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Sampling Soils in A Heterogeneous Research Plot

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1 **TITLE:**
2 **Sampling Soils in a Heterogeneous Research Plot**
3

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12 **KEYWORDS:**
13 Soil heterogeneity, clustered sampling, sample size requirement (SSR), sampling accuracy, soil
14 organic carbon (SOC), soil microbial biomass carbon (MBC)
15

16 **SUMMARY:**
17 The traditional soil-sampling procedure determines the number of soil samples arbitrarily. Here,
18 we provide a simple yet efficient clustered soil-sampling design to demonstrate soil spatial
19 heterogeneity and quantitatively determine the number of soil samples required and the
20 associated sampling accuracy.
21

22 **ABSTRACT:**
23 Soils are highly heterogeneous. In general, the number of soil samples required for soil research
24 has always been determined arbitrarily and the associated accuracy is unknown. Here, we
25 present a detailed protocol for efficient and clustered soil sampling in a research plot and, relying
26 on a pilot sampling using this design, for demonstrating soil spatial heterogeneity and informing
27 reasonable sample sizes and associated accuracy for future study. The protocol mainly comprises
28 four steps: sampling design, field collection, soil analysis, and geostatistical analysis. The step-by-
29 step procedure is modified according to former publications. Two examples will be presented to
30 demonstrate contrasting spatial distributions of soil organic carbon (SOC) and soil microbial
31 biomass carbon (MBC) under different management practices. In addition, we present a strategy
32 to determine the sample size requirement (SSR) given a certain level of accuracy based on the
33 plot-level coefficient of variation (CV). The field sampling protocol and the quantitative
34 determination of the sample size will assist researchers in seeking feasible sampling strategies to
35 meet research needs and resources' availability.
36

37 **INTRODUCTION:**
38 Soils are highly heterogeneous biomaterials[1,2]. Soil sampling is conducted to collect the most
39 representative samples and characterize the nutrient status of a field as accurately and
40 inexpensively as possible. Variability in a soil lies in soil spatial heterogeneity and accuracy of
41 quantification. When spatial variation in soil is not taken into consideration, typical soil sampling
42 can result in a substantial departure from the true mean value of a soil variable, even if the soil
43 analysis itself is highly accurate[3]. For a heterogeneous research plot, variability is frequently of
44 more importance than means[3]; that is, a sampling design that can accurately measure both

45 variability and mean will be preferred.
46

47 When soil spatial variation is further altered due to land management practices[4-6], it is more
48 difficult to conduct soil sampling in an accurate manner. Nevertheless, concerns also arise with
49 regard to the large variations in key soil variables (*e.g.*, SOC and MBC)[7] that are propagated to
50 cause poor constraints of key model parameters which are critical for long-term global soil model
51 projections under climate change[8-10]. As the cost of soil sampling to characterize field variability
52 is a key problem, a simple, reliable, and efficient soil sampling strategy is sought.
53

54 There are many different approaches to collecting representative soil samples in a research plot,
55 and their advantages and disadvantages are summarized in **Table 1**. In a traditional soil sampling
56 (*i.e.*, simple and random sampling), a random collection of a few to more than 10 soil samples is
57 performed in a research plot. In particular, the number of samples in a traditional soil sampling
58 design is always determined arbitrarily and the associated sampling error (*i.e.*, accuracy) remains
59 unknown.
60

61 [Place **Table 1** here]
62

63 As compared with simple and random sampling or compositing, systematic and stratified
64 sampling designs can achieve means with high accuracy along with associated variability (**Table
65 1**). However, they will require intensive soil sampling (*e.g.*, a few 100 samples). Although the
66 accuracy of, and confidence in, a soil test level increases with more soil samples collected per
67 plot[11], the requirement for a large number of soil samples is generally only applicable for a large-
68 scale study[5,11]; it is well beyond the affordability of most soil research projects conducted at the
69 scale of field plots due to constraints in resources. A sampling design is preferred to balance the
70 tradeoffs of these different methods.
71

72 A key issue for a soil sampling design is to determine the number of soil samples required and
73 the associated accuracy given the research questions and field conditions. For instance, a
74 reduction in the number of soil samples is possible in less disturbed sites while still achieving the
75 same level of precision[6], suggesting a need to explicitly quantify the spatial heterogeneity (*i.e.*,
76 nature and occurrence of soil variability) prior to soil sampling[3]. In fact, no such pilot sampling is
77 recommended in most soil sampling designs. Field scientists frequently fail to recognize the
78 importance of estimating statistical power when they design experiments.
79

80 To improve the experimental rigor in soil sampling, a simple and efficient sampling method is
81 presented in this study. The new design shall not only enable the accurate characterization of soil
82 nutrient levels and variability but also, by accounting for soil spatial heterogeneity, provide a
83 quantitative way to inform the number of soil samples and the associated sampling accuracy for
84 future research. The new soil sampling design should help researchers identify optional strategies
85 that fit their sampling and research needs. The overall goal of this method is to provide soil
86 biogeochemists and ecologists with a quantitative and manipulative approach to optimize soil
87 sampling strategies in the context of field research.
88

PROTOCOL:

1. Clustered Sampling Design in a Plot

1.1. Identify sampling zones within a research plot. Determine the number of square grids with equal length (*i.e.*, **Figure 1**; **Figure 3**). Based on the size and shape of the research plot, the target number of square grids is expected to be six to ten so that the total number of soil samples is controlled below 30 within a plot (see step 1.3).

