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An Accurate and Efficient Method for Sorting Biomass Extracted from Soil Cores Using Point-Intercept Sampling

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ABSTRACT

We describe a point-intercept sampling technique that reduces the time and therefore the cost associated with hand sorting biomass extracted from soil cores. Typically, organic material that has been extracted from soil cores is painstakingly separated into categories such as roots, leaves, and unidentifiable organic matter so that each can be weighed. With the point-intercept method, we spread the extracted organic material over a grid and record the category of randomly located point intercepts within grid cells. The proportion of each category determined via point intercepts is then attributed to the total dry mass of the organic material. With a subset of our data, we determined ordinary least squares regression relationships between hand-sorted (census) and point-intercept (sample) estimates of the belowground biomass components roots, aboveground detritus, and soil organic matter. We then applied these regression models to the remainder of our data, which had been hand sorted to serve as a validation dataset. Using bootstrapped 95% confidence intervals of the ordinary least squares (OLS) bisector slope estimate, we found no significant differences between the point-intercept and hand-sorted values for all three belowground biomass components. The time saved sorting belowground biomass by the point-intercept method (~15 min core^{-1}) allowed us to process 43% more cores during the same period. We applied the same technique to components of aboveground herbaceous biomass, but with less success because these pools tended to be less uniformly distributed throughout the sample layer. We recommend the approach for sorting belowground biomass components from soil cores, but the method requires more development before being used to sort other ecosystem components.

Fine root production is a major component of net primary productivity (NPP) in most ecosystems. Fine roots account for 30 to 50% of the annual C contribution to NPP in forests (Grier et al., 1981; Jackson et al., 1997) and an even higher percentage in grasslands (Schlesinger, 1997; McNaughton et al., 1998; Tufekcioglu et al., 1998). Net primary productivity is an important metric of ecosystem response to climate change and disturbance (Grier et al., 1981; Jackson et al., 1997; Millikin and Bledsoe, 1999; Johnson and Matchett, 2001). The amount of biomass fixed by primary producers and available to consumers drives trophic dynamics and biogeochemical cycling (Schlesinger, 1997). Measuring belowground biomass is difficult and time-consuming (Vogt et al., 1998). Despite the promise of indirect approaches such as N budgeting (Aber et al., 1985) and isotopic tracers (Bledsoe et al., 1999; Fahey et al., 1999), most estimates of root production still depend on conventional biomass assessment (e.g., sequential coring and ingrowth cores) (Bledsoe et al., 1999; Fahey et al., 1999; Lauenroth, 2000). The most daunting practical problem with biomass assessment of roots is the high labor cost associated with washing and sorting the samples (Persson, 1990). Moreover, the tremendous variability in the spatial distribution of fine root biomass compels the collection of many cores to accommodate this inherent heterogeneity. Typically the soil cores (usual volume <300 cm^3) are washed over screens to remove soil, rocks, and debris leaving a mass of organic matter spanning the continuum of decomposition from large partially decayed leaves and twigs to fine particulate matter whose original form is indeterminate. Separating fine roots (<2 mm diam.) from other categories of organic matter is a tedious, time-consuming process most often performed by undergraduate assistants. Turnover of these assistants is usually very high, thereby raising the costs associated with training and quality control.

Here we describe a point-intercept approach to measuring the fine root mass in washed soil cores. Using the complete census of fine roots as the standard, we calculate the accuracy and efficiency of the point-sampling technique. We also explore the general utility of this approach by applying it to other cryptic components of ecosystems biomass budgets.

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MATERIALS AND METHODS

Study Site

We conducted this work at the University of California Sierra Foothill Research and Extension Center, located approximately 32 km northeast of Marysville, CA (39°15′ N, 121°17′ W) in the Sierra Nevada foothills. The climate is Mediterranean; cool, wet winters and hot, dry summers predominate. Mean annual temperature is 15.8°C and mean annual precipitation is 71 cm. Soils in this area are generally shallow and classified as Auburn (loamy, mixed, superactive, thermic Lithic Haploxerepts) and Argonaut (fine, mixed, superactive, thermic Mollic Haploxeralfs) series (Jackson and Allen-Diaz, 2002). Soils are derived from metavolcanic greenstone bedrock (Herbert and Begg, 1969).

