U.S. Fish and Wildlife Service
U.S. Department of the Interior

National Wildlife Refuge System

National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions

Version 1.0 **December 2019**

ON THE COVER
Monitoring vegetation with nested plots in a prairie reconstruction.
Photograph by: Pauline Drobney, FWS

NWRS Survey Protocol Signature Page

Protocol Title: National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions	
Version[1]: 1.0	
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Approvals

Action	Appropriate Signature/Name	Date
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Zone I&M[3] or equivalent Approval:		
Regional I&M[4] Approval:		
National I&M[5] Approval:	**Jana Newman, National I&M Manager**	12/12/2019

Version[1]	Date	Author	Change Made	Reason for Change

[1] Version is a decimal number with the number left of decimal place indicating the number of times this protocol has been approved (e.g., first approved version is 1.0.; prior to first approval all versions are 0.*x*; after first approval, all minor changes are indicated as version 1. *x* until the second approval and signature, which establishes version 2.0, and so on). Only two signatures are required: one from the submitter (lead author)[2] one from the approving official, which is dictated by the scope of the protocol[3,4,5].

[2] Signature of station or I&M representative designated lead in development of a site-specific survey protocol.

[3] Signature signifies approval of a site-specific survey protocol.

[4] Signature by Regional I&M Coordinator signifies approval of a protocol framework to be used at multiple stations within a Region.

[5] Signature by National I&M Coordinator signifies approval of a protocol used at multiple stations from two or more Regions.

Survey Protocol Summary

Prairie reconstruction, or the process of planting and managing prairie through time, is an important management practice throughout the tallgrass and mixed grass prairie region in the Midwest and Great Plains. The Prairie Reconstruction Initiative (PRI) has developed a monitoring protocol framework with two complementary floristic monitoring methods for assessing outcomes of reconstructions: a meandering walk and nested frequency plots. The meandering walk allows users to generate a more complete species list and have a relatively quick overview of the floristic community. The nested frequency plots allow users to generate quantitative data to compare among sites and over time. These methods were developed in response to the prairie reconstruction and monitoring needs in the Midwest and Great Plains.

In addition to guidance on implementing the two monitoring methods, this protocol framework explains specifics for sampling, managing data, reporting, qualifications for data collectors, and operational requisites like time and costs. These methods are useful for prairies but would need to be adapted for other natural communities or for areas outside the originally intended geographic scope. The methods may also be useful in assessing habitat for monarchs or other species that occur in prairies.

This monitoring protocol is designed to complement the PRI Management Database (Database). This database allows land managers and practitioners to systematically document their reconstruction site characteristics, site history, seed mix, planting methods, and ongoing management actions to better understand how these factors may influence the outcome of a planting. Users of the protocol are not obligated to participate in the Database, and Database participants are not obligated to monitor their reconstructions. Visit the PRI website to learn more about PRI, the Database, and how to contact current PRI Coordinators.

This framework should be used to create a site-specific protocol (SSP) for monitoring outcomes of prairies reconstructed on National Wildlife Refuge System and other lands. Users should add their site-specific information to the template in Appendix A following the template instructions. Links have been inserted throughout to take the reader to relevant site-specific guidance added to Appendix A.

Add SSP summary

Suggested citation:
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Add SSP Acknowledgments

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Element 1: Introduction

Background

Prairies once dominated large portions of North America but are now among the most endangered ecosystems globally. Historically, Euro-American settlement initiated the conversion of prairie to cropland in the Midwest and Great Plains, a phenomenon that still continues today (Smith 1992, Faber et al. 2012). In addition, native prairie has been extensively converted to or invaded by non-native species. Efforts to conserve remaining native or remnant grasslands are a priority for conservationists, but the extreme loss of these grasslands requires that prairie is also reconstructed to achieve conservation goals. Cropland and exotic grass plantings in areas formerly occupied by prairie provide opportunities for prairie reconstruction, or the process of planting and managing prairie through time. Reconstruction of prairies may be the key to increasing quality habitat for species of concern like grassland birds (Igl and Johnson 1997) or endemic pollinators (Grixti et al. 2009) and to reducing extinction risk of iconic species like the monarch butterfly (*Danaus plexippus*; Thogmartin et al. 2017). From an ecosystem perspective, floristically diverse reconstructed prairies can help to conserve native biodiversity and ecological function (**Figure 1.1**).

In recent decades, the US Fish and Wildlife Service (FWS) and other federal, state, local, non-governmental, and private land managers have expended significant resources to reconstruct diverse plant communities of native prairie species. Prairie reconstructions have been established across the prairie region under a wide range of conditions using a variety of methods and often with considerable effort and expense. Improving the prairie reconstruction process and associated techniques is critical to increase efficiency and effectiveness. Therefore, a group of individuals from several organizations held a structured decision making workshop in 2012 and formed the Prairie Reconstruction Initiative (PRI). A core subset of individuals provides leadership as the PRI Advisory Team (PRIAT).

PRI is a collaborative effort to identify and mitigate uncertainties inherent in the process of prairie reconstruction to ensure that future efforts are cost-effective and meet management objectives. This diverse group of public and private practitioners and researchers seeks to understand and improve practices or conditions that lead to long-term success in prairie reconstruction. PRI shares the collective knowledge of the larger community of practice, investigates problems through scientific experimentation, and has developed the standardized monitoring protocol described here, as well as an associated prairie reconstruction management database. For more information about the PRI Management Database (Database), see Element 4.

Monitoring is necessary to objectively evaluate the success of prairie reconstruction. It enables us to document outcomes and, when coupled with planting and management data, allows us to identify factors that maximize traits we value in a prairie reconstruction. Important measures of the outcomes are noted in **Table 1.1**. Practitioners may track these and other measures to identify trends within a reconstruction that guide management decisions.

Table 1.1. Some important measures of outcomes of prairie reconstructions.

Measure of outcome
Fraction of species expressed to species planted
Dominance of:
Native vs. invasive
Cool-season vs. warm-season
Grass vs. forb
Herbaceous vs. woody
Indices of plant community quality:
Species richness and diversity
Floristic quality index (FQI)
Mean coefficient of conservatism (CofC)

The PRI monitoring protocol development team considered a variety of different monitoring methodologies that would provide different data, answer different questions, and be subject to different observer biases. Recognizing that no single method could do everything needed, PRI developed two methods for monitoring reconstructions: a meandering walk and nested frequency plots (**Table 1.2**). These methods are useful for prairies but would need to be adapted for other natural communities or for areas outside the originally intended geographic scope.

The meandering walk method provides the most complete list of species occurring at a site; the drawback is that spatial and temporal comparisons are limited. One of the primary values of a meandering walk is that it allows early detection of problems within a reconstruction as well as indicates areas of stunning success. Including general abundance provides an estimate of the balance of diversity and alerts a land manager to particular phases of prairie reconstruction or to concerns such as undesired dominance of particular species. The nested frequency plots provide quantitative data for comparisons across reconstructions or over time and allow calculations of metrics, such as diversity and evenness. We avoid using estimates of cover, which have high inter-observer and intra-season variability. The shortcoming of any plot-based method is that inevitably some species will be missed because the plots encompass a small part of the entire area under consideration.

The two methods are designed to be complementary; we envision that a manager or biologist would conduct both methods, though a meandering walk would happen more frequently (preferably annually), and the nested frequency plots could be done at longer intervals. Both methods depend on observers with good botanical skills. While the methods are designed to be used in conjunction with the Database, either can be used independently of it. When combined with site history, site characteristics, planting, and management data (e.g., via the Database), floristic monitoring data will highlight favorable (or unfavorable!) practices for prairie reconstruction.

Table 1.2. Comparisons between the ways that the meandering walk and nested plot monitoring methods address key prairie reconstruction questions.

Question	Meandering Walk	Nested Plots
What native species, whether deliberately planted or not, are establishing?	Relatively complete list of observed species; can be compared with a list of planted species.	List of observed species is likely incomplete and weighted toward common species because sampling is systematic and only a small portion of the site is sampled.
What are the emerging invasive species threats?	Greater potential to detect incipient invasion.	Incidental detection of incipient invasive species; only detects invasive species that occur within plots.
What are the differences in occurrence of species or suites of species (whether desirable or undesirable) through time?	Data are based on subjective abundance categories, can identify large differences but not smaller ones, different observers "catch" different species and estimate abundance differently.	Can statistically compare frequencies measured in each year.
What is the proportional abundance of suites of species (exotic to native, grass to forbs, etc.) through time?	Low precision to assess the question.	Can compare frequency among different suites of species. Usefulness of the analysis is amplified over time.
What is the proportional species richness of suites of species (exotic to native, grass to forbs, etc.) through time?	More complete list of species can allow better comparison of species richness among suites of species.	Can develop species accumulation curves for suites of species of interest.
What is the floristic quality of a planting at various stages and through time?	FQI, mean CofC, and other measures of floristic quality can be calculated in any given year or compared among years.	FQI, mean CofC, and other measures of floristic quality can be calculated in any given year or compared among years for species in plots.
What is the richness, evenness, or diversity of a planting?	Species richness for a given site can be compared across years, assuming the walk is comprehensive within the designated sample area. However, richness cannot be compared among sites because they might be different sizes.	Can calculate all measures of richness, evenness, and diversity; can construct species-area curves to better estimate richness.
Are some species more likely to establish, persist, or increase in dry, mesic, or wet portions of the seed-mix area (if more than one soil moisture type is included in the seed-mix area)?	The walk is intended to be stratified by soil moisture type, so it is possible to compare what was planted with what was observed within moisture categories.	Nested plots are intended to be stratified by soil moisture type (see SOP 2 for information on appropriate sample size). Statistical models can be used to compare among soil moisture types, or multivariate statistical techniques can be used to visualize plant community associations with respect to soil moisture type.

Developing site-specific protocols for FWS stations

This document is an official FWS National Wildlife Refuge System (NWRS) national protocol framework, which is available to anyone. This protocol is national in scope because it includes a significant portion of the historic tallgrass and mixed grass prairie range including multiple FWS regions. Local adaptations may be necessary beyond FWS Midwest and Great Plains Regions where it was originally developed. To fully implement this framework, each participating FWS station is required and other users are encouraged to write a site-specific protocol incorporating station details such as survey locations and local monitoring objectives. FWS stations should integrate their site-specific protocols with their station Habitat Management and Inventory & Monitoring Plans. In Appendix A, we provide guidance for creating a site-specific protocol.

Add SSP Background

Objectives

This protocol framework addresses two kinds of objectives, detailed below.

Management objectives

Management objectives are statements detailing the resource outcomes a practitioner plans to achieve. Before using this protocol framework, we recommend that users clearly define site-specific management objectives to ensure this monitoring approach meets their needs (Appendix B).

To guide its collaborative efforts, PRIAT has defined objectives related to prairie reconstruction (**Figure 1.1**). We determined that the overall objective (i.e., fundamental objective) is to create healthy prairie; this protocol provides a means for assessing progress toward that objective. A sub-objective (i.e., means objective) in striving to create healthy prairie is that the resultant plant community is similar to a corresponding remnant community. Although many components of a reconstruction could be monitored, we concentrated on the plant community because it is the direct result of the planting event, is the basis for other conservation targets, and is the focus of most prairie management. We then developed a more detailed tier of means objectives addressing various aspects of the plant community. **Figure 1.1** can be used to help develop individual objectives for a site-specific monitoring plan based on this protocol framework.

Sampling objectives

While management objectives describe the desired resource outcomes, sampling objectives provide the specifics for measuring those outcomes. Measurable attributes should be identified for each means objective. These attributes then become the focus of the monitoring strategy and facilitate assessment and inference about outcomes of a prairie reconstruction project. See Appendix B for guidance in developing sampling objectives.

Both the meandering walk and nested frequency plot methods described in this protocol address the detailed means objectives for plants shown in the bottom row of **Figure 1.1**. The primary measurable attributes of the meandering walk include a list of plant species (native and non-native) and categorical abundance and distribution of each observed species (SOP 1). The measurable attribute for the nested frequency plots is frequency of occurrence for each plant species (SOP 2).

Add SSP Management and Sampling Objectives

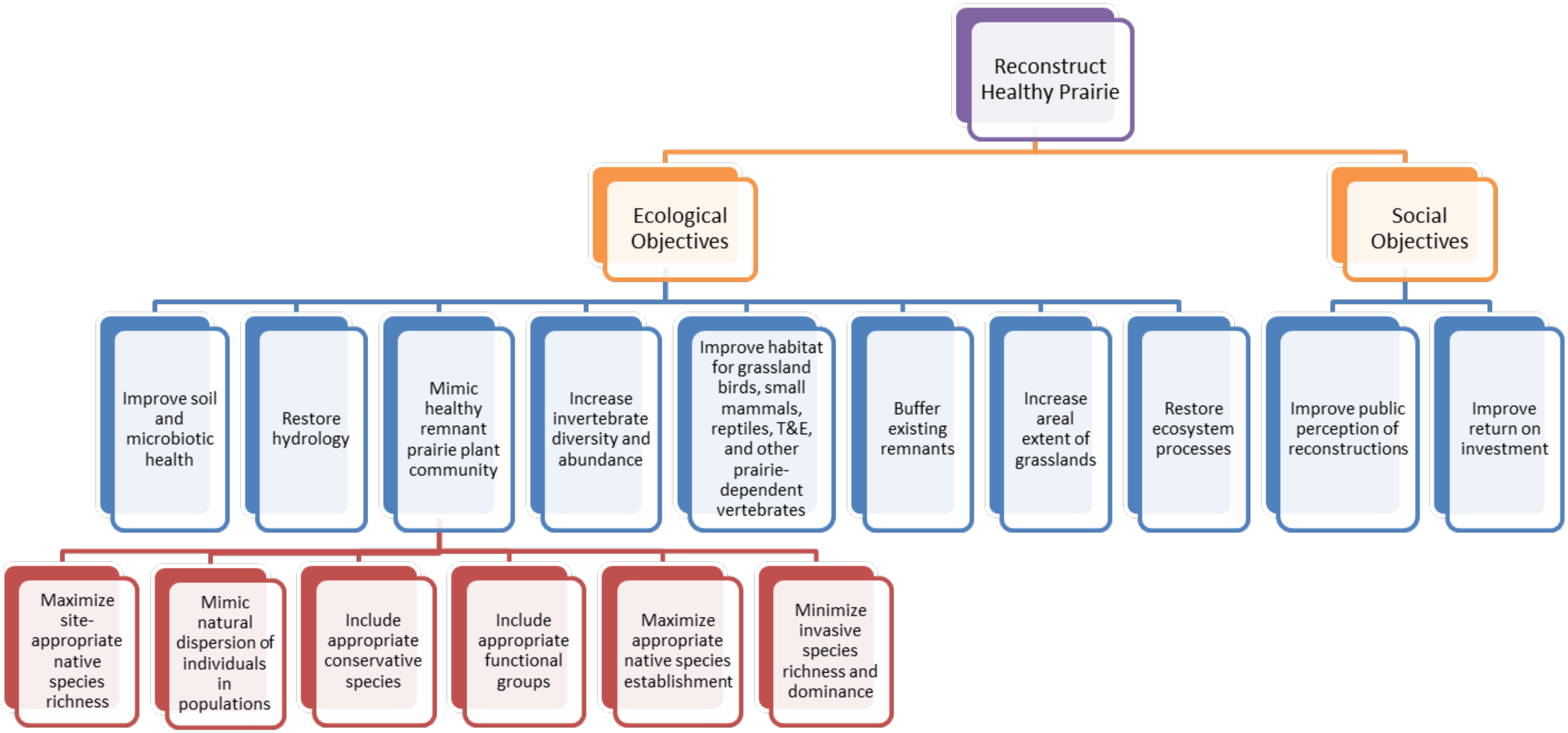


Figure 1.1. Objectives hierarchy for prairie reconstructions. While healthy prairie has many components, our focus in this protocol framework is on the plant community. Top level = fundamental objective, middle levels = ecological and social means objectives, bottom level = detailed means objectives for the prairie plant community.

