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Research

Methods

A method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy

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Summary

- This study evaluates a novel method for extracting roots from soil samples and applies it to estimate standing crop root mass (± confidence intervals) in an eastern Amazon rainforest.
- Roots were manually extracted from soil cores over a period of 40 min, which was split into 10 min time intervals. The pattern of cumulative extraction over time was used to predict root extraction beyond 40 min. A maximum-likelihood approach was used to calculate confidence intervals.
- The temporal prediction method added 21–32% to initial estimates of standing crop root mass. According to predictions, complete manual root extraction from 18 samples would have taken *c.* 239 h, compared with 12 h using the prediction method. Uncertainties (percentage difference between mean, and 10th and 90th percentiles) introduced by the prediction method were small (12–15%), compared with uncertainties caused by spatial variation in root mass (72–191%, for nine samples per plot surveyed).
- This method provides a way of increasing the number of root samples processed per unit time, without compromising measurement accuracy.

Key words: Amazon tropical rainforest, maximum-likelihood approach, methodological evaluation, root sampling method, standing crop root mass, temporal prediction method.

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Introduction

Trees allocate a considerable portion of carbon (C) fixed through photosynthesis to fine roots (4–69%; Vogt *et al.*, 1996 and references therein), and the amount of C and nutrient inputs to soil via root mortality and decay often equals or exceeds that of leaf litter fall (Nadelhoffer & Raich, 1992; Hendrick & Pregitzer, 1993; Roderstein *et al.*, 2005). Root growth, mortality and decay are also dynamic processes that are highly sensitive to environmental change (Gill & Jackson, 2000; Majdi & Ohrvik, 2004). Yet despite their importance to our understanding of ecosystem nutrient cycling and global biogeochemistry, there is relatively little information about the amount and spatial distribution of roots in terrestrial ecosystems. For example, Houghton *et al.* (2001) state that, ‘Given the Kyoto Protocol and imminent need to determine sources and sinks of carbon resulting from land-use change (and, perhaps, from natural processes as well), methods that can determine biomass accurately, repeatedly, and inexpensively are desperately needed.'

This gap in our knowledge partly results from the large amount of effort, in terms of time and labour, required to extract roots from the surrounding soil (particularly non-woody, fine roots). For example, Bernier *et al.* (2005) report that complete manual extraction of roots from soil cores (with a diameter of 4.5 cm, to a depth of 25 cm) takes up to 24 h per core. There is a clear trade-off between investing sufficient effort in each soil sample to derive an accurate measurement of root mass and taking enough samples to capture the majority of spatial and temporal variation in root mass.

The most common approach for isolating roots is to extract a soil core and then separate roots from the surrounding soil over a sieve, either by hand (Prathapar *et al.*, 1989) or using some type of elutriation system (Chotte *et al.*, 1995; Benjamin & Nielsen, 2004). However, all these methods are likely to underestimate the amount of root material in soil samples, because a proportion of the roots inevitably pass through the sieve, or remain uncollected by hand (Sierra *et al.*, 2003).

Using sieves with finer mesh diameter will isolate more root material, but then a relatively larger quantity of mineral grains and organic detritus will not pass through the sieve, and so the researcher is still left with the difficult task of separating roots from detritus. For example, Benjamin & Nielsen (2004) designed an automatic root sieve-washing system which processes up to 24 samples in 1.5 h. However, after washing, samples are still contaminated with detritus, and to isolate roots from detritus manually takes an additional 20 h per washed sample.

One method of compensating for root mass underestimates is to exhaustively extract all root material from a subset of soil samples, and then to use these data to derive a generic correction factor which is applied to the rest of the dataset (see, for example, the recommended protocol in MacDicken, 1997). A key problem with this approach is that the degree of underestimation is likely to vary between samples and locations, and therefore applying a generic correction factor will lead to inaccurate estimates of root mass. To our knowledge, no current methods provide a simple and quick way of quantifying, and correcting for, root mass underestimates on a sample-by-sample basis.

Finally, with current methods it remains difficult to determine whether observed differences in root mass between studies and sites reflect not only real biological differences, but also differences in site characteristics (e.g. soil texture) and equipment (e.g. sieve mesh diameter). For example, soil clay content could affect root structure and function (Silver *et al.*, 2005), but additionally it may also alter the efficiency of root sample extraction from the soil matrix. Thus, the confounding influence of site and equipment differences hinders attempts to interpret and understand the role that roots play in different ecosystems.