The study area was an oak savanna consisting primarily of deciduous blue oak (*Quercus douglasii* Hook. & Arn.) and annual grasses (Shlisky, 2001). Other trees and shrubs included

Abbreviations: aHS, actual hand-sorted biomass; AIC, Akaike's Information Criterion; HS, hand-sorted biomass; NPP, net primary productivity; OLS, ordinary least squares; pHS, predicted hand-sorted biomass; PI, point intercept estimate; SOM, soil organic matter; TOT, total core biomass.

evergreen interior live oak (*Quercus wislizenii* A. DC.), gray pine (*Pinus sabiniana* Douglas), poison oak [*Toxicodendron diversilobum* (Torr. & A. Gray) Greene], California coffee berry (*Rhamnus californica* Eschsch.), and buckbrush [*Ceanothus cuneatus* (Hook.) Nutt.]. Dominant annual species were dogtail (*Cynosurus echinatus* L.), Italian ryegrass (*Lolium multiflorum* Lam.), soft chess (*Bromus hordeaceus* L.), red brome (*Bromus madritensis* L.), ripgut brome (*Bromus diandrus* Roth), and rose clover (*Trifolium hirtum* All.).

Soil Core Sampling and Processing

As part of a study to quantify the net primary productivity of these oak savannas (UC Integrated Hardwood Range Management Program, Project no. 00–1), sequential coring was used to measure the fine root contribution in 36 circular plots (11-m radius) evenly distributed across three watersheds (35, 80, and 116 ha in area). For two sampling periods, early summer and fall, three systematically located soil cores (15-cm depth, 5-cm i.d., AMS Core Sampler, American Falls, ID) were collected per plot for a total of 108 cores. For this analysis we used cores collected in October 2002 immediately following the first winter rainstorm, and we used cores taken at peak standing crop in June 2003. Cores were transported to the laboratory in a cooler and then stored at 5°C for up to 3 mo. Initially we used a commercial elutriator (Bel Art Products, Pequannock, NJ) to wash soil and rocks out of the cores. We collected all root and organic matter fragments left on a 1-mm mesh screen. Washing cores with the elutriator took from 20 to 30 min per core; we found that washing samples by hand over a 1-mm mesh screen took only 10 to 15 min per core. Thus all the cores used in this study were first washed by hand. The washed organic matter was stored in plastic bags at 5°C for up to 1 mo until further processing.

In effort to speed the sorting of fine roots, we devised a point-intercept approach. Each sample of washed organic matter was spread out evenly on a 12.5 by 20 cm clear plastic tray. Beneath the tray was a grid of fifty 2.0 by 2.5 cm cells. The tray was passed under a dissecting microscope set to 0.7× magnification with a pointer in one eyepiece. The tray was placed under the dissecting scope such that the initial cell would be within the scope's viewer. Subsequently, the tray was systematically moved under the microscope so that the pointer would land in an arbitrary location within each cell. We recorded the first object in the pointer's path for each box, resulting in 50 hits per sample. Categories recorded were aboveground detritus, roots, or soil organic matter. Aboveground detritus included oak leaves, grass stems, grass seeds (>1 cm in any one direction), and twigs <2-mm diameter. Roots included all fine roots <2-mm diameter. Soil organic matter (SOM) was composed of aboveground detritus and roots that were too small to be hand sorted (i.e., <0.5 cm long). Twigs and roots >2-mm diameter, moss, bark, and pine cones were removed from the sample and discarded. The sample was then gathered off the tray, and oven dried at 65°C for 48 h to determine the total dry weight of the sample. Biomass of estimates of detritus, fine roots, and SOM based on the point-intercept technique were calculated as the product of the total dry mass of each sample and the proportion of the 50 sample points intercepted by each category.

