Vegetation Sampling Concepts for Compensatory Mitigation Sites

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INTRODUCTION

In 2018, the Norfolk District of the U.S. Army Corps of Engineers (USACE) and the Virginia Department of Environmental Quality (DEQ), in their roles as co-chairs of Virginia's Interagency Review Team (IRT), proposed modifications to the Virginia Mitigation Banking Instrument (MBI) Template (Template). The Template is a document used to establish compensatory wetland and stream mitigation banks in the state, serving as a planning-level tool with minimum standards and design criteria for that purpose. Prospective mitigation banks are approved via IRT ratification of an acceptable MBI, which provides the necessary documentation for the "…establishment, use, operation, and management…" of mitigation banks in Virginia (USACE and DEQ 2018). At each stage in this progression, a bank must meet performance standards for the IRT to approve the release of bank credits, and ultimately for those credits to be used as compensatory mitigation under Section 404 of the Clean Water Act and the Virginia Water Protection Permit Program. The 2018 Template revisions were adopted by the IRT and are available on the USACE Regulatory In-lieu Fee and Bank Information Tracking System (RIBITS; USACE and DEQ 2018).

The overall condition of the vegetation community is an important component of performance standards, and during the 2018 Template review the IRT was considering revisions that would clarify vegetation sampling protocols and encourage the use of specific types of vegetation data in demonstrating ecological performance. The revisions were considered necessary because previous versions of the Template (see USACE and DEQ 2010, 2017) included sampling recommendations that were ambiguous and/or inconsistent with ecological sampling theory (see discussion below). Revisions were also timely because recent research on vegetation development in compensatory wetland mitigation revealed alternative sampling strategies that are more aligned with measurements of wetland function (e.g., stem area at groundline; see Hudson and Perry 2018).

The purpose of this project was to review the scientific literature on vegetation sampling and provide background information on sampling protocols to be incorporated into the 2018 Template revisions. The information provided here was used, in part, to supplement Exhibit J (Monitoring and Reporting Requirements) of the 2018 Template (USACE and DEQ 2018).

PURPOSE OF SAMPLING

One of the most importance considerations in vegetation sampling is to define what is meant by the term "sample". For most scientific measurements of vegetation communities, a **sample** is defined as a collection of **sample units** (SU), the latter of which can be defined as discrete portions of an aggregate (i.e., community) from which repeatable observations can be made (Pielou 1984, Ludwig and Reynolds 1988, Krebs 1999). **Sampling** is therefore defined as the collection and analysis of data from SUs to make informed assumptions about the overall community (Ludwig and Reynolds 1988). This definition of sampling distinguishes it from an ecological **census**, which is defined as the counting of *all* individuals belonging to a group of interest within an area (Henderson and Southwood 2016). In complex ecosystems, ecological census would be nearly impossible and certainly cost-prohibitive; therefore, sampling is seen as an optimal approach to data collection in most ecological studies.

Given the above discussion, it can be said that the purpose of sampling vegetation communities is to develop summary data about the sample based on statistics calculated from measurements or observations of the SUs (e.g., "central-tendency" statistics like arithmetic mean, etc.). Although these summary data represent the sample, they are assumed to also be representative of the overall community *as long as certain assumptions of ecological sampling theory are upheld*. The most important of these are listed below (Krebs 1999):

The purpose of sampling…is to develop summary data about the sample based on statistics calculated from measurements or observations of SUs (e.g., plots). If the data are collected in accordance with ecological sampling theory, the summary statistics for the sample are assumed to be representative of the overall community.

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1. All SUs should have an equal chance of being selected.
2. The sample (collection of SUs) should not cross community boundaries (i.e., the sample should be taken from a relatively homogeneous cover type).
3. Sample adequacy should be demonstrated (see discussion on Sample Adequacy: Species-Area Curve below).

If the above assumptions are met, a sample (and its associated statistical derivations) can be said to represent the underlying community with respect to the measurements or observations collected in the field. Vegetation sampling strategies on compensatory mitigation sites are conformable to the above criteria as long as locations of SUs are randomized (see Sampling Strategy: Random vs. Systematic below), the site is "stratified" (i.e., divided) by planting zone or community type with respect to sample area (see Stratified Random Sampling below), and sample adequacy is evaluated via the species-area relationship or equivalent technique (see Sample Adequacy: Species-Area Curve below).