Element 2: Sampling

Sample design

Data collection and recording are structured according to the spatial hierarchy shown in Figures 2.1 and 2.2. Both monitoring methods require selection of seed-mix areas where observations or measurements will be taken within a pre-defined overall site. We define “seed-mix area” as an area that was planted simultaneously using the same methods and seed mix. This distinguishes it from broader scale sites that can include several planting events and seed mixes. The site selection process is rarely random because the choice of sites is usually based on specific interests and needs of the station or manager. Consequently, inferences are generally limited to the seed-mix area being monitored, and the manager should be aware that the results may not apply to all seed-mix areas.

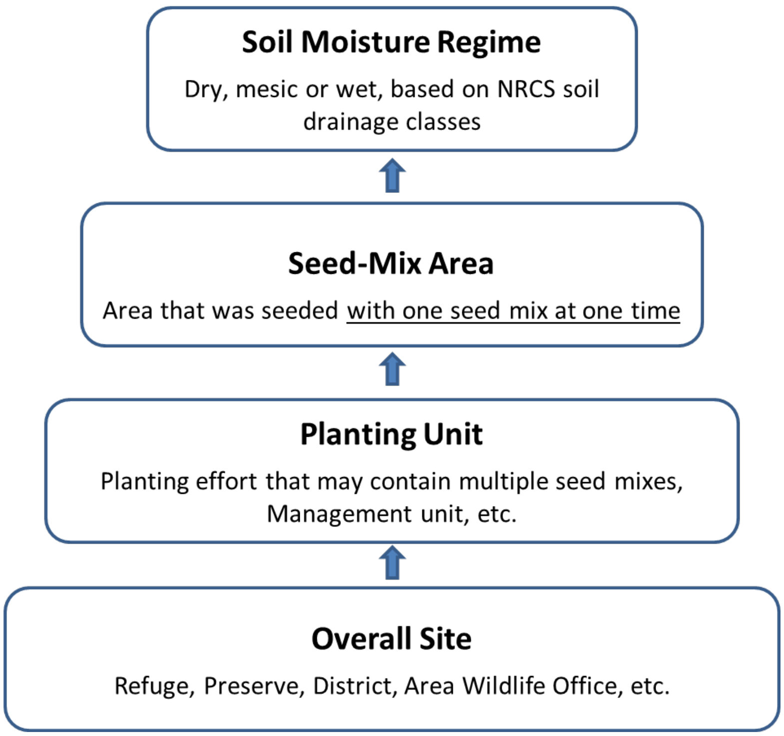


Figure 2.1. Schematic of nested spatial hierarchy. Scale proceeds from broader at bottom to narrower at top. The Database includes the first three levels (Element 4). If a seed-mix area contains more than one soil moisture type, follow the guidance in Elements 2 and 3.

The meandering walk is designed to census as many plant species within a seed-mix area as possible in each year it is performed. As such, sampling is throughout the entire site and does not have discrete sampling plots or areas. Nested frequency plots, on the other hand, are arrayed on a systematic grid that is overlain on the entire seed-mix area and, in essence, represents all possible points within a seed-mix area. This provides unbiased estimators of species frequencies that can be analyzed to understand floristic condition in a given year or changes through time. The systematic sampling can, however, underestimate species richness because the plots only sample a small proportion of the total area.

Add SSP Sample Design

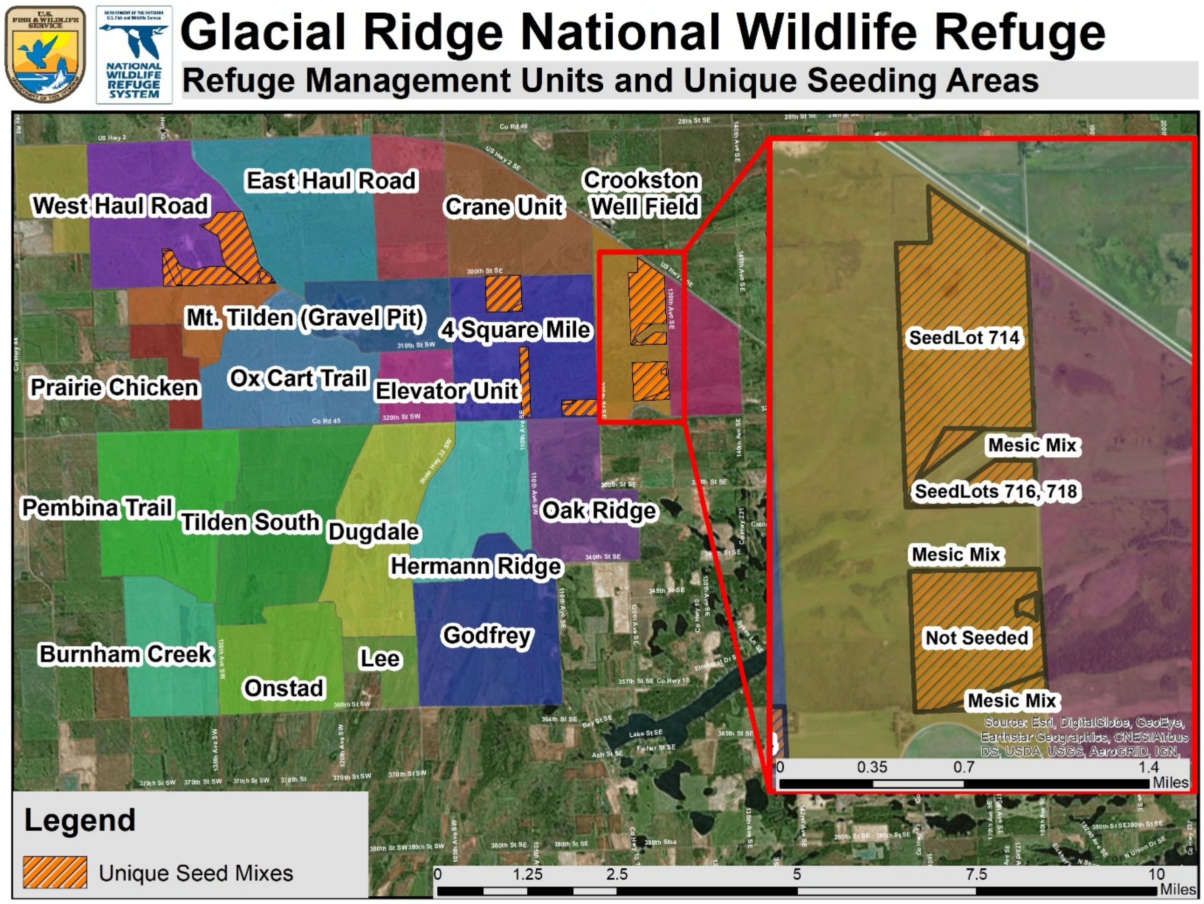


Figure 2.2. Example of nested spatial structure. In this case, staff at Glacial Ridge National Wildlife Refuge (GRNWR) designated GRNWR as the "Overall Site" and the management units (different colored blocks) as the "Planting Units." Each Planting Unit contains multiple unique "Seed-Mix Areas" (orange hatching) depending on the prairie vegetation type targeted during the reconstruction effort. Only a few of the existing Seed-Mix Areas are shown.

Sampling units, sample frame, and target universe

Ultimately, for a given individual seed-mix area, we want to use each method to relate the plant response back to the seed mix, planting techniques, and management. Hence, the sample unit is a seed-mix area. The plant community in the seed-mix area is the target population for any inferences.

Sampling is stratified by broad soil moisture types within each seed-mix area. In many cases, a seed-mix area will encompass one soil moisture type (dry, mesic, or wet; SOP 3). In other cases, however, a single seed mix may be applied to an area that contains more than one moisture type. We expect different species to be adapted to different soil moisture types. However, we recognize that in prairie reconstructions, the expected assemblages of plant species do not necessarily match what is achieved in the resulting plant community. Assemblages may be similar to a prairie remnant analogue, or they may be a mix of species that normally are not found together in prairies but that co-occur on a planting simply because they were in the seed mix and survive in the environment where they established, at least for a period of years or decades. We also recognize that differences in soil moisture types can influence the survival or vigor of some species. By tracking different soil moisture categories, we can better interpret and compare the results and understand nuances associated with custom designing seed mixes for different soil moisture types. The data can also be correlated with other variables of interest to practitioners such as slope, aspect, or topographic position.

Sample selection and size

For the meandering walk method, sampling is guided by a map that includes moisture categories (**Figure 2.3**). For the nested plot method, we sample on a systematic grid to ensure sampling locations are distributed throughout the site to more likely capture variability (**Figure 2.4**). At the same time, a systematic layout allows the observer to walk a straight line from one point to the next rather than having to wander around to random points.

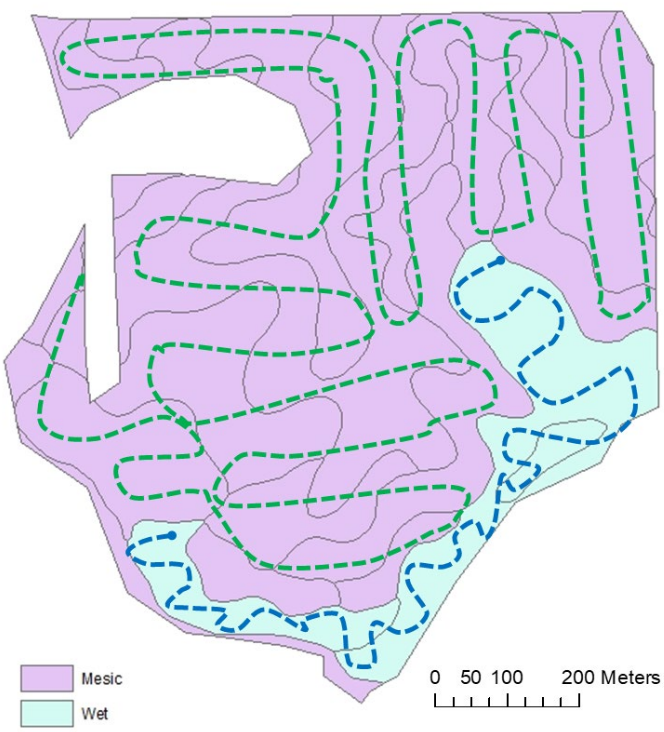


Figure 2.3. Example seed-mix area comprised of two soil moisture types overlain by example meandering walk routes (dashed lines) in both wet and mesic areas. The solid lines are boundaries of soil map units.

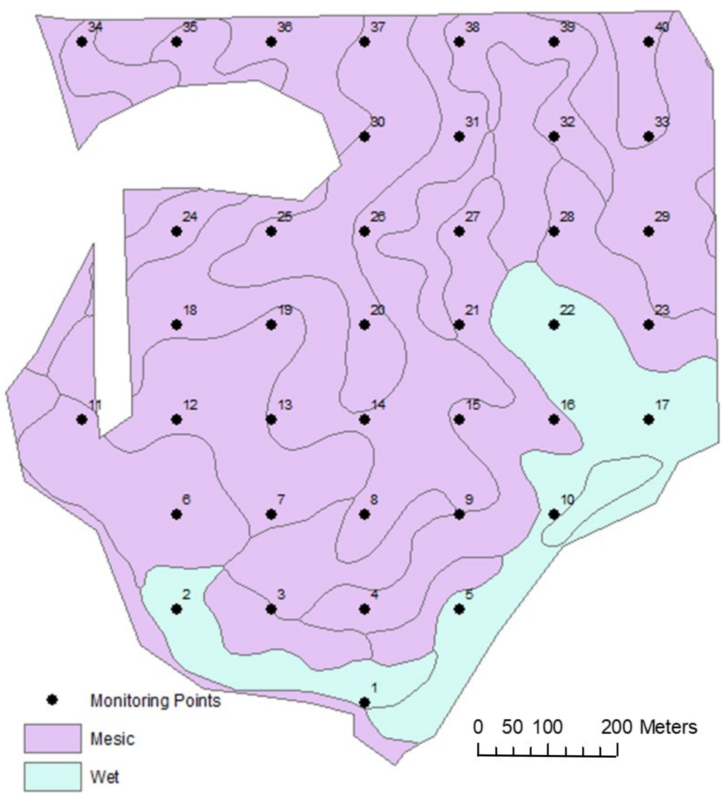


Figure 2.4. Example seed-mix area comprised of two soil moisture types overlain by a sampling grid. Approximately 15% of the seed mix-area is wet and contains 6 of the 40 points. The solid lines are boundaries of soil map units.

The rectangular (50-cm x 200-cm; 1-m^2) nested frequency plots within a given seed-mix area are considered subunits, which taken together give frequency of occurrence within that seed-mix area for a given species. The number of plots in each seed-mix area depends on its size. Based on a series of simulations to estimate sample size needed to detect differences in frequency, we recommend using 3 plots/ha with a minimum of 10 and a maximum of 40 total plots sampled per seed-mix area (Appendix C). In other words, if a site is 3 ha (~7.4 acres) or less, use 10 plots. For sites from 4–13 ha, use 3 plots/ha, and if a site is more than 13 ha (~32 acres), use 40 plots. In addition, the number of plots within each moisture category should be proportional to the area of that moisture category (Element 3, SOP 3). SOP 4 contains guidance for creating the systematic grid within ArcMap. No minimum distance is required between plots because the seed-mix area is the experimental unit of interest, and the plots are used just to get a frequency estimate for the seed-mix area. The 10 to 40 nested plots are not treated as independent samples in analyses. The percentage of plots with a given species present is computed and is not affected by plot correlation or distance between plots. What is important is that the plots are representative of the whole seed-mix area, which is why they are laid out on a grid.

In simulations (i.e., testing if there is a difference in frequency between two treatments), we found that using a maximum of 80 plots did not substantially increase power over having 40 plots (Appendix C). However, if someone had the time and resources, it is allowable to include more than the recommended number of plots. We do not believe this will have any effect on analyses of frequencies of species, and the benefit would be getting a more accurate estimate of the frequency for that seed mix area. However, if using more than 40 plots, all plots should be added when laying out the grid for the entire seed-mix area and not added in the field by the observer.

Add SSP Sample Selection and Size

Survey timing and schedule

Information about frequency of monitoring among years and seasonal timing of monitoring should be included in the site-specific protocol.

Frequency of monitoring among years

Initial monitoring when planting into existing vegetation ideally should occur in the growing season prior to planting to document baseline floristic characteristics. For newly planted sites, we recommend implementing both monitoring methods during the first three growing seasons after planting to capture nuances that relate to site, weather, planting practices, or other conditions. Also consider monitoring in any other year when there is reason to believe that the vegetation is undergoing dramatic change.

After the initial phase, annual surveys are highly encouraged for the meandering walk, but practitioners may decide to conduct surveys at some other predetermined interval (e.g., every two or three years). Conducting the nested frequency plots survey can be relatively time consuming, and practitioners should consider objectives and resources available in developing a monitoring plan. We recommend implementation of the nested plot method at least every 3–5 years after the initial establishment phase. Practitioners may decide to use nested plots more often, depending on site objectives and whether the results of a meandering walk give rise to concerns that warrant collection of quantitative data for analysis.

When beginning monitoring on a planting that happened several years earlier, survey frequency should match the phase of the planting and station objectives. For example, a five-year old planting can sometimes remain in what might normally be considered typical of the second or third year of planting. Practitioners should develop monitoring intervals appropriate to plant community development and local objectives.

