NEON TOS DESIGN OPTIMIZATION: ROOT BIOMASS AND CHEMISTRY, PERIODIC - FIELD SAMPLING REPLICATION

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1 Introduction

1.1 Description of sample design

The TOS Science Design for Plant Biomass and Productivity (RD[03]) defines key vegetation components, defines spatial and temporal criteria for sampling vegetation components, evaluates available sampling methods, and identifies mechanisms for spatial and temporal integration of biomass and productivity sampling with other NEON subsystems - e.g., the Airborne Observation Platform (AOP) and the Terrestrial Instrument System (TIS). The analyses presented here focus on testing the spatial components of the field sampling design and the ability to detect changes in fine-root belowground biomass using data from the NEON 'Root biomass and chemistry, periodic' data product (DP1.10067.001).

Here, we sought to identify whether within-plot replication (i.e., the number of cores per plot) or the number of plots sampled per site could be reduced while maintaining the ability to detect change with desired levels of confidence. Currently, sites with large-stature vegetation are sampled with up to twenty 40m x 40m plots. Within each plot, two 20m x 20m subplots are randomly selected for sampling, and within each of these subplots, a random "sampling cell" location is chosen from which two cores are collected - i.e., four cores are collected per plot, resulting in a maximum of n=80 cores per large-stature site. At sites with small-stature vegetation, up to thirty 20m x 20m plots are sampled. Two cores are collected from one sampling cell per plot, generating a maximum of n=60 cores per small-stature site.

1.2 Analytical Goals

Statistically rigorous analyses are needed to assess the capacity of NEON data to address Observatory goals and to guide sampling design optimization efforts. Initial spatial and temporal sampling designs for the NEON Terrestrial Observation System (TOS) and Aquatic Observation System (AOS) were developed in collaboration with Technical Working Groups (TWGs) comprised of community experts, and these sampling designs were captured in Science Design documents (RD[01], etc.). The initial designs relied on analysis of published datasets (where relevant), analysis of NEON prototype data collection efforts, and subject matter expertise.

Now that the NEON Observatory has matured and moved into full operations, it is critical for TOS and AOS initial design assumptions to be tested with multiple years of data collected from NEON sites. Analysis and evaluation of the data provides a feedback loop that enables assessment of the Design relative to Observatory goals. Moreover, results of these analyses allow the NEON TOS and AOS to effectively prioritize sampling in the face of uncertain budgets and labor availability.

Key questions to address for 'Root biomass and chemistry' sampling during this phase of design optimization are:

1. Where is the variability in the data? Specifically, what proportion of the variance at each site can be attributed to year, NLCD Class, plot, or sampling cell?

   Understanding the components of variance provides a critical component of the actionable directions for modifying sampling size by providing answers to questions such as:

   - At what spatial scale does the sample design capture the most variation?

- How do variance components differ among sites?
- Does a site's NLCD land cover classification predict variance components?

For example, it may be possible to eliminate within-plot or within-cell replication if variance partitioning indicates this component of the spatial design consistently fails to explain variation in the data across sites.

2. Can field replication (either within- or across-plots) be reduced without adversely affecting site-level biomass means within each diameter size category of fine-root biomass?
3. Is it possible to detect meaningful inter-annual change in fine root biomass across each size category?

If the current sampling effort has the statistical power to detect naturally occurring change over multi-year intervals, then it is possible the sampling effort may be reduced while maintaining the ability to detect change. In this context, we investigated the following related questions:

1. Can the current design detect a 20% year-over-year change in belowground biomass at a given site?
2. What sample size is needed to detect a 20% year-over-year change in total fine root mass?
3. Is there sufficient power to detect year-to-year changes (20%) in belowground biomass in each size category after reducing within-plot sampling effort from 2 cores per sampling cell to 1 core per sampling cell, or from 2 sampling cells per plot to 1 cell per plot (at large-stature sites)?
4. Can the number of plots sampled per site be reduced from 30 to 20 or less at sites with small-stature vegetation, or from 20 plots to 15 or 10 plots at sites with large-stature vegetation?

Here, we assume that a design capable of detecting 20% year-over-year change at a site will also be well-poised to detect change of a similar magnitude over longer time periods. We do not have sufficient data at this time to parameterize longer-term temporal variability in belowground biomass, and we have therefore not quantitatively tested this assumption with more complex simulated time series.

1.3 Reference Documents

RD[01]	NEON.DOC.000001	NEON Observatory Design
RD[02]	NEON.DOC.002652	NEON Level 1, Level 2, and Level 3 Data Products Catalog
RD[03]	NEON.DOC.000914	TOS Science Design for Plant Biomass and Productivity
RD[04]	NEON.DOC.014038	TOS Protocol and Procedure: BGB - Plant Belowground Biomass Sampling

1.4 Acronyms

Acronym	Definition
BGB	Below-Ground Biomass
AOS	Aquatic Observation System
NLCD	National Land Cover Database
TOS	Terrestrial Observation System
TWG	Technical Working Group

1.5 Data Acquisition

Below-ground fine root biomass data from the NEON 'Root biomass and chemistry, periodic' data product (DP1.10067.001) were collected following the standard NEON protocol for this data product (RD[04]). All data for these analyses are published and publicly available via the NEON Data Portal. Data from 2016 – 2023 were retrieved from the NEON Data Portal on 2024-07-18 using the `R` `neonUtilities::loadByProduct()` function.

1.6 Data preparation

We removed 'samplingImpractical' records from the `bbc_percore` table, and we removed 'qaDryMass' records from the `bbc_rootmass` table. We joined `bbc_percore` and `bbc_rootmass` records, calculated mass (g) per m^2, and removed records without a mass per m^2. To ensure complete datasets were used for statistical tests, we removed sampling cells with only one core, and we removed plots from large-stature sites with only one sampling cell. We also did not consider sites for which the full sampling design has not been implemented, which removed the following sites from consideration: D05 TREE, D10 RMNP and STER, D12 YELL, D16 ABBY, and D20 PUUM.

1.7 Response variables

The response variables are belowground fine root biomass sorted to 0-1 mm, 1-2 mm, and 2-10 mm diameter size categories. In our analyses we considered a pooled 0-2 mm size category, the 2-10 mm size category, and the total fine root biomass across all size categories.

2 Methods and Results

2.1 Variance components analysis of fine root biomass

For each site with data meeting completeness criteria, the analysis partitions the variance of belowground fine root biomass and illuminates patterns of variability across spatial scales.

2.1.1 Methods

We used separate linear mixed-effects models for each site (`lme4` package for R) to partition variance in observed belowground fine root biomass among spatial grouping factors (`nlcdClass`, `plotID`, `clipID`;

in the text that follows 'clipID' is equivalent to 'sampling cell', as these cells also support herbaceous clip harvest). This analysis was carried out independently at each site for each fine root size category (0-2 mm and 2-10 mm) as well as the total of these size classes. If more than 1 year of complete data were available for a site, only data from the first year for which complete data were available was used. The analysis was based on the following model:

$$Y \sim 1 + (1|nlcdClass) + (1|plotID:nlcdClass) + (1|clipID:plotID:nlcdClass)$$

Where:

- `Y = Log(dryMass + 1)` is the natural log-transformed belowground fine root biomass, and
- `nlcdClass`, `plotID`, and `clipID` are all random factors among which variance was partitioned.