1.2. Mark the center of each square grid (*i.e.*, centroid) and create a circular sampling area with a diameter equal to the side length of the square grid.

1.3. Stand on the centroid in the circular zone with closed eyes and throw a small stone (or another object with weight) in a random direction and distance from the centroid.

1.3.1. If the stone is dropped outside of the circular area, do it again until the first sampling location is identified.

1.4. Repeat step 1.3 until three random sampling locations are obtained in the circular zone.

1.5. Put flags on the three sampling locations and number each flag (*i.e.*, 1, 2, and 3).

1.6. Repeat steps 1.3 - 1.5 in all other circular sampling zones until all locations are determined and numbered in a sequential order (*i.e.*, 4, 5, 6, *etc.*).

2. Distance Measurements and Soil Collection in a Plot

2.1. Choose one corner point and identify it as the origin for the sampling area in the plot.

2.2. Measure horizontal and vertical distances of each flagged location relative to the origin and record them in a field notebook as *x* and *y* coordinates.

2.3. Use a soil auger to take a soil core (0 - 15 cm) from each flagged location and label the bag based on the flag number. Repeat this step until soil cores are taken at all flagged locations.

2.4. To minimize the influence of sampling (*e.g.*, trampling on plants and soil in the plot), ensure that the bags with the soil samples inside stay with their respective flag until assembling all bags in the plot at once at the end of the collection.

2.5. Transport the soil samples in coolers to the laboratory and process each soil core on the same day.

2.6. Remove roots from each core, sieve it through a 2 mm soil sieve, and thoroughly homogenize each core sample prior to any analysis.

133

134   2.7. Determine soil moisture content in each sample by oven-drying subsamples for 24 h at 105
135   °C and ground the air-dried soil subsamples to a fine powder for a total carbon (C) analysis using
136   an elemental analyzer[4]. SOC is derived based on the moisture and C contents.

137

138   2.8. Weigh fresh soil subsamples (of 10 g each) and quantify the soil MBC by chloroform
139   fumigation-K_2SO_4 extraction and potassium persulfate digestion methods[5].

140

141   2.9. Combine the SOC and MBC dataset with *x* and *y* coordinates based on the flag numbers in
142   the plot.

143

144   **3. Descriptive and Geostatistical Analyses in a Plot**

145

146   3.1. For each variable of SOC and MBC, calculate the minimum, maximum, mean, median, and
147   standard deviation, as well as the coefficient of variation (CV).

148

149   3.2. For each variable, conduct a set of geospatial analysis (*i.e.*, trend surface analysis,
150   autocorrelation, and kriging map) to depict the primary surface pattern, fine-scale variability, and
151   spatial distribution. The details of the approaches of geostatistical analyses can be found in
152   former publications[4,5].

153

154   **4. Exploration of SSR and the Associated Sampling Accuracy in a Plot**

155

156   4.1. Plot the SSR and relative error (γ) based on the CV obtained in a plot. Within each plot, the
157   log-transformed SSR and relative error (γ) have a negative linear relationship (equations 1 - 3).
158   Based on the relationship (equation 3), the number of samples required for the specified
159   accuracy can be determined:

160

161
$$\mathrm{CI} = \bar{X} \pm t_{0.975} \times \frac{s}{\sqrt{n}} \quad (1)$$

162
$$\gamma = \frac{t_{0.975} \times \frac{s}{\sqrt{N}}}{\bar{X}} = t_{0.975} \times \frac{CV}{\sqrt{N}} \quad (2)$$

163
$$\ln(\mathrm{N}) = -2 \times \ln(\gamma) + 2 \times \ln(t_{0.975} \times \mathrm{CV}) \quad (3)$$

164

165   Here, $\mathrm{CI}, \bar{X}, s, n, N, CV$, and γ denote the confidence interval, plot mean, plot standard
166   deviation, sample number, coefficient of variation, and relative error, respectively; $t_{0.975}$ = 1.96.
167   The log-transformed sample size requirement (N) has a negative linear relationship (*i.e.*, slope =
168   -2) with the log-transformed relative error (γ).

169

170   4.2. Apply the above relationship for future sampling in a plot by calculating N in equation 3

171   under a desired accuracy (*e.g.*, relative error [γ]). Or, for a given number of soil samples already

172   collected in a plot, apply the relationship to derive at the associated accuracy.