To evaluate the performance of the point-intercept technique, we randomly selected 50 cores from two sample dates (October 2002, June 2003). After recording the point interceptions, the sample was completely hand sorted into the same three categories: detritus, fine root, and SOM. The tissue in each category was oven dried and weighed, as above. The time taken to process each core was recorded.

Comparing Hand-Sorted and Point-Based Biomass Estimates

We explored the ability of point-intercept estimates of fine root biomass to predict hand-sorted biomass by modeling hand-sorted biomass (HS) as a function of the point-intercept estimate (PI) and the total core biomass (TOT). We used a random subset of 30 dual samples to develop our models and reserved the remaining 20 dual samples for validation. We used ordinary least squares regression to fit a full model, HS = $a + b$(PI) + c(TOT), and a reduced model, HS = $a + b$(PI). We selected the best model using Akaike's Information Criterion (AIC), which accounts for the tradeoff between explanatory ability of a model (i.e., residual deviance) and the number of model parameter estimates (i.e., degrees of freedom) (Burnham and Anderson, 1998). When the two models were significantly different ($P < 0.05$), the model with the lowest AIC was selected as the most parsimonious model; otherwise the simplest model was chosen. Models with $R^2 < 0.1$ were not considered in the ensuing validation phase.

We used the subset of 20 reserved samples (i.e., samples not used to develop the model) to validate the predictive ability of the best point-intercept model. If the model is an accurate predictor, then the slope of the relationship between the predicted hand-sorted mass (pHS) and the actual hand-sorted biomass (aHS) should not significantly differ from 1. Because uncertainty exists in both pHS and aHS, it is not a straightforward decision whether aHS should be regressed on pHS or vice versa. Hence, we used the OLS bisector method to estimate intercepts and slopes (Isobe et al., 1990), which effectively "splits the difference" between the regression lines generated by alternately regressing aHS on pHS and pHS on aHS. We then calculated bootstrapped 95% confidence intervals (Crawley, 2002) for the OLS bisector slope estimate to determine whether it bounded the 1:1 line.

Extending the Point-Intercept Method

Sorting the contents of litter collection and herb clippings is another tedious chore commonly encountered in plant and ecosystem ecology. The fundamental challenge is the same as soil cores—small pieces of tissue need to be sorted into categories. Following the approach used for the soil cores, we collected 40 random samples from our study plots. For each sample, we collected aboveground herbaceous biomass in paper bags by clipping a 0.0625-m^2 quadrat to ground level. In the same bag, we put all the leaf litter from the surface of the same quadrat. Bags were dried at 65°C for 48 h before the contents were evenly spread across a 29 by 45 cm tray divided into twenty 7.25 by 9 cm cells. This grid was larger than that used for the belowground biomass because the total sample volume was greater for the aboveground biomass. Five hits were recorded in each box, for a total of 100 sample points, to estimate the proportion of live grass, live forb, live legume, dead herb, twig, pine needle, and oak leaf litter. The entire sample was then hand sorted into the same categories, weighed, and recorded. A random subset of 23 quadrat samples were used for model development: the remaining 17 were reserved for validation. We used the same procedures and criteria described for the roots to fit, choose, and validate the point-intercept technique for sorting the quadrat samples.

RESULTS

For the three ecosystem components in the soil core, there was a strong linear relationship between the PI

Table 1. Summary of results for linear models predicting actual biomass as a function of point-intercept estimate (PI) + total core biomass (TOT). If full and reduced models for a given ecosystem component were not significantly different ($P < 0.05$) the reduced model was selected (selected models shown in italics).