For small-stature sites, the `plotID` was removed since the spatial sampling design in these plots employs a single sampling cell per plot. For each site-specific model, the `nlcdClass` variable was removed if there was only one `nlcdClass` or if `nlcdClass` variance was close to 0 and prevented the model from converging. Likewise, the `plotID` and/or `clipID` variables were removed if the variance for either of these factors was close to 0 and prevented the model from converging.

For sites with more than one year of data and more than one NLCD class, we analyzed the relative importance of spatial variables (`nlcdClass`, `plotID`) and the temporal variable `year` (i.e., first versus second of two sampling events separated by a 5-year interval).

$$Y \sim 1 + (1|nlcdClass) + (1|year)$$

2.1.2 Results

The total variance in belowground fine root biomass was highly variable across sites and size classes, as was the proportional variance explained by random factors in the model at sites with both large-stature vegetation (Figure 1) and small-stature vegetation (Figure 2). Notable spatial patterns for biomass summed across size classes include:

- The `NLCD class` was the most important variance component of belowground biomass in 5 of 26 sites with more than one NLCD class.
- The spatial `plotID` variable was the most important variance component of belowground biomass at 12 of 23 large-stature sites.
- The spatial `clipID` (sampling cell) variable was the most important variance component of belowground biomass at 21 of 39 total sites.

Models that converged for summed biomass did not always converge for individual size classes, particularly the 2-10 mm size class. However, the relative importance of the different spatial random factors was similar for the individual size classes, with site-to-site variation in which spatial random factors explained the most model variance.

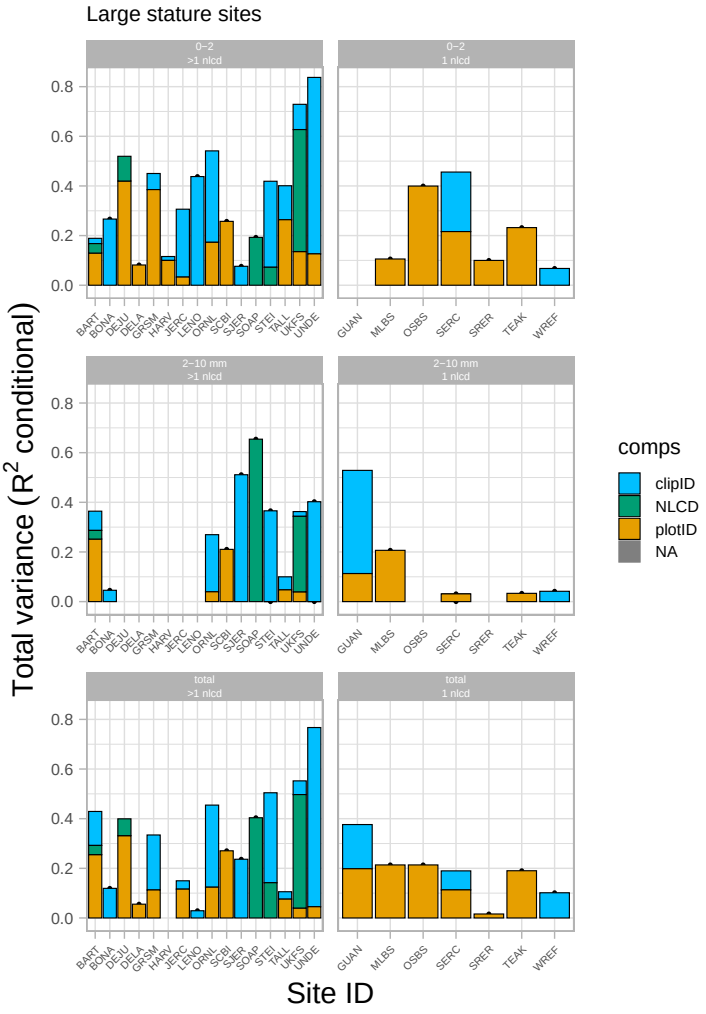

Figure 1: Proportion of the total model variance in belowground fine root biomass explained by ‘clipID‘ (blue), ‘plotID‘ (orange), and ‘nlcdClass‘ (green) at each site and across fine root diameter size classes at large-stature sites.

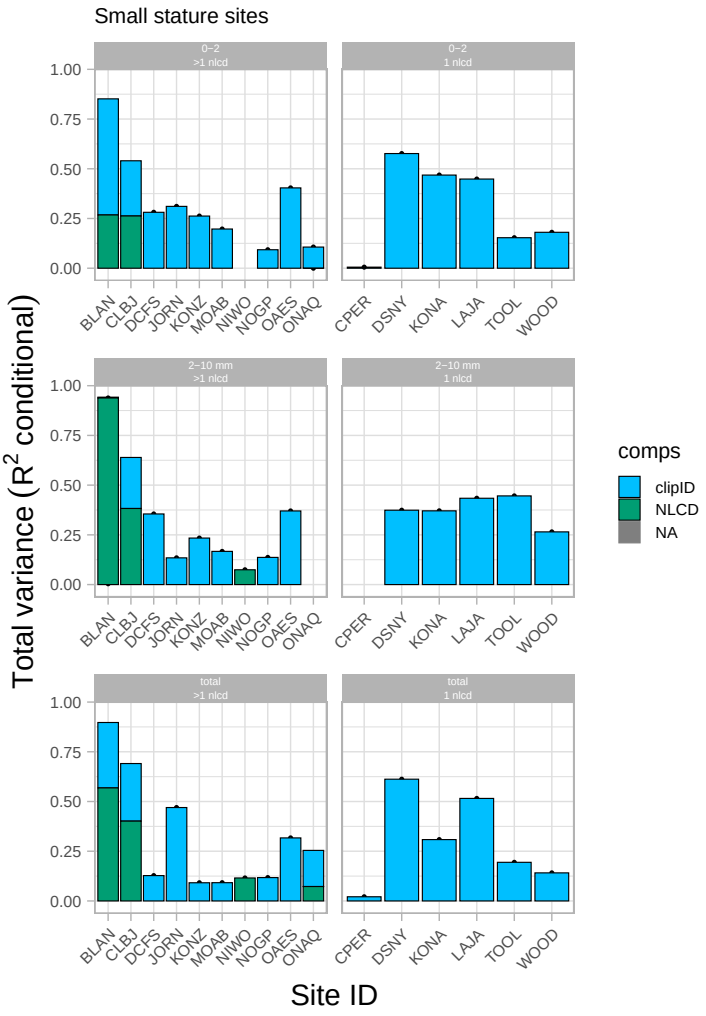

Figure 2: Proportion of the total model variance in belowground fine root biomass explained by ‘clipID‘ (blue) and ‘nlcdClass‘ (green) at each site and across fine root diameter size classes at small-stature sites.

