

Quantifying Deep-Soil and Coarse-Soil Fractions: Avoiding Sampling Bias

R. B. Harrison,* A. B. Adams, C. Licata, B. Flaming, G. L. Wagoner, P. Carpenter, and E. D. Vance

ABSTRACT

Forest soils are often deep and/or coarse-textured, which does not always lend itself to easy unbiased sampling. Two important Pacific Northwest (PNW) forest soil series that are deep and coarse-textured were studied to evaluate methods of estimating soil C: (i) a loamy sand glacial outwash soil (Indianola series, mixed, mesic Dystric Xeropsammments) and (ii) a very gravelly sandy loam glacial outwash soil (Everett series, sandy-skeletal, isotic, mesic Vitrandic Dystraxerepts). Four methods were compared for estimating soil C, including: (i) large pit (0.5 m²) excavation, (ii) dug pit with 54-mm hammer-core bulk-density sampling, (iii) 31-mm soil push sampler, and (iv) clod method. Coarse (>2 mm) fragments were also collected, processed, and analyzed for soil C. Extending soil sampling deeper than 15 cm increased soil C estimates by as much as 120%. The pit excavation method with sand-displacement volume measurements, which is by far the most labor-intensive and time-consuming, was considered the "standard" by which other methods were compared, as it didn't contain any obvious biases. Soil core methods overestimated the <2-mm soil fraction (samples taken between large rocks). Biased methods are often accepted as the "best available" due to the high time requirement of pit excavation. The 31- or 54-mm soil core methods often didn't work due to the high rock content (>50%) of the Everett soil. Including C analysis of the >2-mm soil fraction increased soil C estimates by 170% for the Everett series soil (due to organic C contained in the rocks; there were no carbonates) but did not substantially increase the estimate in the Indianola series soil.

ACCURATE ASSESSMENTS of forest soil C pools for their responses to management practices requires soil sampling and analytical approaches that accurately and precisely represent the sites of interest. In particular, sampling which introduces a consistent bias toward higher or lower C concentration can result in tight confidence intervals around inaccurate values. Such sampling bias may or may not be a serious problem depending on the hypotheses tested in a study. If bias is consistent, soil samples may be undersampled or oversampled by the same amount, and accurate conclusions of the effects of forest management drawn. However, studies that require determination of accurate soil C pools will be adversely affected by any introduced sampling bias.

Soil is the primary repository for long-term C storage in forest ecosystems, yet represents the most difficult forest C pool to estimate (Homann et al., 2001). Many published studies of forest ecosystem C only sample the very surface of a deeper soil resource. Soil sampling depths are often shallow (e.g., <15 cm; Brady and Weil,

2002; Staley et al., 1988). Clearly, when the contribution of soils to forest ecosystem C is being assessed, soil-sampling depths should correspond to the realities of the ecosystem being studied (Staley et al., 1988; Prichard et al., 2000).

A study of the effects of N fertilization on soil C (Canary, 1994; Canary et al., 2000) found that 37% of C in the profiles of three forest soils was found at 25 to 85 cm, when the soil was sampled to a total depth of 85 cm. The study also found that conclusions about the effects of N fertilization on soil also depended on sampling depth. For instance, the apparent soil C increase from N fertilization would have been 20% if only the forest floor was sampled and 11% if the sample included mineral soil to a depth of 25 cm. The positive effect of N fertilization on soil C shrank to only 4% when the sample included mineral soil to a depth of 85 cm. In this study, it was concluded that redistribution of C was the main effect of N fertilization in these soils, and increases in total soil C were not as large as the increase in the forest floor and surface mineral soil would imply. The study emphasized that soils should be sampled to the maximum depth possible or practicable (Canary et al., 2000) to prevent reaching inaccurate conclusions on the effects of forest management on soil C.

In a study of the effects of biosolids application on soil C on two coarse-textured soils, Harrison et al. (1994) found that 26% of C was found in the soil profile at depths of 27 to 190 cm. The biosolids treatment significantly increased C throughout the soil profile.