173

174   **REPRESENTATIVE RESULTS:**

175   The above approach has been employed in two case studies, one in a Southern US rural region

176   and another in Middle Tennessee.

177

178   In the rural Southern Piedmont region, three land-use types were selected, including 1)

179   uncultivated oak-hickory hardwood forests, 2) cultivated fields where conventional tillage and

180   fertilization are used annually to produce wheat, sorghum, and corn, and 3) old-field pine forests

181   that are each about 50 years in age since the last cultivation[4]. Three independently replicated 30

182   x 30 m plots were identified from the area for each land use. In each plot, a cluster soil sampling

183   design was applied (**Figure 1**). Each circular zone had a 5 m radial distance from each centroid.

184   Twenty-seven cores were collected from each of the nine plots, 81 cores per land use, and 243

185   cores in total. SOC was quantified by a CHN analyzer. The major finding was that cultivated land

186   substantially homogenizes the spatial heterogeneity of SOC and other variables[4]. The SSR differed

187   among land uses with a generally ascending order as old-field forest > regenerated pine forest >

188   cultivated cropland (**Figure 2**). Exceptions are that one hardwood forest plot had an SSR as small

189   as the cultivated plot, and one pine plot had an SSR as large as the hardwood plot (**Figure 2**).

190   Taking γ = 0.1 or 10% as an example, SSR was 4, 10, and 30 (cultivated cropland), 80, 85, and 300

191   (pine forest), and 25, 200, and 350 (hardwood). If only three soil samples were collected in all

192   plots, the relative error would have been ~10% - 30% (cultivated cropland), ~50% - 80% (pine

193   forest), and ~28% - 100% (hardwood).

194

195   [Place **Figure 1** here]

196

197   [Place **Figure 2** here]

198

199   In the Tennessee State University (TSU) Main Campus Agriculture Research and Extension Center

200   (AREC) in Nashville, TN, USA (36.12° N, 36.98° W, elevation 127.6 m) in 2011, a field switchgrass

201   experiment was established with three nitrogen (N) fertilization treatments in a randomized

202   block design[5]. The crop type is of the 'Highlander' variety of eastern 'Alamo' switchgrass (*Panicum*

203   *virgatum* L.). The three N treatments included no N fertilizer input (NN), low N fertilizer input (LN:

204   84 kg of N ha-1 in urea), and high N fertilizer input (HN: 168 kg of N ha-1 in urea). Within each

205   plot, a rectangular area of 2.75 x 5.5 m zone was identified and further divided into eight square

206   grids of 1.375 x 1.375 m. Within each circular zone, a centroid was identified, and three cores

207   were collected with a random direction and distance relative to each centroid (**Figure 3**). A total

208   of 24 cores were thus collected from each of 12 plots, yielding 288 soil cores. The MBC in each

209   core was quantified by chloroform fumigation-K2SO4 extraction and potassium persulfate

210   digestion methods. The major finding was that the N fertilization generally enhanced the spatial

211   heterogeneity of MBC in the switchgrass cropland. The SSR was generally greater with

212   fertilization (**Figure 4**). One exception is that the SSR for an HN plot was lower than that of the

213   NN plot (**Figure 4**). Taking γ = 0.1 or 10% as an example, SSR was 10 and 20 in two replicated plots

214 (NN), 30 and 50 (LN), and 15 and 70 (HN). If only three soil samples were collected in all plots,
215 the relative error would have been ~20% - 25% (NN), ~26% - 35% (LN), and ~20% - 40%
216 (hardwood).
217
218 [Place **Figure 3** here]
219
220 [Place **Figure 4** here]
221
222 **FIGURE AND TABLE LEGENDS:**
223
224 **Figure 1: An illustration of a clustered random sampling design within a 30 x 30 m research plot**
225 **at the Calhoun Experimental Forest, SC, USA[4].** The filled circles represent centroids (n = 9). The
226 large dashed circle represents the sampling area around one centroid (radius = 5 m). Xs represent
227 sample locations determined from randomly chosen directions and distances from a centroid.
228 This figure has been modified from Li *et al.*[4].
229
230 **Figure 2: Plot of the sample size requirement (SSR) and relative error (γ) for SOC of hardwood**
231 **forest, pine forest, and cultivated cropland.** The log scale was applied on both axes. The dotted
232 lines represent cultivated soils, the grey lines pine-forest soils, and the dark lines hardwood forest
233 soils. Three different lines for each land use correspond to three replicate plots. This figure has
234 been modified from Li *et al.*[4]
235
236 **Figure 3: Illustration of a clustered random sampling design within a 2.75 x 5.5 m plot in a**
237 **fertilization experimental site at the Tennessee State University (TSU) Agricultural Research**
238 **Center in Nashville, TN, USA.** The filled circles represent centroids (n = 8) and each plot consisted
239 of eight centroids in each square grid (of 1.375 x 1.375 m). In each subplot, a circular area was
240 determined for soil sampling. Xs represent sample locations determined from random directions
241 and distances from a centroid within each circular sampling area (dashed circle). This figure has
242 been modified from Li *et al.*[5]
243
244 **Figure 4: Plot of the sample size requirement (SSR) and relative error (γ) for MBC under three**
245 **fertilization treatments.** The log scale was applied on both axes. The dotted lines represent
246 cultivated soils, the grey lines pine-forest soils, and the dark lines hardwood forest soils. NN = no
247 N fertilizer input; LN = low N fertilizer input; and HN = high N fertilizer input. Two different lines
248 for each land use correspond to two replicate plots. This figure has been modified from Li *et al.*[5].
249
250 **Table 1: The advantages and disadvantages of major soil sampling designs adopted in the soil**
251 **research community.** The table has been summarized from Tan *et al.*[3], Jones[12], and Swenson *et*
252 *al.*[11]
253
254 **DISCUSSION:**
255 The traditional soil sampling method lacked a quantitative basis and led to unknown accuracy,
256 whereas the more advanced sampling strategies involved intensive soil collections and induced
257 unaffordable costs for most soil research at the field plot scale. A simple, efficient, and reliable