Seasonal timing of monitoring

Phenology will differ among species, and the biologist or manager should consider the best timing within the growing season to maximize the number of species that can be successfully identified for each particular planting. In many cases, early to mid-summer (approximately between when sedges bloom to when early composites such as false sunflower [*Heliopsis helianthoides*] begin to bloom and cool-season grasses are beginning to produce seed) is a good time to survey early-season species that have not quite senesced as well as later emerging species that are big enough to be identified even if they are not yet in bloom. Timing of monitoring should be based on the same phenological cues among years on a given site, although monitoring in the year the site was planted may be done later in the season when a large number of plants become apparent. Keep in mind that first observation often occurs in different years among species.

If resources permit, we recommend a second meandering walk later in the season to allow identification of species with characteristics that are only apparent in late season to improve understanding of the developing plant community. This will facilitate development of a more complete initial species list that can also be used as a reference in subsequent years. A second meandering walk in a season can be added either annually or only in some years. However, the primary walk should be done at the same phenologic time in every monitoring year. For analysis, data from each walk should be kept separate.

Add SSP Survey Timing and Schedule

Sources of error

In a collaborative monitoring effort where many different observers will collect data, one source of error is inconsistent application of the survey methods. The specific details and graphics provided in this protocol framework will help to reduce this error. Proper training and yearly refreshers are critical. Consistency in surveyors from year to year will also help to reduce error. In addition, the PRI team may periodically provide field training on the methods.

Plant misidentification can be a major source of error in any floristic monitoring scheme. Because of the difficulties of attempting to survey plants of varying phenologies, it is possible that plants will be misidentified or missed altogether if sampling occurs only once during the growing season. Graminoids, for example, can be especially difficult to identify without inflorescences or following senescence, requiring expertise in use of diagnostic vegetative characteristics. While there are some species with easily identifiable seedlings, many are difficult, and attempts at identification can result in frustration rather than usable data. Seedlings that can be identified with confidence should be. Those that are either too small or too damaged to identify are recorded as an unidentified graminoid or forb seedling. It can be useful to note suspected identity or at least genus in "comments" if possible. Seedling information can provide general indications of recruitment. However, **surveyors should never guess at identity when entering data in the "species" field; it is much better to have an unknown in a collection than to have a misidentified species.** If possible, flag an unknown plant species, and return later when diagnostic characteristics are apparent, allowing the addition of that species to the data.

Reducing the effect of misidentification error requires that experienced botanists who are knowledgeable about the local flora perform the survey. Referring to a planted species list per seed-mix area can further help to reduce this source of error. This becomes more difficult when using bulk harvest (harvest of many species together), but seed tests, lists of species known from the collection site, or other information on added species can help fill out a list of expected species.

There is a danger in providing a species list from prior surveys to a field crew, and the manager or biologist should weigh the tradeoffs. The primary negative issue is the likelihood of propagating identification errors through time. If certain species are difficult to tell apart, and an earlier determination had been made, the new surveyor may be comfortable accepting the prior species identity, rather than trying to key out the plant. If the prior determination was incorrect, the error will be repeated. On the plus side, however, a list of species found in prior surveys can encourage surveyors to search out species they have not yet found, especially when conducting the meandering walk. The identification of a species should not be based on the list; it should be independent from the list. The list provides some possibilities, but confirmation of a species must be based on its characteristics.

One source of error specific to the meandering walk method is that rare or cryptic species will be under-observed, whereas showier species, especially when in bloom, will be more noticeable. Multiple walks in a season could help remedy this. Nested frequency plots will miss uncommon species, overestimate randomly distributed species, and underestimate clonal species (Elzinga et al. 1998). In addition, with any plot-based approach, determining which plants are inside the frame is a common source of error. Prior to examining species composition within a frame, rooting locations of plants on the edge should be determined, and only those rooted within the frame should be recorded.

We recognize that GPS use in the field includes some error depending on the type of GPS unit used, atmospheric conditions, cover, and satellite position. This imposes limits on the resolution of data analyzed in a spatial context but does not influence estimates for the seed-mix area, unless the GPS location error is related to or confounded with frequency of some species, which is unlikely. If the GPS error can be considered to be random with respect to mapped locations and different plant species, it simply adds to the measurement error in time series analysis. We accept that there will be random spatial errors in exact relocation of mapped sampling points.

Add SSP Sources of Error

Element 3: Field Methods and Processing of Collected Materials

This protocol framework consists of two monitoring methods including the meandering walk and the nested frequency plots. The two methods differ in types of data collected and in degree of analytical rigor possible, but they are complementary, and both are necessary to capture key information (**Table 3.1**).

Pre-survey logistics and preparation

Meandering Walk

MAPPING: Create a map with the boundary of the seed-mix area targeted for survey. The meandering walk is stratified by soil moisture types that correspond to dry, mesic, or wet conditions. See SOP 3 for guidance on how to use the Natural Resources Conservation Service Web Soil Survey to include these soil moisture categories on your map. We recognize that the extent and location of mapped soil units are approximations. Nonetheless, the NRCS soil survey is a standard tool we can use to better understand how soil moisture type influences the outcomes of plantings.

It is preferable to conduct the meandering walk throughout the entire seed-mix area unless conditions prevent total coverage. If you have a very large or challenging area, creating separate polygons and searching a few different areas is acceptable. The designated search polygon(s) in aggregate should include the diversity of plant communities, topography, and soil types on the entire site. If the seed-mix area is partially sampled, subareas should be determined prior to the field survey, and a map with the appropriate subarea boundaries should be generated. Search these areas every time you sample.

Table 3.1. Brief overview of the two sampling methods.

Attribute	Meandering Walk	Nested Plots
Interval	Annually or less often, depending on objectives; surveys can be repeated in a single year	Every 3–5 years or more often, depending on objectives
Field time[1]	2–4 hours	0.5–3 days
Metrics	Species list, general abundance and distribution; FQI calculated	Species frequency of occurrence; diversity, evenness, optimal plot size calculated
Species list	More comprehensive	Less comprehensive
Analysis	Subjective: qualitative and limited quantitative comparisons over time at a site	Quantitative: statistically compare over time and among sites and methods
Botanical skill	High	High

[1]Time required to implement a method varies depending on skill level, type of seed mix planted, size and complexity of site, and weather conditions leading to monitoring. In this estimate, we assume an 80-acre seed-mix area with a relatively high diversity seed mix.

If management concerns dictate addition of other subareas at a later date, delineate those areas so they can also be repeated in subsequent years. However, keep the list generated from the original subareas separate from the added subareas so that data from the larger survey area can still be summarized for just the original subareas.

Create a final digitized polygon of the area(s) to be searched at the site (including the soil moisture category boundaries), and export a map to the GPS unit, or print one for use in the field. This will be the permanent meandering walk search area for this site (but see above about sampling subareas). It can be useful to include topography and/or a satellite imagery basemap for use in the field.

Add SSP Meandering Walk Mapping

PLANTED AND PREVIOUSLY OBSERVED SPECIES LIST: If desired, use species lists that include both planted and previously surveyed species on the site. Such lists can alert observers to be on the lookout for certain species, but be aware that previous surveyors may have made identification errors (Element 2). The list could include appropriate abbreviations to use in the field datasheet.

Add SSP Meandering Walk Planted and Previously Observed Species List

Nested frequency plots

MAPPING: Sampling is stratified by broad soil moisture type (dry, mesic, wet). See SOP 3 for guidance on how to use the Natural Resources Conservation Service Web Soil Survey to include these soil moisture categories on your map. We recognize that the extent and location of mapped soil units are approximations. Nonetheless, the NRCS soil survey is a standard tool we can use to better understand how soil moisture type influences the outcomes of plantings.

Sampling occurs on a systematic grid, and the number of points needed depends on the size of the seed-mix area (Element 2, **Figure 2.4**). Apply a grid for the appropriate number of sample points in proportion to the area of each soil moisture type. SOP 4 contains guidance for creating the systematic grid within ArcMap. Points that fall in locations that are permanently anomalous and cannot be adequately monitored through time can be moved slightly. Examples include points located within a permanent fireline that is regularly mowed or along a border where planting equipment might not have consistently delivered seed due to irregular-edge characteristics. However, if the vegetation at the sampling point is only temporarily changed, for example, areas mowed to manage invasive species or close cropped or rutted areas caused by grazing, then sample this point.

Prior to the field season, create a map that includes the sampling grid and boundaries of seed-mix areas and soil moisture categories. It can be helpful to include topography and/or satellite imagery for use in the field. Use the same set of points for subsequent surveys at the site. Apart from the systematic grid of sampling points, this is the same map used in a meandering walk for that particular seed-mix area (unless the meandering walk occurs on a subset of the entire seed-mix area). Download the points to a GPS unit.

If GIS technology and a GPS unit are not available, the systematic grid stratified by soil moisture type will need to be laid out by hand on an appropriate satellite image or other map of the site. An alternative method of navigating to each point will need to be developed (e.g., using compass direction and distance from an obvious starting point) and documented.

Add SSP Nested Frequency Plots Mapping

PLANTED AND PREVIOUSLY OBSERVED SPECIES LIST: If desired, use species lists that include both planted and previously surveyed species on the site. Such lists can alert observers to be on the lookout for certain species, but be aware that previous surveyors may have made identification errors (Element 2). The list could include appropriate abbreviations to use in the field datasheet.

Add SSP Nested Frequency Plots Planted and Previously Observed Species List

PLOT FRAME: The total area sampled by the plot frame doubles as each larger subplot is surveyed (**Figure 3.1**, **Table 3.2**). For Database-wide analyses, only these original subplot sizes will be used.

Although we recommend using the dimensions indicated in **Table 3.2**, the size of the subplots within the plot frame can be modified, if necessary, by continuing to divide the smallest subplot into two equal-sized halves (**Figure 3.2**) for sites with highly dominant vegetation. For example, if a species is nearly always present in the smallest subplot, it will be difficult to observe an increase in frequency of that species in subsequent monitoring events. In this case, creating a smaller subplot will be useful. Contact the PRI Coordinator for guidance if needed.

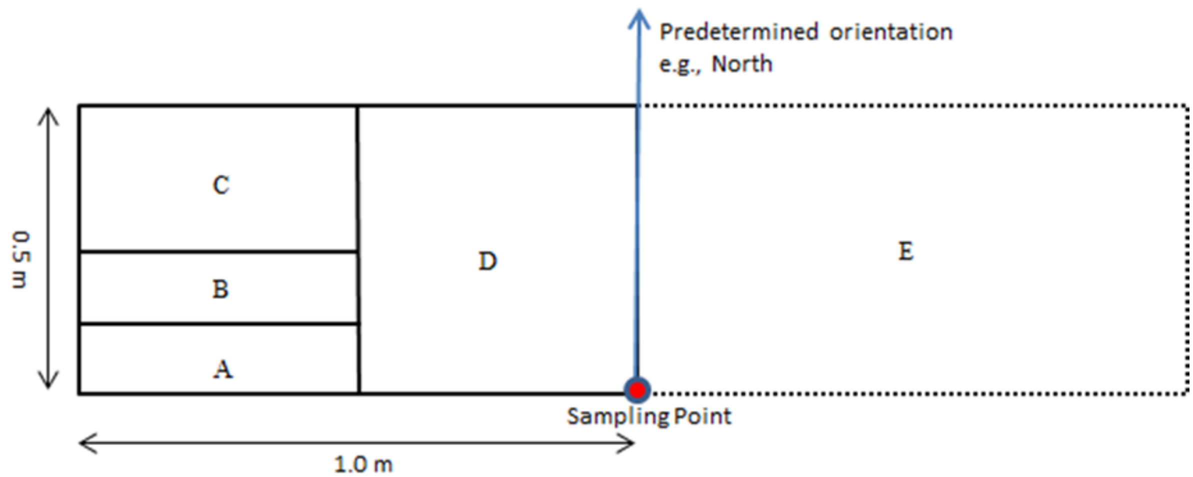


Figure 3.1. Layout of subplots within plot.

Table 3.2. Composition, size, and dimensions of nested plots. Each 1-m^2 sampling plot is divided into five subplots (A–E), which are combined sequentially to create the five nested plots (1–5). Area examined doubles as nested plot number increases.

Nested Plot Number	Composed of These Subplots	Area	Dimensions
1	A	1/16 m^2	12.5 cm x 50.0 cm
2	A, B	1/8 m^2	25.0 cm x 50.0 cm
3	A, B, C	1/4 m^2	50.0 cm x 50.0 cm
4	A, B, C, D	1/2 m^2	50.0 cm x 100.0 cm
5	A, B, C, D, E	1 m^2	50.0 cm x 200.0 cm

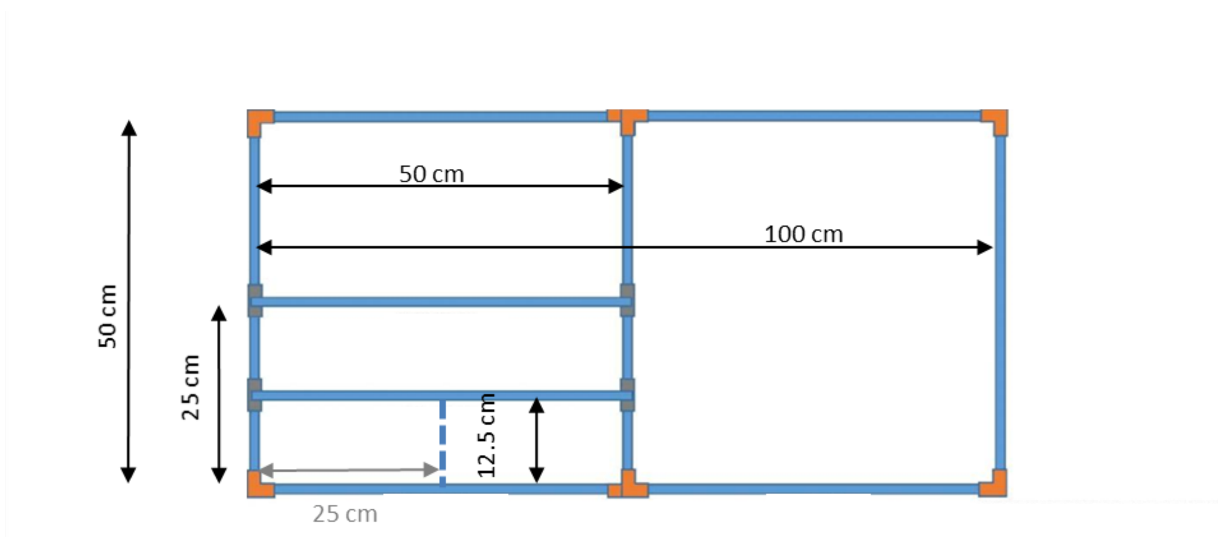


Figure 3.2. Internal dimensions of the nested plot frame. The dotted line indicates where an additional subdivision could be made.

Keep in mind that species' frequencies can change over time, so optimal nested plot size (which is species-specific) may also change. Therefore, it is important to always keep track of the subplot in which each species was first observed in each plot in each monitoring event; in this way, it will always be possible to translate the frequency to a different nested plot size that is more appropriate in the future (Element 4).

In tall vegetation, it is useful to be able to remove one side of the frame so that it can be slipped into the base of the vegetation. Nested frequency plot frames are constructed using instructions in Appendix D. We have had success using a frame constructed from PVC pipe, but users are free to use other materials as long as the internal dimensions are preserved.

Finally, prior to beginning data collection, develop and document a consistent scheme for orienting the plot frame in the field (SOP 2). It is essential that an observer place the frame without inadvertent bias in the field.

Add SSP Nested Frequency Plots Frame

Establishment of sampling units

See SOP 1 (meandering walk) and SOP 2 (nested frequency plots) for instruction about how to establish the sampling unit in the field. Care should be used when monitoring near the boundary of adjacent seed mixes, because there may be a region of overlap (or a gap in application) of seed mixes in a given planting unit. If seed-mix areas are highly interspersed (e.g., in ridge and swale topography) or if seed mixes are applied in very patchy or complicated ways such that monitoring them separately would be impractical, they may be monitored as one seed-mix area and treated as a single sample unit.