These problems can be minimized, however, if current methods are modified so that root collection per sample is divided into separate time intervals to reveal the pattern of root extraction over time. If the amount of roots extracted over time changes in a predictable way, then, even after sample processing has finished, the amount that would have been extracted if processing had continued may be estimated. For prediction to be accurate, within-sample processing effort should remain as constant as possible over the entire period of processing. This 'temporal prediction' method potentially increases the rate of sample processing without compromising measurement accuracy, and may also correct for the confounding effect of variation between samples in terms of root extraction rate caused by site and equipment differences. Since a different curve is calculated for every single soil sample, based upon the unique pattern of root extraction observed from each sample, the temporal prediction method should prove applicable for a wide range of vegetation and soil types. If root extraction were performed several times over, for the same sample, it is likely that there would be some variation in the amount of roots collected between iterations, even if processing conditions remained identical. In this study, this source of variation was referred to as within-sample measurement error. Using a maximum-likelihood approach, this measurement error can be incorporated into an estimate of the total amount of root matter in soil samples, and thus provides confidence intervals on the estimate. The objectives of this study therefore were: (i) to evaluate whether root mass extraction from soil cores can be accurately predicted; (ii) to quantify within-sample measurement error for root mass collected at each time interval; and (iii) to use the maximum-likelihood technique to estimate mean (± confidence intervals) standing crop root mass (t ha^{-1}) in two rainforest plots in the eastern Amazon.

In this study, the temporal prediction method is applied to provide estimates of root mass, but there is nothing to prevent application of the same approach to estimate root length, surface area or volume from soil samples. The main change necessary is that roots collected from soil samples, instead of being weighed, should be scanned and analyzed with commercially available software to record root morphology. In addition, root samples collected in each time interval may be subdivided into categories (e.g. live/dead, mycorrhizal/non-mycorrhizal, fine/coarse, different species) to derive a more detailed assessment of root material present in soil samples.

Materials and Methods

Field site and sampling

The experimental site is located in the Caxiuanã National Forest, Pará state, eastern Brazil (1°43′3.5″S, 51°27′36″W). The forest is a lowland *terra firme* rainforest with high annual rainfall (*c.* 2500 mm) but a pronounced dry season (Fisher *et al.*, 2006). The most widespread soil type is a highly weathered yellow Oxisol (Brazilian classification: Latosol),

Table 1 Soil and vegetation characteristics at each plot surveyed

Plot characteristics	Plot A	Plot B
Vegetation		
Tree number (individuals ha^{-1})	434	419
Stem basal area (m^2 ha^{-1})	23.9	25.1
Leaf area index (m^2 m^{-2})	5.1 ± 0.6	5.5 ± 0.7
Soil		
pH	4.5 ± 0.3	3.8 ± 0.1
Water content (%)	17.5 ± 1.3	27.8 ± 3.1
Cation exchange (mol dm^{-3})	0.8 ± 0.2	1.3 ± 0.3
Clay content (%)	15.2 ± 3.9	44.5 ± 1.8
Carbon content (g kg^{-1})	9.6 ± 0.3	19.3 ± 0.9

Values are means ± SD.
Tree number and basal area represent all individuals > 10 cm diameter at breast height, measured in January 2005. Leaf area index data were captured with hemispherical images of the canopy, and analyzed with commercially available image analysis software. Leaf area index values presented are means of 25 replicate images recorded in November 2001, 2002 and 2003. Values for soil characteristics are means of four replicate measurements at each plot, with the exception of soil moisture values, which are derived from 25 replicate measurements in June 2005.

although there is substantial spatial variation in the relative proportion of sand (> 0.05 mm particle diameter) and clay (< 0.002 mm particle diameter). Two 1 ha (100 × 100 m) plots were established at locations with different vegetation and soil characteristics (Table 1). For further details of soil texture and chemistry at the site, see Ruivo & Cunha (2003).

