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SPECIAL FEATURE: UNCERTAINTY ANALYSIS

Sampling and processing roots from rocky forest soils

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Abstract. Quantifying root biomass in rocky forest soils is challenging. This report provides practical advice for field sampling and laboratory processing of root biomass in these settings. Manual coring is the most efficient method for sampling fine root biomass in the upper soil profile (we sampled to 30 cm). However, careful correction for coarse fragment volume is needed because manual coring is impeded by rocks. Unbiased estimation of root biomass below obstructions requires either excavating a pit or power coring. We recommend power coring because of the very high field labor costs of pit excavation. Roots can be separated from soil either by dry picking or by wet sieving. For surface organic matter-rich horizons typical of many forest soils, only dry picking is feasible. A timed interval approach can greatly reduce laboratory processing time. Because sorting live from dead roots is necessarily subjective, efforts to avoid fragmentation of root systems obtained from cores are strongly recommended. Sample size requirements for detecting changes or differences in root biomass at the stand level are presented based on extensive sampling in northern hardwood forests. Detecting 20% differences in fine root (<1 mm) biomass in 0–30 cm soil using ten 5-cm manual cores generally would require about nine sample plots in a stand, whereas detecting such differences in deep soil (30–50 cm) would be virtually impossible because of extreme spatial variation. Power analyses such as these can help improve experimental designs, as the spatial intensity of sampling determines the detectable difference, which can in turn guide decisions about the temporal frequency of sampling.

Key words: bias; biomass; carbon; error; fine roots; power analysis; soil coring; Special Feature: Uncertainty Analysis; uncertainty.

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Introduction

Obtaining accurate and precise estimates of the biomass and nutrient content of tree roots is an important objective of many studies of primary production and biogeochemistry of forest ecosystems. Roots are a large and dynamic soil pool, and they serve key functional roles including anchorage and the acquisition and transport of water and nutrients. Characterizing the distribution and biomass of tree roots is challenging because of high spatial variability and difficult access. Indeed, measurement of root biomass and nutrient content is typically among the most expensive procedures in forest ecosystem studies, and the inability to detect differences or changes in root biomass is a common limitation in comparative and experimental research (Park et al. 2008). Uncertainty in root biomass data may constrain the assignment of carbon (C) offset crediting associated with forest management (Fahey et al. 2010).

Variation in root biomass across forest landscapes results from such influences as stand age and species composition; soil properties including soil depth, parent material composition, texture, and fertility; and topography, drainage, and microclimate (Vitousek and Sanford 1986, Cairns et al. 1997, Tateno et al. 2004). Within sites, root biomass also exhibits high variation as a result of microtopography, position of individual tree stems, and small-scale soil variation, both horizontal and vertical (Cavelier et al. 1996, Jones et al. 1996). In addition, the biomass of fine roots will often vary temporally, both across years and across seasons (Makkonen and Helmisaari 1998). These sources of variation can confound even carefully designed and well-funded attempts to detect patterns or treatment responses.

Rocky forest soils present particularly difficult conditions for root biomass measurement. Many northern forest soils are derived from unsorted glacial drift containing coarse fragments that limit easy access below the surface horizons. In addition, slow decomposition of plant detritus in cold climates and limited faunal mixing in acidic soils result in the accumulation of soil organic matter, either as surface organic horizons or as true Histosols, and it is very difficult to separate fine roots from this material because they are similar in density, particle size, and appearance.

Studies of root dynamics and belowground C storage are often hampered by poor statistical power, largely because of high spatial variability of soil characteristics, but also by low replication when effort per sample is high. For example, Yanai et al. (2003) surveyed studies of the mass of surface organic horizons in northern forest soils and concluded that most were unable to detect changes <15–20%. Mineral soils are more difficult to sample than surface horizons and the detectable differences are even greater. Thus, responses of root biomass and other soil C pools to climate change and forest management activities are likely too small to detect with current data sets and approaches. Any sampling method that can reduce minimum detectable differences for a given level of investment should be of great interest.

Root sampling approaches

The most common approaches for field sampling of root biomass are soil excavation and soil coring (Bledsoe et al. 1999). Soil excavation using quantitative soil pits reduces uncertainty caused by small-scale spatial variation by sampling a larger soil volume than coring techniques, but soil pits are more labor-intensive and destructive, and thus the number of samples obtained per site is necessarily limited. For example, in rocky glacial till soils, excavation of a single quantitative soil pit of 0.5 m^2 to a depth of 1 m typically takes up to two person-days including field sample processing (Park et al. 2007). This approach involves sequential removal of all soil from depth increments in a known area, sieving coarse fragments in the field, weighing, and subsampling for laboratory analysis. Sampling time is greatly reduced for smaller soil pits; a 0.3 × 0.3 × 0.3 m pit requires about 2–3 person-hours (Fahey et al. 2013; Table 1), but depth of sampling is restricted by coarse fragments and large woody roots. Another advantage of excavation approaches over soil coring is that biases associated with soil compaction and edge effects (see *Root collection methods*) can be greatly reduced.

Soil coring methods are many and varied, but they all provide greater numbers of samples than excavation methods for the same effort and thus allow greater spatial coverage of a study site or research plot. For manual methods, a common technique uses 5-cm diameter, sharpened and split polyvinyl chloride pipe, pounded into the soil with a rubber mallet. In rocky soils, these corers often break so that extra corers are needed. With this technique, sampling time can average about 0.5 person-hours per core (Table 1), and sampling to 30–40 cm depth is feasible except on high bulk density soils where steel corers are needed. A rugged steel corer driven by a slide hammer can be effective

Table 1. Estimated time for field sampling and laboratory processing of fine root biomass samples in rocky northern forest soils.

Sampling approach	Depth	Area	Person-hours
Field collection			
Soil pit excavation	1 m	0.5 m^2	15–20
Soil pit excavation	30 cm	0.1 m^2	2–3
Manual coring	30 cm	20 cm^2	0.5
Power coring	0.5 m+	79 cm^2	2
Laboratory processing			
Handpicking forest floor, using timed interval†	6 cm	20 cm^2	1
Handpicking mineral	30 cm	20 cm^2	1
Wet sieving mineral	30 cm	20 cm^2	0.5

† Metcalfe et al. (2007).

in some difficult settings (Jurgensen et al. 1977); however, as detailed in *Root collection methods*, large rocks and woody roots prevent unbiased sampling.