Add SSP Establishment of Sampling Units

Data collection procedures (field, lab)

Meandering walk

During a meandering walk, one or more observers walk a meandering path throughout the site, visiting all noticeable vegetation zones associated with variations in soil moisture, topography, and other spatial and vegetative features (**Figure 2.3**). Observers document all plant species seen, including an abundance estimate based on an ordinal scale (sparse, occasional, frequent, very common) and an indication of distribution (localized, widespread). The result is a list of plant species that is more complete than is possible by monitoring plots. These data are critical for understanding the results of prairie reconstruction techniques. The duration of the walk is also recorded. Details about field methods for the meandering walk are found in SOP 1.

Add SSP Meandering Walk Data Collection Procedures

Nested frequency plots

The nested frequency plots method entails documenting the presence of plant species within a specified number of 50-cm x 200-cm plots distributed in a systematic grid (**Figure 2.4**) throughout the survey area (Element 2). The number of plots varies from 10 to 40, depending on the size of the site. Each plot is subdivided into a series of progressively smaller subplots

(**Figure 3.1**). Each species that occurs in a plot is recorded once by documenting the smallest subplot in which it occurred. Details about field methods for the nested frequency plots are found in SOP 2.

Add SSP Nested Frequency Plots Data Collection Procedures

Processing of collected materials

See Appendix E for guidance about collecting and photographing plants. All collected or photographed unknown plants should be examined as soon as practical during or after the field season to try to identify them. The appropriate datasheets and digital data records should be promptly amended to reflect the correct identity.

Add SSP Processing of Collected Materials

End-of-season procedures

See Element 4 for guidance on data entry (into the monitoring module of the Database when it becomes available, local data files, or a mobile data-collection device) and on developing appropriate metadata. If applicable, all paper datasheets should be stored in a secure location after entry and quality assurance/quality control processes are completed. Datasheets should also be scanned locally for long-term storage.

All electronic data collection and navigation devices, including cameras, should be checked to ensure all data on each device have been downloaded and all devices have been cleaned using the manufacturer's recommended cleaning solution.

Add SSP End-of-Season Procedures

Element 4: Data Management and Analysis

Data management

Data management is key to the long-term success of a monitoring project. This section describes data management for those entering monitoring records into the customized Database. In following this protocol, you may choose to contribute to the Database and make use of its analysis package, or you can opt to enter data independently in an Excel spreadsheet or similar software and conduct your own analyses. If you choose not to use the Database, equivalent data management steps should be taken to protect your records.

Add SSP Data Management

The PRI Management Database

The Database provides a single site for practitioners to systematically document their reconstruction site characteristics, site history, seed mix, planting methods, and ongoing management actions to better understand how these factors may influence the outcome of a planting. By pooling data from many sites, we hope to identify key elements in the prairie reconstruction process that consistently lead to a floristically diverse prairie that establishes quickly, has minimal weed pressure, and maintains its integrity. Several documents related to the database are publicly available at our FWS Service Catalog (ServCat) "Database Project" site.

The Database itself is hosted on a password-protected Department of Interior SharePoint site, which our collaborators may access upon request to one of the PRI Project Coordinators. The user guide provides detail about how to enter data. Appendix F of this document contains a list of fields currently in the Database, which will be modified in the near future to accept monitoring data based on the protocol methods described here.

The Database incorporates a nested spatial structure consisting of overall site, planting unit, and seed-mix area (**Figures 2.1** and **2.2**). The overall site is a broad ownership designation. Each overall site will generally contain several planting units**,** which in turn may each contain one or more seed-mix areas. Within the Database, each documented management action applies to a seed-mix area (although it may be recorded as spot, partial, or complete coverage of that seed-mix area).

The two monitoring methods detailed here are designed to accompany the Database, but they may also be used independently of it. Furthermore, Database participants are not obligated to monitor reconstructions; however, if they choose to monitor, they should monitor each seed-mix area separately as described here to link effectively with the Database. It is imperative that seed-mix area names and extents exactly match in both the monitoring and the planting and management portions of the Database. If seed mixes are highly interspersed, participants should consult with the Database Manager.

As the Database becomes fully developed, the Database Managers will develop a full data management plan and store it in our ServCat "Monitoring Project" along with other project information. In addition, we plan to develop a mobile data entry application using Collector or Survey123 for ArcGIS.

Concurrent with the PRI project record update, the Database Managers and PRI Science Team will perform annual quality control on records stored in the Database and review metadata for necessary updates.

Add SSP PRI Management Database

Data entry, verification, and editing

Appropriate datasheets for both monitoring methods are provided in SM 1. Ideally, observers should review datasheets at the end of each day to correct potential errors when they are still fresh in the observer's memory. If a mobile data-collection device is used in the field, it is important to verify the accuracy of the data as it is collected and at the end of each day, including any automatically filled fields.

Paper datasheets should be filed in a consistent place, and data should be entered in electronic form as soon as possible (to be able to do quality control by memory if field notes are unclear) into the Database on the PRI SharePoint site or into an appropriate local data file. Participants are advised to scan their datasheets at the end of the season and store them in a secure location in case future questions arise. Electronic records should be checked in to the Database daily, or at minimum, the records should be exported to a server or a separate hard drive.

Add SSP Data Entry, Verification, and Editing

Metadata

Metadata provide the context for current and future researchers to interpret a dataset and perform analyses. In the near term, metadata also allow for improved quality control of data. Data standards and other metadata will be reviewed annually concurrent with the PRI project record update. Revisions will be recorded here in subsequent versions of this protocol framework. When a data management plan is developed, this section may refer to that document.

Here we describe metadata at higher levels of the project. When developing a site-specific protocol, you will need to document any additional metadata required for your overall site and seed-mix areas.

Add SSP Metadata

PRI-level metadata

Information on the Prairie Reconstruction Initiative is available to the public in ServCat under reference number 67947. Annual updates to this record should include, at minimum, updating relevant contact information.

Database-level metadata

Metadata for the Database are available to the public in ServCat under reference number 58469. When fully developed, this will include a data management plan for this Database. The current database is temporarily in stored in SharePoint, which does not have traditional data field types typically captured in a data dictionary. However, the "Data fields details" sections of SOPs 1 and 2 provide guidance in the meantime. As we move to a more permanent database, we will develop a full data dictionary for the database.

Site-level metadata

For those using the Database for monitoring records, the site history, planting process, and management should also be entered into existing portions of the Database. A user guide for the existing database can be found in our ServCat project. These metadata will be vital to interpreting the success or failure or restoration projects both at a local level and in broader analyses.

Record-level metadata

The datasheet for each monitoring method (SM 1) contains some basic information that provides context for the records recorded that day. In some cases, observer and date might provide context while performing data entry and quality control (e.g., who can I ask about this record?), while in other cases these fields may be pulled directly into an analysis (e.g., control for observer effects). Take care to fill in all of these fields when conducting monitoring. The "Data fields details" sections of SOPs 1 and 2 provide instructions about how to fill out these fields.

Data standards

The adoption of data standards makes records easy to understand, decreases the need for metadata maintenance, and allows records to be easily integrated with other data sources for analysis. Here is a list of current standards for the Database, which will be revised over time as the Database is updated.

ORGANIZATIONAL CODES (FWS ONLY): An identifier selected from the FWS's Corporate Master Table will be used to identify the organizational unit responsible for reconstructions associated with the FWS.

SPATIAL DATA—METADATA STANDARD: Any spatial data associated with the Database is required to have Federal Geographic Data Committee (FGDC) compliant metadata.

SPATIAL DATA—COORDINATE SYSTEM: In preparation for ArcGIS Online tools, spatial data is preferred in latitude and longitude using the World Geodetic System 1984 (WGS84) datum. However, any fully specified coordinate system that can be projected into the WGS84 system will be accepted. For example, if coordinates are provided in a Universal Transverse Mercator (UTM) coordinate system, the zone must be specified. Nearly all GPS or GIS created files will meet this standard.

SPECIES CODES: The FWS Data Standard for species is the Integrated Taxonomic Information System (ITIS). On paper datasheets, participants are free to use their preferred species codes, but they will be required to select scientific names when records are entered in the database. The Database Managers will be responsible for maintaining the official crosswalked list between species scientific names and their ITIS Taxonomic Serial Number (TSN). This list will be updated at the end of each field season.

SOIL MOISTURE TYPES: Three soil moisture types used to stratify monitoring are derived from the attribute "drainage class" in the Natural Resources Conservation Service Web Soil Survey (SOP 3, in particular **Figure SOP 3.1**).

Planning and Review of Inventory and Monitoring on Refuges (PRIMR)

For FWS participants, a template named "PRI Vegetation Monitoring" is available in PRIMR to help get your record started. You will need to fill in some fields that are specific to your location and your objectives. If you cannot see the template in PRIMR, please ask the R3 Data Manager. Please be sure to link your PRIMR record to this protocol in ServCat.

Data security and archiving

For participants who contribute monitoring data to the Database, all data will be stored on the centralized SharePoint Database. Department of Interior security protocols protect against unauthorized access, and daily backups protect against data loss from disk failure. Additionally, users can only view and modify their own records, protecting against accidental overwriting or deletion of others' records. At the end of each field season, the full dataset will be archived by the Database Manager and stored as an "internal" document associated with the Prairie Reconstruction Initiative ServCat record. Private records will be safeguarded as a part of this process. When a mobile data collection application is developed, this section will be revised.

Add SSP Data Security and Archiving

Analysis methods

The Database Managers and the PRI Science Team will periodically conduct and summarize the analyses from a Database-wide perspective for data entered into the Database to gain a broader understanding of prairie reconstruction. However, biologists or managers may choose to review their own data at the local-level perspective (seed-mix area or overall site) more frequently to respond promptly to management triggers. Database Managers will develop a process to allow contributors to generate an automated report for their own sites incorporating the analyses below. A biologist or manager could also execute these analyses independently. To be clear, many in-depth questions can be answered by a concerted research effort, but this discussion is intended to describe the analyses and interpretations that can be done by most practitioners.

For all analyses, it is important to understand the distribution of your data before selecting a statistical approach. In addition, when looking at multiple years, analyses need to account for correlation among years within an observational unit. For these analyses, an observational unit is defined as a seed-mix area. As long as this protocol is followed, temporal correlation (and spatial correlation, if any) is accounted for, and the distribution of the response is properly modeled (e.g., counts usually follow a Poisson or Negative Binomial distribution rather than Normal), no assumptions are likely to be violated.

Seed-mix area—Meandering Walk

The primary information generated from the meandering walk is a list of all species encountered with their categorical abundance and distribution in each moisture category within a seed-mix area (SOP 1). The species list generated can be compared to the list of planted species as one measure of success, and a floristic quality index (FQI) can be calculated where coefficients of conservatism (CofC) are available (Swink and Wilhelm 1994, https://universalfqa.org). Some analyses can be conducted, however, with care. Species richness and maybe some other variables are area dependent, so if comparing among seed-mix areas or projects of different sizes, these differences would need to be accounted for in analyses of these variables. In addition, this sampling method is subjective, i.e., observers are free to walk where they want, for however long they want. Therefore, effort may vary from observer to observer.

If comparing sites or years surveyed by the same observer, statistical analyses should be fine because it could probably be assumed that all areas were surveyed with a similar amount of effort. However, if comparing two projects that have different observers, it may not be possible to determine if differences are due to environmental conditions, reconstruction methods, observer effort differences, or observer skill differences. Therefore, formal statistical analyses can be conducted, but those doing these analyses need to understand that with this method, the data collected at a particular site are somewhat confounded with the observer that surveyed that site, and it may not be possible to determine if changes observed are real or due to observer differences. We encourage Site Survey Coordinators to keep notes about observer skill level, extreme weather patterns, or other things that could provide insights about differences in data among years.

Even if one decides not to do statistical analyses, summaries of the results of meandering walks can be compared subjectively over time to alert managers to trends that are not in the expected direction. See **Table 1.2** for a comparison of questions answered by the meandering walk and the nested plots methods.

There are many choices of analyses, but we recommend at least performing the following analyses:

- Compare the list of species encountered to the list of known species that were planted (by seed or plug) for this site to get:
    - the proportion of planted species that were expressed
    - the proportion of planted species that failed to be expressed (and which species these were)
    - a list of species that were observed but were not known to be planted
    - species expression through time within and among seed-mix areas
- A list of non-native species with their categorical abundances will alert the manager to possible problems that need to be addressed.
- Estimate species richness (number of species observed), abundance, and distribution.
- A floristic quality assessment using the coefficients of conservatism (CofC) for the appropriate state (a website such as https://universalfqa.org may be used) to produce a FQI and mean CofC for the species list.
- Other analyses may include computing the proportion of grass species to forb species and the proportion of cool-season species to warm-season species.
- Each of these metrics may be compared to previous years' surveys to understand trends among different stages of reconstruction.

If a second meandering walk was conducted in a given year (Element 2), a combined species list may be compiled; however, comparisons among years should be based only on similarly timed surveys.

Add SSP Seed-Mix Area—Meandering Walk Analysis

Seed-mix area—Nested Frequency Plots

For each plot visited during the nested frequency plots survey, species presence within a series of nested plots ranging in size from 1/16 m^2 to 1 m^2 is documented by recording the smallest subplot in which each species is observed (**Figure 3.1**, **Tables 3.2** and **4.1**, and SOP 2). If a species is documented in the smallest subplot (A), it is present in all nested plot sizes (1–5), whereas a species documented in only the largest subplot (E) is considered absent from nested plots 1–4 and present only in nested plot 5 (**Table 4.1**).

Frequency has been long recognized as a reliable and repeatable measure of plant abundance for purposes of monitoring (Curtis and McIntosh 1950, DeBacker et al. 2011). Frequency also avoids observer, seasonal, and weather-related biases that influence cover estimates. In addition to monitoring, frequency has also been used to compare plant species occurring in different treatments, such as disturbance types (i.e., burning, mowing, grazing, spraying), and within vegetation types (Larson et al. 2001, Larson 2003). Indices, such as species diversity and evenness, can be calculated from frequency data. This protocol will allow managers and biologists to assess and compare various practices that are used to implement and manage reconstructions over the long term.

Table 4.1. Species presence/absence for each nested plot is determined based on the smallest subplot in which that species was observed. For each nested plot size, compute frequency for a given species by dividing the number of plots in which the species was present by the total number of plots.

Smallest Subplot Species Was Observed In	Status for Nested Plot 1	Status for Nested Plot 2	Status for Nested Plot 3	Status for Nested Plot 4	Status for Nested Plot 5
A	Present	Present	Present	Present	Present
B	*Absent*	Present	Present	Present	Present
C	*Absent*	*Absent*	Present	Present	Present
D	*Absent*	*Absent*	*Absent*	Present	Present
E	*Absent*	*Absent*	*Absent*	*Absent*	Present

Frequency is sensitive to the overall distribution of each species, but simulation (Heywood and DeBacker 2007) and field (DeBacker et al. 2011) studies have demonstrated that a plot size that results in frequencies of 20–50% provides the greatest statistical power for assessing change over time. This frequency refers to the percentage of plots in which a species is detected within a seed-mix area. By nesting plots of different sizes, frequency can be used to assess trends in species that vary substantially in relative abundance. For example, big bluestem may be dominant in a seed-mix area, resulting in detection in every 1-m^2 plot (nested plot 5), making it impossible to assess any increase. However, it may be detected less often (i.e., in the 20–50% range) in a smaller plot size, perhaps 1/8 m^2 (nested plot 2). To assess changes in frequency of big bluestem over time in this example, the smaller nested plot size should be used to calculate frequency. The optimal nested plot size is the one that yields approximately 35% frequency for that species. A smaller optimal nested plot size indicates greater abundance (Curtis and McIntosh 1950). The nested feature of the plots should be retained throughout monitoring so that optimal nested plot size can be reassessed each sample period.