Quantifying prediction accuracy

Eight soil cores (diameter, 14 cm; depth, 30 cm) were extracted from an area adjacent to plot A with matching vegetation and soil characteristics, using opposable semicircular cutting blades. A potential disadvantage of this approach is that there may be minor variations in the volume of soil extracted as a core, which could contribute to apparent spatial variation in root mass. Conventional cylindrical soil corers were not used because they could not sever some of the coarse roots encountered, and caused considerable soil compaction. The opposable semicircular cutting blades were retracted, in order to extract discrete portions of the core at a time (thus minimizing compaction), and a knife was used to sever coarse roots encountered within the core hole. The soil in each core was homogenized, and roots were removed from the soil cores by hand over a period of 120 min, which was split into 10 min time intervals. Subsequently, roots extracted at each interval were cleaned of residual soil and detritus, dried at 70°C to constant mass and weighed. Cumulative sample dry root mass extracted at each time interval was plotted against time for each core. Two different curve types (saturation and logarithmic) were fitted to the first 40 min of manual root extraction, and used to predict the pattern of extraction up to 120 min. Predicted root mass collected between 50 and 120 min was then compared with the actual amount of root material manually collected over the same period. The saturation curve is described by the following equation:

$$R_t = R_c t/(k_r + t)$$

(R_t, root mass extracted at time t; R_c, total root mass in the sample; k_r, a half saturation constant). The logarithmic curve is described by the following equation:

$$R_t = a \log(t) + b$$

(a, a constant defining the shape of the curve; b, the intercept). Other curve formulas (exponential, power, second-order polynomial) were also tested, but were found to be unsuitable for this particular situation.

Estimating within-sample measurement error

There is likely to be some uncertainty around root mass extracted at each time interval for each soil sample, caused by within-sample measurement error. This measurement error cannot be assessed with live root material because root tissue dries, and therefore loses mass, over time. To avoid this problem, the following experiment was devised. A single soil core was extracted (diameter, 14 cm; depth, 30 cm), the majority of roots were removed with a sieve, and the soil was homogenized. Forty-five grams of wire segments of different colors (black, brown, and white), thicknesses (0.5, 1, 2 and 5 mm diameter) and lengths (0.5, 1, 3 and 5 cm) were thoroughly mixed into the soil sample. Wire segments were then manually removed from the soil over a period of 40 min, which was split into 10 min time intervals. Segments extracted from each interval were weighed. At the end of the collection period, the segments extracted were then mixed back into the same soil sample, and the process was repeated a further nine times. These data were used to estimate sample-specific mean and variation in the cumulative mass of segments collected at each time interval.

Field application and data analysis

In June 2005, nine soil cores (diameter, 14 cm; depth, 30 cm) were removed at locations along a regular grid within each plot, using opposable semicircular cutting blades. The soil in each core was homogenized, and roots were removed from the soil cores by hand over a period of 40 min, which was split into 10 min time intervals. The cumulative increase in roots extracted over time was used to fit a curve which predicted root extraction rate. There was some within-sample measurement error around mass collected at each time interval. There were therefore multiple parameter combinations and curves that fitted within the error limits of the observed data for each

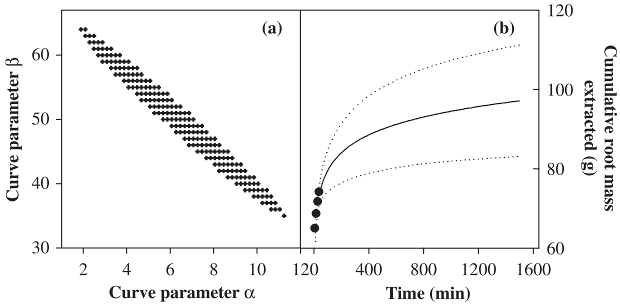

Fig. 1 (a) Parameter combinations that adequately describe the observed pattern of root extraction within specified within-sample measurement error limits; (b) the resulting range in predicted cumulative mass collected until the cut-off point at 740 min. Data are taken from core 4, plot A. Crosses, parameter combinations; closed circles, manually extracted mass; solid black line, mean predicted extraction curve beyond 40 min; dotted black lines, 10th and 90th percentiles around mean predicted curve. Means and percentiles are calculated from the range of curves specified by the parameter combinations in (a).