Evaluating total fine root biomass summed across all size categories at sites with two complete years of data, the temporal variance was greater than the spatial variance at 3 sites out of 14 (DEJU, ONAQ, WOOD). Temporal variance was also greater than spatial variance in the summed 0-2 mm size class at D02 SCBI and D09 DCFS (Figure 3).

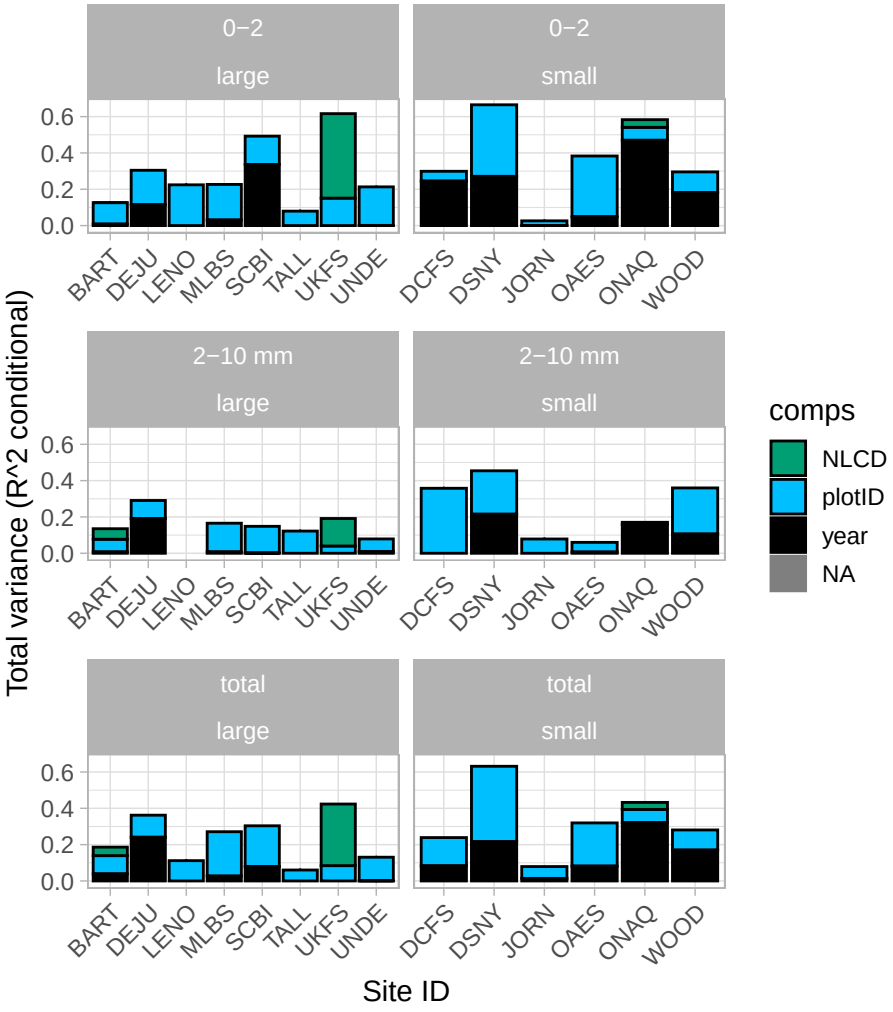

Figure 3: Proportion of the total model variance in belowground fine root biomass explained by 'year' (black), 'plotID' (blue), and 'nlcdClass' (green) across fine root diameter size classes at sites with two complete years of data.

2.2 Spatial sampling effects on belowground fine root biomass estimates

2.2.1 Methods

To evaluate whether existing site-level means of fine root biomass could be confidently generated with fewer within-plot field replicates, we performed a bootstrapping exercise using sampling with replacement (n = 300 iterations per site). We analyzed data separately for sites with large-stature (40m x 40m plots) versus small-stature vegetation (20m x 20m plots) due to differences in spatial sampling design.

For large-stature vegetation sites we evaluated three levels of within-plot replication:

1. Sample 2 cells per plot and 2 cores per cell, as in the current sampling design (total of 4 cores per

plot). For the bootstrapping, we created a list of unique sampling cells (clipIDs) and we then sampled 2 cores from within each cell with replacement. This procedure guaranteed that both cells within a plot contributed to the site-level estimate, and sampling with replacement allowed for the possibility of either drawing the same north or south core twice, or randomly re-selecting 1 north and 1 south core as in the original data.

2. Sample 2 cells per plot and 1 core per cell (total of 2 cores per plot). For the bootstrapping, we created a list of unique sampling cells (clipIDs) and we then sampled 1 core from within each cell.
3. Sample 1 cell per plot and 1 core per cell (total of 1 core per plot). For this lowest level of within-plot replication, we randomly selected one core from each plot.

For small-stature vegetation sites we evaluated two levels of within-plot replication:

1. Sample 1 cell per plot and 2 cores per cell, as in the current sampling design (total of 2 cores per plot). To accomplish this, we sampled 2 cores from the single cell (clipID) in each plot, with replacement. Similar to large-stature sites, this procedure allowed for the possibility of either drawing the same north or south core twice, or randomly re-selecting 1 north and 1 south core as in the original data.
2. Sample 1 cell per plot and 1 core per cell (total of 1 core per plot). To accomplish this lowest level of within-plot replication, we randomly sampled one core from each plot.

For each of the 300 bootstrap iterations, we tallied whether the re-sampled site-level mean was within +/- 10% of the observed site-level fine root biomass mean. Across all 300 iterations, we then calculated the percentage of re-sampled means that were within +/- 10% of the observed mean (i.e., confidence). This analysis was performed for each fine root diameter size category and for the total fine root biomass summed across size categories.

2.2.2 Results

At sites with large-stature vegetation, for 15 of 23 sites it was possible to reproduce the observed mean total fine root biomass (sum of all size categories) with 90% confidence after reducing to 1 core per sampling cell (D01 BART, HARV; D02 SCBI, SERC; D03 JERC; D04 GUAN; D05 STEI; D06 UKFS; D07 GRSM, MLBS, ORNL; D08 DELA; D16 WREF; D19 BONA, DEJU) (Figure 4). However, bootstrapping indicated that it was not possible to reproduce the observed mean total fine root biomass with 90% confidence at any large-stature sites when only 1 core from a single cell was re-sampled.