Sampling is stratified by soil moisture type within a seed-mix area to help ensure representative sampling, and all plots are pooled together for analysis. There are many choices of analyses, but we recommend at least performing the following analyses:

- Calculate the percentage of plots in which a species was observed for each nested plot size to estimate frequency (**Figure 3.1**, **Table 4.1**).
- Determine the optimal nested plot size for the more abundant species (the size that yields closest to 35%). If the initial frequency of a species of interest is between 20% and 50%, it should be possible to track change in a species' frequency over time, whether it increases or declines. The majority of species will always be assessed using the largest plot size.
- Estimates of species richness, evenness index, diversity index, FQI, and mean CofC can be made based on species frequencies in the largest subplot size (Elzinga et al. 1998).

- The fraction of species expressed to species planted should be assessed.
- The proportion of native vs. invasive, cool season vs. warm season grasses, and grass vs. forb can be calculated.
- The relative proportions of the expressed species can be compared to relative proportions in the seed mix.
- Compare changes in frequency of a given species (or suite of species) through time:
    - within a site
    - among sites
- Compare different methods and phases of reconstructions using above metrics.

Note that species richness increases with area monitored. Therefore, it is not correct to simply compare the number of species on seed-mix areas that vary in size unless the same number of nested frequency plots are used at each site. Statistical methods (e.g., rarefaction or extrapolation) exist to compare species richness across seed-mix areas of different sizes where different number of plots are used, but they are beyond the scope of this protocol (see Gotelli and Colwell 2011).

Add SSP Seed-Mix Area—Nested Plot Analysis

Database-wide analysis
When sufficient management data have accrued from participants in the Database, an independent research effort will be conducted to monitor, analyze, and interpret outcomes of many reconstructions. These will be reported in peer-reviewed research papers. The PRI Science Team will work with researchers to identify needed analyses.

Preliminary power analysis shows that, for the nested plots method, a minimum of 15 seed-mix areas in each of two treatment categories being compared will be required to be able to detect a difference in species frequency of 0.15 (illustrated in the graphs in Appendix C). This difference applies to frequency of particular species or functional groups between treatments, which might include seeding method, season of seeding, or other categorical variables that apply to a seed-mix areas as a whole.

Software
For Database-wide analysis, SAS (SAS Institute Inc. 2015) will be used and possibly R package Vegan (R Core Team 2017, Oksanen et al. 2018) to create species-area curves. For the local level, analyses can be conducted in Excel. ArcMap (Esri 2015) is used to generate maps for use in the field.

Add SSP Software

Element 5: Reporting

For participants in the PRI Database, PRI is developing tools to generate simple summary reports from the Database automatically; these reports may also include some of the analyses described in Element 4. The general focus of each report is outlined below. For those not using the database, similar reports may be generated independently.

Survey completion in PRIMR

When monitoring is conducted on an NWRS station, the Site Survey Coordinator should report progress in PRIMR in the annual survey tracking section.

Implications and application

Reports from the PRI Management Database

OVERALL SITE REPORT: A site-specific report can be generated from the Database that summarizes characteristics of seed-mix areas, species planted, planting method and timing, and management practices by year. This report could be useful as an annual management planning tool. Reports will be available from the Data Manager (in the short term) or automatically (eventually) from the Database upon request by the user.

DATABASE-WIDE SUMMARY: A report summarizing the total number of seed-mix areas, the number with monitoring data using each method, and their distribution geographically and by type of organization can be derived from the Database. This report will be generated by the Data Manager and is useful in understanding aggregations of participation among geographic regions, user categories, or types of reconstructed prairie. This information can also indicate when a large scale research effort among sites is appropriate.

Monitoring reports

Users can include their management objectives in the Database, and we encourage inclusion of these objectives in reports. Users will have access only to reports relating to their individual sites and to aggregated data when it is available as part of a Database-wide analysis. Users can create their own reports or, if using the Database, will have access to automatically generated reports.

SEED-MIX AREA REPORT—MEANDERING WALK: Over time, a report based on the meandering walk method would comprise a species list, the sample period(s) in which each species was found at the site, the categorical abundance and distribution of each species at each sample period, and the results of analyses identified in Element 4. The report would allow managers and biologists to track newly observed species, species that have been lost from the site, and changes in species richness of suites of species of interest including native/non-native, grass/forb, etc.

Add SSP Seed-Mix Area Report—Meandering Walk

SEED-MIX AREA REPORT—NESTED FREQUENCY PLOTS: A report based on nested plot data would include the list of species encountered, their frequency by nested plot size during each sample period, and the results of analyses identified in Element 4. The report would allow managers and biologists to compare results over time and among sites.

Add SSP Seed-Mix Area Report—Nested Frequency Plots

DATABASE-WIDE SUMMARY: A report summarizing Database-wide analyses will be produced as such analyses occur. Results may be disseminated via peer-reviewed papers. These data can lead to comparisons among stations in order to gain a broader perspective about characteristics of sites in a geographic area or similar sites within the Database. For example, reconstructed sand prairies could be targeted for more specific information about response to site history, planting techniques, and management. Such analyses can also stimulate development of hypotheses for focused research projects useful to managers.

Reporting schedule

Reports will be generated for each seed-mix area during the winter after the survey. However, biologists and managers may wish to review their own data prior to receiving a report to more promptly respond to threats such as an infestation of an undesirable species. Database-wide reports will be generated approximately every two years.

Add SSP Reporting Schedule

Report distribution

Overall site and seed-mix area reports are available to users for their own sites on request. The Database Manager may issue notifications of the availability of these reports to the designated contact person. With the contact person's permission, reports may also be shared with a wider audience. Database-wide summaries will be available to all interested parties though all personal information will be removed in these summaries. Those not using the Database will be responsible for distributing their own reports.

Add SSP Report Distribution

Element 6: Personnel Requirements and Training

Roles and responsibilities

The Site Survey Coordinator of each overall site is responsible for hiring, training, and supervising the field crew at that site, including data collection in the field, data entry, and proofreading. We expect 1–2 staff will be required for each survey, though this can vary depending on site characteristics and associated management objectives. The Site Survey Coordinator is also responsible for building the plot frame required for the nested frequency plots. For each overall site represented in the Database, there should be one primary contact person, generally the person who is charged with oversight of the data entry. The Database Managers and PRI Project Coordinators are responsible for providing access to the Database, if desired, and guidance for implementing this protocol framework. In addition, the Database Managers and Science Team will develop tools to automatically generate reports based on the summaries described in Element 5 and the analyses described in Element 4; the Database Managers will maintain the Database and the SharePoint site.

Add SSP Roles and Responsibilities

Qualifications

Field personnel

Field personnel should have the training and knowledge to readily identify native and non-native plant species in vegetative condition that are likely to be encountered. Those using this protocol should also have expertise with plant identification keys and methods to collect and press specimens for later identification. The ability to navigate to specific locations using a GPS or by other means is required.

GIS/Mapping

Intermediate GIS or good mapping skills are needed to determine sample points for the nested plots, prepare maps, and upload points to a GPS unit (if used) or paper maps for field navigation.

Add SSP Qualifications

Training

Periodic training in the nested plot and meandering walk field methods may be offered by PRI. Several plant identification sessions are offered across the project area each year; participants can contact PRI Coordinators to learn more. In addition, periodic training in using the Database may be offered via webinar. Training materials will also be available for use of electronic data entry using applications such as Survey123, when the application is available. The local Site Survey Coordinator should assure that field staff are capable of implementing SOP techniques prior to field work.

Prior to beginning surveys, and possibly midway through the season, it is important to have the field crew spend time studying the list of potential species at seed-mix areas with special attention to species that may be difficult to distinguish from one another. They should examine these species in keys and field guides so that they are aware of the likely challenges in identification and understand which characteristics are important to distinguish the more difficult species. It is appropriate to provide the crew with a list of the species planted, as this is a key response variable, and they should be rock solid in identification of these species. Likewise, a list of invasive species in the region will help surveyors become familiar with species they might not otherwise expect to see.

Add SSP Training

Element 7: Operational Requirements

Budget

Implementing this protocol requires staff time but limited equipment. The total budget for a station or organization will depend on how many seed-mix areas are monitored, their size and complexity, which method is used, whether a mobile data-collection device or paper datasheets are used in the field, the experience of the monitoring crew, and the pay rate of each crewmember. **Table 7.1** provides estimates of staff time and equipment cost to implement each method based on prior experience of the authors. There will be cost and time efficiencies if more than one seed-mix area is monitored or both methods are used (e.g., in design/planning, training, and analysis). Additional PRI organizational expenses exist, including developing and managing the Database, coordinating participants, and generating Database-level reports.

Add SSP Budget

Table 7.1. Estimates of staff time and costs for phases of meandering walk and nested plot surveys. Estimates are per year, per person, and per seed-mix area unless otherwise indicated. Equipment costs are likely incurred every few years.

Phase	Staff time Meandering Walk	Staff time Nested Plots	Equipment
Design and Planning	0.5–1 day	1–2 days	
Equipment		0–3 hours[2]	$30 (plot frame—nested plots) $200–300 (mobile device; optional)
Training	1 day * # staff	1 day * # staff	
Field Work	2–4 hours	1–3 days	
Plant ID/Verification	1–8 hours	1–8 hours	
Data Entry and Checking	0.5–2 hours	1–8 hours	
Analysis	0.5–8 hours	0.5–8 hours	
Interpretation and Reporting	0.5–3 day	0.5–3 day	
TOTAL[1]	2.5–7.75 days	2.8–12.4 days	$30–330

[1]Total depends on many factors, including number, size and characteristics of seed-mix areas monitored (see text), number of staff per survey.
[2]Initial assembly of plot frame may take up to 3 hours.

Staff time

Staff time has several components: preparation time in the office, implementation in the field, identification of unknown species, data entry and checking after the monitoring event, and data analysis and interpretation. Preparation including design, planning, plot frame construction, and field crew training will require more time in the initial year of monitoring than in later years.

If using the Database, additional time should be budgeted for data entry. The amount of time it will take to enter the site history and planting data for each site into the Database is variable and depends on the complexity of the seed mix and how easy it is to assemble the various pieces of data. Time is also required to enter ongoing management data into the Database, an essential part of understanding the outcome of a reconstruction.

Estimates of time per survey in **Table 7.1** are based on initial testing of the methods for an 80-acre plot. Nested plot estimates, however, do not include time to walk to and between plots, which can vary greatly depending on terrain and vegetation characteristics. Keep in mind that size and complexity of the site and expertise of the observer(s) influences time needed in the field.

Time needed to resolve unknown species based on photographed or collected specimens and plant identification resources depends on personnel expertise. Based on monitoring experiences, entering and proofreading data from datasheets will take approximately three times longer than entering and checking data on electronic devices. Analysis and interpretation time varies depending on whether the practitioner chooses to depend on automatic reporting from the database or to perform independent analyses. Data analysis and interpretation are the responsibility of the site staff if they are performing monitoring independently of a larger research effort.

A PRI Data Manager and Project Coordinator are needed to oversee the Database and project participation. The Prairie Zone Biologist for FWS R3 is the point of contact for work on NWRS stations and Partners for Fish and Wildlife lands. These positions are currently funded through the FWS Region 3 Division of Natural Resources and Conservation Planning. Current contact information is on the PRI website.

Add SSP Staff Time

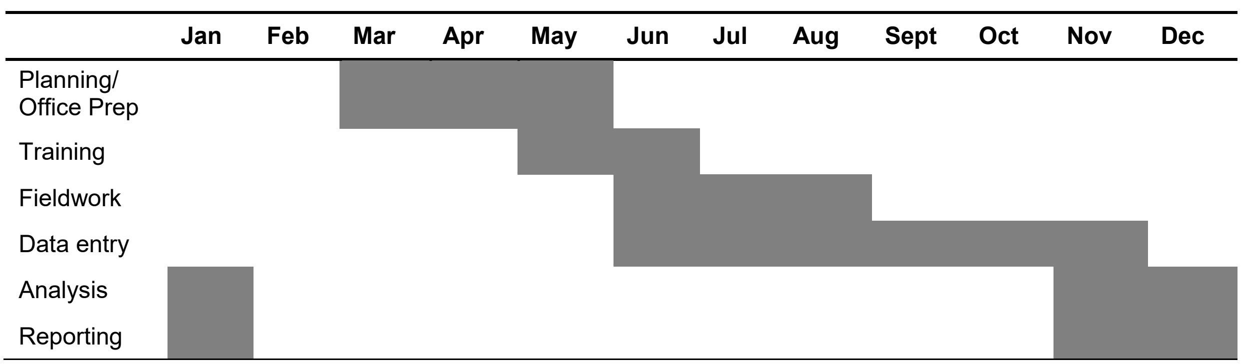
Schedule

A meandering walk is ideally conducted yearly for each seed-mix area to track development. Nested frequency plots could be conducted every 3–5 years after the establishment phase, but a manager or biologist may decide to do it more often, depending on management priorities and needs associated with each seed-mix area. See Element 2 for detail.

Annual field work generally will be completed during early to mid-summer, with planning, office preparation, and training occurring prior to that; data entry, analysis, and reporting occur afterwards (**Table 7.2**). Activities will take place during the window indicated but will not take the entire time. Exact timing of fieldwork should be adjusted by following the guidance in Element 2. Note that for stations or organizations participating in the Database, the Database Managers and Science Team will develop tools to automatically generate analyses and reports.

Add SSP Schedule

Table 7.2. Example annual schedule of activities for monitoring vegetation in prairie reconstructions in the Upper Midwest.



Annual tasks for Database Managers and PRI Coordinators

- Update the project record
- Update data management plan
- Review metadata (all levels)
- Review and update data standards (including adding new species to ITIS crosswalk)
- Quality control on records in Database

Coordination

The Site Survey Coordinator is responsible for local coordination of monitoring. Each overall site participating in the Database should work with the PRI Project Coordinators and Database Managers to ensure data integrity. The PRI Science Team will provide coordination for a larger analysis of multiple sites at appropriate intervals. This protocol framework may also be used independently without participating in the Database.

Add SSP Coordination

Element 8: References

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Add SSP References

Appendices

Appendix A: Guidance for Creating a Site-Specific Protocol

Each US Fish and Wildlife Service (FWS) station using this Protocol Framework for Monitoring Vegetation in Prairie Reconstructions (framework; McColpin et al. 2019) needs to create a stepped down site-specific protocol (SSP). The following guidance provides a standard format for documenting appropriate site-specific information that aligns with the framework.

To begin the process, download the Word version of the complete framework. This version will already have template styles and be 508c compliant. Refer to the companion template instructions document for guidance about 508c compliance and to resolve formatting problems. Any tables added in this appendix should use the "Protocol Table Title – Appendix" style for the title; any figures should use the "Protocol Figure Caption – Appendix" style for the caption. These styles will automatically number the tables and figures. When the list of tables and list of figures are updated, the new tables will appear there. After adding all site-specific information, the table of contents should be updated.

All site-specific information will be detailed in Appendix A with convenient links from the framework to the appropriate place in Appendix A so that methods and rationale are easily accessible. These links throughout the framework prompt a user to add SSP information. The user can link back and forth. This keeps all the site-specific information in one place and keeps the framework verbiage intact. To see the bookmarks, go to File->Options->Advanced->Show document content, and check the box for Show bookmarks. Take care not to delete the bookmarks in this appendix.

We encourage non-FWS organizations to adapt the site-specific template based on specific organizational objectives and guiding documents. You may use this guidance for that purpose by deleting any FWS logos and language and including details appropriate to your organization.