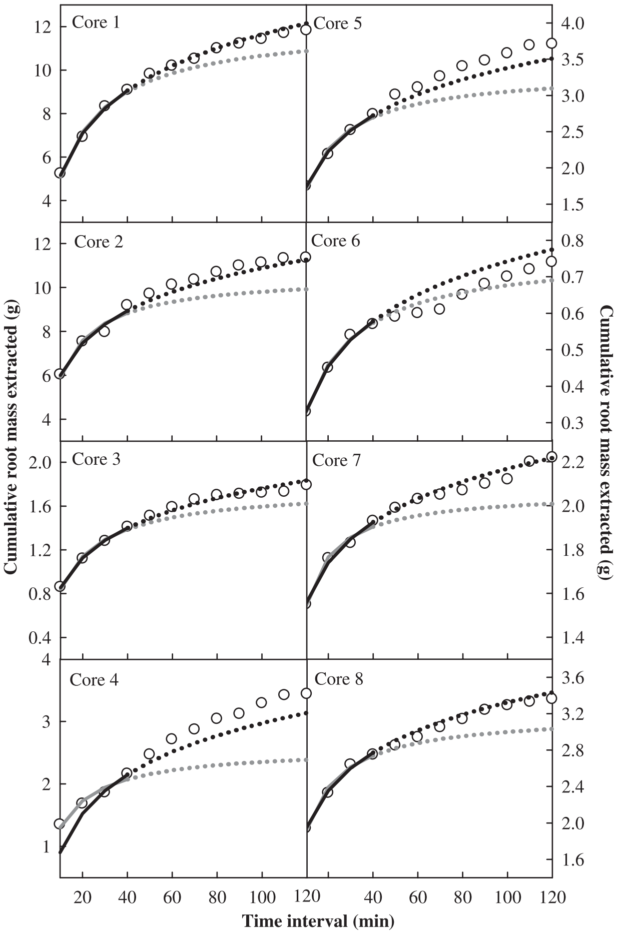

Fig. 2 Observed and predicted cumulative root mass extraction over a period of 120 min, from eight different soil cores. Predictions are based on the pattern of extraction observed between 0 and 40 min. Open circles, manually collected root mass; black line, mass extraction predicted by the logarithmic equation; gray line, mass extraction predicted by the saturation equation.

sample (Fig. 1). A maximum-likelihood approach was used (van Wijk *et al.*, 2002; Williams *et al.*, 2006) to fit a range of acceptable curves, based upon the observed cumulative increase in roots collected over 40 min for each sample together with estimates of within-sample measurement error around each data point (see the previous section). Based upon curves fitted by the maximum-likelihood approach, root mass extraction (± confidence intervals) beyond 40 min was predicted. The optimal parameters were found by minimizing the following objective function ($O(p)$):

$$O(p) = \sum_{i=1}^{n} \frac{1}{\sigma_{yi}^{2}}\left(y_{i,\mathrm{meas}}(x_i) - y_{i,\mathrm{mod}}(x_i : p)\right)^2$$

(n, total number of measurements; p, number of model parameters; $y_{i,\mathrm{meas}}(x_i)$, measured value of output variable y at the value x_i of the driving variable x; $y_{i,\mathrm{mod}}(x_i : p)$, modeled value of the output variable at the value x_i of the driving variable x given the parameters p; σ_{yi}^2, within-sample measurement error variance for each of the observations). The minimal sum-of-squares followed a chi-squared distribution with $n - p$ degrees of freedom. A Monte-Carlo approach was used to generate parameter confidence regions, varying the two unknown parameters at 100 points linearly arranged between specified maximum and minimum values ($8 < R_c < 80$ and $0.01 < k_r < 10$; $0.1 < a < 20$ and $0.0 < b < 100$). A chi-squared test was used to determine which of the 10 000 parameter combinations that could possibly explain the pattern of root extraction from each soil sample lay within a 95% confidence interval of the observations.

Owing to the nature of a logarithmic curve, the predicted amount of root material extracted never saturated and it was therefore necessary to select a cut-off point to determine the maximum root biomass. In this study, this point was when root mass extracted in a single 10 min time interval was less than 1% of the cumulative total mass already collected. Differences in mean uncorrected (roots manually collected within the first 40 min) and corrected (roots manually collected plus the predicted amount of roots gathered until the cut-off point) masses were assessed with the paired sample *t*-test (output = test statistic *t* and significance *P*-value). Mass values were square-root-transformed to conform to the assumptions of parametric analysis. Statistical analysis was carried out using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Prediction accuracy assessment

The curve equations fitted to the first 40 min of root extraction from homogenized soil samples showed a close fit to the pattern of extraction between 50 and 120 min (Fig. 2;

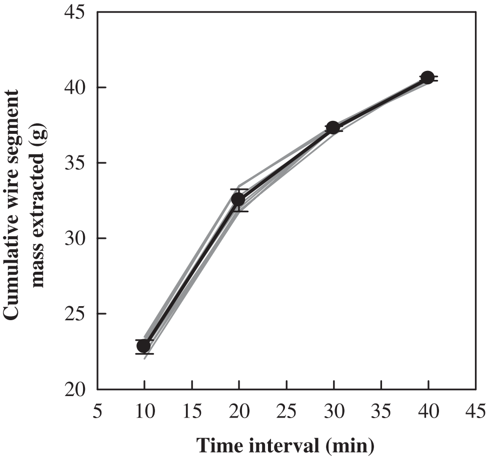

Fig. 3 Cumulative wire segment mass extraction over 40 min from 10 replicated measurements for the same soil sample. Gray lines, individual measurements; black open circles and black line, mean of 10 measurements. Error bars, SD of the mean.