With respect to individual fine root size categories (0-2 mm, 2-10 mm diameter), we found that mean 2-10 mm fine root mass could be reproduced with 90% confidence at only 3 sites (D01 BART; D16 WREF; D19 DEJU) when within-plot replication was reduced to 2 cells per plot and 1 core per cell. For the 0-2 mm size category, created by pooling 0-1 mm and 1-2 mm diameter root mass in the original data, it was possible to reproduce the observed mean with 90% confidence at 18 of 23 sites (D01 BART, HARV; D02 SCBI, SERC; D03 JERC, OSBS; D05 STEI; D06 UKFS; D07 GRSM, MLBS, ORNL; D08 DELA, TALL; D16 WREF; D17 SOAP; D19 BONA, DEJU) (Figure 4).

Taken together, the results suggest that variability in the 2-10 mm size category hinders confident reproduction of bootstrapped site-level means for total fine root biomass.

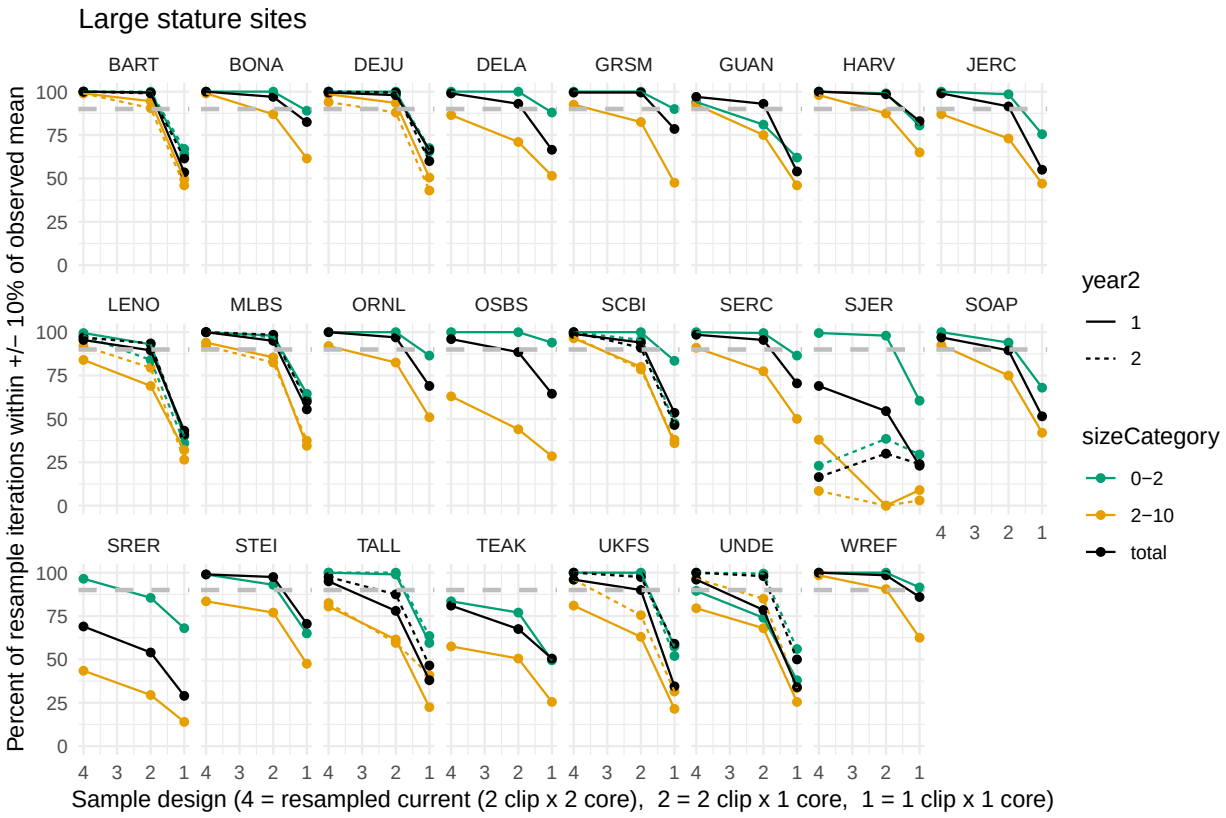

Figure 4: Percent of resample iterations for each site and size category that are within +/- 10% of the observed mean at sites with large-stature vegetation (40m x 40m plots).

At sites with small-stature vegetation, for 3 of 16 sites it was possible to reproduce the observed mean total fine root biomass with 90% confidence after reducing to 1 core per plot at three sites (D09 NOGP; D10 CPER; D11 CLBJ) (Figure 5). Similar to sites with large-stature vegetation, it was again difficult to reproduce biomass means in the 2-10 mm size category with 90% confidence once sampling was reduced to 1 core per plot, and we observed no sites where this was possible. For the 0-2 mm size category, it was possible to reproduce the observed mean fine root mass at 4 sites when only 1 core per plot was re-sampled (D06 KONZ; D10 CPER; D13 NIWO; D11 CLBJ) (Figure 5).