A COMMON MISTAKE: "SAMPLE" VS. "PLOT" PERFORMANCE

In vegetation community analysis, the typical sample unit is a **plot**, which can be defined as any two-dimensional area of any size or shape (e.g., quadrats, rectangular plots, circular plots, belt transects, etc.; Mueller-Dombois and Ellenberg 1974, Gage and Cooper 2010). When plots are arrayed on a site in observance of the above assumptions (independent SUs, homogeneous sample area, and sample adequacy), then any central-tendency data calculated to summarize the sample would be considered an adequate representation of the overall community. In the case of compensatory mitigation, if data such as stem density or overall cover (for example) are collected and presented in this manner, then the average values for those metrics should be taken as representative of the overall community. Therefore, if a performance standard has been developed for these measurements, then it should be evaluated on a mitigation site using the *average value calculated from the SUs* rather than on a plot-by-plot basis. This is because a plot is a SU, which *by definition* should only be considered as a discrete portion of an aggregate sample (recall that this is the purpose of sampling). As long as proper ecological sampling techniques are observed, then it is the *summary statistics for the sample* and not for the individual plots that should be analyzed against performance standards.

Assuming an appropriate ecological sampling strategy was used to study plants on a compensatory mitigation site, it is a mistake to pronounce the entire site – or even a portion of it – as failing to meet a performance standard based solely on the results of one plot.

To illustrate with an example: A common vegetation performance standard for forested wetland mitigation sites is to achieve a minimum of 400 stems per acre (a density measurement that can easily be evaluated using plots). Let's assume that an appropriate ecological sampling strategy was employed to measure stem density across a forested planting zone within a mitigation site (i.e., plots were randomized, sample area was homogeneous, and sample size was adequate). If the average overall stem density for the sample was greater than 400 stems per acre (performance standard met), but one of the plots *by itself* didn't meet the density requirement, *then it is a mistake to pronounce the entire site – or even a portion of it – as failing to meet the performance standard*. An individual plot (SU) should not be used or evaluated in this manner.

SAMPLING STRATEGY: RANDOM VS. SYSTEMATIC

There are many different vegetation sampling strategies that have been developed over the past century for different purposes (for a comprehensive review, see Mueller-Dombois and Ellenberg 1974, Krebs 1999). Most sampling strategies may be divided along two primary lines: 1) random sampling and 2) systematic sampling. **Random sampling** is typically carried out by using plot locations determined from a randomization procedure such as a random numbers generator to assign X,Y coordinates or random distances along a transect. **Systematic sampling** is based on a layout of plots that are evenly spaced along a transect or grid across the site (Henderson and Southwood 2016). Random sampling has the benefit of satisfying the first condition of ecological sampling theory above, namely, that all SUs (plots) have an equal chance of being selected. Also, because randomization minimizes sampling bias in plot selection, statistical error terms may be assigned to mean values of the sample (Mueller-Dombois and Ellenberg 1974). By contrast, systematic sampling may be appropriate in situations where organismal distribution across an environmental gradient is of interest, or where mapping the exact locations of community changes across a landscape is important; however, common parametric statistical analyses cannot be completed on systematic sampling data without some form of data transformation (Henderson and Southwood 2016). This is due to the fact that the systematically collected SUs are not independent and are therefore subject to sampling bias.

For most compensatory mitigation sites, a systematic sampling strategy would result in *oversampling* the vegetative community. This occurs because the number of plots is predetermined based on the sampling grid, and plots are sampled irrespective of the relative distribution of species across the site. In effect, oversampling results in a situation where *rare species are mathematically reduced to zero* (or nearly so), because plots are indiscriminately added based on their location along the sampling grid rather than the overall relative distribution of species within the community (McCune and Grace 2002). In addition, oversampling often creates the problem of reducing variance to the point that even minuscule differences can generate very small p-values when comparing group means statistically (e.g., Analysis of Variance, ANOVA), which could lead an observer to determine that those differences are statistically significant and mistakenly conclude that they are biologically relevant (Steel et al. 2013). Oversampling may also be undesirable in applied ecology studies because of the additional time, effort, and expense required to collect the data (Henderson and Southwood 2016). For these reasons, oversampling is every bit as problematic as *undersampling* (or sampling too few SUs), in which case rare species in the sample become mathematically much larger than they should be or are missed altogether, or variances are too large to detect biologically relevant differences. Note that requiring a categorical plot density (such as 3 plots per acre) without sample adequacy analysis could result in either scenario but would most likely result in oversampling of the vegetative community.