In this appendix, black font indicates standard headings and suggested standard information; red font indicates information needed from your overall site. In your final document, all site-specific information should be recorded in Appendix A and clearly stand out from the original framework; we suggest using red font. Blue font in this appendix indicates formatting instructions and should be deleted.

We are developing a template for the Planning and Review of Inventory and Monitoring on Refuges (PRIMR) database, which will provide a standardized format for FWS stations to enter a survey based on this protocol. Work with the Prairie Reconstruction Initiative (PRI) Coordinator and/or your Zone Biologist when developing a site-specific protocol and entering survey information into PRIMR.

For FWS, instructions for entering site-specific protocols into ServCat may be found at https://ecos.fws.gov/ServCat/DownloadFile/102128

The rest of this appendix has headings consistent with the framework and guidance about details that should be included in a site-specific protocol.

Title, Authors, and Signature Page

The SSP must include a title page including authors, and a signature page; refer to the companion template instructions document for guidance.

Add title page including authors and a page for signatures of approving officials.

SSP Summary

SUGGESTED STANDARD SUMMARY (may be altered): This site-specific protocol is based on the framework, which contains the overall background, design, and methods used. The framework contains two complementary vegetation monitoring methods for assessing outcomes of reconstructions: a meandering walk and nested frequency plots. This document includes details particular to Station Name, including any departures from the framework.

Add a short paragraph summarizing how this protocol relates to the station Inventory and Monitoring Plan (IMP).

Limit the Protocol Summary to approximately 400 words.

Acknowledgments

Add acknowledgements if desired. There is no need to acknowledge the framework or its authors.

Narrative

Element 1: Introduction

Background

SUGGESTED: This SSP is stepped down from the framework. The framework was developed in response to a need articulated by the Prairie Reconstruction Initiative, a diverse group of public and private practitioners and researchers seeking to understand and improve practices or conditions that lead to long-term success in prairie reconstruction.

Insert refuge-specific information providing an overview of refuge location, history, purpose, and extent. Include background about why reconstructing prairie is important in the context of that refuge. FWS staff can copy and paste from the Inventory and Monitoring Plan (IMP), Habitat Management Plan (HMP), or the Comprehensive Conservation Plan (CCP) with some editing to make it coherent as a stand-alone section. Keep it less than one page.

Add vicinity map (from HMP or CCP or other) showing where the refuge sits in the landscape.

Objectives

Management objectives

Add any management objectives (from the station HMP for FWS) that are relevant to prairie reconstruction.

Sampling objectives

Sampling objectives provide the specifics for measuring the resource or related indicator targeted in the management objectives. These sampling objectives should reflect rationales for surveys in the station Inventory and Monitoring Plan (IMP) for FWS. For this protocol to be appropriate, the meandering walk or the nested frequency plots (or both) should be able to measure the metric(s) identified in the sampling objectives.

For each management objective identified above, the refuge needs to specify at least one appropriate sampling objective.

See Appendix B in the framework for guidance in writing objectives.

Element 2: Sampling

Sample design

Followed design as described in the framework. However, indicate if there are changes.

Sampling units, sample frame, and target universe

The sample unit is each seed-mix area, stratified by soil moisture type as described in the framework. For the nested plots, the rectangular (50 cm x 200 cm) nested frequency plots within a given seed-mix area are considered subunits, which taken together give frequency of occurrence within that seed-mix area for a given species.

Sample selection and size

Indicate which seed-mix areas will be monitored using each method (meandering walk, nested frequency plots); include their sizes, and insert a map. There should be some justification why particular plantings were chosen for monitoring. A general sampling scheme can be designated, such as all areas planted after a certain year, all reconstructions on the refuge, or reconstructions planted with a seed mix having more than a certain number of species.

Survey timing and schedule

Indicate what phenological or other cues will be used, if any, to trigger the survey. Indicate why this timing was chosen. Note approximate start date, estimated end date, and survey frequency (when you intend to repeat the survey) for each method in each seed-mix area. It is useful to develop an annual monitoring schedule for a period of years (e.g., 10 years) and note changes to that schedule through time and reasons for changes.

Sources of error

As described in the framework. Add any additional sources of error that might be unique to this station.

Element 3: Field Methods and Processing of Collected Materials

Pre-survey logistics and preparation

Meandering walk

MAPPING: As described in the framework. Indicate any changes in mapping methods unique to this station. If maps will be produced manually, describe the methods used for creating the maps and for finding sampling points.

The final GIS layer(s) for each seed-mix area surveyed, including coordinate system, datum, and types of features used, should be appropriately archived. Indicate where those layers will be stored and who created them. Also indicate where the other details below will be stored and the procedures for updating that document.

- For the meandering walk the layers include:
    - Survey polygons
    - Soil moisture polygons

If an entire seed-mix area will not be surveyed, explain why the entire area is not included. If sampling in subareas, document how they will be identified and delineated.

PLANTED AND PREVIOUSLY OBSERVED SPECIES LIST: For each seed-mix area, if a list of planted or previously observed species will be used during monitoring, indicate how it will be developed, where it will be stored, and the process for updating it as new species are observed. Describe how and when it will be used, such as prior to the field season for review, or during data collection. (Note that the use of previously observed species lists during sampling can bias species identifications.)

Nested frequency plots

MAPPING: As described in the framework. Indicate any changes in mapping methods unique to this station. If maps will be produced manually, indicate the methods used for creating the maps and for finding sampling points.

The final GIS layer(s) for each seed-mix area surveyed, including coordinate system, datum, and types of features used, should be appropriately archived. Indicate where those layers will be stored and who created them. Also indicate where the other details below will be stored and the procedures for updating that document.

- For nested frequency plots the layers include:
    - Survey polygons
    - Soil moisture polygons
    - Nested frequency plots grid points

Indicate the method used to lay out the grid points, and the process for eliminating or moving points, if necessary (Element 3). For each seed-mix area, document that the total number of plots follows the sampling scheme indicated in the framework or justify why a different number of plots was chosen. Use caution if reducing the number of plots to assure that data analysis is valid. For each seed-mix area, document that the plots are distributed proportionally in each soil moisture type.

PLANTED AND PREVIOUSLY OBSERVED SPECIES LIST: For each seed-mix area, if a list of planted or previously observed species will be used during monitoring, indicate how it will be developed, where it will be stored, and the process for updating it as new species are observed. Describe how and when it will be used, such as prior to the field season for review, or during data collection. (Note that the use of previously observed species lists during sampling can bias species identifications.)

PLOT FRAME: For nested frequency plots, indicate whether the standard plot frame with five subplots will be used (1/16 m^2 up to 1 m^2) or whether additional (or fewer) subplots will be used (Elements 3 and 4 in the framework).

Establishment of sampling units

Indicate what type of GPS unit or mobile device will be used to locate plots in the field. If none is used, describe method to locate plots. Consider acceptable range of error when choosing a GPS unit.

Data collection procedures

The field methods will be implemented as described in Element 3 and SOPs 1 and 2 of the framework. Indicate changes to the field methods, if any, and explain why changes will be implemented.

Indicate whether paper datasheets or a mobile data collection device and application will be used.

For nested frequency plots, indicate the predetermined method for orienting plots in the field.

Indicate the taxonomic reference used for plant names (e.g., USDA Plants, Flora of North America, ITIS). If abbreviations such as those used for FQA will be used, indicate how a crosswalk between abbreviations on the datasheets and scientific names will be created and where it will be stored.

Processing of collected materials

Indicate any site-specific guidance about processing collected materials.

End-of-season procedures

As described in the framework. Add any site-specific details.

Element 4: Data Management and Analysis

Data management

Indicate site-specific guidance for data management.

PRI Management Database

Add information about participation in the PRI Management Database such as seed-mix areas, rationale for selection, and other relevant details.

Data entry, verification, and editing

As described in the framework, with site-specific details indicated below.

Identify which of the following methods will be used for data entry:

- Enter data electronically in the field onto a tablet, smartphone, or other device equipped to access the PRI map within Collector for ArcGIS (when this tool becomes available); indicate application used such as Survey123 (when this tool becomes available).
- Record data on paper forms using the versions of the datasheets available in SM 1 of the framework. Data on paper datasheets will be entered by participants into the PRI online database (when it becomes available) or into a local electronic format [indicate what format, e.g., Excel, Access]. If using monitoring data in conjunction with the Database, check with PRI Coordinator to assure format compatibility.

Identify any other data management procedures that will be used at the refuge (other than what is described in the framework).

Metadata

As described in the framework. Some details are documented in the various sections of this site-specific protocol. Indicate other site-specific details, if necessary.

Indicate where a list of the people (and their roles) who collected and managed the data for each survey will be stored and how it will be updated.

Indicate where notes about the reliability of the data from each survey will be stored and how they will be updated as new surveys are conducted.

Data security and archiving

As described in the framework. Indicate site-specific details.

PRIMR

For FWS Stations: Provide the PRIMR reference for this survey.

ServCat

For FWS Stations: Indicate ServCat location where this document and associated data will be archived.

Analysis methods

As described in the framework. Indicate site-specific details.

Seed-mix area—Meandering Walk

As described in the framework. Indicate site-specific details.

Seed-mix area—Nested Frequency Plots

As described in the framework. Indicate site-specific details.

Software
As described in the framework. Indicate site-specific details.

Element 5: Reporting

As described in the framework, except as detailed below.

Implications and application
Seed-mix area report—Meandering Walk
Add site-specific details as necessary.

Seed-mix area report—Nested Frequency Plots
Add site-specific details as necessary.

Reporting schedule
Add site-specific details.

Report distribution
Indicate site-specific details.

Element 6: Personnel Requirements and Training

As described in the framework, except as detailed below.

Roles and responsibilities
Indicate who serves as the Site Survey Coordinator. Also indicate observers, data recorders, data enterers, proofreaders, etc., and how this information will be updated in the future.

Qualifications
Indicate any site-specific details.

Training
Briefly describe any training that is particular to that refuge (e.g., at Cypress Creek, training in species identification was conducted by XX with both a lecture/sample portion and a field portion of the day).

Element 7: Operational Requirements

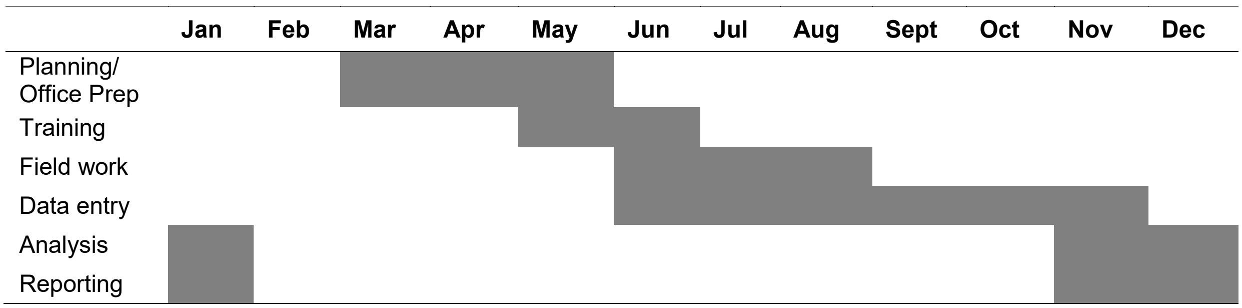
Budget
Provide an estimated annual budget for the anticipated number of seed-mix areas that will be monitored using each method.

Staff time
As described in the framework. Indicate site-specific details.

Schedule

As described in the framework. Indicate site-specific details.

Table A.1. Example annual schedule of activities for monitoring vegetation in prairie reconstructions at Station Name. [Revise schedule to fit station needs.]



Coordination

As described in the framework. Insert any site-specific details.

Element 8: References

Citations should be listed in alphabetical order by primary author's last name. For FWS Site-Specific Protocol, formatting for citations within the text and references section should follow the style requirements outlined in the *Journal of Fish and Wildlife Management* online guide for authors.

McColpin AC, Drobney PM, Larson DL, Ahlering MA, Buhl DA, Dixon CS, Vacek SC, Walker BA. 2019. National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions. US Fish and Wildlife Service, National Wildlife Refuge System, Division of Natural Resources and Conservation Planning. Available: https://ecos.fws.gov/ServCat/Reference/Profile/113970 (Month and year accessed in parentheses)

Insert any other references cited (HMP, CCP, etc.).

Appendices, Standard Operating Procedures, Supplemental Materials

Follow the instructions in the protocol template (see first page of this appendix) for adding Appendices, SOPs, or Supplemental Materials, if needed. They should be added in the appropriate place in this document after Appendix F, SOP 4, or SM 1.

Appendix B: Developing Objectives

In order to successfully monitor and evaluate the condition of prairie reconstructions, both management and sampling objectives must be articulated. Each objective should have Specific, Measurable, Achievable, Results-oriented, Time-fixed (SMART) elements that relate to the protocol (FWS 2004). Objectives are important because they articulate questions to be answered by monitoring. The documents referenced at the end of this appendix (FWS 2004, FWS 2013) contain detailed information about how to construct objectives.

The below discussion includes examples of measurable management and sampling objectives to illustrate the relationship between management and monitoring. In our examples below, a manager is interested in developing high quality prairie that will also serve as habitat for a diversity of prairie invertebrates. Key questions about the floristic results of prairie reconstruction might be as follows:

Given our planting and management methods:

- Will planting a highly diverse seed mix during the initial planting event result in a highly diverse plant community through time?
- How long does it take before maximum establishment of floristic diversity is expressed?
- Will conservative species show up in the first year or later? How successful will they be in establishing overall, and, floristically, how will the planting compare to a remnant prairie?
- To what degree will exotic species inhibit our objectives?

An example of a management objective could be:

Create quality pollinator habitat and connect native prairie remnants by planting 200 acres of prairie within 2 years from present on former cropland at Prairie Haven NWR, using a diverse seed mix (at least 100 species), to establish a prairie community including all of the native planted species, with 30% of the species having a coefficient of conservatism of 5 or above, 60% of vegetation being forbs, less than 30% of exotic cover or density and a Floristic Quality Index (FQI) of at least 30 within 8 years.

Associated sampling objectives could read as follows:

On 200 acres of newly planted prairie, record establishment of species richness annually to detect if and when all native planted species establish and in what year each species is first observed.

With 95% confidence, detect whether 30% of established species have a coefficient of conservatism of 5 or above within 8 years, with no more than a 10% chance of concluding that these conditions were not met (i.e., not rejecting null hypothesis) when in fact they were met.

With 95% confidence, detect whether 60% of established native species are forbs and if the plant community attained a FQI of 30 within 8 years, with no more than a 10% chance of concluding that these conditions were not met when in fact they were.

With 95% confidence, detect if exotic species cover or frequency (index of density) is 30% or less, with no more than a 10% chance of concluding that these conditions were not met when in fact they were.

In this example, the following characteristics necessary for monitoring and analysis were addressed as follows:

1. What will be surveyed – plant species
2. Where the survey will be conducted – at Prairie Haven NWR on 200 acre planting
3. The attribute actually measured or estimated –
    - plant species richness
    - frequency (index of density) of the 100 planted prairie species expressed
    - frequency (index of density) of additional (not planted) native and exotic species
4. The measurable state of the attribute – species richness, % forb species, % natives vs. exotic species, FQI
5. The time frame of the survey – within 8 years of planting
6. The target response from management – establishment of 100 planted native species

Sampling objectives should also include the following to guide analysis:
7. Desired accuracy of estimates – 95% confidence
8. Magnitude of change one wants to detect – increase from 0 to 100 native species; FQI of 30
9. Chance of error you are willing to accept - 10% or less
10. Power to detect a change of a specified magnitude – 90%

References
[FWS] US Fish and Wildlife Service. 2004. Writing refuge management goals and objectives: a handbook. Washington, DC: US Department of Interior, Fish and Wildlife Service. Available: https://www.fws.gov/refuges/policiesandbudget/pdfs/writingrefugegoals_022504.pdf (July 2019).