The soils in the Canary et al. (2000) and Harrison et al. (1994) studies were relatively infertile. Characterization of a highly fertile soil in the Fall River Long-term Site Productivity Study in Washington State showed that even more of the soil C pool was located at depth, with 60% of total soil C (forest floor + mineral horizons) located below the 18- to 150-cm depth (Flaming, 2001). These and other studies (Huntington et al., 1988; Federer et al., 1993; Fernandez et al., 1993; Hammer et al., 1995; Cromack et al., 1999) show that systematically excluding the contribution of lower soil horizons can result in substantial underestimates of whole-ecosystem C and inaccurate conclusions about the effects of forest management practices on soil C.

A second potential source of sampling bias in forest soils is results from exclusion of C in the large diameter fraction (>2 mm) of soil (Corti et al., 1998; Fernandez et al., 1993). In most studies this fraction is effectively considered a void. Following standard soil methods of analysis (Gee and Bauder, 1986), which typically include screening and discarding the >2-mm soil fraction, can result in underestimates of soil C. For instance, Whitney and Zabowski (2001) found 66 and 33% of total soil C was in the >2-mm soil fraction in Alderwood and

R.B. Harrison, A.B. Adams, C. Licata, B. Flaming, G. Wagoner, P. Carpenter, College of Forest Resources, Box 352100, University of Washington, Seattle, WA 98195-2100. E.D. Vance, National Council for Air and Stream Improvement, P.O. Box 13318, Research Triangle Park, NC 27709-3318. Received 7 Jan. 2002. *Corresponding author (robh@u.washington.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: PNW, Pacific Northwest.

Dingleman soil series, respectively. Unfortunately, very few studies of forest soils have determined the contribution of the >2-mm soil fractions to total soil C as organic C, and this bias has likely led to underestimates of total soil C in many studies of rocky forest soils, regardless of the sampling depth of soil.

Unless the entire volume (all size fractions) of soil is collected, processed, and analyzed, three parameters that must be well-estimated to accurately calculate soil C include: (i) horizon depth, (ii) bulk density, and (iii) representative subsamples for C analysis. In many cases, the soil profile is sampled by horizon due to the relative constancy of soil C within an individual soil horizon relative to other soil horizons, though this is not necessarily required for an accurate quantification of total-profile soil C. Acquiring these estimates can be complicated by the inherent variability of soil, the presence of roots and trees, large rocks, etc., but also by biased sampling procedures for estimating bulk density. For instance, bulk density is often estimated using core-sampling devices or soil clods, which may not incorporate rocks. This would typically result in an underestimate of whole-soil bulk density in rocky soils with coarse fragments larger than the cores, because they cannot be incorporated into the cores or clods. When cores or clods are also used to take samples for chemical analysis, the estimate of C concentration could also be biased, as typically the fine fraction of soil contains a higher C concentration (Whitney and Zabowski, 2001).

The purpose of this study was to determine if there is a sampling bias using widely accepted methods of determining bulk density, including (1) large pit excavation (0.5 m²), (2) hammer-core bulk-density sampling (54-mm diam.), (3) soil push sampler (JMC backsaver N-3 handle with 31-mm diam., Clements Associates Inc., Newton, IA), and (4) clod method from pit excavation. To determine potential sampling bias in selecting subsamples for C analysis, soil samples for particle-size estimations were also selected using either, (i) a subsample of material excavated in the 0.5-m² excavation, or (ii) the 54-mm hammer-core sample.

MATERIALS AND METHODS

Study Location

Two soils were studied in the Cedar River Watershed, which is located 60 km southeast of Seattle, WA. The area has a maritime climate, with an annual average of 132 cm of precipitation, most falling as rain. The two soil series sampled included an Everett very gravelly sandy loam and an Indianola loamy sand. Descriptions of a soil profile located at each site are given in Table 1.

Soil Sampling and Bulk Density

This study was designed to compare methods of soil sampling for estimating soil C. Three pits within each soil type in a single forest stand were located approximately 10 m from each other in a triangular pattern to give a reasonably consistent soil series for which each sampling method would be compared. Soil was sampled using the following methods: (1) dug pit (0.5 m²) excavation, (2) dug pit using a 54-mm hammer-core bulk-density sampler, (3) 31-mm soil push sampler (JMC

Table 1. Depths of horizons and profile description of soils.