STRATIFIED RANDOM SAMPLING

A **stratified random sampling** design is one in which the study area is divided into a number of non-overlapping subdivisions (or strata[2]) and samples are randomly selected from each subdivision (Gage and Cooper 2010, Manly 2015, Henderson and Southwood 2016). The benefit of this approach is that investigators are able to sample the plant community in a non-biased manner (due to the randomization component) while also ensuring that the sampling effort adequately covers the entire study site (due to the stratification component) (Mueller-Dombois and Ellenberg 1974, Tiner 2017, Henderson and Southwood 2016). In most circumstances involving ecological sampling, a stratified random approach represents the single most powerful sampling design that ecologists can adopt in the field to represent the overall community and, as Krebs (1999) states: "Stratified [random] sampling is almost always more precise than simple random sampling, and every ecologist should use it whenever possible." An example of a stratified random plot layout is provided in Figure 1.

> ***"Stratified random sampling is almost always more precise than simple random sampling, and every ecologist should use it whenever possible." (Krebs 1999)***

In most applications, community stratification occurs in two steps: 1) stratification by community type; and, 2) stratification within homogeneous cover types. This approach works well on compensatory mitigation sites because most vegetation "zones" correspond to planting zones or proposed habitat types in the mitigation design for the site.

SAMPLE ADEQUACY: SPECIES-AREA CURVE

Typically, a stratified random sampling approach is coupled with a sample adequacy determination. Sample adequacy is most frequently evaluated using the species-area relationship (Scheiner 2003), though other methods can be used (e.g., standard error ≤ 10% of the mean, McCune and Grace 2002). In species-area analyses, the cumulative total number of species is tracked as plots are sampled, and researchers develop a graph with cumulative species richness (total number of species) on the Y-axis and cumulative area sampled on the X-axis (which can be approximated by cumulative number of plots). The curve generated by this approach is an example of a "species-area curve," and it is considered to be stabilized when the curve flattens out toward the top right-hand side (as if to approach an upper asymptote). In practice, the inflection point of the curve is used to approximate a minimum adequate sample size for vegetation research (McCune and Grace 2002). During sampling, scientists create a species-area curve after the initial sampling effort (the initial number of plots can be estimated from the literature; see How Many Plots? below). By entering cumulative species richness and plot number into a simple graphing program (Excel, etc.), a species-area curve can be generated "on the fly" as a simple scatterplot/trendline graph and interpreted in the field, and scientists can add plots as necessary until the curve stabilizes. An example of a species-area curve generated for data collected from the above sample site is shown in Figure 2.

10% Effort Line: In general, the species-area curve can be inspected using a simple trendline function available in most graphing programs (e.g., Excel). A rule of thumb is to determine the "10% effort line", which is a line tangent to the curve and parallel to a line drawn from the origin of the graph to the outermost sample point. This is referred to as the 10% effort line because it is the exact point along the species-area curve where a 10% increase in effort only yields a 10% increase in species richness, and any additional sampling along the curve to the right of this point results in fewer and fewer new species encountered (Mueller-Dombois and Ellenberg 1974). In practice, the point of the