To obtain soil cores that encompass most or all of the soil profile, power coring techniques can be employed. Rau et al. (2011) used a motorized device powering a 10-cm diameter diamond drill bit to obtain cores as deep as 1 m in rocky soils. This device is portable, weighing approximately 29 kg, can be transported on a frame over large distances and rough terrain, and can be assembled using commercially available components and easily manufactured parts (Rau et al. 2011). The time for coring averages about one person-hour per core (Table 1), but the transport and handling of the heavy equipment add to the sampling effort. Because the power corer is able to cut through rocks and large roots, error and bias due to selective rejection of sampling locations can be reduced in comparison with manual cores. When soil sampling is desired in conjunction with root sampling, it is important to recognize that this method may give biased estimates of base cation availability, due to the grinding action of the corer (Levine et al. 2012).

Another approach to root biomass estimation in forests applies allometric equations relating an easily measured dimension of individual trees (e.g., stem diameter) to root biomass, as determined by excavation of the whole tree root system. For example, Whittaker et al. (1974) excavated the root systems of 81 northern hardwood trees at Hubbard Brook Experimental Forest, New Hampshire ("with the encouragement of dynamite," p. 235), obtaining r^2 values of about 0.99 for the logarithmic regression of whole root system dry weight for three dominant species using tree diameter as the predictor variable. This approach has been validated for lateral roots (<10 cm in diameter) measured in soil pits at the same site (Fahey et al. 1988, Vadeboncoeur et al. 2007). Unfortunately, the effort required to develop allometric relations for roots is very high, and these relations may vary significantly among sites and species combinations. Further evaluation of error associated with application of allometric relations for roots across forest sites is needed.

Objectives

The overall goal of this report is to provide practical advice to research scientists undertaking measurement of root biomass in northern forest ecosystems. First, we evaluate approaches for field collection of samples from sites with varying soil characteristics. We explain the challenges of separating roots from soil in a reproducible way and sorting live and dead roots. We also present a power analysis comparing three field techniques based upon root biomass data collected in glacial soils in New Hampshire, USA. Finally, we provide summary recommendations for obtaining reliable root biomass data in different soils and for different purposes. We hope these insights based on long experience of root sampling will prove useful to others working in northern forest ecosystems.

Advice on Root Sampling Approaches

Root collection methods

Many forest soils exhibit high accumulations of surface organic matter, and fine roots are often concentrated in organic horizons (Vogt et al. 1983, Fahey and Hughes 1994). An important decision facing researchers measuring root biomass in soils with organic horizons is whether to sample these horizons separately from roots in the mineral soil or to sample by depth increments measured from the surface of the organic horizon. This decision will depend largely on the purpose of the study. For example, at the Hubbard Brook Experimental Forest (HBEF), fine root biomass has been measured for the organic horizon in part because our approach to quantifying and modeling water and nutrient cycling distinguishes mineralization, leaching, and root uptake between the forest floor and mineral horizons (Yanai 1992, Fitzhugh et al. 2001). Similarly, in studies of earthworm effects on soil biogeochemistry, it has proven helpful to quantify roots in the forest floor in the absence of earthworms because the principal effect of earthworm invasion is elimination of the forest floor (Bohlen et al. 2004, Fahey et al. 2013). However, the separation between forest floor organic horizons and underlying mineral soil is subjective in the field and hard to standardize (Federer 1982); thus, sampling by depth from the surface is more repeatable.

A second decision is whether to use an excavation method or soil coring, and if coring, what core size. Small diameter cores (e.g., <5 cm) cause

more soil compaction, resulting in greater amounts of roots being collected from a presumed soil volume (Park et al. 2007). It is important to measure the soil depth sampled based on the depth of penetration by the corer rather than the thickness of the compacted core, but this approach still does not account for compaction below the corer. Errors associated with the cutting edge are also relatively larger with smaller cores. Roots pulled into or out of the core sample along the cutting plane may account for the larger variability found with smaller diameter corers (Park et al. 2007). The larger the sample area, the smaller the error or bias from these edge effects. Moreover, larger samples have a smaller proportion of small root fragments that are very difficult to quantify accurately (see *Separating roots from soil*).

Soils in many forest ecosystems are derived from substrates that contain a significant proportion of coarse fragments, such as glacial drift or volcanics. Soils with few coarse fragments in the root zone (those formed in glacial outwash, lacustrine sediments, clay plains, etc.) usually can be sampled readily using inexpensive coring devices whereas rocky soils present different challenges for accurate root biomass estimation.

In particular, manual coring to the depth of obstruction allows the collection of many samples in the most densely rooted horizons (e.g., 0–30 cm), but this approach does not provide accurate or unbiased estimates of total fine root biomass. Manual coring cannot measure root biomass underlying coarse fragments, and even for shallow soil layers, a correction for the volume of the rooted profile occupied by rocks must be determined. Coarse fragment volume often exhibits high spatial variation in glacially derived soils; for example, Fahey et al. (2005) observed a range of 5.8–39.5% coarse fragment volume across eight study plots in the HBEF. Manual coring also is limited in the vicinity of tree boles by large woody roots. Although tree stems and root crowns typically occupy <2% of forest area, not sampling near tree crowns could result in bias if fine roots are concentrated in the vicinity of root crowns (Büttner and Leuschner 1994).

Separating roots from soil

To quantify biomass and chemistry, roots must be separated from the soil. For fine roots, this step in the process can be more time-consuming than field sampling (Table 1). The two principal approaches for separating fine roots from soil are handpicking (without sieving), and wet or dry sieving.

Handpicking.—In northern hardwood and conifer forests, fine roots often are highly concentrated in a thick organic horizon; half or more of the total fine root biomass may occur in these layers (Fahey and Hughes 1994). As noted earlier, separating fine roots from organic soil horizons is particularly difficult because the partially decayed organic matter is similar in density and particle size to the roots and is not easily removed from the root surfaces. Based on long experience with this problem, we believe that the most reliable approach currently available is tedious handpicking of roots from soil. Quantitatively separating living roots from the tangle of organic debris in varying states of decay is difficult to automate. Sieving procedures (see *Sieving and washing approaches*) can remove some of the fine particulate organic matter, but larger fragments of leaves, dead roots, and decomposing wood are much more difficult to remove, as fine roots are often intimately connected to these materials. The skeletal remains of decomposing leaves can look similar to fine root branches. The time required to sort live roots from organic horizons varies markedly among samples, depending on root-length density as well as the physical characteristics of the matrix. An experienced worker will average 2–4 h to completely sort all fine roots from a 5 cm diameter core of organic horizons that average 4–8 cm depth in Spodosols of northern hardwood forests (Table 1).

