Testing collection-time reduction in fine-root biomass estimation in Atlantic Forests

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METHODS IN VEGETATION SCIENCE

Testing collection-time reduction in fine-root biomass estimation in Atlantic Forests

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Abstract

Aims: 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.

Location: Brazilian Atlantic Forest.

Methods: 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). A further 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.

Results: We found that Weibull was the best-fit model, and it performed well for modeling 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.

Conclusions: 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 | INTRODUCTION

Fine roots comprise an important plant photosynthate and resource investment (Jackson et al., 1997; McCormack et al., 2015; Yuan & Chen, 2012). 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 (McCormack et al., 2015; Yuan & Chen, 2012). 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 often been ignored in field studies or estimated as a theoretical proportion of above-ground values (Clark et al., 2001; 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, seasonal soil 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) (Addo-Danso et al., 2016; Livesley et al., 1999; 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 (Addo-Danso et al., 2016; Clark et al., 2001; Levillain et al., 2011; 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 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 at observed collection times shorter or longer than 40 min.

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 model selected in question 1 capable of making good and reliable estimations of the total root mass for collecting reference samples at 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 estimations associated with the reference collected mass; and (5) does the relative 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 the 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 biomass estimated by the same model in short observed collection times.
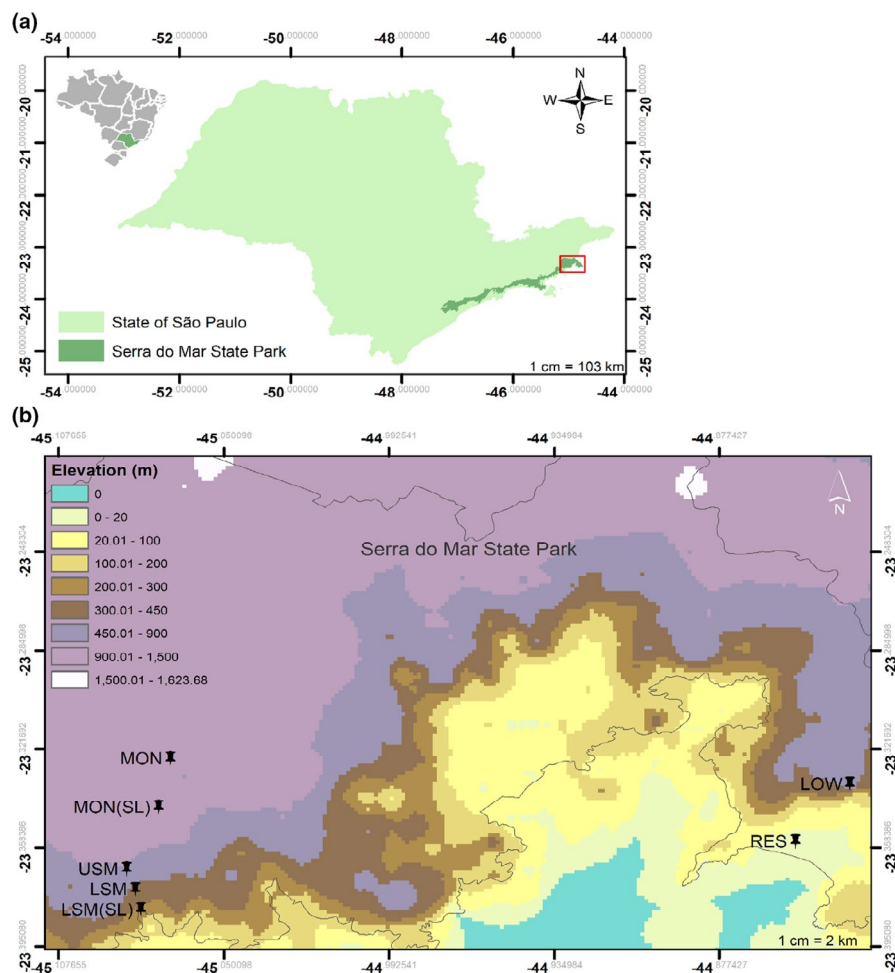
2 | METHODS