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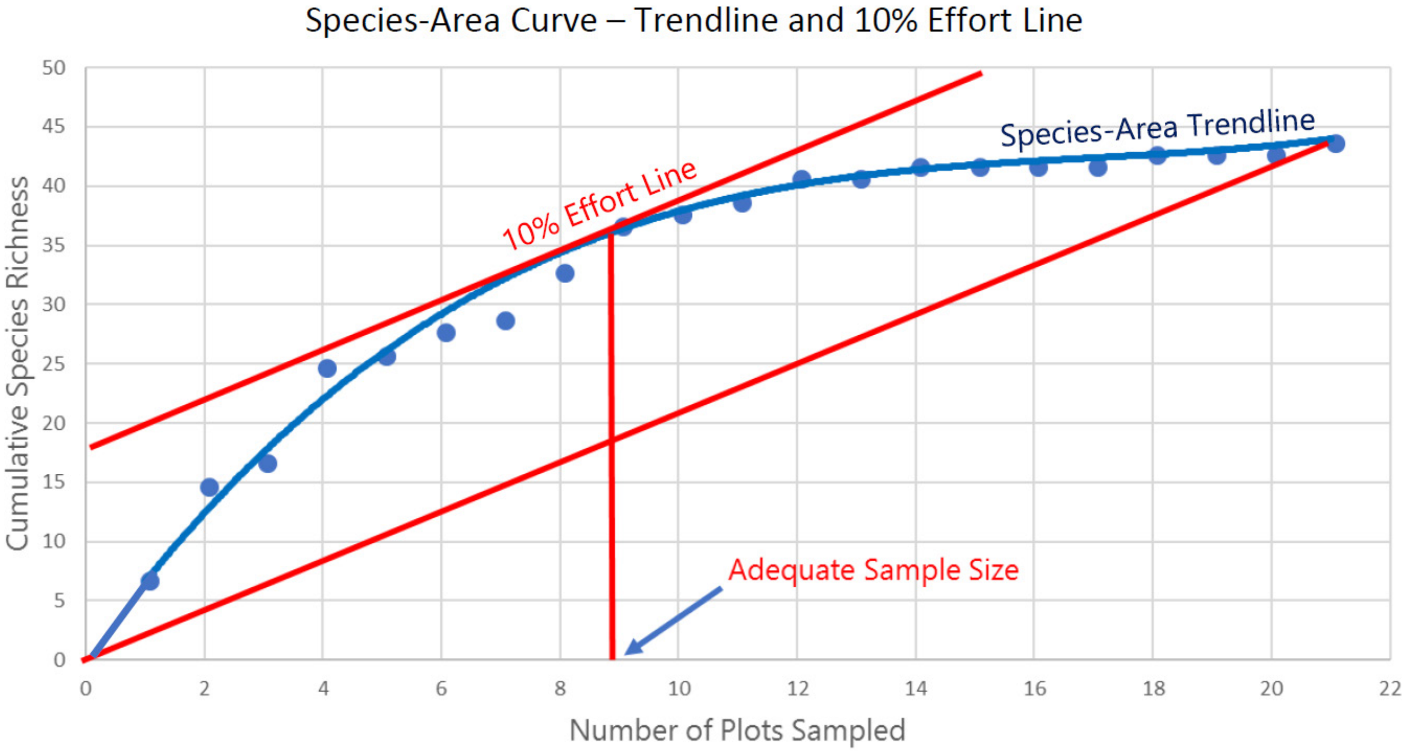
[2] The use of the term "strata" in the literature to describe the subdivisions arrived at when using this approach is somewhat unfortunate given the frequency with which that same term is used to refer to vegetation layers (e.g., tree, sapling, shrub, and herbaceous "strata"). For the purposes of clarity, when stratified random sampling is being used or described, it is recommended that investigators adopt the term "layer" when referring strictly to vegetation layers (in lieu of "stratum" or "strata").

10% effort line tangent to the curve is projected down to the X-axis and the corresponding plot number is considered to be an adequate minimum number of SUs (or minimum area) for the sample. In the example provided in Figure 2, this would coincide with approximately 9 total SUs (vegetation plots). It is important to note that for the purposes of monitoring vegetation in compensatory mitigation sites, it is *not necessary to discard any data* from vegetation plots that have been sampled in excess of the minimum adequate sample size. The goal of the species-area analysis is simply to demonstrate that a minimum adequate sample has been achieved with the sampling effort.

Basic Graph: Although the graph overlay features such as the trendline and 10% effort line provide researchers with a benchmark upon which to judge sample adequacy, for most applications researchers can simply plot

FIGURE 1: Example of a stratified random sampling design on a 3-acre restoration site (VHB, Inc.; used with permission). The study area is delimited by the red boundary line, and a baseline along the northern property boundary was established and subdivided into six equal "strata", each 140 feet in width. In this example, a single transect was positioned perpendicular to the baseline in each section, originating at a randomly-selected point (determined using a random numbers generator with minimum and maximum values set at 1 and 140, respectively). Plots were then randomly placed on each transect using the same randomization procedure described above but taking the overall transect length as the maximum value for the random numbers generator. In this manner, three plots were established on longer transects and one on the shortest transect. (T = transect; PS = photostation).