[FWS] US Fish and Wildlife Service. 2013. How to develop survey protocols, a handbook (Version 1.0). Fort Collins, Colorado: US Department of Interior, Fish and Wildlife Service, National Wildlife Refuge System, Natural Resource Program Center. Pages 11-14. Available: https://ecos.fws.gov/ServCat/DownloadFile/19346 (July 2019).

Appendix C: Power Analysis Simulations for Nested Frequency Plots

A series of simulations was performed to estimate power to detect a specified difference in mean frequency between two groups (e.g., two seed mixes, two planting methods, two locations) with specified sample sizes (i.e., number of seed-mix areas in each group and number of nested plots within each seed-mix area).

The first set of simulations assumed a set number of plots per seed-mix area, regardless of size of the seed-mix area. Analysis was done by simulating data for *n* seed-mix areas with *m* plots within each seed-mix area for two groups. The response variable is a frequency as would be computed from a nested plot design, i.e., the proportion of the m plots in which a species was observed. This proportion was simulated for each seed-mix area by drawing from a binomial distribution with probability of *p*. The probability for the second group differed from the first group by a difference of *d*. The simulated data were then analyzed using logistic regression. This simulation was repeated 1000 times, and the power was computed as the proportion of runs in which a significant difference was detected between groups at a significance level of 0.05.

The simulation was run over a range of values for: the number of seed-mix areas (*n*), number of plots within each seed-mix area (*m*), average frequency (*p*), and differences between average frequencies (*d*).

- *n* = number of seed-mix area; used 5, 10, 15, 20, 25, and 30
- *m* = number of plots within each seed-mix area; used 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100
- *p* = average frequency; used 0.1, 0.3, and 0.5
- *d* = difference between average frequencies; used 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3.

However, binomial data collected in the field (such as this frequency data), are often overdispersed (i.e., the data exhibit greater variation than is assumed under the binomial distribution). Overdispersion is often caused by clustered or correlated data. Ignoring overdispersion can result in underestimated standard errors, underestimated p-values, and inflated type I error rates. Diane Larson (Research Wildlife Biologist, USGS) provided some data in which the nested plot sampling method was used to collect frequency data; analysis of these data revealed that they were slightly overdispersed. Therefore, the simulation described above was rerun but this time simulating overdispersed data.

The analysis was repeated by simulating data for *n* seed-mix areas with *m* plots within each seed-mix area for two groups. The response variable is a frequency as would be computed from a nested plot design, (i.e., the proportion of the *m* plots in which a species was observed). This proportion this time was simulated for each seed-mix area by drawing from a beta-binomial distribution (which will result in overdispersed binomial data) with probability of *p* and variance of *v*. The probability for the second group differed from the first group by a difference of *d*. The simulated data were then analyzed using logistic regression with an overdispersion correction. This simulation was repeated 1000 times, and the power was computed as the proportion of runs in which a significant difference was detected between groups at a significance level of 0.05.

The simulation was run over a range of values for: the number of seed-mix areas (*n*), number of plots within each seed-mix area (m), and differences between average frequencies (*d*). To generate beta-binomial data, an average frequency (*p*) and a variance (*v*) need to be specified.

n = number of seed-mix areas; used 5, 10, 15, 20, 25, and 30

m = number of plots within each seed-mix area; used 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100

d = difference between average frequencies; used 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3

p = average frequency; used 0.1, 0.3, and 0.5

v = variance; used 0.01, 0.03, and 0.05.

A plot of power versus number of plots (*m*) for each difference (*d*) was generated for each level of number of seed-mix areas (*n*). We examined all the plots generated; for simplicity, we present here only the most relevant. Plots are given for an average frequency $p = 0.3$ and variance $v = 0.01$ (**Figure C.1**). This variance was most similar to the overdispersion of the data provided by Diane Larson. The frequency of 0.3 is near the optimal frequency for detecting change (Element 4).

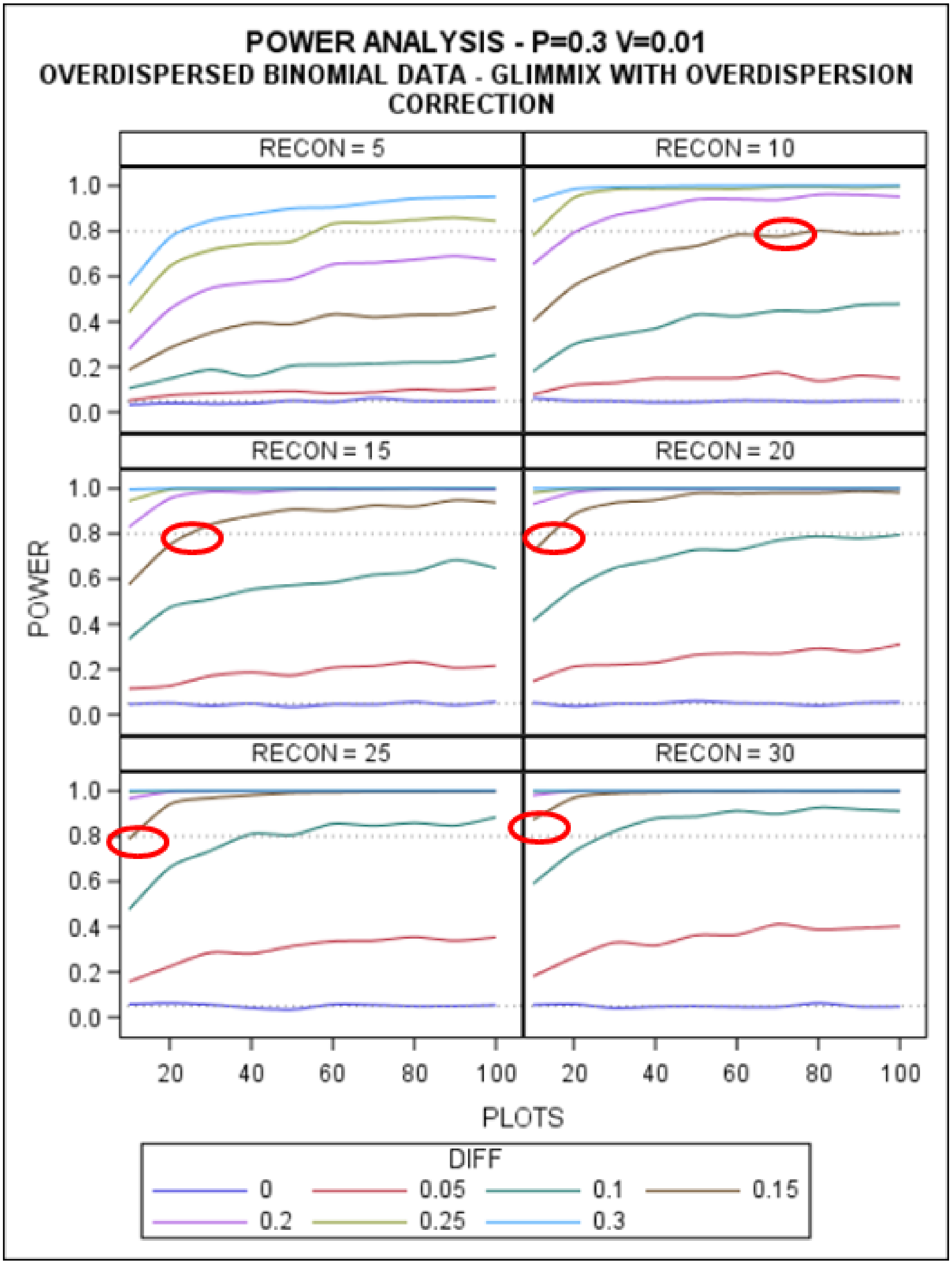


Figure C.1. Results of power analysis for a set number of plots per seed-mix area. Shown is the relationship between power and the number of plots per seed-mix area. Red ovals indicate the point at which it is possible to detect a difference in frequency of 0.15 at power = 0.8 for differing number of seed-mix areas (RECON) in each group being compared.

The second set of simulations incorporated a variable number of plots depending on the size of the seed-mix area. The simulations were first run assuming binomial distribution of frequency data (as for the first set) and then repeated assuming overdispersed data as described below.

The analysis was done by simulating data for *n* seed-mix areas. The number of plots (*m*) per seed-mix area is allowed to vary by some rate (*r*) that is based on size of the seed-mix area (e.g., 2 plots per hectare, 1 plot per 2 hectares) but with a minimum (min) and maximum (max) number of plots per seed-mix area. The area of each seed-mix area was simulated using a lognormal distribution with a minimum size of 1 ha (2.5 acres) and a maximum size of 81 ha (200 acres). The response variable is a frequency as would be computed from a nested plot design, (i.e., the proportion of the *m* plots in which a species was observed). To simulate overdispersed binomial data, this proportion was simulated for each seed-mix area by drawing from a beta-binomial distribution (which will result in overdispersed binomial data) with probability of *p* and variance of *v*. The probability for the second group differed from the first group by a difference of *d*. The simulated data were then analyzed using logistic regression with an overdispersion correction. This simulation was repeated 1000 times, and the power was computed as the proportion of runs in which a significant difference was detected between groups at a significance level of 0.05.

The simulation was run for various levels of each of: the number of seed-mix areas (*n*), differences between average frequencies (*d*), plot sampling rate (*r*), minimum number of plots per seed-mix area (min), and maximum number of plots per seed-mix area (max). To generate beta-binomial data, an average frequency (*p*) and a variance (*v*) need to be specified.

n = number of seed-mix areas; used 5, 10, 15, 20, 25, and 30
d = difference between average frequencies; used 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3.
r = plot sampling rate; used 3 plots per hectare, 2 plots per hectare, 1 plot per hectare, 1 plot per 2 hectares, 1 plot per 3 hectares, and 1 plot per 4 hectares
min = minimum number of plots per seed-mix area; used 10 and 20
max = maximum number of plots per seed-mix area; used 40 and 80
p = average frequency; used 0.3
v = variance; used 0.01 and 0.02.

A minimum and maximum number of plots per seed-mix area were included for two reasons. First, for really small seed-mix areas the number of plots would end up too small to get a reliable estimate of frequency. Therefore, setting the minimum equal to 10 or 20 assures there is an acceptable sample size to compute frequency for all seed-mix areas. Second, for some of the sampling rates (e.g., 3 plots per hectare), the number of plots needed in a very large seed-mix area would get large (e.g., 240 plots in a seed-mix area of 80 ha) and may not be feasible in the field. So setting a maximum number of plots per seed-mix area would keep the numbers of plots at reasonable levels.

A plot of power versus number of seed-mix areas (*n*) for each difference (*d*) was generated for each level of the plot sampling rate (*r*). We examined all the plots generated; for simplicity, we present here only the most relevant. Plots are given for an average frequency *p* = 0.3, variance *v* = 0.01, minimum of 10 plots and maximum of 40 plots (**Figure C.2**). This variance was most similar to the overdispersion of the data provided by Diane Larson. The frequency of 0.3 is near the optimal frequency for detecting change (Element 4). The graphs not depicted here showed minimal increase in power by raising the minimum number of plots to 20 or the maximum to 80.

These simulations may be overly cautious because the range and distribution of seed-mix area sizes assumed for the power analysis resulted in most of the seed-mix areas being small. Therefore, for most plot sampling rates considered in the simulation, the majority of the seed-mix areas would have had the minimum sample size. These seed-mix areas with the minimum sample size seem to be having the most influence on the power so that the few large seed-mix areas with lots of plots are having little effect on power. In reality, we expect most seed-mix areas to be large enough to require more than the minimum sample size.

Based on these simulations, we recommend a sampling rate of 3 plots/ha, with a minimum of 10 plots and a maximum of 40 plots.

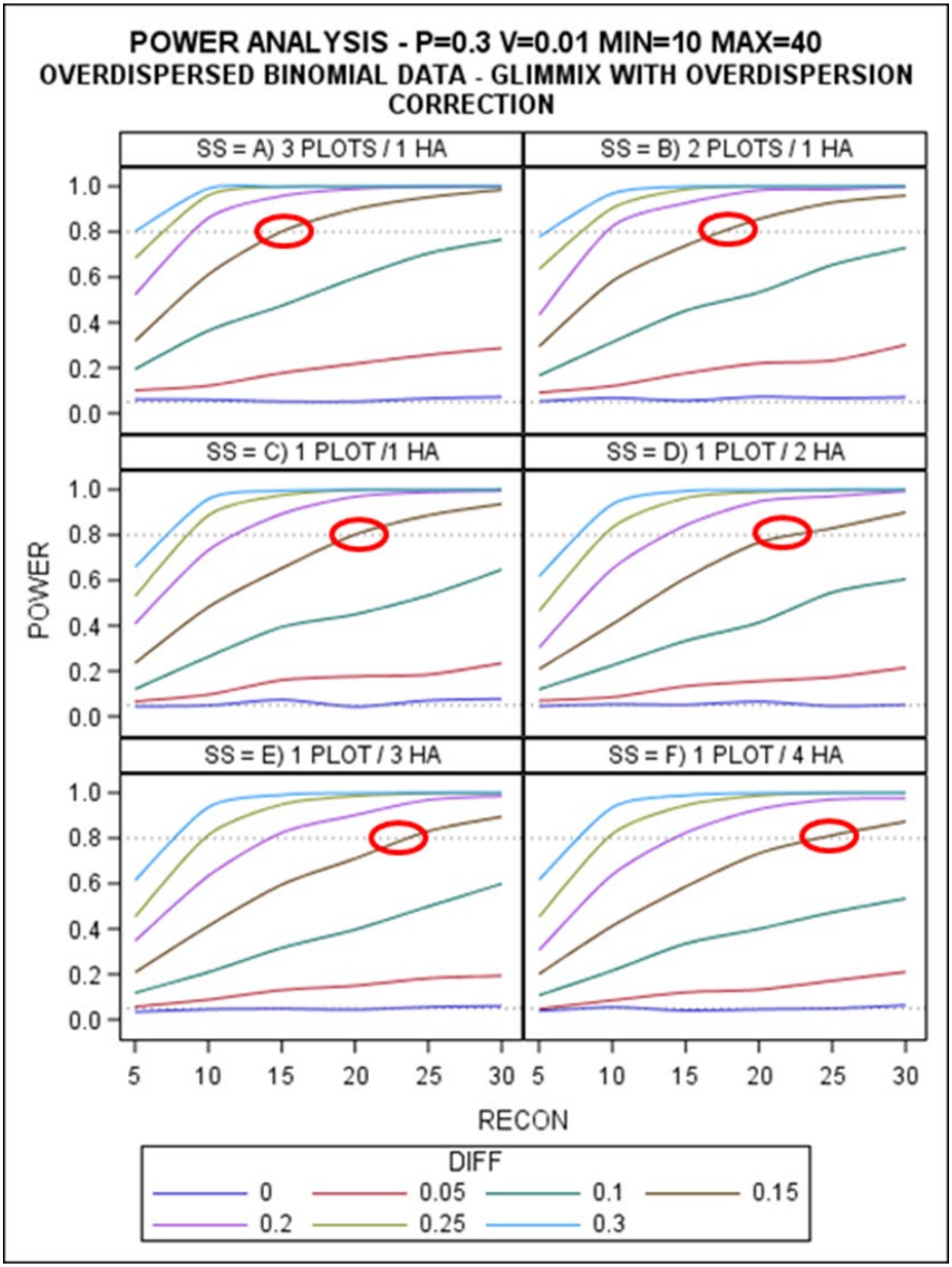


Figure C.2. Results of power analysis where number of plots varies with size of the seed-mix area. Minimum of 10 plots, maximum of 40 plots/seed-mix area, and starting frequency of 0.3. Shown is the relationship between power and number of seed-mix areas (RECON) sampled. Red ovals indicate the point at which it is possible to detect a difference in frequency of 0.15 at power = 0.8 for differing sampling intensities. The highest sampling intensity (3 plots/1 ha) is in the upper left, the lowest (1 plot/4 ha) in the lower right.