Horizon	Depth	Color	Texture
cm			
Everett very gravelly sandy loam soil			
A	0–15	10YR 2/2	very gravelly sandy loam
Bs	15–32	10YR 3/4	very gravelly sandy loam
BC	32–48	5YR 4/4	extremely gravelly sand
C1	48–85	2.5Y 4/4	extremely gravelly sand
C2	85–105	2.5Y 4/3	extremely gravelly sand
Indianola loamy sand soil			
A	0–20	5YR 3/3	loamy sand
Bw1	20–50	5YR 3/4	loamy sand
BC	50–80	10YR 4/3	loamy sand
C1	80–120	10YR 4/4	sand
C2	120–180	2.5Y 4/3	sand

“backsaver” sampler), and (4) clod method (Blake and Hartge, 1986). These coarse-textured soils did not form clods, so Method 4 was dropped. Samples were taken to the depths shown in Table 1, where possible.

The clod method did not work for either soil, as they were coarse-textured and no clods could be collected. It was impossible to take a 31-mm core sample in the Everett very gravelly loamy sand soil, as the small size of the core sampler would not incorporate the abundant large rocks. The other methods worked in all cases, though it was obvious that the 54-mm hammer core could not be placed randomly within the pit face to collect representative samples. Instead, it was necessary to sample between large stones. Larger samples have been designed to deal with this problem, but they are often difficult to utilize (Jurgensen et al., 1977) and still have problems with large rocks. Sampler placement was not a problem with the Indianola soil, where 54-mm cores could be taken nearly anywhere within the pit face. The excavated-pit method worked well for the Indianola soil, provided that a sheet of plywood with a 0.5-m² square cut out of it was placed over the pit to prevent the weight of the excavator from knocking soil at the edge back into the pit and breaking down the edge of the pit. There was a tendency for the Everett soil to collapse, and it was helpful to support the walls of the pit with plywood during excavation. Two methods were utilized for determining the volume of soil excavated, including (i) sand displacement, and (ii) measuring the volume of the pit using the ruler method of Huntington et al. (1989) and Canary et al. (2000), where a grid frame was placed over the pit, and measurements taken from multiple fixed points at the surface of the soil to the bottom of the pit. The methods did not result in any significant differences in volume estimates for the first pits sampled. Because using the sand displacement method was difficult to use at depth, the ruler method of Huntington et al. (1989) and Canary et al. (2000) was subsequently used.

To collect soil material for C analysis, subsamples were collected either by using the entire contents of three soil cores taken per horizon, or by taking a subsample of the material excavated from the pit by horizon. This was accomplished by placing the entire volume on a plastic tarp, mixing it well, dividing it in half, and making further divisions until a sample of 10 kg or less could be taken. This material was then transported to the University of Washington College of Forest Resources analytical lab for processing and chemical analysis.

For this study, the pit excavation method with sand displacement, which is considerably more time-consuming than the other methods and is generally not used in studies of C content of rocky forest soils, was considered the “standard” by which the other methods were compared since it had no obvious bias (Hamberg, 1984; Huntington et al., 1988; Richter et al., 1989). Measurements of bulk density by horizon from each

of the three soil pits were averaged to estimate means of each sampling method.

Soil Analysis

Soil material was air-dried to constant weight, and then screened to 2 mm. The soil was not wet-sieved. The <2-mm portion was ground to a fine powder using a mortar and pestle, and analyzed for total C. Samples were analyzed for C on an automated CHN analyzer (Perkin-Elmer 2400, Norwalk, CT). A subsample was oven-dried at 105°C to determine moisture content of the air-dried sample.