A time-saving approach to the handpicking problem was devised by Metcalfe et al. (2007) who employed a temporal prediction method to estimate fine root biomass in Amazonian forest soils. The diminishing root mass recovered during sequential, timed picking intervals was used to estimate total root biomass in soil core samples. This method assumes that reduced recovery rate through time results from the decline in root biomass remaining in the sample. Koteen and Baldocchi (2013) argued that part of the decline in recovery rate probably results from greater difficulty in recovering smaller and smaller fragments. Nevertheless, empirical trials suggested that this timed picking interval approach can greatly reduce sample processing time without

undue loss of accuracy (Metcalfe et al. 2007); for example, with five picking intervals of 10 min each, the total time would be about one person-hour (Table 1).

Subsampling.—Another approach to minimize processing time and overcome high spatial variation in fine root biomass sampling is to collect large or numerous samples, thoroughly mix the samples, and then subsample. When processing roots collected by pit excavation methods, subsampling is required. After hand mixing of large samples, root-length density in replicate subsamples often differs by 50% or more (data not shown), which suggests that multiple subsamples would be needed to obtain a representative result. One challenge with this approach is that thorough mixing of the densely rooted forest floor is virtually impossible without breakage and fragmentation of the fine roots, thereby creating more difficulty for sorting live from dead roots. One possible solution would be to sort all roots, both live and dead, from the mixed subsamples and obtain an accurate live:dead ratio on these subsamples.

Sieving and washing approaches.—For many soils and applications, mesh sieves can be effective for separating roots from soil. In general, the mesh size for sieving approaches should not be much larger than the fine root dimensions; for example, a 1-mm mesh sieve recovered only 34% of the total root length of crop roots as compared with a 0.2-mm mesh (Amato and Pardo 1994). Of course, the smaller the mesh, the more particles of organic matter and coarse sand will be retained, necessitating more laborious handpicking. A variety of time-saving, automated devices for wet sieving fine roots from mineral soil samples has been developed (Smucker et al. 1982, Benjamin and Nielsen 2004) such that multiple samples can be processed simultaneously. For all these approaches, the problem of efficiently sorting live from dead roots must be confronted, and fragmentation of root systems should be minimized.

Image analysis.—Benjamin and Nielsen (2004) developed an image analysis approach for quantifying fine root length in the mixed roots and debris of sieved samples, and Dowdy et al. (1995) utilized a shape algorithm to distinguish roots from debris. These methods may be effective for determining root length but not biomass. Representative samples could be analyzed for specific root length to convert length to biomass. For some purposes, such as for estimating water or nutrient uptake capacity, length may be more useful than biomass.

Sorting live and dead roots

Reliable approaches for distinguishing live and dead roots are needed to estimate living root biomass. For some purposes, it is also useful to measure dead root biomass; for example, in the sequential coring method for estimating fine root production, both live fine root biomass and necromass can be useful in calculations (Vogt and Persson 1991). An additional benefit of measuring both live and dead roots is that, having sorted roots from soil, workers may be reluctant to discard the dead roots. Obviously, including dead roots in the live root category would lead to overestimation of live root biomass.

The limitation of accurately sorting live and dead roots is among the most important sources of uncertainty in fine root biomass and production measurements, a claim that is as true today as 35 yr ago (Bohm 1979). The ratio of live:dead fine roots can vary markedly among forest types, sampling dates and soil depths, reflecting unknown contributions of differences in root turnover and decay rate (Silver and Miya 2001, Persson and Ahlstrom 2002, Park et al. 2008). In addition, distinguishing dead roots from coarse particulate organic matter is difficult in principle and in practice, and visual criteria for this distinction are subjective and variable. Root death is often a gradual (Comas et al. 2000) rather than discrete event (in contrast with leaf abscission), and repeated observations of individual fine roots using minirhizotrons commonly note the "resurrection" of roots previously defined as dead (Johnson et al. 2001).

Two general approaches for distinguishing live from dead roots have been employed: subjective observation and vital staining. A variety of observational criteria have been employed including tensile strength, brittleness, color of the surface and stele, and integrity of the root apex (Vogt and Persson 1991). In practice, it is necessary to apply these criteria in numerous, somewhat subjective decisions, leading to inevitable variation among workers and samples. The problem is especially difficult for small root fragments that are broken off during sample collection and handling. Distinguishing live from dead roots is

most efficiently accomplished for second- or higher-order roots, and attached lower-order roots are assumed to share their status. Thus, field sampling and handling that minimize fine root fragmentation will certainly improve the accuracy of root vitality assessment.

Vital stains can be employed to distinguish live from dead roots (Fahey et al. 1999). Stains such as rhodamine, fluorescein diacetate, and tetrazolium chloride target enzyme systems of metabolically active plant cells to indicate fine root vitality. These approaches are effective for root tissues when samples are processed immediately after collection (Comas et al. 2000, Cleavitt et al. 2008). Although routine use of vital stains is impractical for sorting live and dead roots collected quantitatively from soil cores, they may be useful for better standardizing the criteria employed in visual sorting. Comas et al. (2000) used this approach to determine that "browned" roots of grape, while still retaining some living tissue, were functionally dead, whereas black and shriveled roots were completely dead and had undergone varying degrees of decomposition.

Consideration of root function: root order sampling

Roots have commonly been classified on the basis of diameter; for example, fine roots often are defined as those <2 mm or <1 mm in diameter. However, the development and function of roots may be better represented by root order (Fitter 1982, 1987), where the most distal root tips are defined as first order and connection between two lower-order roots leads to a root of the next higher order. Recent studies have demonstrated the value of root order in characterizing fine root dynamics and function (Pregitzer et al. 1997, Guo et al. 2004, 2008, Valenzuela-Estrada et al. 2008, McCormack et al. 2015). Quantitative sampling of root systems by root order necessitates the collection of large soil samples (~500 cm^2 in area; Guo et al. 2004). In the laboratory, intact root branch networks are gently teased from bulk soil and subsequently immersed in water and stirred to remove most adhering soil. Residual soil, organic particles, and dead root fragments are removed with forceps. Then, a combination of dissection and image analysis can be used to quantify biomass, length, and diameter of each root order.