258   sampling design should be a useful tool to balance both aforementioned methods and, more
259   importantly, inform a quantitative way to determine the number required under certain accuracy
260   for the sake of future sampling needs. However, such a sampling design is still missing. Here, a
261   method for manipulating a clustered sampling procedure to quantify soil spatial heterogeneity
262   was presented and, relying on this design, to inform the number of soil samples required for
263   future sampling under specific accuracy. There are two critical steps within the protocol. The first
264   is to determine the sampling area and identify the sampling zone in a given plot area. Because
265   the dimension and shape of a specific research plot can vary from one study to another, the
266   number and length of the square grid representing the sampling zone should be modified to best
267   fit the plot characteristics and cover the plot area as much as possible. In general, the number of
268   square grids should be limited to eight to ten so that 24 - 30 soil samples will be collected in a
269   given plot. This less intensive sampling requirement is acceptable for a pilot study in a plot. The
270   second critical step is to determine the sample number required under specific accuracy.
271   Although the number of soil samples under a desired accuracy can be derived at based on the
272   pilot sampling strategy, other available resources need to be accounted for (*e.g.*, labor, cost, and
273   personnel). If the number of soil samples required for a desired accuracy exceeds the affordability,
274   the desired accuracy should be lowered so that the number of soil samples can be recalculated.
275   The recalculations should be repeated until the best fit is achieved to balance the desired
276   accuracy and the available resources.

278   The protocol can be easily modified to fit the specific shape, area, and location of a research plot.
279   Even within an irregular plot or a very large or small plot area, the procedure can be performed
280   by controlling the size of the square grid to cover most of the plot area. On the other hand, when
281   soil samples are collected beyond the circular sampling zone in the plot, they can be still
282   accounted for in the descriptive and geostatistical analysis. The flexibility of the protocol in this
283   regard is advantageous as it can, thus, reduce the cost of sampling.

285   An important limitation of this method is that the number of soil samples required for certain
286   accuracy will depend upon the plot level CV determined by a group of 24 - 30 soil samples in the
287   pilot soil sampling. For a highly heterogeneous plot, 30 samples or less can produce a larger CV
288   than that based on a greater number of samples (> 30). As a result, the number of soil samples
289   calculated with the same accuracy will be larger. That is, the number of soil samples required for
290   the same accuracy will be overestimated in the plot. For a highly homogenous plot, a smaller
291   number of samples will produce a plot level CV similar to 30 samples, thus, resulting in an
292   overestimation of the resource need. Therefore, for these extremely heterogeneous or
293   homogeneous plots, the soil sample number (*i.e.*, 30 or less) proposed in the pilot sampling
294   design may cause unnecessary investment either in the pilot sampling stage or in future sampling.

296   We demonstrate significant advantages of the clustered soil sampling strategy. It provides a
297   reliable and affordable soil sampling strategy to obtain soil spatial heterogeneity and offers a
298   quantitative way to derive the number of soil samples required for a certain desired accuracy.
299   Although the intensive strip or stratified sampling may provide a better description of spatial
300   variation, the cost of conducting such sampling is too high for most soil studies. The traditional
301   sampling is arbitrary and lacks any quantitative basis for sampling accuracy. The current protocol