FIGURE 2: Species-area curve generated for samples collected on the site shown in Figure 1. See text for explanation of trendline, 10% effort line, and adequate sample size determination.



an X-Y scatterplot or line plot with markers in Excel (or equivalent graphing application) and interpret the graph as shown in Figure 3. The species-area relationship is clearly demonstrated without the need for either overlay. The important aspect of this process is to demonstrate the "flattening out" of the curve to the right (i.e., the sample is adequate as fewer and fewer new species are encountered with additional sampling effort).

If the Curve Doesn't Stabilize: On sites with high species richness, it is possible that the species-area curve will not flatten out to the right after completing the minimum number of sample plots (see How Many Plots? below). When this occurs, one plot should be added to each stratum (transect) using the stratified random approach describe above. Once these plots have been sampled, species-area data from the additional plots should then be added to the curve and inspected to determine if an adequate sample size has been achieved. Using the example in Figure 1, if the initial curve did not produce an adequate sample, six additional plots (one per transect) would be added to the sampling effort, any additional species encountered would be added to the cumulative species richness total and included on the species-area graph, and the curve would be re-evaluated. This iterative process may be repeated until the curve levels off.

"Stairstep" Curves: In other cases, the species-area curve may produce a "stairstep" pattern such as the one show in Figure 4. A stairstep pattern typically means that the species-area phenomenon has been tracked across community boundaries (Scheiner 2003), thereby violating sampling rule #2 above ("The sample should not cross community boundaries"). When this occurs, researchers should re-stratify the site into discrete, homogeneous cover types and re-sample using the stratified random approach described above. In most cases, plots already sampled may be retained in the data sets for the remapped community types.

PLOT SIZE AND SHAPE

A review of the literature on vegetation sampling methods reveals one seemingly eternal truth: there is no such thing as a "standard" plot size or shape (Mueller-Dombois and Ellenberg 1974, Krebs 1999). Although this topic has been assessed by recent authors (Kenkel and Podani 1991, Chytrý and Otýpková 2003, Lichvar and Gillrich 2014), the seminal and most instructive work on plot size and shape in ecological sampling was completed in the early- to mid-20th Century by authors like Clapham (1932), Oosting (1942, 1948), Bormann (1953), and Greig-Smith (1957). Their work, and that of other ecologists, plant biologists, agricultural scientists, and foresters, was focused on the goal of *optimization* in plot-based plant community sampling.

FIGURE 3: The same species-area curve plotted in Figure 2 but based on a simple line graph with markers created in Excel. This graph is easily interpreted as leveling off in the upper half, suggesting that a sample size of 9-11 plots represents the minimum adequate number of SUs for this site (corresponding to the inflection point on the graph shown by the red circle).