Quantifying coarse root biomass: quantitative soil pits and allometric equations

For some purposes, such as estimating forest C sequestration, total root biomass is a more important measurement than fine root biomass, which usually comprises only a small proportion of the total (e.g., <2-mm fine roots constituted only about 10% of total root biomass in mature northern hardwood forest at HBEF; Fahey et al. 2005). Soil coring methods, even power cores, are not efficient for sampling biomass of large woody roots in mature forests because the probability of encountering the largest roots with a small core is low, and many samples would be required. Soil excavation approaches have been employed to quantify total lateral root biomass in forests. From a labor cost standpoint, the profile wall method (Bohm 1979, Pearson et al. 1984) utilizing a backhoe to excavate trenches is most efficient. The diameter classes of all roots intersecting a trench wall are measured, and root biomass can be estimated based upon the tissue density. Because of difficulty in recovering small roots, this method is effective only for large woody roots (>1-cm diameter). A more accurate approach utilizes quantitative soil pits, but as detailed earlier the labor costs are very high (Table 1). Neither of these excavation approaches is effective for measuring biomass of root crowns. Root crowns are best estimated by excavation or by allometric models based on excavation, as described in *Root sampling approaches*.

Uncertainty in predicting coarse root biomass based on allometric relationships with dbh is similar to that for aboveground biomass, based on goodness of fit (Whittaker et al. 1974, Drexhage and Colin 2001, Wang 2006). Some studies suggest that uncertainty is associated mostly with the method and ease of excavation, but variation in allometry for a species across sites also has been indicated (Bolte et al. 2004). The accuracy of allometric approaches for estimating total root biomass can be evaluated by comparison with field measurements. For example, Fahey et al. (1988) compared coarse root (>1-cm) biomass of a 22-ha mature northern hardwood forest watershed estimated by quantitative pit excavation and by allometric models. The estimates agreed within 8%. Notably, in this case the sampling effort for both approaches was exceptionally high: A total of 58 soil pits (0.5 m^2 each) was excavated, root

systems of 81 trees were excavated to develop allometric equations, and diameters of all stems >5-cm diameter were measured in the 22-ha forest. In another study with only three 0.5-m^2 soil pits in 10 stands, measured biomass of roots <10-cm diameter differed by about 25% from allometric predictions (Vadeboncoeur et al. 2007). Inaccuracies were greater in two young stands (14 and 16 yr), presumably because root systems remained from the pre-harvest forest, as many stems originated as stump sprouts.

Evaluation of Root Sampling Intensity

The question of how many samples to collect must be confronted at the initiation of any sampling campaign. In the absence of quantitative information on spatial variation and other sources of error, researchers often choose a somewhat arbitrary sampling intensity based largely on available funding. We have conducted extensive sampling of root biomass using coring and excavation methods in a series of northern hardwood forests on rocky soils. These data sets provide a basis for judging the adequacy of sampling intensity to meet research needs, and we illustrate approaches for evaluating root sampling intensity on the basis of this work.

Site description

We analyzed root biomass data from three sites in the White Mountain National Forest, New Hampshire, USA. Quantitative pit samples were collected from the Bartlett Experimental Forest (BEF; Richardson et al. 2007) and at HBEF (Bormann and Likens 1979). Power core and manual core samples were collected at HBEF and at a third site, Jeffers Brook (JB) located near Warren, New Hampshire (Bae et al. 2015). Soils at all the sites are primarily isotic, frigid Haplorthods of sandy loam to loamy sand texture derived from glacial drift. Forests at all the sites have a history of logging beginning in the late 19th century, but no history of soil cultivation or agricultural activity. At all three sites, we sampled roots in both mature forests (>85 yr old) dominated by sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt) and in successional forests (20–40 yr old) that also included paper birch (*Betula papyrifera* Marsh), pin cherry (*Prunus pennsylvanica* L.f.), red maple (*Acer rubrum* L.), and aspen (*Populus grandidentata* Michaux).

Root sampling

Quantitative soil pits were excavated in six stands at BEF (C1, C2, C4, C6, C8, C9; Park et al. 2007) in midsummer 2004, with one 0.5-m^2 pit located in each of three 30 × 30 m plots in each stand. The organic layer was sampled by Oie and Oa horizons, and the mineral soil was sampled by depth increments (cm) of 0–10, 10–30, 30–50, and 50 C horizon (Vadeboncoeur et al. 2012). Intact root branches were recovered when soils were sieved through a 12-mm screen in the field; the sieved soil was also subsampled for root picking. Roots were characterized by diameter class: 0–1, 1–2, 2–5, 5–10, and 10–20 mm (Park et al. 2007).

Manual cores were collected using 5-cm diameter PVC corers in successional and mature stands in late summer in 2008 at HBEF and JB sites and in 2010 at BEF. Ten cores were collected in each of four plots in each stand to 30 cm depth, separated into 0–10 and 10–30 cm depth increments, measured from the surface of the organic horizon. Roots were dry-picked from these samples and divided into 0–1 and 1–5 mm diameter classes.

Power cores (10-cm diameter) were collected in late summer 2010 using a gas-powered rotary drill (Rau et al. 2011) from the stands at the HBEF and JB sites that were not sampled with quantitative pits in 2004. Five cores were taken in each of four plots in each stand, and roots were quantified for the 30–50 cm depth increment, measured from the top of the mineral soil, because this depth was not accessible by manual coring. Roots were divided into 0–1 and 1–5 mm diameter classes.

Statistical analysis

We compared the power of the various root collection methods to estimate root biomass at the stand level as a function of sample size, for various combinations of soil depth and root diameter class. We combined biomass for the 0–10 and 10–30 depth increments in manual cores and in quantitative pits, and we grouped the 1–2 and 2–5 mm diameter classes in pits so they were comparable to the 1–5 mm diameter class in manual and

power cores. Sample size requirements were not significantly different between these depths and size classes (data not shown).

To determine the number of samples required to detect a change between pre- and post-treatment for the three collection methods, we calculated the sample size needed to detect a difference of 20% of the mean root biomass using a two-sample *t* test:

$$n = 2\left(\frac{s^2}{d}\right)^2 \times (Z_{1-\alpha/2} + Z_{1-\beta/2})^2 \quad (1)$$

where s^2 is the pooled variance, d is the detectable difference (20% of the mean), Z is the critical value from the standardized normal distribution, $\alpha = 0.05$, and $\beta = 0.2$ (i.e., power is 0.8). Pooled variance can be calculated as:

$$s^2 = \frac{s^2_{\text{pre}} + s^2_{\text{post}}}{n_{\text{pre}} + n_{\text{post}}}. \quad (2)$$

Since we had no estimate for post-treatment variability, we assumed the pre- and post-treatment variances to be equal.

For each depth and diameter class, the number of samples required to detect a 20% difference in root mass was calculated at the stand level (i.e., number of plots within stand) for all three methods, and at the plot level (i.e., number of cores within plots) for two methods (manual and power cores). There was only one soil pit per plot, which precluded an estimate of variability within plots for the pit method.