mean R^2 of 0.97 and 0.96 for the logarithmic and saturation equations, respectively). The logarithmic equation underestimated manual extraction by 1.9%, while the saturation equation underestimated it by 8.2%. For the saturation equation, the extent of the underestimate increased with each consecutive time interval (Fig. 2). In contrast, there was no systematic change in the fit between the logarithmic predictions and observed data over time (Fig. 2). The logarithmic curve equation was chosen to predict root extraction from all soil cores extracted from plots A and B, although the equation parameters varied between soil cores.

Within-sample measurement error assessment

There was little within-sample measurement error around replicated measurements of wire segment extraction from a homogenized soil sample (Fig. 3, Table 2); standard deviation as a percentage of the mean mass across all time intervals was 1.3%. Measurement error decreased over time (Fig. 3, Table 2), as each repeated extraction of wire mass converged towards the upper limit of wire mass present in the sample. A within-sample measurement error of 3% was assigned around values of mass extracted at every time interval, for all soil cores extracted from plots A and B. This error value was greater than that calculated directly from the measurement error assessment but ensured that uncertainty around predicted mass was not underestimated.

Table 2 Results of within-sample measurement error assessment

Time interval (min)	Mean wire mass (g)	Range	Variance	SD
0–10	22.80	1.46	0.21	0.45
10–20	32.52	1.79	0.54	0.73
20–30	37.27	0.62	0.03	0.17
30–40	40.59	0.44	0.02	0.15

Mean and variation of cumulative wire segment mass collected for each time interval. Means and measures of variation are derived from 10 replicated measurements for the same soil sample, for each time interval.

Field application of method

The number of curves that could account for the observed pattern in cumulative root mass extracted from each sample ranged between three and 429 out of the 10 000 parameter combinations tested. Considering only root mass extracted in the first 40 min, estimated mean standing crop root masses in the top 30 cm of soil were 38.7 and 32.6 t ha^{-1}, for plots A and B, respectively (see estimates from individual cores in Fig. 4). Incorporating the predictions of the curves significantly increased these initial estimates of mean plot standing crop root mass by 21% (47.0 t ha^{-1}) for plot A and 32% (43.2 t ha^{-1}) for plot B ($t = 10.1$, d.f. = 16, $P < 0.001$). According to the temporal prediction method, it would have taken, on average, an additional 12.6 h per sample (ranging between 1.0 and 18.3 h) to collect this extra root material manually.

The range of acceptable parameter combinations and curves per sample resulted in a range of values for the predicted root mass extracted (see error bars in Fig. 4). However, the uncertainty (quantified as the percentage difference between the mean and 10th and 90th percentiles) caused by

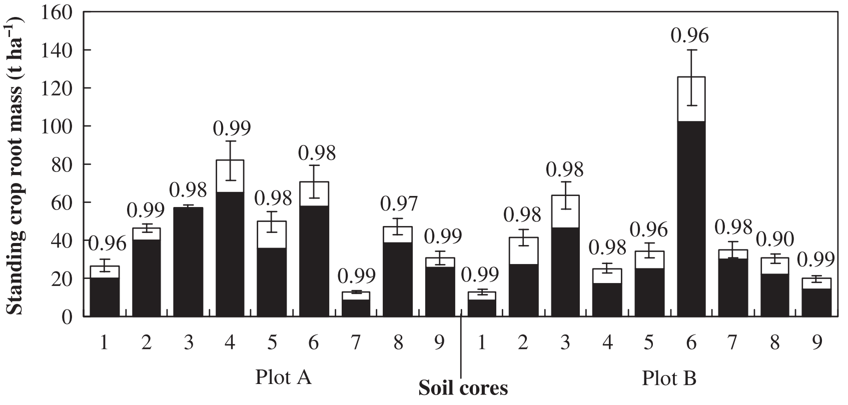

Fig. 4 Standing crop root mass estimated from soil cores extracted in each plot. Values above each bar represent the R^2 of the fit between the predicted and observed root masses extracted in the first 40 min, for each sample. Closed columns, uncorrected mass (roots manually collected within the first 40 min); open columns, additional mass from incorporating the temporal prediction method. Error bars, 10th and 90th percentiles around mean predicted mass collected.

within-sample measurement error was relatively small (3% for uncorrected mass, and 12–15% for corrected mass) compared with the uncertainty introduced by spatial heterogeneity in standing crop root mass (72–191%; see the variation in standing crop root mass between cores in Fig. 4).