Ecosystem component	Linear model	Parameter estimates: Intercept	Slope	Total	Model comparison	AIC†	R^2	F	P
Belowground									
Roots	PI + TOT	0.027	0.649	−0.030		−57.83	0.80		
	PI	0.013	0.577		PI + TOT vs. PI	−57.14	0.78	2.53	0.12
Soil organic matter	*PI + TOT*	−0.077	0.699	0.365		−41.10	0.98		
	PI	0.152	1.085		PI + TOT vs. PI	−16.39	0.94	38.76	0.000001
Aboveground	*PI + TOT*	0.051	0.297	0.066		−51.26	0.74		
	PI	0.087	0.494		PI + TOT vs. PI	−47.93	0.69	5.25	0.03
Aboveground									
Oak leaves	PI + TOT	1.232	0.919	−0.014		75.03	0.96		
	PI	1.146	0.899		PI + TOT vs. PI	74.14	0.96	0.10	0.75
Pine needles	PI + TOT	0.403	0.315	0.002		66.35	0.09		
	PI	0.421	0.321		PI + TOT vs. PI	64.36	0.09	0.01	0.94
Twigs	PI + TOT	−0.005	1.785	0.007		25.55	0.73		
	PI	0.058	1.876		PI + TOT vs. PI	24.14	0.73	0.52	0.48
Herbaceous litter	*PI + TOT*	−0.096	0.862	0.168		104.52	0.88		
	PI	−0.067	1.142		PI + TOT vs. PI	107.32	0.85	4.63	0.04
Live grass	*PI + TOT*	−0.099	0.578	−0.026		25.51	0.76		
	PI	−0.191	0.458		PI + TOT vs. PI	29.59	0.68	6.05	0.02
Live forbs	PI + TOT	0.011	0.474	−0.001		−22.77	0.84		
	PI	0.004	0.474		PI + TOT vs. PI	−24.74	0.84	0.03	0.87
Live legumes	PI + TOT	0.047	0.015	0.005		−8.71	0.09		
	PI	0.102	0.070		PI + TOT vs. PI	−9.03	0.02	1.52	0.23

† Akaike's Information Criterion (AIC) accounts for the tradeoff between the explanatory ability of a model (i.e., deviance reduction) and model complexity (i.e., the number of parameters estimated).

and HS estimates of biomass (Table 1). For fine roots, the inclusion of the TOT term did not significantly improve the model. In the quadrat sample, the results were mixed. The PIs of pine needles and live legumes were a poor linear fit of the HS values ($R^2 < 0.1$). The other categories in the quadrat sample performed more like the soil cores (Table 1).

The PI model accurately predicted HS root mass (Fig. 1). The slope of the validation line was 1.02 and the 95% confidence interval bounded the 1:1 line. The predictive equations for most ecosystem components were validated as well. However in two cases (twigs and detritus), there was substantial scatter around the 1:1 line (i.e., $R^2 < 0.35$).

The point-intercept method greatly improved the efficiency of sorting soil cores. Based on our experience (more than 10 technicians processing thousands of cores from the oak savanna), the time needed to hand sort all the fine roots from a washed core ranged between 10 and 60 min with 90% of the cores sorted within 25 min. In contrast, the time needed to complete the point-intercept method was never more than 10 min. Thus, even a conservative estimate of the efficiency results in a 60% reduction in the time spent sorting cores.

DISCUSSION

The uncertainty added by point-intercept sampling for fine roots seems justified by the large gain in efficiency. The overall fit in the validation set ($R^2 = 0.61$) was lower than hoped, but much of this residual variation was due to the leverage of two points. Since the results from individual soil cores are typically aggregated (e.g., in our case, we take the mean of the three cores to estimate plot-level fine root mass), the errors will tend to average out. Moreover, the extra variation introduced by the point sampling is small compared to the variability between cores. Fine root mass can vary by more than 20-fold in a typical collection. For us, the time saved with the point-intercept sampling translated into being able to process 43% more cores. In other words, we could process 154 cores using point-intercept sampling in the time it took us process 108 cores using hand sorting (time spent washing, weighing, handling, and recording was held constant). For a small sacrifice in accuracy of sorting, we can increase the intensity of our field sampling to accommodate the spatial heterogeneity inherent in fine root biomass.