These sample sizes were used as the dependent variable in ANOVA. The model comparing manual cores and pits included stand (11 levels) and diameter (2 levels). The model comparing pits and power cores for 30–50 cm depth included stand (9 levels) and diameter, but because the same stands were not sampled by these two methods, we relied on Tukey's test of means separation to evaluate the significance of method in the model. This comparison of sampling approaches presumes that the variability in root biomass, but not root biomass itself, is due to the sampling method (pits, manual cores, or power cores), although our data were collected at different dates and somewhat overlapping locations.

To evaluate the distribution of sampling effort between the number of plots and the number of samples per plot, we calculated the standard error (SE) of the mean $(\text{SE}(\bar{y}_i))$ for different combinations of sampling intensities, using the following formula:

$$\text{SE}(\bar{y}_i) = \sqrt{\frac{s^2_d + ns^2_e}{rn}} \quad (3)$$

where s^2_d is the variance component among cores within a plot, s^2_e is the variance component among plots within a stand, n is the number of plots, and r is the number of cores.

Results of the power analysis

In general, the estimates of stand-level fine root biomass (<1 mm) for 0–30 cm depth obtained with ten 5-cm manual cores were similar to those for soil pits in six stands at BEF; the largest difference was for stand C4 (30% higher for pits than for cores; Fig. 1). We compared the number of plots required to detect a difference of 20% in root mass at the stand level for samples collected by manual coring (10 cores per plot) and quantitative soil pits (1 pit per plot) for 0–30 cm depth (Fig. 1). We expected these approaches to have similar statistical power, because one soil pit samples a larger volume of soil than 10 manual cores (Table 1). Instead, the variance among plots based on pits was higher than for cores, and the sample size requirements (numbers of plots with one pit or 10 cores) were about twice as high for pits as cores, a significant difference according to ANOVA (Table 2). Using the manual coring method, the average number of plots required was similar across the stands with an overall average of nine plots required to detect a 20% difference in mass for 0–1 mm roots. Estimates of sample size requirements for 1–5 mm roots were about twice as high for 0–1 mm roots, a difference that was also statistically significant (Table 2). Notably, these main effects of method and diameter were driven by a strong interaction of diameter and method in the ANOVA resulting from a much higher sample size requirement for 1–5 mm roots in pits than in cores.

Sample size requirements at the stand level for fine roots may be higher in these soils than in similar forests on less rocky sites. A useful comparison is available from two sugar maple stands on sandy soils with few stones, which were sampled with similar methods (eight 5-cm cores per plot and three plots per stand; Hendrick and Pregitzer 1993). For <0.5-mm roots, we calculated

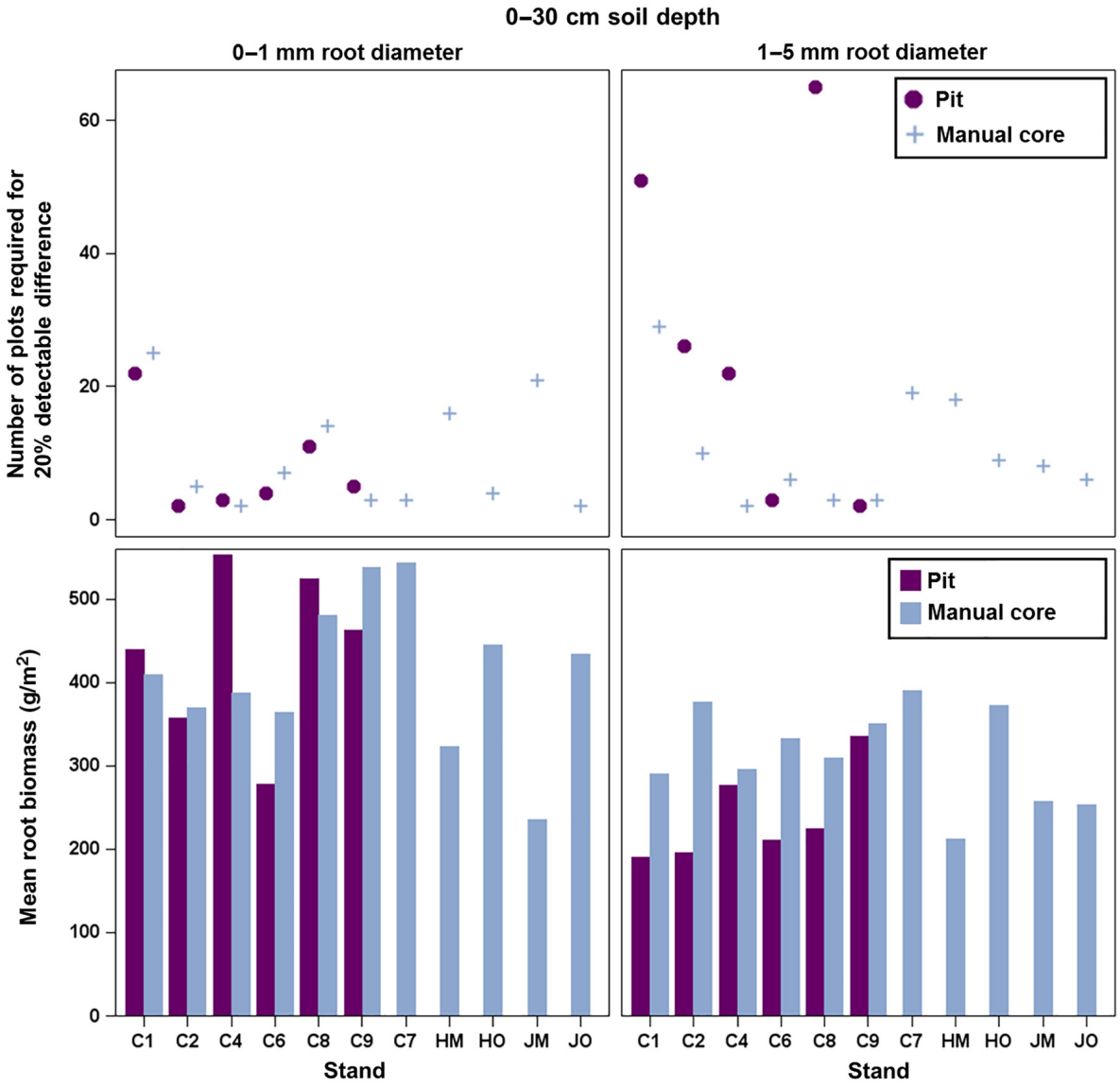

Fig. 1. Stand-level fine root biomass (<1 mm and 1–5 mm) for 0–30 cm soil depth in six stands at Bartlett Experimental Forest, based on 5-cm diameter soil cores (10 per plot) and 0.5-m^2 soil pits (1 per plot), and the number of plots required to detect a 20% difference in fine root mass by these two methods.