2.1 | Study region

To answer the questions in this study, we carried out our fieldwork along an elevational gradient of the Atlantic Forest from 10 to 1000 m above sea level (a.s.l.). This is the second-largest rainforest on the South American continent and an important biodiversity hotspot (Colombo & Joly, 2010; Myers & Mittermeier, 2000; Rezende et al., 2015). Specifically, we worked in seven sites in Serra do Mar State Park, São Paulo, southeastern Brazil (Figure 1a): an old-growth seasonally flooded forest — *Restinga* (RES, ±13 m a.s.l.); an old-growth lowland forest (LOW, ±70 m a.s.l.); a lower submontane forest post selective logging (LSM(SL), ±150 m a.s.l.); an old-growth lower submontane forest (LSM, ±248 m a.s.l.); an old-growth upper submontane forest (USM, ±370 m a.s.l.); a montane forest post selective logging (MON(SL), ±1031 m a.s.l.); and finally, an old-growth montane forest (MON, ±1038 m a.s.l.) (Figure 1b). We took advantage of the fact that, in each of these places, there were 1-ha plots (a grid of 100 m × 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).

2.2 | 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 (submontane 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-mm 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.

2.3 | Testing the shortest observed collection time under different conditions

We carried out further 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 (not for the absolute mass) because the absolute mass would vary

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

Parameter	Atlantic Forest physiognomy			
	Restinga	Lowland	Submontane	Montane
Elevation (m)	0–50	50–100	100–500	500–1200
Rainfall (mm)[a]	2146	2146	2146	1975
Temperature (°C)[a]	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)	63.4	102.6	126.2	139.5
N (Mg/ha)	3.7	7.6	10.0	10.6
P (mg/kg)	11.5	17.6	12.9	21.2
K (mmol$_c$/kg)	1.0	1.8	2.2	2.1
Mg (mmol$_c$/kg)	1.1	4.6	7.2	5.3
Ca (mmol$_c$/kg)	1.5	5.4	12.0	7.2
Al (mmol$_c$/kg)	15.6	14.3	25.1	26.7
pH	3.6	4.6	3.8	3.7
CEC (mmol$_c$/kg)	85.4	110.9	160.2	121.3
Sum of bases (mmol$_c$/kg)	3.0	7.2	15.3	8.1
Above-ground biomass				
Trees (Mg/ha)	163.5	204.8	247.7	271.3
Palms (Mg/ha)	2.8	3.9	6.0	11.3
Ferns (Mg/ha)	0.0	0.2	0.1	0.8

Abbreviation: CEC, cation exchange capacity; mmolc, millimoles charge per kg.

[a]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 submontane forests for the period from March 2013 to February 2014.

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 (Figure 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 longer 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.

2.4 | 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 R package *AICcmodavg* (Table 2). Only models having ΔAIC ≤ 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.

To analyze the performance of the best-fit model at shorter collection times, shorter 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) (original approach), 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 for 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 (Appendix S1). 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 R package *ehaGoF* (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 ≥10% and <20%; fair when ≥20% and <30%; and poor when RRMSE was ≥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 field-observed 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 × 100. The test has the purpose of evaluating if the model's error is associated with the observed mass, i.e., 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.

TABLE 2 Models tested for best fit for fine-root mass accumulation. Root samples were collected over 120 min in time intervals of 2 min each in submontane and montane forests (540 root samples per area) in Serra do Mar State Park, southeastern Brazil

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

Note: 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.

We fitted the best model selected from previous analyses to the observed fine-root 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 analyze if the estimated mass differed between ecosystems (Restinga, lowland forest, lower submontane forest, lower submontane forest post selective logging, upper submontane forest, montane forest post selective 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 statistical analyses.
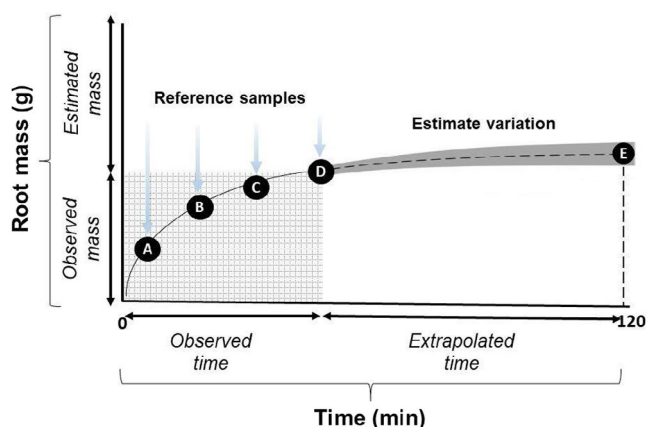

FIGURE 2 Hypothetical representation of how the best-fit model was applied to the temporal prediction method. The chequered background represents the observed mass collected in the field. The white background represents the mass estimated by the model. A is the fine-root mass accumulated over the first 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)

3 | RESULTS

3.1 | 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 ($\Delta AIC \leq 2$), but with a low fit percentage were Chapman–Richards (25.2%), power (8.1%), logarithmic (4.5%), and hyperbolic (3.6%) (Table 3).