A concern in moving from censusing to sampling is the degree of precision with the sampling approach. We tested the precision of the point-intercept technique by comparing results between the lead author (RW) and two trained undergraduate assistants in two separate tests. For each test, seven samples were spread out on the grid. Each person then recorded 50 point intercepts and used the equations from Table 1 to predict the mass of each ecosystem component. For fine roots, the root mean square error was <0.01 g in both tests (Table 2). For all categories, the point-intercept estimates of mass did not significantly differ among researchers (Table 2).

In our point-intercept design, we empirically chose a grid size large enough to accommodate a diffuse, uniform distribution of the sample (<3-mm-thick layer of root core sample and <1-cm-thick layer of litter sample) and recorded enough points to reduce the influence of any single point. In general, the approach worked best when the sample was uniformly distributed in three dimensions (SOM, herbaceous litter, oak leaves, and roots) while less uniform material such as twigs, pine needles, and live forbs, which all tended to sort toward the bottom or top of the sample layer, resulted in poor performance of this approach.

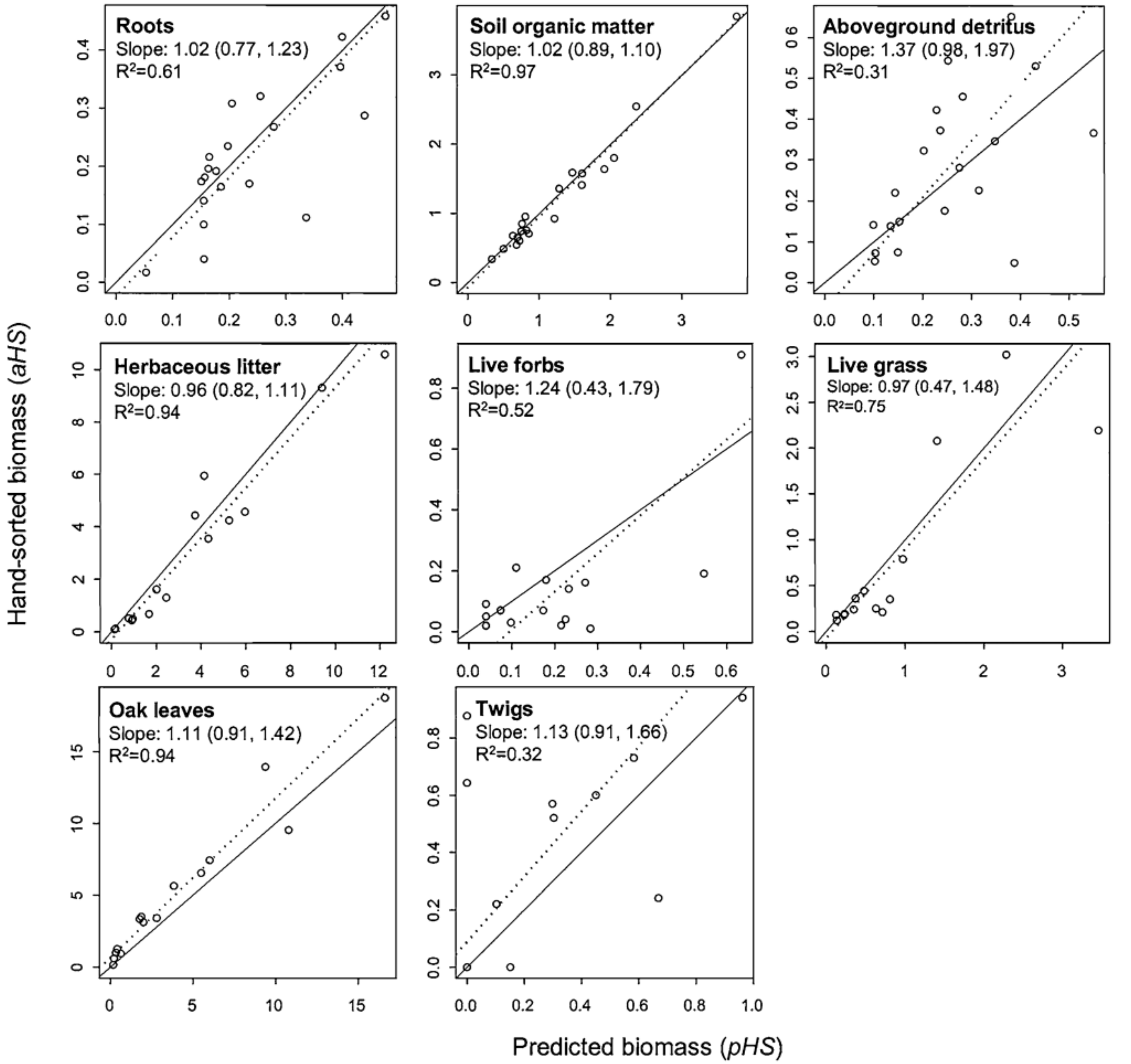

Fig. 1. Scatterplots of the actual hand-sorted biomass (aHS) and the predicted hand-sorted biomass (pHS) using models developed from original data. Solid lines indicate 1:1 and dotted lines the ordinary least squares (OLS) bisector regression line. Ordinary least squares bisector slope estimates (along with their bootstrapped 95% confidence intervals) were used to validate models.

While the scale of the grid and the number of points is somewhat arbitrary, several general concepts must be considered when using point-based sampling (Wensel et al., 1980; Jukola-Sulonen and Salemaa, 1985; Husch et al., 2003). The sample must be spread thin enough to prevent multiple hits at any one point. In practice, we found that about 50% white space on the grid was sufficient to reduce the problem of multiple intersections, which results in roughly a 1:3 sample volume/surface area ratio. At the same time a sufficient number of points must be recorded to minimize the bias introduced by mistaken identification. Since the time needed to measure additional points was small, we