Table 2. Results of analysis of variance of sample sizes (number of plots) required to detect a 20% difference in fine root biomass (0–1 and 1–5 mm diameter) at the stand level (11 stands) for 0–30 cm soil depth using two methods, 5-cm diameter manual cores (10 per plot) and 0.5-m^2 soil pits (1 per plot).

Source	df	Type III SS	Mean square	*F*	Pr > *F*
Stand	10	2854.16	285.42	2.76	0.03
Diameter	1	883.45	883.45	8.54	0.01
Method	1	477.04	477.04	4.61	0.04
Diameter × Method	1	725.57	725.57	7.02	0.02

Note: SS = sum of squares.

sample size requirements for detecting a 20% difference that ranged from three to five plots per stand, generally fewer than in our stands (Fig. 1). Like ours, their forests had higher spatial variation for larger diameter roots (2–10 mm), with sample size requirements roughly similar to those for coarse roots in our stands.

Roots at depth are much more difficult to characterize precisely than more superficial roots. We compared the number of plots required within stands for detecting a 20% difference in fine root biomass at depth (30–50 cm) between soil pits and power cores. Although these methods were applied in different stands, both approaches indicated widely varying statistical power across stands as well as very high sample requirements owing to high spatial variation (Fig. 2). Detecting changes in fine root biomass at depth in response

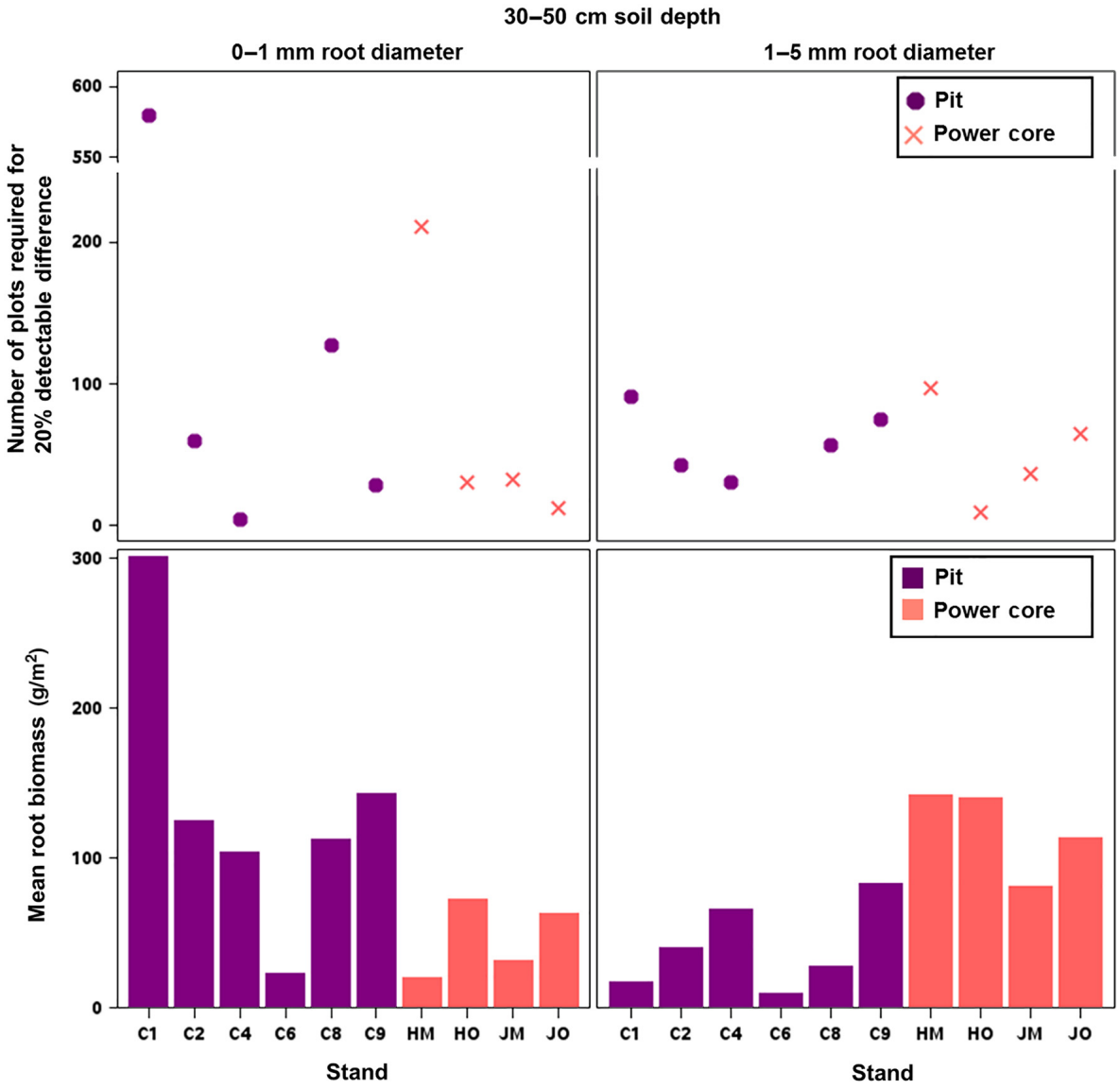

Fig. 2. Stand-level fine root biomass (<1 mm and 1–5 mm) for 30–50 cm soil depth in 10 stands, and sample size requirement (number of plots) to detect a 20% difference in root biomass using soil pits (1 per plot) and 10-cm diameter power cores (5 per plot). There is no sample size estimate for site C6 because only one pit was deeper than 30 cm; thus, we have no estimate of variance.

to treatments or through time will be challenging by either method. Because of the much greater time and effort required for soil pit excavation than power coring, we conclude that for purposes of measuring fine root biomass at depth in rocky soils, the power coring technique is clearly preferable. Moreover, we observed that the sample size requirements for detecting a 20% change in mean root biomass with the soil pit method was highly variable among six stands (Fig. 3); thus, the advantage provided by soil pits for sampling roots at greater depths is compromised by high variation in many stands. Finally, we note that sample size requirements for the entire soil profile are generally similar to those for the Oa horizon and the 0–30 cm mineral soil depth, as most of the roots occur in these soil layers in these forests (Fig. 3).