We found an excellent fit ($R^2 \geq 93\%$) 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, there was strong evidence that the relative estimated mass did not differ among the different observed collection times ($F_4 = 0.08$; $p = 0.99$). 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 time (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.

3.2 | 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

TABLE 3 Absolute (*n*) and relative (%) frequency for models with the best fit ($\Delta AIC \leq 2$) for 1080 cumulative fine-root mass samples collected for different durations of observed time over 120 minutes, and at 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)

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 gray. RRMSE = Relative Root-Mean-Squared Error
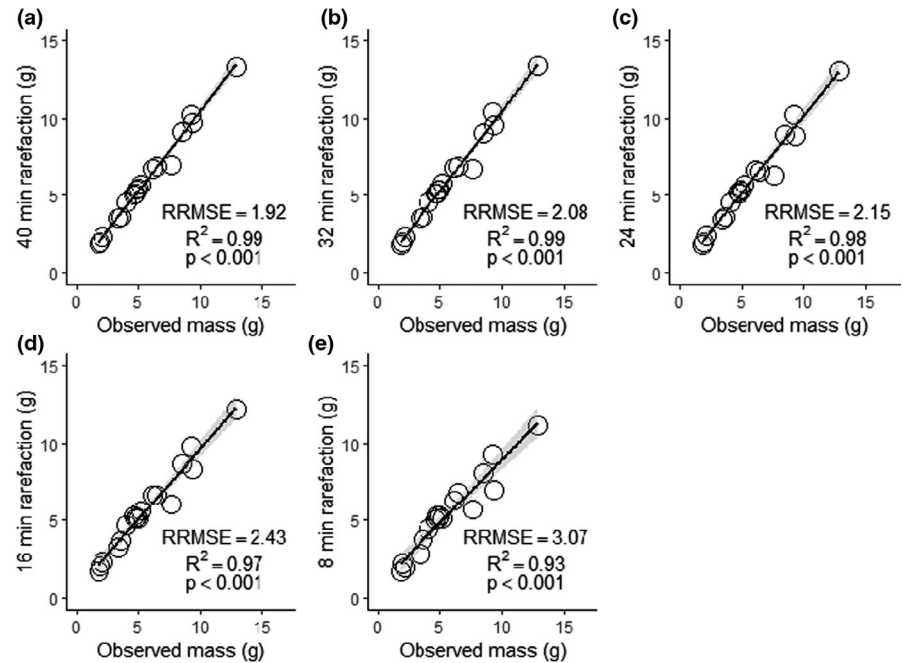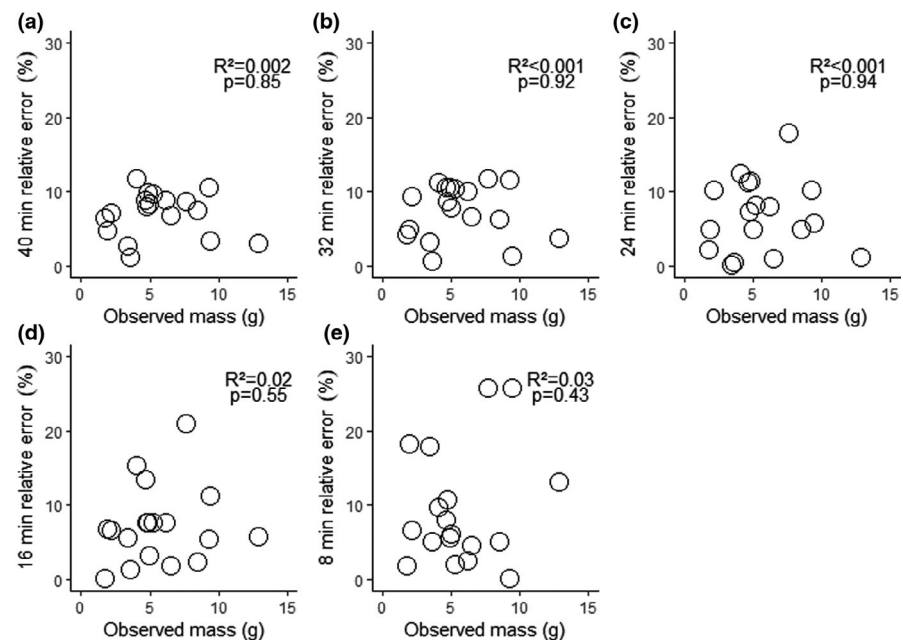

FIGURE 4 The Weibull model estimation relative error at different rarefaction times (40, 32, 24, 16, and 8 min) as a function of the observed fine-root mass. There was strong evidence of no relationship between the relative error and observed mass for any collection times (*p*-value > 0.05)

(Figure 5). As expected, the ecosystems' absolute mass varied, but there was strong evidence that the percentage of the observed mass collected over 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).