302 is superior due to its less intensive sampling requirement, ease in operating it in the field, power
303 to reveal spatial patterns using rigorous geostatistical analysis methods, and capacity to
304 quantitatively determine the sample size given any desired accuracy. The knowledge of the
305 sample size required for a specific sampling accuracy will allow researchers to strategize their
306 investment in soil sampling efforts.
307
308 Employing the efficient clustered sampling procedure allows rigorous testing of soil spatial
309 heterogeneity and improves scientists' capacity to conduct soil sampling with accuracy. The less
310 intensive and quantitative nature of the soil sampling strategy will enable its wide application in
311 soil research communities. Given the likely altered soil spatial heterogeneity under rapid global
312 changes, the soil sample requirement for the same sampling accuracy in a research plot may vary
313 over time. The proposed sample number in the pilot sampling design may vary with different
314 soils and ecosystems. Future applications that could emerge from this work include determining
315 the sample number for specific soils or ecosystems. Thus, further empirical work is needed on
316 the application and identification of the method in various soils and ecosystems. Long-term and
317 wide applications may help identify a generic sample size requirement for specific ecosystems,
318 which can be recommended for soil researchers.
319

327 **DISCLOSURES:**
328 The author has nothing to disclose.
329

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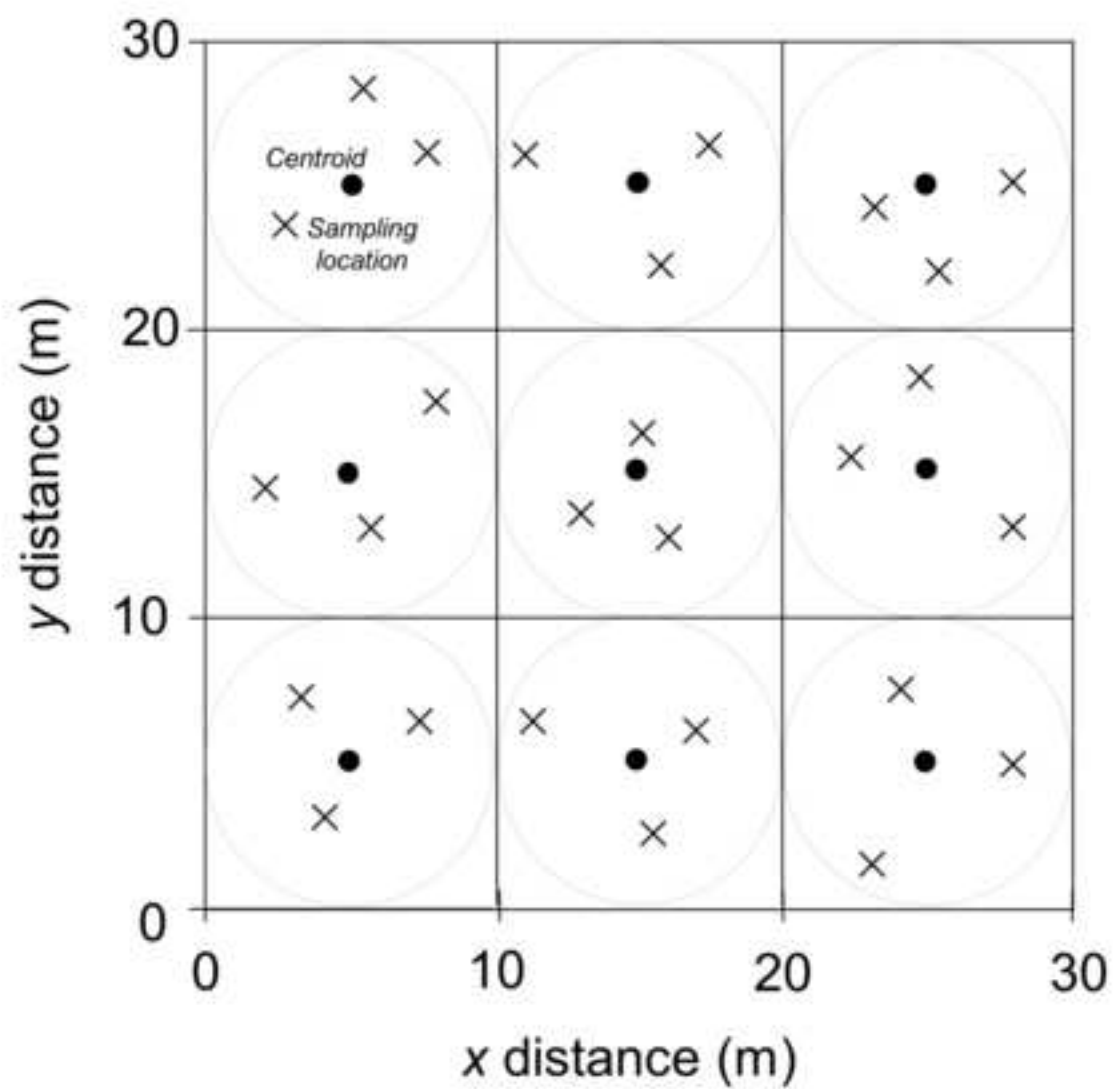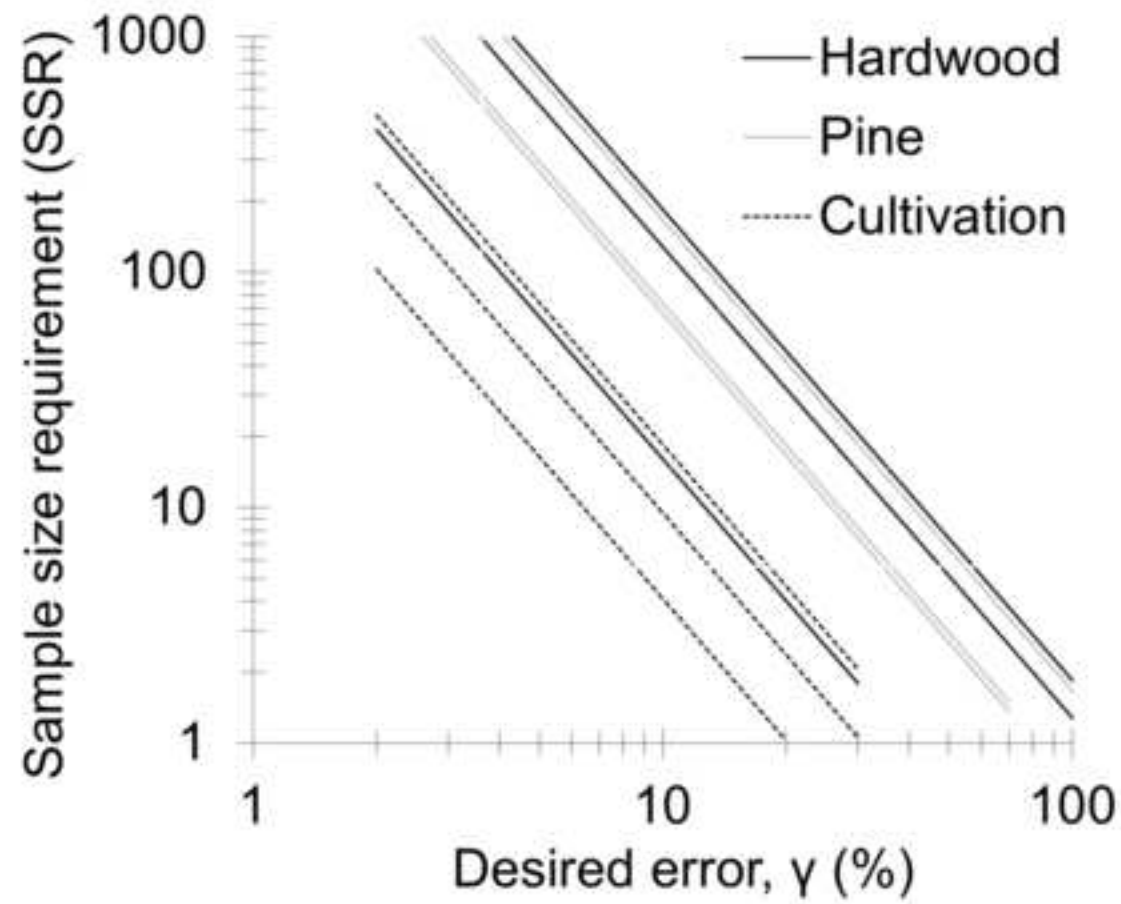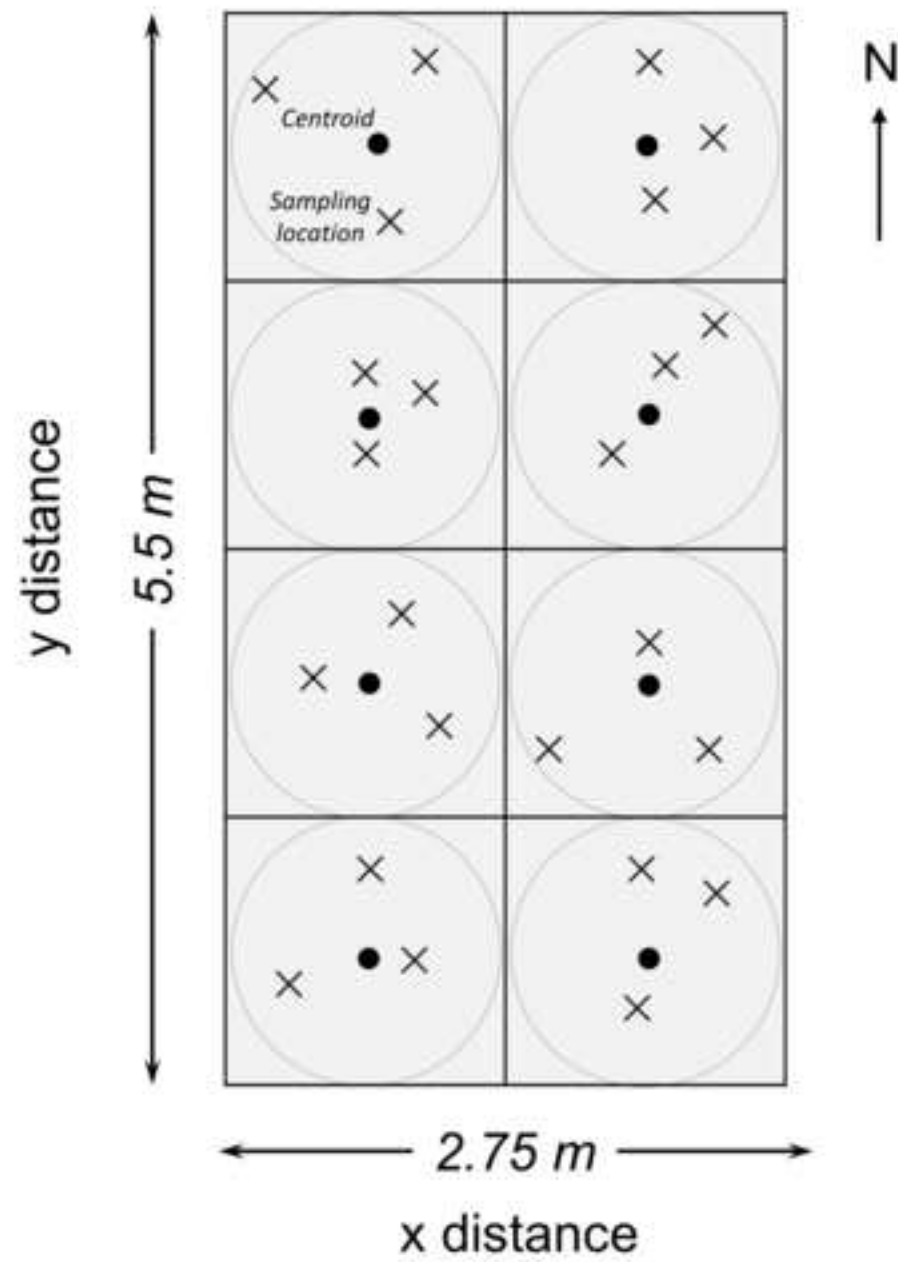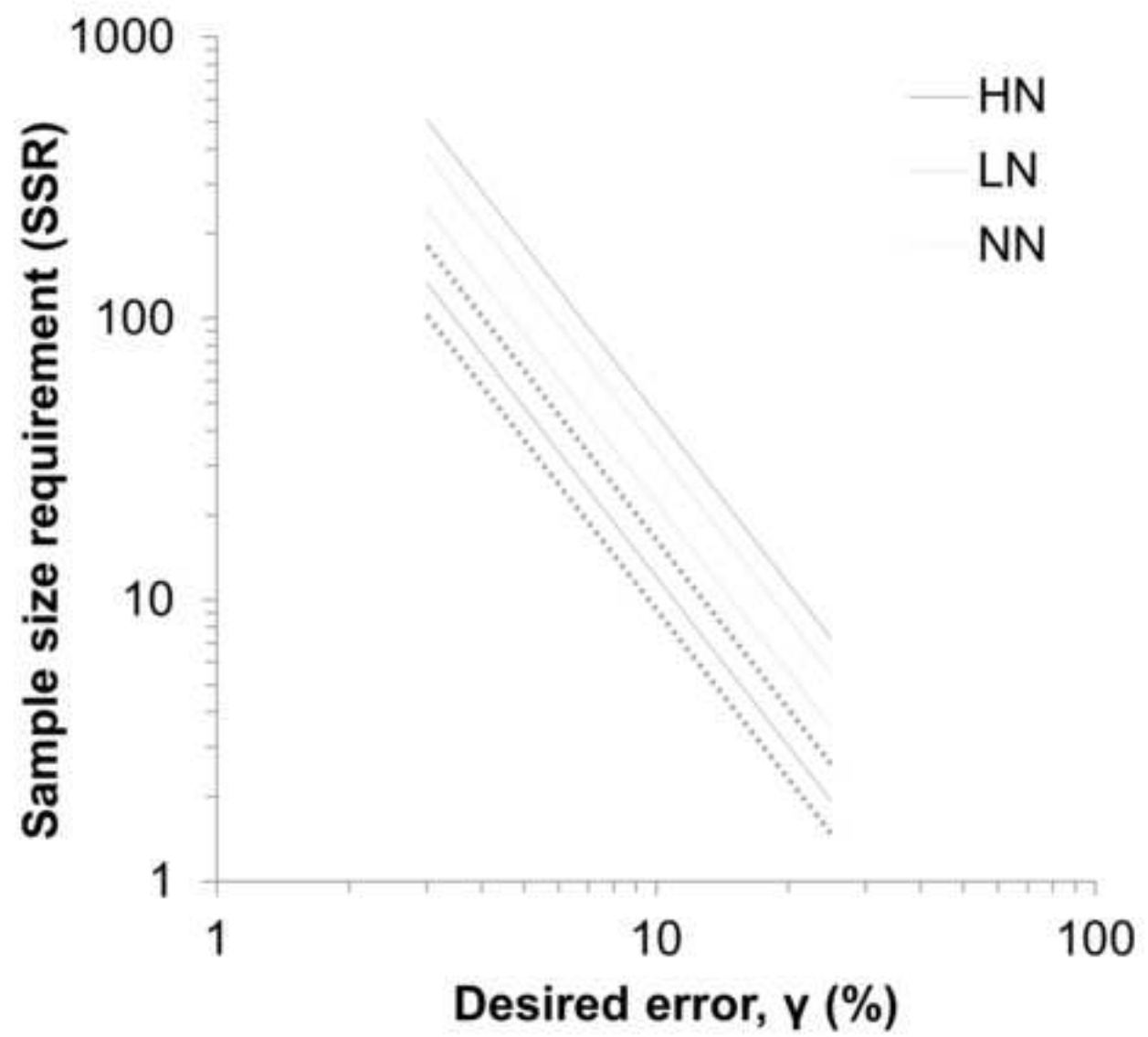368 FL (2001).