The preceding power analyses addressed the number of experimental plots required to achieve a detectable difference, holding constant the number of samples in a plot (10 manual cores, five power cores, or one quantitative pit). We also estimated the number of cores needed within plots to detect a 20% difference in mean root biomass between plots (Fig. 4). We could not directly compare methods because manual and power cores were done over different depth intervals. Therefore, although sample size requirements were greater at deeper soil depths,

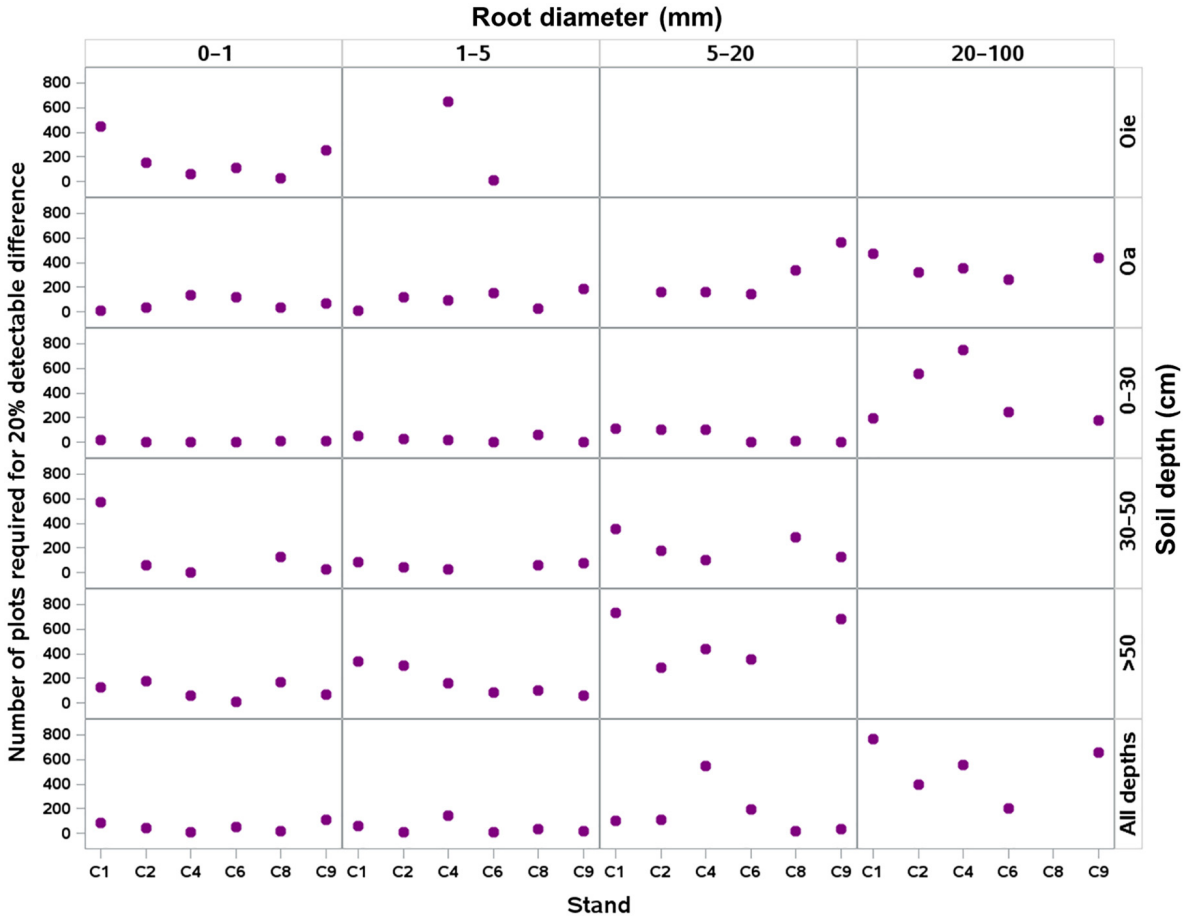

Fig. 3. Sample size requirement (number of sample plots) using soil pits to detect a 20% difference in mean root biomass (0–1, 1–5, 5–20, and 20–100 mm) for each soil horizon to the C layer in six stands and for the entire soil profile. There are no sample size estimates for cases where there was no estimate of variance (i.e., fewer than two pits had roots in these size and depth categories).

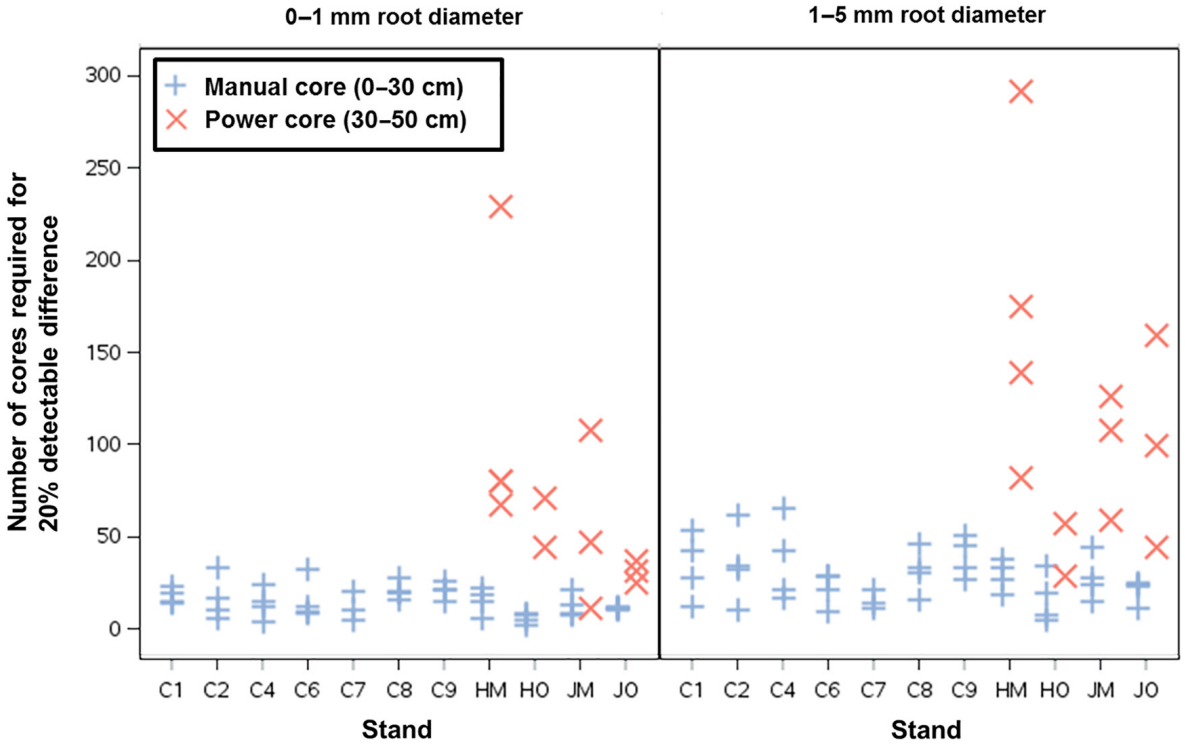

Fig. 4. Number of cores required within plots to detect a 20% difference in mean root biomass using 5 cm diameter manual cores and 10 cm diameter power cores in 11 stands.

it is unclear whether this is attributable solely to the higher variability of root biomass at depth (Fig. 4). For both methods, fewer cores were needed within plots to detect a difference in biomass in the smaller (0–1 mm) than in the larger (1–5 mm) roots (Fig. 4).