Discussion

Method assessment

Division of the processing period into time intervals provided a simple way of checking how thoroughly a chosen processing method removed root material from the soil. Furthermore, results of the prediction accuracy assessment indicate that this method can also be used to correct for mass underestimates when extracting roots from soil (Fig. 2). Although it was only possible to verify predictions over a time period of 120 min for eight soil samples, further work, checking predictions against root collection over longer periods of time, could reinforce the preliminary conclusions of this study.

While a logarithmic curve formula accurately describes root mass extraction in this study, it is possible that other curve formulas may be more appropriate for predicting extraction of root length and surface area; in which case, the best-fitting curve formula may be identified by following the curve prediction accuracy assessment procedure outlined in the Materials and Methods section. We explicitly present the saturation curve formula, even though it underestimates root mass extraction at our field site, because it directly predicts the upper limit of roots collected (R_c, see description of the curve formula in the Materials and Methods section) and may be more applicable in other situations, or when using more extraction time intervals. The specific combination of curve formula and time interval length and number may be modified to adapt to a wide range of research situations.

To our knowledge, this study provides the first approximate estimate of within-sample measurement error in root mass estimates. We propose that this estimate of measurement error is not likely to vary substantially between soil samples, as long as the myriad factors that affect root (or wire segment) extraction for each individual sample remain relatively constant across repeated measurements of the same sample. However, the degree of variation in within-sample measurement error between different samples could be directly addressed by repeating the measurement error assessment (Fig. 3) on soil or wire samples with different characteristics (e.g. soil texture, wire diameter distribution). The measure of within-sample measurement error used in this study (standard deviation as a percentage of mean mass) corrects for the confounding influence of variation in mass between time intervals and cores. We conclude that further work is required to test and refine the temporal prediction methodology under different field conditions, and to examine different root characteristics (e.g. length, surface area).

Estimates of standing crop root mass

In this study, estimates of mean standing crop root mass in the top 30 cm soil layer are 47.0 and 43.2 t ha^{-1} in plots A and B, respectively (see estimates from individual cores in Fig. 4). To estimate standing crop root mass for the entire soil column, we used data and equations derived from root profiles taken from tropical evergreen forests and estimated that 28% (in between the values of 31 and 24% reported by Jackson *et al.*, 1996 and Schenk & Jackson, 2002, respectively) of the total root mass present at this site occurs below the depth sampled. Thus, estimates of standing crop root mass for the entire soil column are 60.2 t ha^{-1} on plot A and 55.3 t ha^{-1} on plot B. These estimates are higher than most values reported from similar ecosystems (mean of 49 t ha^{-1}, Jackson *et al.*, 1996) This difference may be partly the result of underestimates of root mass in previous studies which do not use the temporal prediction method proposed here, although the extent of this effect is difficult to assess because of additional differences among studies in terms of vegetation type and methods. In this study, standing crop root mass estimates are likely to be conservative because logarithmic curves generated from 40 min of manual sampling consistently underestimated the actual amount of root material extracted (Fig. 2).

At this site, a large number of samples are required to capture spatial heterogeneity in standing crop root mass. For example, 119 and 157 soil core samples are required to estimate standing crop root mass in plots A and B, respectively, within 10% confidence intervals with 95% probability (D. B. Metcalfe, unpublished). To achieve these recommended sample sizes would take one person approx. 66 and 87 d (sample size multiplied by mean sample processing time per person, estimated using the temporal prediction method, of 13.3 h) of manual root collection per person for plots A and B, respectively. By contrast, to process the same number of samples using the combined manual collection and subsequent temporal prediction approach would take approx. 3 d per person for plot A and 4 d per person for plot B (sample size multiplied by 40 min). Additional work necessitated by the use of the temporal prediction method (e.g. assessment of curve prediction accuracy and within-sample measurement error) adds approx. 1 d more. The temporal prediction method proposed here therefore provides a means to obtain the large sample sizes required to quantify standing crop root mass, without compromising measurement accuracy.

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