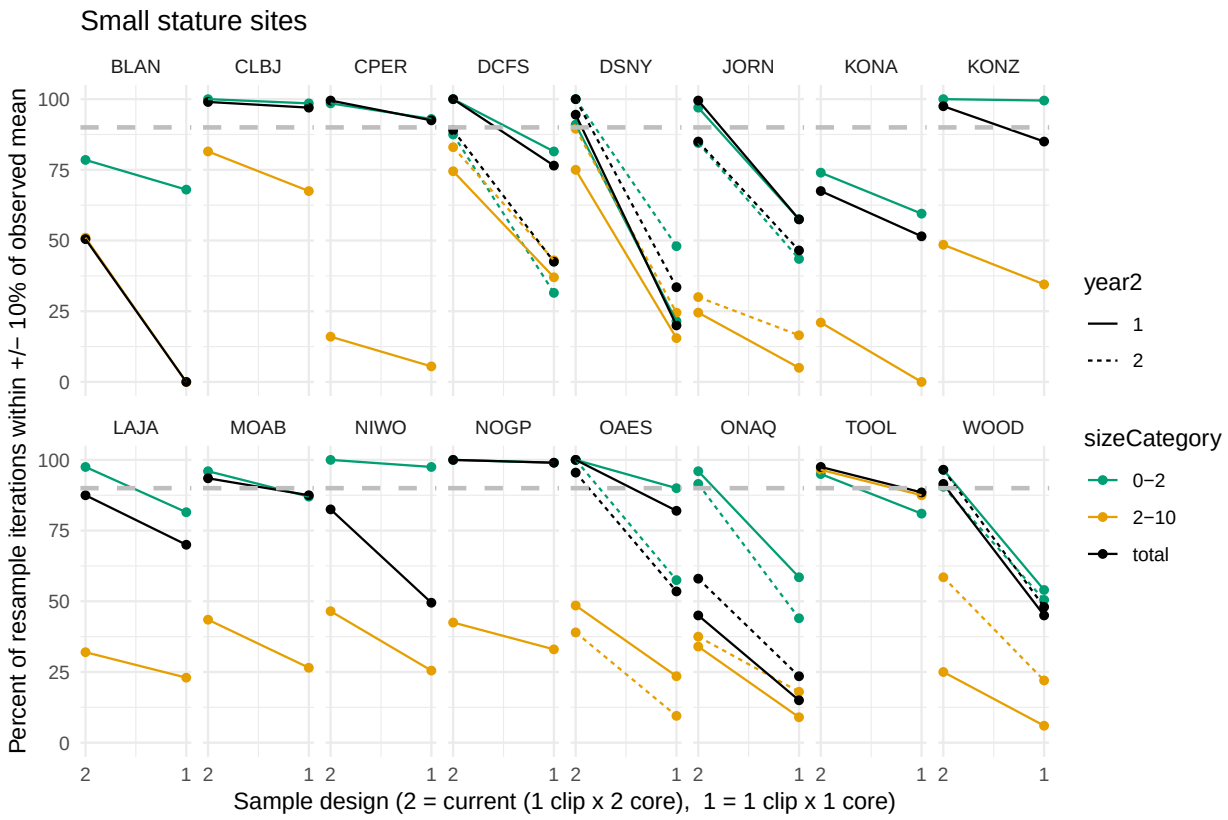

Figure 5: Percent of resample iterations for each site and size category that are within +/- 10% of the observed mean at sites with small-stature vegetation.

2.3 Year effect at sites with multiple years of complete data

2.3.1 Methods

For the 14 sites with complete sampling in two different years (fine roots are sampled every 5 years at each site, and complete sampling is not always attainable due to logistics), we investigated whether a significant `year` effect ($p < 0.05$) could be opportunistically detected for each fine root size category (0-2 mm, and 2-10 mm diameter) as well as the total fine root biomass. There is no *a priori* reason for a significant `year` effect to exist, but if a `year` effect is detected it suggests that the current sampling design is potentially adequate to detect a +/- 10% change in fine root mass with 90% confidence, which was the original design criterion.

The analysis to detect `year` was based on the following model:

$$Y \sim 1 + year$$

Where:

- `Y = Log(dryMass + 1)` is the log-transformed belowground fine root biomass, and

- `year` is the fixed temporal variable

For each site with a `year` effect in at least one size category, or the total fine root biomass, we quantified the percentage of bootstrapped re-samples that still had a `year` effect once within-plot replication was reduced. We used the same re-sampled datasets for this analysis that were used earlier for the bootstrapping analysis of site biomass means.

2.3.2 Results

There are 14 sites with two different years of complete sampling. Six of these sites (D05 UNDE; D06 UKFS; D07 MLBS; D08 LENO; D11 OAES, and D14 JORN) showed no significant `year` effect ($p > 0.05$) for either the 0-2 mm size category, the 2-10 mm size category, or for total fine root biomass. Four of the 14 sites (D03 DSNY; D09 WOOD; D15 ONAQ; and D19 DEJU) did show a significant `year` effect for all of the original size categories (0-1 mm, 1-2 mm, and 2-10 mm), and an additional two sites (D02 SCBI and D09 DCFS) showed a significant `year` effect for both the pooled 0-2 mm size category and total fine root biomass. In addition, the D08 TALL site showed a significant `year` effect for just the pooled 0-2 mm size category, and D01 BART had a significant `year` effect only for total fine root biomass. Altogether, 8 of 14 sites showed a significant `year` effect in at least one size category or for total fine root biomass. The subsequent analysis of the impact of sampling reductions on observed `year` effects is focused on these 8 sites.

For the 8 sites with a significant `year` effect ($p < 0.05$) for at least one size category or total fine root biomass, a bootstrapped reduction in within-plot sampling replication frequently resulted in losing the ability to detect year-over-year change (Figure 6). At sites with small-stature vegetation, reducing sampling to one core per plot resulted in loss of the significant `year` effect at all sites except D15 ONAQ for the 0-2 mm size category. At sites with large-stature vegetation, reducing sampling to 2 cells per plot and 1 core per cell resulted in loss of a significant `year` effect for 4 of 7 sites, and further reducing sampling to 1 core per plot resulted in loss of all significant `year` effects (Figure 6).

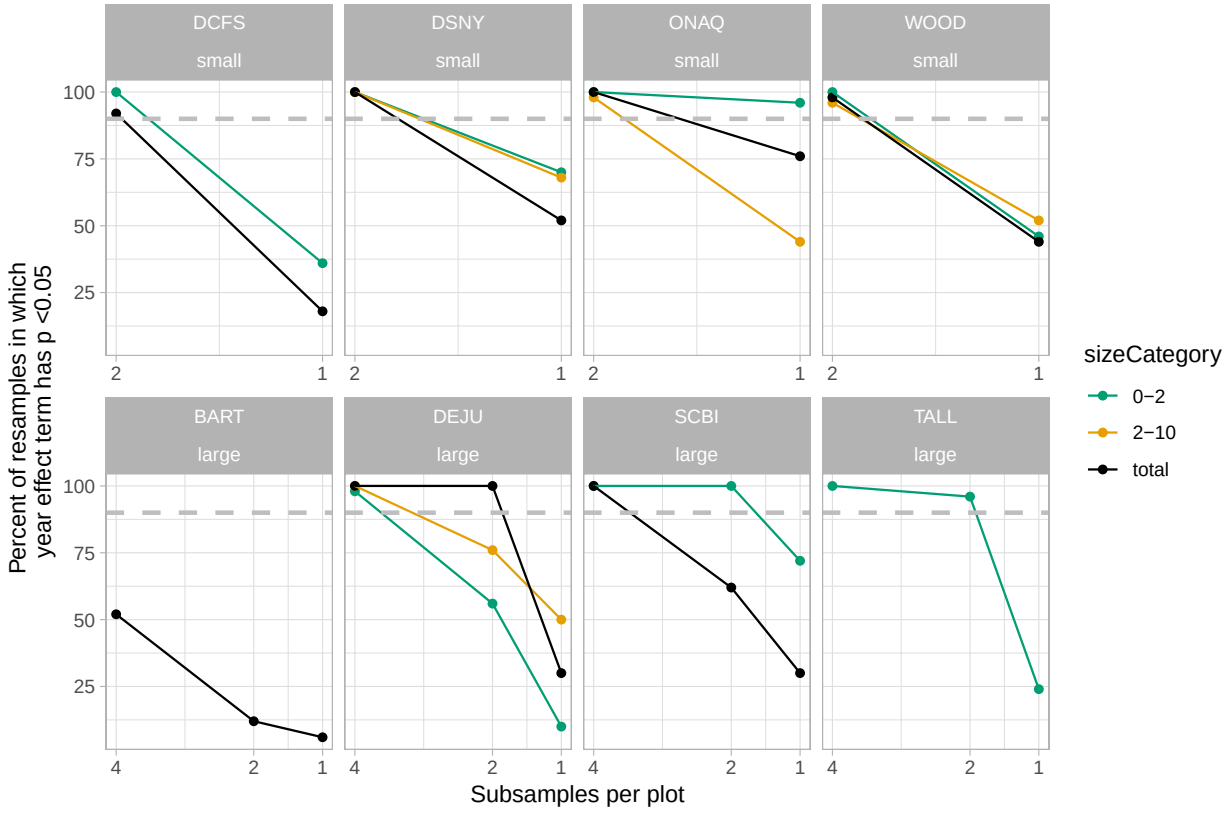

Figure 6: Year effect for 10 sites with full sampling in two different years. Only site by size category combinations with a significant year effect in the original data are displayed.