erred on the side of oversampling (50–100 points). Obviously, the number of point intercepts necessary for reliable estimates will depend on the variability inherent to the population of interest. In our case, regression relationships may have improved for some ecosystem pools with a greater number of points, however, we were mainly interested in belowground pools where our approach performed acceptably.

Table 2. Comparison of biomass estimates made by lead author (RW) and two trained undergraduate assistants.

	RW vs. worker 1		RW vs. worker 2	
Category	RMSE	*P* (paired *t* test)	RMSE	*P* (paired *t* test)
Aboveground detritus	0.0113	0.9019	0.0212	0.6587
Roots	0.0089	0.9849	0.0083	0.2462
Soil organic matter	0.0127	0.9981	0.0056	0.1235

Finally, it is best not to adjust simultaneously grid size and the number of points between samples. Varying both increases the likelihood of introducing a significant bias (Wensel et al., 1980). A better strategy is to change only one dimension. In our case, we occasionally needed to adjust the grid size for very small samples.

Point-intercept sampling has a long history in natural resource assessment. For example, it has been in use for over 50 yr as a way to measure species composition in rangelands (Heady et al., 1959; Cook and Stubbendieck, 1986) and is a core concept in forest inventory (Husch et al., 2003). However, we are unaware of this approach being applied to quantify the mass of various ecosystem pools. Based on our results, we recommend the approach for sorting fine roots from soil cores. On the other hand, the method requires more development before being used to sort other ecosystem components. Pine needles were underestimated by >50% by the point-intercept method and live legumes were overestimated; fits to the best model in both cases were $R^2 < 0.1$ (Table 1). In contrast, the mass of herbaceous litter and oak leaves were predicted with great accuracy by the point-intercept model (Fig. 1). These mixed results probably reflect the disparate size of the material. Pine needles were by far the largest and live legumes leaves (mostly leaves from *Trifolium*) were by far the smallest components in the sample. As noted above, the performance of point-intercept sampling depends on appropriately scaling both the size of the grid and the number of points.

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