A considerable amount of the Everett soil was in the >2-mm soil fraction. Rock types were mixed, as these are glacial outwash soils, but were primarily granites and andesites. Testing for carbonates were made, but none were found. Since one of the purposes of this study was to determine the amount of C in this fraction, which is not normally sampled or analyzed, the following method was used to acquire a fine powder sample representative of the coarse fraction. The soil was further screened into the following size fractions, 2 to 5, 5 to 10, 10 to 20, 20 to 40, and >40 mm. These fractions were weighed, and the 2- to 5-, 5- to 10-, and 20- to 40-mm samples were further reduced in size by using a rock hammer and anvil. A fine powder was created from all of the >40-mm rocks using a masonry drill cooled and lubricated with deionized water to drill a hole through their center. Care was taken not to include significant amounts of silicon carbide into the sample by microscopic analysis of the drill bit after each drilling. If the drill bit showed any chipping visually, that sample was discarded. Slow drilling and constant water cooling generally prevented chipping of the drill bit. This method was far more practical than pulverizing the whole sample. A composite of the >2-mm fraction of soil was then created by mixing weighted amounts of each size fraction to form a single sample representative of the >2-mm fraction. This sample was further ground to a fine powder using a mortar and pestle and analyzed for total C.

Statistical Analysis

Estimates of mean differences were calculated using the pit average. This gave a sample size of $n = 3$ for each measurement. A paired Student's t test analysis was performed on the data (SYSTAT, 1990) to compare bulk density for each sampling method, with samples from each pit paired for analysis.

RESULTS AND DISCUSSION

Bulk Density

Measures of bulk density, which accurately represent field conditions, are extremely important for calculating soil C content and its response to management practices (Van Remortel and Shields, 1993; Page-Dumroese et al., 1999). Although generally well suited for medium- and finer-textured soils, the clod method didn't work for either the Everett or Indianola series soils studied since no soil clods were present in these coarse-textured soils. We were also unable to collect cores with the 31-mm soil probe in the Everett very gravelly sandy loam. Measures of bulk density across all methods and both soils ranged from 0.81 to 1.34 g cm⁻³ and generally increased with depth (Table 2). For the pit excavation methods, the volume of each soil horizon was estimated using two approaches: (i) the displacement method and

Table 2. Estimate of total soil bulk density for an Everett series soil and Indianola series soil using several different methods of volume and soil sampling.†

Soil depth cm	0.5-m ² excavation method‡			
	displacement	frame	54-mm core	31-mm probe
	g cm ⁻³			
	Everett very gravelly sandy loam soil			
0–15	1.11a§	1.13a	0.96a	¶
15–32	1.18b	1.14b	0.89a	¶
32–48	¶	1.22b	0.81a	¶
48–85	¶	1.34b	1.02a	¶
85–105	¶	1.26b	0.95a	¶
	Indianola loamy sand soil			
0–20	0.93a	0.92a	0.96a	0.97a
20–50	1.05a	1.10a	1.02a	0.99a
50–80	¶	1.06a	1.11a	1.16a
80–160	¶	1.10a	1.08a	1.05a
160–180	¶	1.14a	1.11a	1.09a

† Estimate taken from three samples.

‡ Method of Canary et al. (2000).

§ Different letters indicate that means in rows are significantly different (0.05 level).

¶ Indicated that samples could not be taken using this method.

(ii) the frame measurement method. The displacement method could be easily used for surface horizons, but proved difficult in the subsoil horizons. Comparisons of the displacement and frame method showed no significant difference ($P = 0.05$) and an average error of only 0.52% for the three pits measured for each soil series (Table 2), indicating that large rocks sticking out of the sides of the pits didn't change the volume calculations substantially.

In the Everett soil, it was difficult to use the 54-mm hammer core sampler due to the prevalence of large rocks, making it necessary to take core samples between rocks. This compromise is often made when using core samplers in coarse-textured soils and resulted in underestimates of bulk density of the Everett soil. For instance, the average total-profile bulk density estimated by the 54-mm hammer core bulk density sampler was 24% lower than for the pit excavation method (0.93 vs. 1.22 g cm⁻³, respectively). These mean differences were statistically significant ($P = 0.05$) for all but the 0- to 15-cm horizon (Table 2).