Finally, we considered both sampling intensity within a plot and the number of plots, which is important to optimizing allocation of sampling effort. Combinations on the upward diagonals of Fig. 5 represent equal sampling effort (numbers of cores times numbers of plots). We found, based on SE at the stand level, averaged across 11 stands, that it was generally more beneficial to have more cores within a plot than fewer cores distributed across more plots. The only exception to this generalization occurred with 1–5 mm roots in the 30–50 cm depth.

Increased power to detect treatment effects on fine root biomass can be obtained by employing a before–after control impact (BACI) experimental design (Stewart-Oaten et al. 1986). Before–after control impact designs account for temporal changes in a response variable that are not related to the treatment, and the impact of the treatment is quantified as the difference in response between study plots observed over time.

$$\text{Treatment Impact} = (\mu_{ca} - \mu_{cb}) - (\mu_{ta} - \mu_{tb}) \quad (4)$$

where μ is the mean of the response variable; c represents the control, a the measurement after treatments, b the measurement before treatments, and t the treatment. A key assumption with this approach is that in absence of the treatment, the differences between the sites would be constant with time (Stewart-Oaten et al. 1986). Linear models can then be employed to evaluate significance of treatment; in the simplest case, the time-by-treatment interaction provides the test of treatment effect (Smith 2002). Fahey et al. (2016) employed a BACI design to demonstrate that Ca addition resulted in a ~30% decline in fine root biomass at HBEF with very high confidence ($P < 0.0001$). Without using the BACI design, the difference between the control and treatment sites was significant but only at $P = 0.05$.

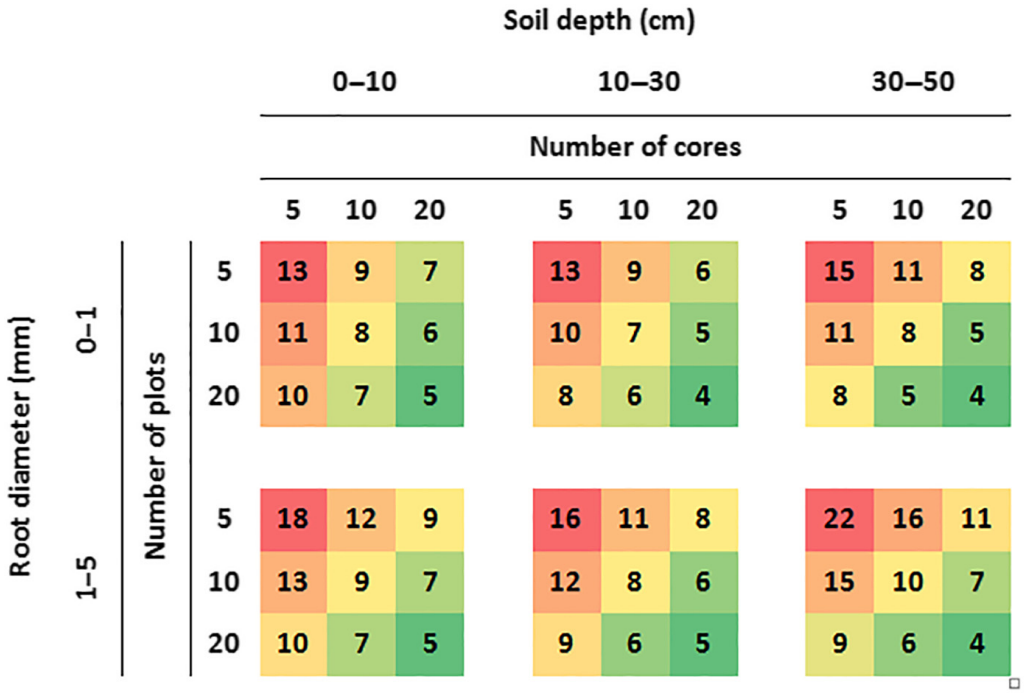

Fig. 5. Standard error of the mean (relative standard error; RSE) associated with allocation of effort to cores within a plot and plots within a stand, based upon samples collected from 11 stands and 33 plots. Values are based on manual cores in 0–10 and 10–30 cm depths and on power cores in the 30–50 cm depth. Combinations on the upward diagonals represent equal sampling effort (numbers of cores times numbers of plots). Confidence intervals are generally tighter (REs are smaller) when effort is allocated to more cores distributed across fewer plots.

Summary and Recommendations

Root biomass measurements are needed for a variety of purposes in forest ecosystem studies, and the needs of the research will influence the choice of methods. Many studies rely on root measurements obtained within 20–30 cm of the soil surface because of the relative ease of sample collection with simple coring devices and because root biomass and production are often concentrated there (Gale and Grigal 1987, Hendrick and Pregitzer 1996). For purposes of accurately quantifying root biomass for the full depth profile, expensive excavation or power coring techniques are often needed, and sampling coarse woody roots for purposes of quantifying total root biomass necessitates different approaches than sampling fine roots. If the objective is to detect a treatment response of fine root biomass or turnover, a large sample size obtained from the densely rooted surface soil may be most efficient (Helmisaari and Hallbäcken 1999).

Sampling designs should be optimized based on the goals of the research, the expected variability of root biomass at the study sites, and sampling costs and budgets. For the purpose of quantifying total fine root biomass in rocky soils using coring approaches (as opposed to excavation), it may be beneficial to use both manual coring (to obtain a large number of shallow cores) and power coring (to obtain samples from sub-soil horizons and beneath coarse fragments). For example, researchers could obtain many (e.g., 15–20) manual soil cores to the depth of obstruction and a few (e.g., 4–5) deep cores (e.g., 50 or 100 cm depth) obtained by more costly power coring. Also, we suggest that roots of very large diameter (>2 cm) should not be estimated by soil pits or cores but by tree inventory and allometry or soil trench excavation.

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