369

Figure 1

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Figure 1

Figure 2

Figure 2

Figure 3

Figure 4

Table 1

Sampling design	Advantage
Simple and random sampling	Cost effective, quick and inexpensive, widely adopted, easy operation, optimal in homogenous site
Systematic sampling	High accuracy and known variation, optimal in large scale heterogeneous site
Stratified sampling	Accurate mean estimate, relatively easy operation, optimal for clustered and stratified region
Compositing	Cost effective, accurate mean estimate, easy operation, optimal in heterogeneous site

Disadvantage
Low accuracy and high variation, <5 samples
Cost ineffective, large sample number
Cost ineffective, large sample number (usually less than systematic/grid sampling)
Unknown field variation, >3 samples for composite

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Soil auger	AMS Fisher	350.05	For soil collection
Screwdriver	Scientific Fisher	19-313-447	For soil collection
Rope	Scientific Home	19-313-429	For delineating sampling zone
FatMax 35 ft. Tape Measure	Depot Fisher	#215880	For measuring distances
Marking flag	Scientific Fisher	S99537	For marking sampling locations
Plastic Zipper Seal Storage Bag	Scientific Fisher	09-800-16	For soil collection
Sharpie	Scientific Fisher	50-111-3135	For soil collection
Marking pencil	Scientific Fisher	50-294-45	For recording data in field
Lab notebook	Scientific	11-903	For recording data in field
ArcGis 10.3	ESRI Fisher		For producing kriging map
Sieve	Scientific	04-881G	For sieving soil sample

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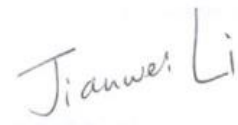Environmental Sciences

September 2, 2018

Dear Dr. Steindel,

   Thank you kindly for the reviews. They were very helpful and have guided me in improving the quality of the paper. I responded to each of the comments in the following page.

   I appreciate your efforts on this manuscript, and I hope that you will find out my revisions satisfactory.

Best regards,

Jianwei Li

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Your manuscript, JoVE58519R1 "Experimental Protocol for Sampling Soils in A Heterogeneous Research Plot," has been editorially reviewed and the following comments need to be addressed. Please track the changes to identify all of the manuscript edits. After revising the submission, please also upload a separate document that addresses each of the editorial comments individually with the revised manuscript.

[Response] The track changes were identified in the revised manuscript and a separate response to editor was attached as well.

Your revision is due by **Sep 06, 2018**.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision".

Best,

Phillip Steindel, Ph.D.
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Editorial comments:

1. It is still difficult to understand the manuscript in places (see notes in text for examples); please proofread further.
[Response] The proofread was conducted by a colleague who is a native English speaker. Clarification and revision were made based on your notes. I hope the revision is satisfactory.

2. Generally, the soil sampling and analysis in step 2 could use more detail. How exactly are analyses done? How exactly should they be organized "based on the flag number and the associated horizontal and vertical distances"? How exactly will you minimize the pilot sampling's influence on future sampling? References to previous work could be used here.
[Response] More details were added in step 2 and I hope your concerns were addressed adequately. One more required equipment (i.e., soil sieve) was added to the Material List.

3. In Figures 2 and 4, why are there multiple lines for each land use type-do they correspond to different replicates? This is unclear.
[Response] Yes, they are replicated plots for each land use. This was clarified in the legends of Figures 2 and 4.

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