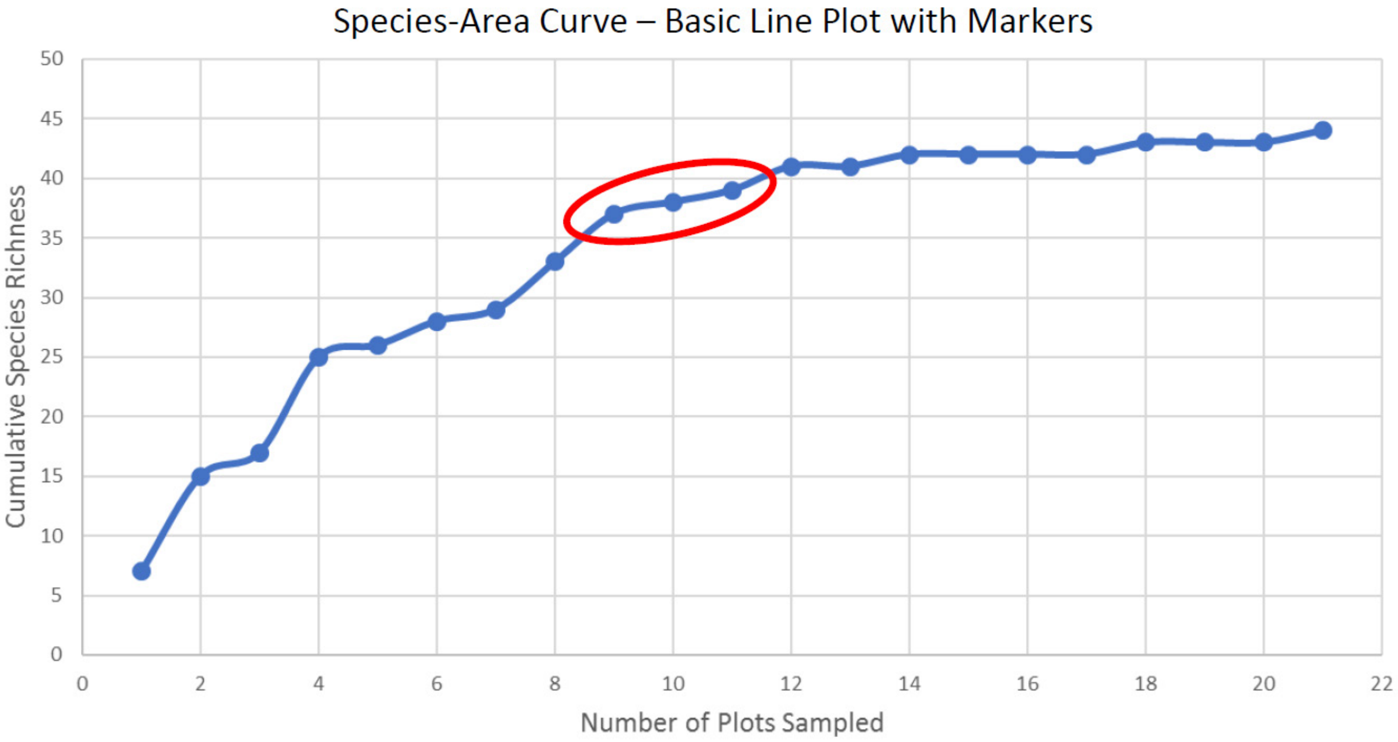
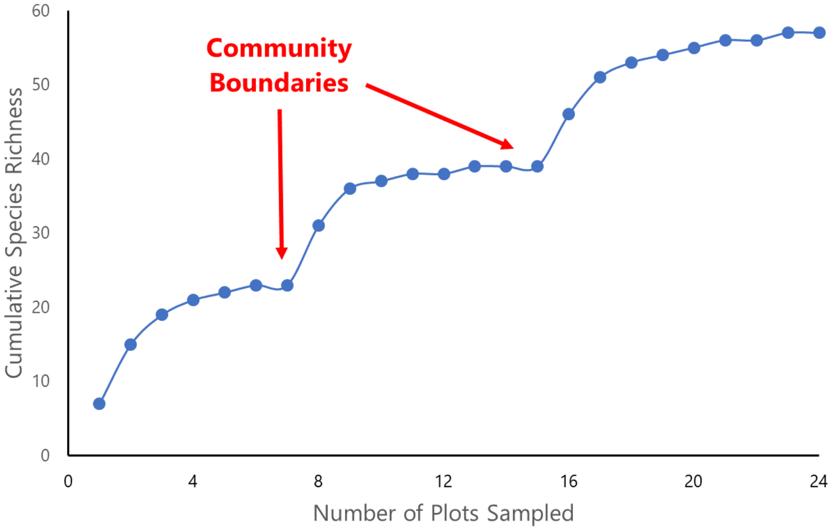


FIGURE 4: "Stairstep" species-area curve, indicating that the species-area phenomenon has been tracked across community boundaries.