2.4 Power analysis: Sampling effort required to detect 20% year-over-year change in fine root biomass

2.4.1 Methods

To further evaluate the ability of the sampling design to detect year-over-year change in fine root biomass with a standardized method at all sites, we performed a power analysis using simulated data generated with mixed-effects models. Using the best fit mixed effects models from the variance partitioning analysis (described above), we simulated a 20% year-over-year change in fine root biomass and iteratively determined whether that known change could be detected with 80% power given varying levels of simulated sampling effort. For example, if the best random effects model for a given site was:

$$Y \sim 1 + (1|nlcdClass) + (1|plotID:nlcdClass) + (1|clipID:plotID:nlcdClass)$$

a constant `year` effect of 0.2 was added to produce a mixed-effects model like:

$$Y \sim 1 + year + (1|nlcdClass) + (1|plotID:nlcdClass) + (1|clipID:plotID:nlcdClass)$$

The starting model varied from site-to-site, but the process of adding a fixed `year` effect was identical for all sites. Once a mixed-effects model containing `year` was selected for a site, we used the variance structure of the model to extract a random effect for each random variable, added these random effects to the mean biomass, repeated for time 1 and time 2, added the simulated year effect of 0.2 to year 2, and then repeated 50 times. The power metric was the percentage of repetitions where the imposed 20% `year` effect could be detected amidst the noise of the random variables. The desired power for supporting any change in the sampling design was ≥80%.

2.4.2 Results

At sites with large-stature vegetation, we simulated a reduced within-plot sampling effort of 2 cells per plot and 1 core per cell while keeping plot number constant (50% sampling effort reduction compared to the original design), and we detected the imposed 20% year-over-year change in total fine root biomass with power ≥80% at 3 of 23 sites (D07 ORNL; D19 BONA, DEJU) (Figure 7). When we simulated a further reduced sampling effort of 1 core per plot (75% sampling effort reduction compared to the original design), we were unable to detect the imposed 20% change in total fine root biomass with power ≥80% at any sites (Figure 7).

Considering only the 0-2 mm size category and the same 50% intra-plot sampling reduction described above, we detected the imposed 20% change in 0-2 mm root biomass with power ≥80% at 10 of 23 sites (D02 SCBI, SERC; D03 JERC, OSBS; D05 UNDE; D06 UKFS; D07 ORNL; D16 WREF; D19 BONA, DEJU), and we were unable to detect the 20% change in 2-10 mm root biomass with sufficient power at any sites (Figure 7). Consistent with the bootstrapping results, significant variability in mass within the 2-10 mm size category appears to be responsible for the inability to detect an imposed 20% change in total fine root mass with the desired power at many sites.

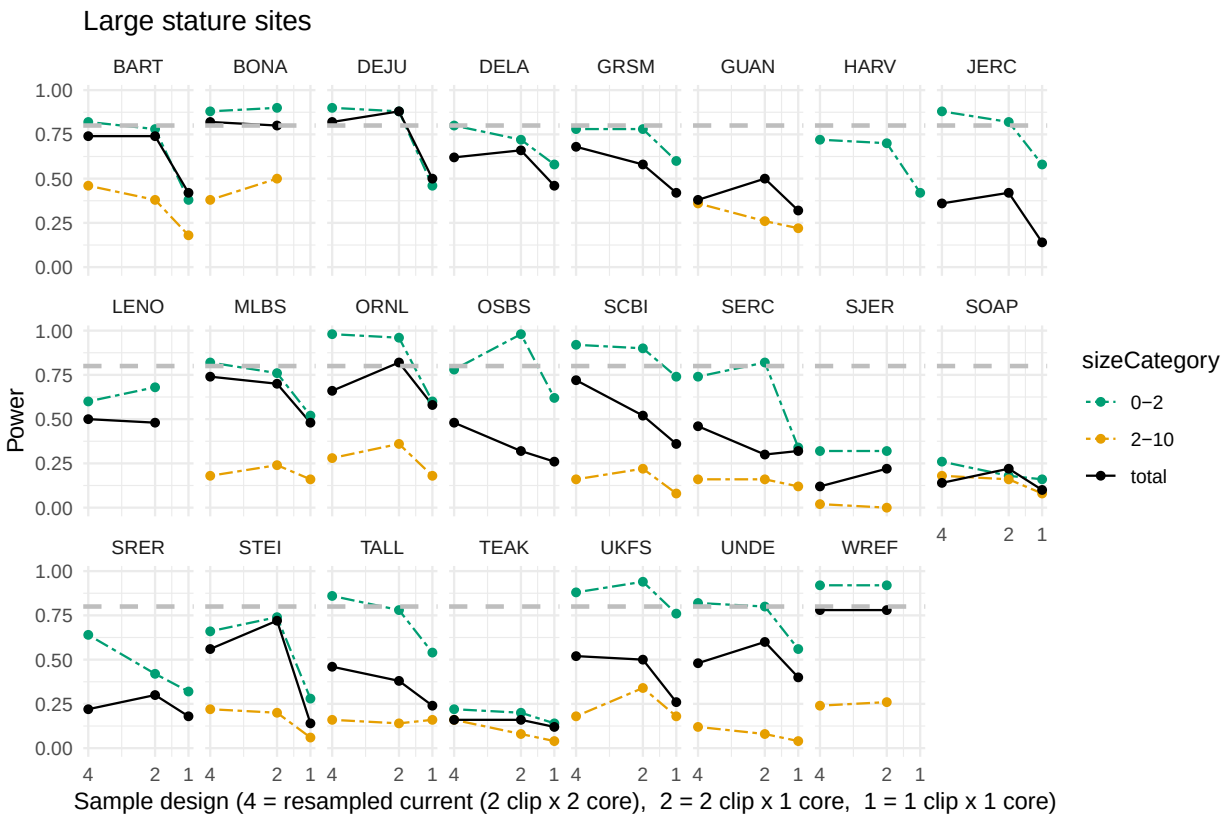

Figure 7: Power analysis of within-plot reductions in sampling effort and the ability to detect an imposed 20% change in fine root mass at sites with large-stature vegetation.

To better understand the contribution of plot-level replication to the ability to detect change, we kept within-plot replication constant and simulated the ability to detect a 20% change in root biomass with reduced numbers of plots compared to the current design. At large-stature vegetation sites, when we reduced plot number from 20 (current design) to 15 or 10 plots, we found no sites at which the imposed 20% change in total fine root mass could be detected with power ≥80% (Figure 8). Considering only the 0-2 mm size category, it was possible to detect a 20% change in mass with power ≥80% at 5 sites (D02 SCBI; D03 JERC; D07 ORNL; D08 DELA; and D19 DEJU), and it was not possible to detect a 20% change in mass for the 2-10 mm size category with power ≥80% at any sites (Figure 8).