4 | 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 mass observed 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 (Marthews et al., 2014; Metcalfe et al., 2007), 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's properties. Weibull is a type of distribution that has been known to be 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).
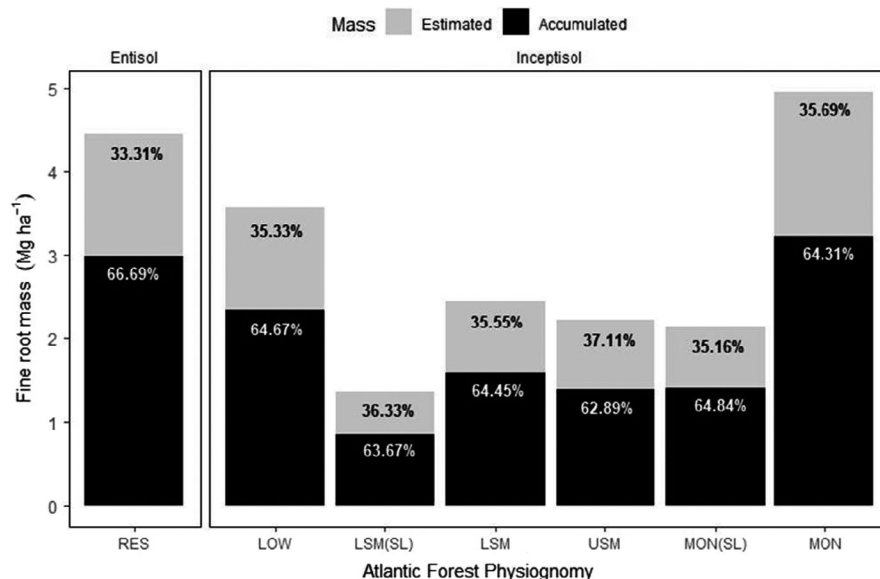
FIGURE 5 Absolute (Mg/ha) and relative (%) fine-root mass quantified for 120 min in seven ecosystems along an elevational gradient in Atlantic Forests. Black bars represent the percentage of the mass collected in the first 8 min (fieldwork with four series of 2 min each). Gray bars represent the percentage of mass estimated (uncollected) by the Weibull model at 120 min. There was strong evidence (p-value = 0.499) that the percentage of collected and estimated mass did not differ among ecosystems, soil types (Entisol vs Inceptisol), and land-use histories (old-growth vs selective-logging). Acronyms: RES: old-growth Restinga; LOW: old-growth lowland forest; LSM(SL): lower submontane forest post selective logging; LSM: old-growth lower submontane forest; USM: old-growth upper submontane forest; MON(SL): montane forest post selective logging; MON: old-growth montane forest

The Weibull distribution has been commonly used in forest science, particularly to predict patterns of above-ground structures, such as tree diameter distributions (Mcgarrigle et al., 2011; Zhang & Liu, 2006) 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 (Guo et al., 2021; Schwarz et al., 2013). We found that Weibull works well for modeling 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 collecting roots in the field. In this study, for example, just by reducing the observed collection time per soil core to 8 min, we spent 88% less time extracting roots per study site — from 1080 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 mass estimated for 120 min was collected in the initial minutes, even at the shortest observed collection time (where the accumulated reference sample masses represented 64.5%, and the remaining mass was estimated by modeling), 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 modeling process, whose final estimate will have the built-in correction.

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 absence 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., 2011; Silva et al., 2020; Sousa Neto et al., 2011), 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.

AUTHOR CONTRIBUTIONS

Cinthia A. Silva conceived the ideas and designed the methodology and collected the data; Cinthia A. Silva, André M. D'Angioli, and Marcos A. S. Scaranello analyzed the data; Bruno Bordron structured the manuscript's draft; Vinícius Londe and Cinthia A. Silva led the manuscript's writing; Carlos A. Joly and Simone A. Vieira obtained funding and supervised the research group.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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