Optimizing Plot Shape: Empirical research on plot shape suggests that sampling efficiency is maximized by using elongated rectangles as sampling plots (Bormann 1953). The rationale for this is that elongated rectangles are more likely to be oriented across "within-community" variation due to environmental changes such as microtopographic relief, soil heterogeneity, or moisture variability. By contrast, circular or square plots are less likely to capture within-community variability; therefore, the sample variance for circles or squares is likely to be higher than elongated rectangles (Clapham 1932). The lower sample variance means that rectangles provide more information per unit area than comparably sized circles or squares. However, there are two major drawbacks to using elongated rectangles: 1) rectangles are slower and more difficult to lay out; and, 2) there is a higher potential for "edge decisions" (i.e., deciding what gets counted in or excluded from the plot) (McCune and Grace 2002). The longer and narrower a rectangular plot is, the greater its periphery in comparison to a circular or square plot of the same area. This results in a greater possibility for making cumulative edge errors with rectangles (Bormann 1953, Krebs 1999).

In terms of sampling efficiency for woody species (trees and shrubs/saplings), *circular plots* are easiest to lay out in the field (only one reference point is needed at the center), and circles minimize the number of edge decisions because they have the lowest perimeter-to-area ratio. In an effort to strike a balance between optimization for reduced sample variance and optimization for field efficiency (as well as other considerations such as cost), it seems prudent to sample woody vegetation on compensatory mitigation sites with *circular plots* and to address community variation with sample adequacy. In other words, provided that the sample can be demonstrated as adequate based on the species-area relationship (see discussion above), within-community variability should be adequately addressed using circular plots (SU-level variance notwithstanding).

Recommended Plot Sizes and Shapes

Woody – 18.5 ft (5.6 m) radius circular plot = 100 m2 (1076 ft2)

Herbaceous – 3.3x3.3 ft (1x1 m) square sampling frame

For herbaceous sampling on compensatory mitigation sites, *standard square sampling frames* are easy to construct and use, and the square dimension offers a compromise between the variance and perimeter effects noted above.

Plot Size – Woody Sampling: The "minimum area" concept in ecological sampling is based on the assumption that a minimum plot size can be ascertained and used in traditional plot-based sampling to adequately represent an ecological community. In a review of the minimum area concept, Mueller-Dombois and Ellenberg (1974) suggested that sample plots in regenerating forest communities should be in the range of 50-200 m^2 (538-2153 ft^2). In Virginia, recommendations for woody species density plots on mitigation sites have typically varied from 30-foot radius circular plots to 20 ft x 20 ft square plots. It is interesting to note that although these two plot sizes have been included in previous guidance documents as comparable options for monitoring purposes (e.g., USACE and DEQ 2004), they are almost an order of magnitude different in size (2827 ft^2 vs. 400 ft^2, respectively).

An appropriate plot size can be derived empirically on a site-by-site basis by sampling nested plots, evaluating cumulative species richness, and using the species-area relationship to identify minimum effective plot size (see Mueller-Dombois and Ellenberg 1974). However, it is not efficient, desirable, or cost-effective to conduct a pilot study on every compensatory mitigation site just to define a plot area that will most likely fall within a common range of values (e.g., 50-200 m^2 as noted above). Therefore, **a plot size of 100 m^2 (1076 ft^2) is recommended for woody species sampling** based on the standardization of this plot size in accepted protocols such as the North Carolina Vegetation Survey (Peet et al. 1998) and the National Wetland Condition Assessment (USEPA 2016). It is important to note that this plot size is only recommended for monitoring if used in combination with a multiple plot sampling design aimed at covering at least a minimum percentage of the sample area (see How Many Plots? below).

The radius for a 100 m^2 (1076 ft^2) circle would be approximately 5.6 m (18.5 ft). This equates to 0.025 or 1/40th of an acre, which provides a convenient multiplier (40x) to express values such as stem counts in per acre units. In addition, a 100 m^2 plot size is consistent with recommendations from Hudson and Perry (2018) for stem area at groundline (SAG) measurements.

Plot Size – Herbaceous Sampling: Although the most commonly used plot size in herbaceous community sampling on Virginia compensatory mitigation sites is the 1 m^2 (10.8 ft^2) square sampling frame (USACE and DEQ 2004, DeBerry and Perry 2004, 2012), a variety of plot sizes and shapes may be used to assess herbaceous vegetation (Mueller-Dombois and Ellenberg 1974, Krebs 1999, Tiner 2017). One concern is that the use of smaller plot sizes on larger sites risks higher sample variances, perhaps to the point that an excessively large number of plots would need to be sampled to capture the overall community variability and minimize sample error (Krebs 1999). However, on most compensatory mitigation sites in Virginia, the emergent (=herbaceous) planting zones are small relative to scrub-shrub and/or forested planting zones (pers. obs.). Therefore, the most commonly used square sampling frame dimension of 1x1 m (3.3x3.3 ft) would be appropriate for most herbaceous community sampling on Virginia mitigation sites.