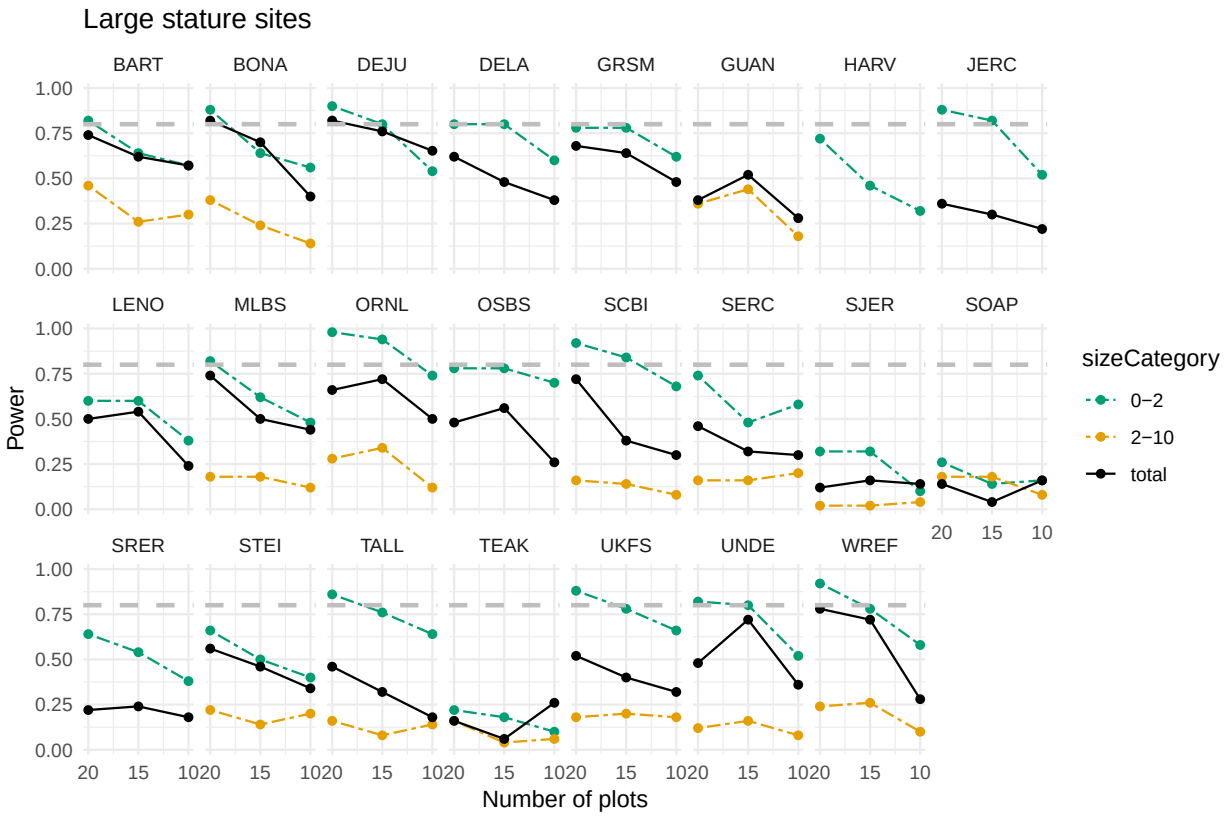

Figure 8: Power analysis of plot number reductions and the ability to detect an imposed 20% change in fine root mass at sites with large-stature vegetation.

At sites with small-stature vegetation, model convergence with an imposed `year` effect term was achieved at only 4 sites. At these sites, power to detect the imposed 20% change in fine root mass was well below 80% for all size categories and for total fine root mass, even with the current sampling design that includes 2 cores per plot (Figure 9) and 30 plots per site (Figure 10).

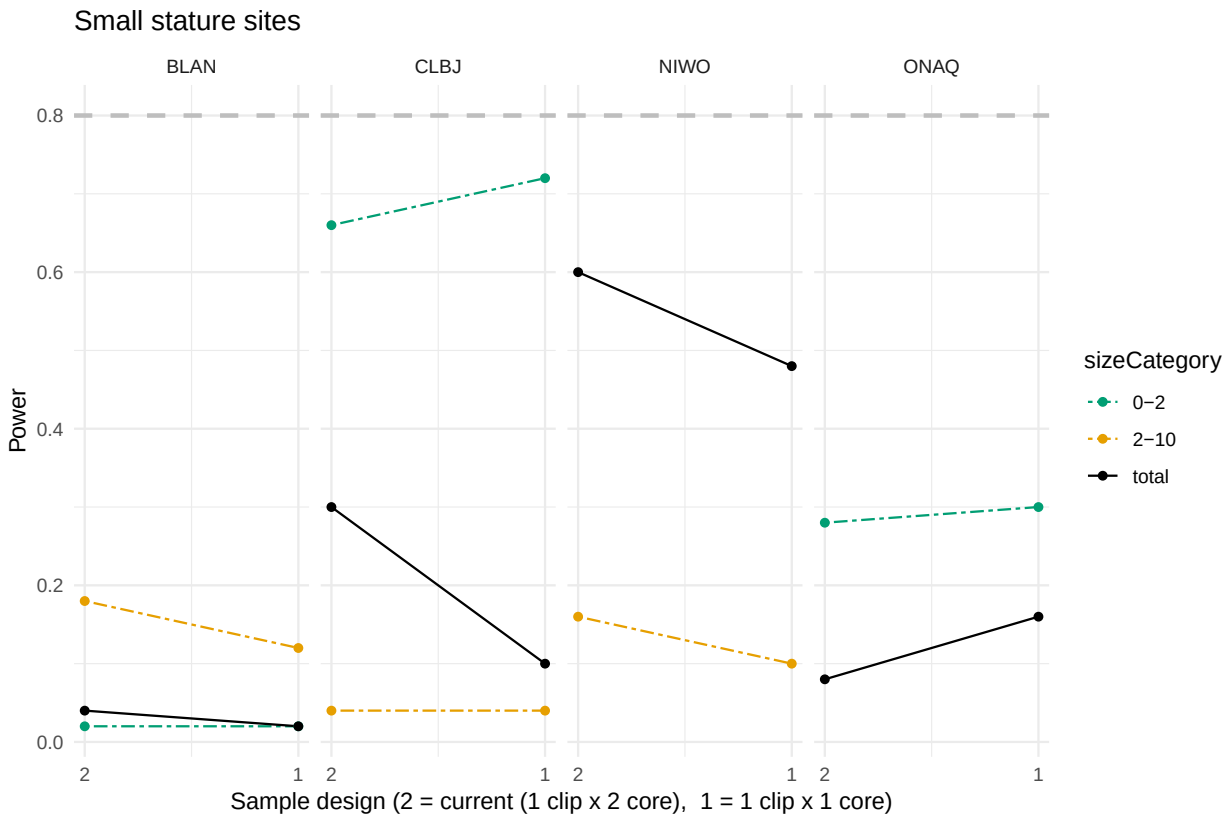

Figure 9: Power analysis of within-plot reductions in sampling effort and the ability to detect an imposed 20% change in fine root mass at sites with small-stature vegetation.