In the Indianola loamy sand soil, 54- and 31-mm cores were taken easily in all horizons and there was no statistically significant difference in mean total-profile bulk density estimates by method ($P = 0.05$). For instance, the means for 0 to 180 cm were nearly identical at 1.06, 1.06, and 1.05 g cm⁻³ for the excavation, 54-mm hammer core, and 31-mm probe methods, respectively (Table 2).

Coarse Fraction Estimate

Traditionally, the soil rock fraction (>2 mm) is screened out and not considered when estimating soil C. Justification for this obviously biased sampling method includes (i) the >2-mm fraction represents a minor soil component, (ii) there is lower C in this fraction compared with the finer fraction, and (iii) this fraction is difficult to subsample and analyze. In the Everett soil, the >2-mm fraction is a substantial portion of the total soil weight and volume, ranging from 620 to 720 g kg⁻¹ of the whole-soil volume with an average of 680 g kg⁻¹

Table 3. Estimate of total-soil coarse fraction for an Everett series soil and Indianola series soil using several different methods of collecting soil material.†

Soil depth cm	0.5-m ² excavation method‡	54-mm core	31-mm probe
g kg ⁻¹			
Everett very gravelly sandy loam soil			
0–15	620b§	410a	¶
15–32	650b	450a	¶
32–48	720b	440a	¶
48–85	690b	470a	¶
85–105	720b	490a	¶
Indianola loamy sand soil			
0–20	33a	43a	29a
20–50	41a	52a	38a
50–80	42a	31a	28a
80–120	21a	37a	24a
120–180	44a	49a	38a

† Estimate taken from three samples.

‡ Method of Canary et al. (2000).

§ Different letters indicate that means in rows are significantly different (0.05 level).

¶ Indicated that samples could not be taken with this method.

soil (Table 3). The coarse fraction estimated using the 54-mm hammer core sampler ranged from 410 to 490 g kg⁻¹ (Table 2), which underestimates that obtained from pit excavations by 33%. The difference between the two methods is statistically significant for all soil horizons sampled ($P < 0.05$). Many of the rocks sampled from the 0.5-m² pit excavation had diameters >54 mm, and couldn't be incorporated into the sample core using this method.

The >2-mm fraction of the Indianola loamy sand soil (21–52 g kg⁻¹) was much smaller than in the Everett very gravelly sandy loam. Though the coarse fraction estimate using the 54-mm core sampler method was 24% higher than that from the excavation method, the range in variation among all horizons and among methods was -26 to +76%, indicating high variation. No differences were statistically significant ($P = 0.05$) due to this high variability within horizons and methods (Table 3). A similarly high variation was observed with the 31-mm probe method, which produced estimates 10% less than (not significant at $P < 0.05$) those from the 54-mm core method. It is possible that the 54-mm hammer core method compressed the samples during sampling due to friction on the sides and pushing rocks through the soil material.

Mineral Soil C Concentrations

Soil samples from the pit excavation method were analyzed for total C. Obtaining and processing representative samples of the >2-mm fraction for C analysis was much more difficult than for the <2-mm fraction. A considerable percentage of the coarse fraction was comprised of transported, chemically unweathered granite rocks at both sites, which are extremely difficult to break down and grind to powder for C analysis. This is one reason why the >2-mm size fraction is often ignored in soil C analyses in such soils.

As expected, the highest C concentrations were in the <2-mm soil fraction and in the surface soil horizons,

Table 4. Total C concentration of size fractions for an Everett series soil and Indianola series soil.†

Soil depth cm	<2-mm soil fraction	>2-mm soil fraction
mg C kg ⁻¹ soil		
Everett very gravelly sandy loam soil		
0–15	67.2b‡	9.9a
15–32	16.2b	2.6a
32–48	11.8b	1.7a
48–85	6.2b	1.1a
85–105	3.9b	0.7a
Indianola loamy sand soil		
0–20	54.0b	12.2a
20–50	12.1b	2.1a
50–80	6.2b	1.2a
80–120	3.1b	0.8a
120–180	1.4a	0.5a

† Estimate taken from three samples.