HOW MANY PLOTS?

Once an appropriate plot size and shape have been determined, sampling may proceed using a stratified random sampling approach in combination with a sample adequacy determination (i.e., species-area curve) as described above. However, the question remains: How many plots should the researcher *start with* in order to initiate the sample on a site? For woody species sampling, several authors recommend establishing a minimum percentage of the entire study area (stratum, or planting zone) as a baseline for determining initial plot number (Mueller-Dombois and

TABLE 1: Minimum number of 100 m^2 woody sampling plots (based on 2% of total sample area).

Sample Area (ac.)	Number of Plots	Sample Area (ac.)	Number of Plots
1-5	**4***	28	**23**
6	**5**	29	**23**
7	**6**	30	**24**
8	**6**	31	**25**
9	**7**	32	**26**
10	**8**	33	**27**
11	**9**	34	**28**
12	**10**	35	**28**
13	**11**	36	**29**
14	**11**	37	**30**
15	**12**	38	**31**
16	**13**	39	**32**
17	**14**	40	**32**
18	**15**	41	**33**
19	**15**	42	**34**
20	**16**	43	**35**
21	**17**	44	**36**
22	**18**	45	**36**
23	**19**	46	**37**
24	**19**	47	**38**
25	**20**	48	**39**
26	**21**	49	**40**
27	**22**	50+	**add 1 plot per 2ac.**

* Note: In order to ensure a sufficiency of SUs to calculate meaningful averages for observations in smaller planting zones or community types, a minimum of 4 woody sampling plots is recommended for sample areas 1-5ac. in size.

Ellenberg 1974, Krebs 1999, Gardener 2017). In North Carolina, the recommended sampling strategy is to have the total cover of vegetation plots make up at least of 2% of the entire sample area, where the sample area usually corresponds to a planting zone such as forested, scrub-shrub, or emergent wetland (USACE and NCIRT 2016). Using this practice for woody sampling plots that are 100 m^2 (1076 ft^2), minimum plot guidelines were developed as shown in Table 1 below. Readers interested in additional information on minimum sampling area are directed to the review in Tiner (2017).

A minimum percent criterion for sample area is less applicable to herbaceous sampling because the smaller plot size (1 m^2, or 10.8 ft^2) would necessitate sampling an excessive number of plots (ca. 80 per acre) to achieve 2% coverage, which would result in community oversampling (see Sampling Strategy above). DeBerry (2006) documented herbaceous communities using 1 m^2 sampling frames arrayed in a stratified random sampling design on fifteen different wetland mitigation sites of different ages in Virginia. In this study, 15 herbaceous plots were sampled within a 1-hectare (2.5-acre) section of each site, and in all cases the herbaceous sample was found to be sufficient based on sample adequacy analysis. Given this result, it seems appropriate to recommend a 5 plot per acre minimum sample size for herbaceous monitoring in emergent planting zones on compensatory mitigation sites in Virginia, coupled with species-area sample adequacy analysis to determine if plots need to be added.

SUMMARY

The review provided above is intended to be used by the IRT as well as any practitioner who is or will be sampling vegetation to determine compliance with performance standards on compensatory mitigation sites. While the body of scientific research on vegetation sampling points to the approaches described herein, it is important to note that there are other techniques consistent with ecological sampling theory that may be used on compensatory mitigation sites. However, for most sites, a sampling strategy with the following characteristics will be sufficient for measuring vegetation attributes against performance standards:

- Stratified random sampling design coupled with sample adequacy determination using the species-area relationship.
- Plot sizes of 100 m^2 (1076 ft^2) for woody species sampling (circular plot) and 1m^2 (10.8 ft^2) for herbaceous sampling (square plot).
- Initial woody species plot density based on a sample size covering approximately 2% of the sample area (stratum, or planting zone) for woody sampling.
- Initial herbaceous species plot density of 5 plots per acre. ■

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