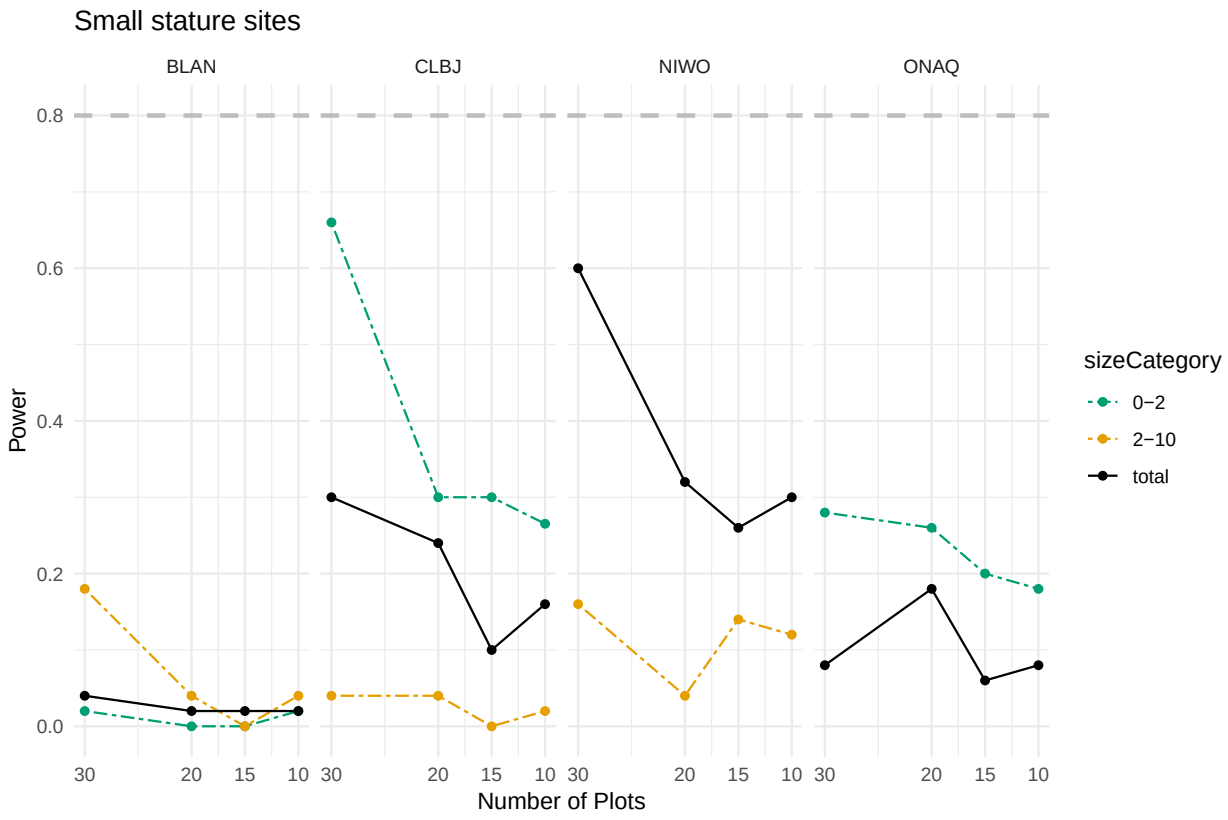

Figure 10: Power analysis of plot number reductions and the ability to detect an imposed 20% change in fine root mass at sites with small-stature vegetation.

For the small subset of sites for which we have full data from two different sampling periods, the results of this power analysis are consistent with the detection of a `year` effect in the original data (see above), increasing our confidence in the power analysis.

3 Discussion

Focusing on total fine root biomass, we found three sites with large-stature vegetation where both the site means bootstrapping analysis and the power analysis supported reducing within-plot replication from the current 2 cells per plot x 2 cores per cell design to a 2 cells per plot x 1 core per cell design (D07 ORNL; D19 BONA, DEJU). Focusing only on the 0-2 mm diameter size category, we identified 8 large-stature vegetation sites where both the site means bootstrapping analysis and the power analysis supported reducing within-plot replication from the current design to a 2 cells per plot x 1 core per cell design (D02 SCBI, SERC; D03 JERC; D06 UKFS; D07 ORNL; D16 WREF; D19 BONA, DEJU). However, reducing within-plot replication to 2 cells per plot x 1 core per cell results in an inability to detect meaningful change in the 2-10 mm diameter size category at all sites.

At sites with small-stature vegetation where the current sampling design specifies one sampling cell per

plot and two cores per sampling cell, our analyses showed that there is already insufficient power for change detection. Simulation results further confirm that reducing within-plot replication to 1 core per plot, or reducing the number of plots sampled, is not supported by the bootstrapping or simulation data.

4 Recommendations

The optimization analyses presented here indicate that the amount of variation both within plots and among plots is sufficiently high that it is only possible to reduce field sampling within-plot replication at 8 sites with large-stature vegetation. We briefly outline three recommendations:

A. If it is important to detect change in fine root biomass within 0-1 mm, 1-2 mm, and 2-10 mm diameter size categories - e.g., due to diameter-specific changes in root chemistry, turnover time, or other considerations - the bootstrapping and power analysis results do not support reductions in field sampling effort. Reducing within-plot sampling or the number of plots would result in reduced power to detect temporal change, especially within the largest 2-10 mm diameter size category.

B. If it is adequate to detect change in fine root biomass in a pooled 0-2 mm size category, as suggested by the Plant Biomass and Productivity TWG, the bootstrapping and power analysis results support reducing within-plot replication from the current design to 2 cells per plot x 1 core per cell at a subset of 8 sites with large-stature vegetation (D02 SCBI, SERC; D03 JERC; D06 UKFS; D07 ORNL; D16 WREF; D19 BONA, DEJU).

C. More conservatively, sampling can be reduced to 1 core per cell at D07 ORNL, D19 BONA, and D19 DEJU (sites with large-stature vegetation) while maintaining the ability to detect a 20% year-over-year change in total fine root biomass as well as in the pooled 0-2 mm size category.

5 Acknowledgements

We are grateful to the NEON Plant Biomass and Productivity TWG members for their critical assessment of optimization analysis goals, and their feedback regarding results, including the suggestion to pool the 0-1 mm and 1-2 mm size categories for analysis. The majority of TWG members concurred that the community is primarily interested in the ability to detect change in the collective 0-2 mm diameter size category.

6 References

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