‡ Different letters indicate that means in rows are significantly different (0.05 level).

with total C ranging from 0.5 to 67 mg C kg⁻¹ (Table 4). The <2-mm soil fraction contained significantly ($P = 0.05$) higher C concentrations than did the >2-mm fraction.

Site-Level Mineral Soil C Estimates

Total mineral soil C expressed on a per-area basis is the most common measure of soil C sought in research studies and for the purposes of measuring and accounting for terrestrial C sequestration. Results of this work show that ignoring the >2-mm soil fraction results in a strongly biased total C estimate in the Everett series soil but a much smaller bias in the Indianola series soil. For instance, soil C in the 0- to 105-cm depth of the Everett series soil was estimated to be 79.7 Mg ha⁻¹ when only the <2-mm soil fraction is considered, compared with 215 Mg ha⁻¹ (a 170% increase) when the >2-mm fraction was included (Table 5). In contrast, including the >2-mm fraction in the Indianola series soil only increased the estimate of C from 180 to 186 Mg ha⁻¹, which was only a 3% increase. These results show that the potential contribution of >2-mm soil frac-

Table 5. Total C content of size fractions and whole mineral profile for an Everett series soil and Indianola series soil.†

Soil depth cm	<2-mm soil fraction	>2-mm soil fraction	Total soil fractions
Mg C ha ⁻¹			
Everett very gravelly sandy loam soil			
0–15	43.3a‡	70.6	114b
15–32	11.0a	20.5	31b
32–48	6.4a	16.5	23b
48–85	9.5a	21.1	31b
85–105	9.5a	7.1	17b
0–105	79.7a	136	215b
Indianola loamy sand soil			
0–20	96a	3.3	99a
20–50	38a	1.6	40a
50–80	19a	0.8	20a
80–120	13a	0.3	14a
120–180	13a	0.4	14a
0–180	180a	6.5	186a

† Estimate taken from three samples.

‡ Different letters indicate that means in rows are significantly different (0.05 level).

tion must be assessed in studies of total soil C in rocky soils.

The depth to which soil was sampled also had a major impact on the estimates of C in these soils. In the Everett series soil, sampling to a depth of 15 cm would have underestimated C by 48% compared with including the 15- to 105-cm depths of soil (114 vs. 221 Mg C ha⁻¹) (Table 5). In the Indianola soil, sampling to a depth of only 20 cm would have underestimated soil C by 53% compared with including the 20- to 180-cm depths of soil (99 vs. 213 Mg C ha⁻¹). These results confirm the importance of deep sampling for obtaining accurate estimates of total C pools in these soils. There is some evidence that forest management treatments may affect different soil horizons variably (Johnson, 1992; Canary, 1994; Canary et al., 2000; Homann et al., 2001), and shallow soil sampling may draw incorrect conclusions of forest management effects on the soil total-profile compared to deeper sampling. There is also the possibility that changes in soil C from forest management effects might be differentially distributed among different size fractions. More work is needed to determine how widespread biased sampling is in studies of forest C sequestration.

CONCLUSIONS

- 1) There was no substantial difference between displacement and frame measurement methods for estimating the volume of excavated pits, but the use of the displacement method for estimating the volume of subsoil horizons required more time, particularly since volumes were estimated by soil depth and required multiple additions and removal of sand.
- 2) Core sampling methods resulted in lower total soil bulk density estimates compared with pit excavations in the rocky Everett series soil.
- 3) Core sampling resulted in underestimates of the soil mass >2-mm fraction compared with pit excavations in the Everett series soil.
- 4) More than half of the soil C (75.2%) was found in the >2-mm soil fraction of the Everett series soil, but only 3.3% in the same fraction of the Indianola series soil. None of the C was from carbonates.
- 5) In the Everett series soil, sampling to a depth of 15 cm would have underestimated total mineral soil C by 48% compared with that from sampling to 105 cm. For the Indianola soil, sampling to a depth of 20 cm would have underestimated total mineral soil C by 53%.
- 6) These results suggest to accurately assess total C pools in these soils, sampling should include both the >2-mm soil fraction and deep soil layers. In soils containing a substantial amount of coarse fraction material, we suggest that excavated pits or a similar sampling approach be used.

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