 7: e48989.
- Zangaro, W., de Assis, R.L., Rostirola, L.V., de Souza, P.B., Gonçalves, M.C., Andrade, G., & Nogueira, M.A. 2008. Changes in arbuscular mycorrhizal associations and fine root traits in sites under different plant successional phases in southern Brazil. *Mycorrhiza* 19: 37–45.
- Zanini, A.M., Mayrinck, R.C., Vieira, S.A., de Camargo, P.B., & Rodrigues, R.R. 2021. The effect of ecological restoration methods on carbon stocks in the

Brazilian Atlantic Forest. Forest Ecology and Management 481: 118734.
Zhang, L., & Liu, C. 2006. Fitting irregular diameter distributions of forest stands by Weibull, modified Weibull, and mixture Weibull models. Journal of Forest Research 11: 369–372.

APPENDIX A - CHAPTER 1

Table S1. Back-transformed equations to obtain the shape parameter of the Weibull model for different time intervals.

Number and duration of each time interval	Intercept	Slope	Residuals	R²	<i>p</i> -value	Weibull shape (α)
12 intervals of 10 min	0.14	1.05	0.08	0.98	<0.0001	$\alpha = \exp(0.14+1.05*\log(\text{cumulative weight}))*\exp(0.08)$
15 intervals of 8 min	0.17	1.06	0.08	0.98	<0.0001	$\alpha = \exp(0.17+1.06*\log (\text{cumulative weight}))*\exp(0.08)$
20 intervals of 6 min	0.20	1.06	0.09	0.97	<0.0001	$\alpha = \exp(0.20+1.06*\log(\text{cumulative weight}))*\exp(0.09)$
30 intervals of 4 min	0.27	1.07	0.11	0.96	<0.0001	$\alpha = \exp(0.27+1.07*\log(\text{cumulative weight}))*\exp(0.11)$
60 intervals of 2 min	0.47	1.02	0.15	0.92	<0.0001	$\alpha = \exp(0.47+1.02*\log(\text{cumulative weight}))*\exp(0.15)$



Figure S1. Fine-root biomass production over three soil depths. Forest-cover types: (a) OG – Old-growth, (b) PSL – Post-selective logging, (c) PCC – Post clear-cut and (d) PP – Post-pasture. The numbers above the bars connecting compared groups, represent the differences found in the Kruskal-Wallis test.
а **Explanatory variable Forest cover** Estimate SE **T-value R**²adj р 1.728 Intercept 0.502 3.444 0.004 -0.011 0.041 0.090 0.459 0.653 Cstockwood **OG1** Intercept 1.719 0.465 3.699 0.002 -0.051 0.131 0.251 0.521 0.611 NPPwood Intercept 1.080 0.491 2.202 0.045 0.068 0.125 0.086 1.450 0.169 Cstockwood OG2 0.011 Intercept 2.175 0.459 4.742 0.000 -0.194 -1.082 0.298 0.179 NPPwood Intercept 1.533 0.459 3.342 0.005 0.077 0.101 0.067 1.498 0.156 Cstockwood OG3 Intercept 2.110 0.254 8.313 < 0.00001 -0.061 0.369 0.717 0.055 0.150 NPPwood Intercept 0.166 0.499 0.332 0.745 0.331 0.270 0.093 2.903 0.012 Cstockwood PSL Intercept 1.720 0.461 3.730 0.002 -0.058 -0.088 0.680 NPP_{Wood} 0.209 -0.422 2.598 6.035 < 0.0001 0.038 Intercept 0.431 Cstockwood -0.152 -1.263 0.227 0.120 PCC < 0.0001 Intercept 1.938 0.305 6.363 -0.045 0.070 0.559 NPPwood 0.117 0.598 Intercept 3.382 0.825 4.099 0.001 -0.065 -0.082 0.294 -0.279 0.784 Cstockwood PP 1.095 0.222 Intercept 0.943 1.160 0.265 0.83 0.36 2.30 0.04 NPPwood

Table S1 – Fine-root biomass productivity (NPP _{FR}) as a function of wood stock (Cstock _{Wood})
and productivity (NPP _{Wood}) in different forest cover types of Tropical Montane Atlantic Forest.



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