Appendix D: Plot Frame Construction

Dimensions for the plot frame are shown in **Figure D.1 (a)**. The plot frame is typically made from 0.5-inch PVC pipe (lengths needed are shown in **Figure D.1b**), which can be obtained at most hardware stores, but you are free to use other materials as long as internal dimensions (**Figure D.1a**) are preserved. The construction depicted allows a user to temporarily remove pieces to facilitate placing the frame in tall vegetation. The frame should be reassembled before reading the plot to accurately determine which individual plants are in each subplot.

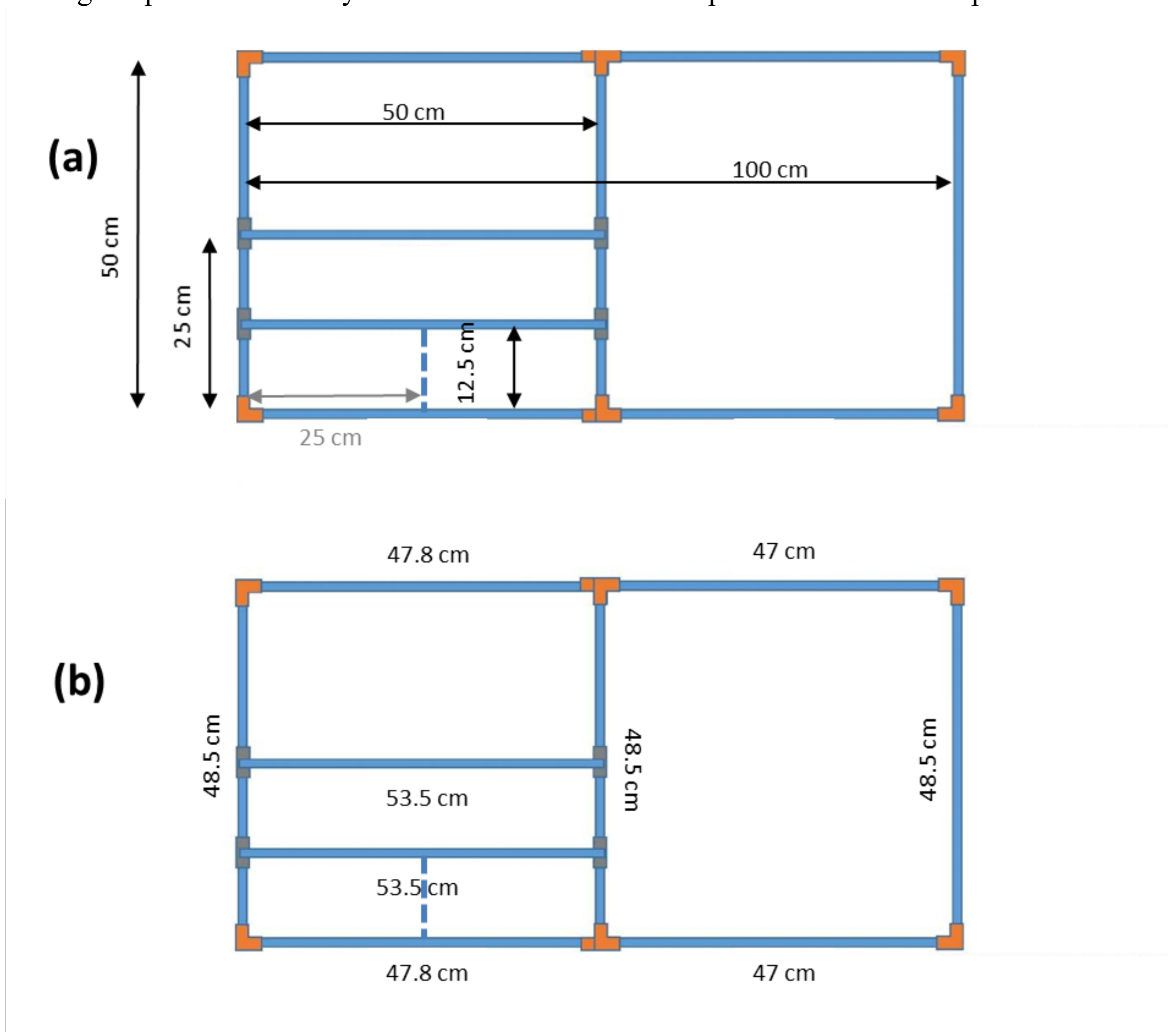


Figure D.1. Nested plot frame. **(a)** Internal dimensions of the nested plot frame. The dotted line indicates where an additional subdivision could be made. The desired overall internal space is 50 cm x 100 cm. **(b)** Measurements when using 0.5-inch-diameter PVC. Measurements for individual pieces are adjacent to that piece. Lengths differ due to differences in the internal length of each side of the T-connector. Also needed are clamps (represented by gray rectangles) and screws to hold them in place. This allows the user to remove these pieces once the plot frame is "flipped." These subplot dimensions have been used in tallgrass prairie reconstructions, but in some cases the smallest subplot may need to be halved (dashed line) if any species are found to occur in more than about 50% of the 12.5 cm x 50 cm subplots (Element 3). Consult the PRI Coordinator if you think you may need smaller subplot sizes.

Appendix E: Photographing and Collecting an Unknown Plant in the Field for Future Identification

If observers are uncertain of the identification of a plant, they are encouraged to take photographs and/or to collect and press a specimen for later identification. In the field, photograph the specimen before collection, being sure to include identifying features of the plant as well as an overall view showing the plant's growth habit and surroundings. Characteristics of importance in plant identification vary by species. Be cautious when collecting plants to avoid collecting a rare native plant. The observer needs to balance the risk of harming a rare or uncommon native plant with the risk of being unable to identify an unknown species. In general, if there are fewer than about 10 plants of that species in the local vicinity, consider only photographing the plant, including a reference for scale (like a ruler), to help in later identification. When photographing, it can be useful to bring a piece of fabric placed in such a way as to isolate details of the plant from the surrounding vegetation in the photograph.

Identifying features often important to include in photographs:

- Stems
    - Amount and/or angle of branching
    - Degree, type, and location of hairiness
    - Presence of bracts or types of nodes
    - Height and width
- Leaves
    - Arrangement on stem
    - Shape, size, and margin type
    - Venation patterns
    - Degree of hairiness, above and below
    - Length or absence of petiole (stalk) and bracts
    - Lower vs. upper leaf characteristics including presence or absence
    - Ligule (Poaceae)
- Flowers/fruits/seeds
    - Petal arrangement, shape, and color
    - Sepal arrangement, shape, and color or absence
    - Ligule (Asteraceae)
    - Degree of hairiness
    - Length of pedicel and/or peduncle (stalk of flower and cluster, respectively)
    - Fruit/seed shape, size, color, and ornamentation

When an unknown species is fairly common, it can be collected. For common species, a specimen including roots may be collected outside the plot; some species require roots for positive identification. For somewhat less common species, collect the specimen by clipping the above ground part at ground level (some characteristics depend on lower leaves). Include tape or tag with collection information (collector name, date, seed-mix area and plot association, suspected identity) and place specimen in a plastic bag with a damp (not wet) paper towel until returning to the office or lab. Specimens keep better if they are kept cool; a nearby cooler where specimens can periodically be deposited in the field is helpful. All collected specimens and photographs (especially the photo numbers) should be noted in the comments fields in the

Database or datasheet; supplement with data in a notebook if necessary. It can be useful to include a note with site/plot information in each photograph to keep photos organized.

Once at the lab, collected specimens should either be identified immediately or be promptly pressed using a standard plant press and pressing process. All photographs and pressed specimens should be labeled with the date, seed-mix area, plot/subplot, and collector to link back to the appropriate survey and reference person. Specimens should be stored in the location designated by the Site Survey Coordinator. Once identified, unknown species that were photographed and/or collected should be updated on datasheets and in digital files with the correct name.

See the below or similar websites for further guidance (accessed November 2018):

Missouri Botanical Garden—Pressing Plants:
http://www.mobot.org/MOBOT/molib/fieldtechbook/pressing.shtml

NRCS—Florida ECS Quick Tips:
https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/flpmctn12688.pdf

The Fabulous Garden Blog—How to Photograph Plants for Identification:
https://thefabulousgarden.com/blog/2018/05/04/how-to-photograph-plants-for-identification/

Appendix F: List of Fields in the PRI Management Database

The Database provides a single site for practitioners to systematically document details about the location, planting, and management of prairie reconstructions to better understand how these factors may influence the outcome of a planting (Element 4). The data described below are not part of the monitoring protocol per se but will be used by a larger effort to relate monitoring results to site characteristics and management history. Many of the following fields are optional and will not be known for some plantings. In addition to these fields, many Database forms have a comments field, a place to attach supplementary information, data quality rating, and a contact person.

- Overall Site
    - Overall Site name
    - Area (acres)
    - Organization type
    - Managing organization/landowner
    - Latitude and longitude of a central point
- Planting Unit Information
    - Planting Unit name
    - Area (acres)
    - Basic objectives for the planting
- Seed-Mix Area
    - Name of this unique seed-mix area (e.g., Unit1-Wet)
    - Area (acres)
    - NRCS soil drainage class(es) (excessively drained, somewhat excessively drained, well drained, etc.)
    - Soil type (clay, clay loam, silty loam, etc.)
    - Target prairie type (dry, wet, mesic, sand, limestone, other)
    - Invasive species present at time of planting
- Seed-Mix Area History
    - Year Seed-Mix Area was planted to prairie
    - General 10-year history of the Seed-Mix Area (# years cropped, # years pasture, # years hay, # years established vegetation)
    - Detailed history of the Seed-Mix Area—for each year, up to 10 years:
        - Cropped? What type? Herbicide resistant?
        - Pasture? Type of vegetation (brome, fescue, native, etc.)? What grazers? How many?
        - Hay? Type of vegetation (brome, fescue, native, etc.)? Number of cuttings?
        - Established vegetation (fallow, native, exotic, trees, etc.)? Enrolled in CRP or other easement program?
        - Herbicide used, if any?
    - Other concern, if any (contaminant cleanup site, landfill, reclaimed gravel mine, reconstruction on roadbed, etc.)
- Seeding Details
    - Date Seed-Mix Area was planted to prairie

- Whether the planting is an interseeding
- Seeds
  - Seed origin (<20 mi from planting site, 20–60 mi from planting site, etc.)
  - Name of established ecotype, if any
  - Whether the mix includes cultivars or horticultural selections (if so, which species)
- Whether any site preparation occurred (fill out management action if so)
- Seeding Actions
  - Planting method (broadcast, drill, etc.)
  - Seed to soil contact techniques, if any (dragging, cultipacking, etc.)
  - Seeding rate (with units pounds/acre or seeds/square foot)
- Costs per acre (if known)—for cover crops, seed, site prep, etc.
- Soil tests, if any (nitrate, phosphorus)

- Seeding Species List
  - Species planted (scientific and common names)
    - For each species, weight planted (grams, ounces, or pounds)
    - Seed mix type—single species collection (i.e., hand collected individually or purchased individually) or bulk collection
    - % viable, % live (if known)
    - Whether cleaned
    - Whether tested and type of test
- Plug Planting, if any
  - Date plugs were planted
  - Species planted
    - For each species, estimated number of plugs planted
  - Method (hand planted, assisted with mechanical planter)
  - Irrigated? Date?
  - Greenhouse? Size and type of container?
  - Salvaged? From where?
  - Costs per acre (if known)
  - Soil tests, if any
- Management Actions
  - Does action apply to entire Planting Unit or only some of that Planting Unit's Seed-Mix Areas?
  - Were management actions taken?
    - Select no, and identify the year if no actions occurred in a given calendar year
    - Select yes to record an action
      - Start and end dates for the action
      - Management triggers
      - Describe action (mowing, herbicide, fire, grazing, etc.) with some details about coverage, equipment, type of herbicide, etc.

Standard Operating Procedures (SOP)

SOP 1: Meandering Walk

SOP 2: Nested Frequency Plots

SOP 3: Using the NRCS Web Soil Survey to Identify Soil Drainage Classes Within a Survey Area

SOP 4: Creating the Systematic Grid for Nested Frequency Plots

SOP 1: Meandering Walk

The meandering walk is used to track the progress of a seed-mix area by attempting to record all species expressed in the planting in a given year and through time. The observer strives to develop as complete a plant species list as possible for a seed-mix area during each meandering walk. This list will be larger than would be possible using a plot- or transect-based method. In addition, expression of exotic and/or invasive species can provide insights about threats through time.

Attributes measured

- Species encountered (presence)
- General abundance (sparse, occasional, frequent, very common)
- General distribution (localized, widespread)
- Duration of the survey (hours, minutes)
- Area surveyed (acres)

Equipment and supplies

- Map showing seed-mix area boundary and soil moisture type boundaries if applicable; satellite imagery or topography is helpful
- GPS unit with map, if used
- Survey protocol
- Datasheets or mobile device equipped for data entry
- Clipboard
- Pencils/pens
- Watch/clock
- List of species planted, if desired
- List of species observed in prior surveys (if any), if desired
- Field references, such as field guides or cheat sheets (hard copy or digital)
- Hand lens
- Plastic bags/cooler and/or plant press for specimen collection
- Camera to photograph unknown plants

Survey timing and schedule

Meandering walks are typically done annually and based on phenological cues. In some cases, two walks may be conducted each year to pick up cool-season and then warm-season plants. Conduct the walk according to the phenological timing and frequency determined for the survey by confirming with the Site Survey Coordinator or by checking archived data for dates and frequency of sampling during previous monitoring visits. See Element 2 for broader details.

Sources of error

See Element 2 for a full discussion of sources of error. A brief list to consider is:

- Inconsistent application of survey methods
- Plant misidentification
- Overlooking rare or cryptic plants, especially when not in bloom

Establishment of sampling units

Each discrete seed-mix area is the sampling unit that should be searched (Elements 2 and 3). Use the map you created (Element 3) as well as a GPS unit, if desired, to search the seed-mix area or designated subareas, as instructed by the Site Survey Coordinator.

Care should be used when monitoring near the boundary of adjacent seed mixes, because there may be a region of overlap (or a gap in application) of seed mixes in a given planting unit. If seed-mix areas are highly interspersed (e.g., in ridge and swale topography) or if seed mixes are applied in very patchy or complicated ways such that monitoring them separately would be impractical, they may be monitored as one seed-mix area and treated as a single sample unit.

Data collection procedures

Within each search area, do a "botanist's walk" to create a full species list for the seed-mix area including relative abundance and distribution category for each species (see Data field details). This entails traversing all noticeable vegetation zones, including parts of the site with different moisture types, topography, or other characteristics that could yield unique species. Botanists are encouraged to follow their instincts with cues from the landscape about where additional species might be found in order to obtain the most complete species list. The biologist or manager may want to guide technicians to be sure to visit certain parts of the seed-mix area known to be of interest. Each soil moisture category that occurs in the seed-mix area (Elements 2 and 3) should be searched and **species recorded separately**.

If subareas have been added after initial sampling, keep the list generated from the original subareas separate from the added subareas so that data from the larger survey area can still be summarized for just the original subareas (Element 3).

The amount of time a walk takes can vary among years but should be recorded. In some years, some species may be more apparent than in other years or in different areas, and thus species lists among years will often be different. Monitoring should generally cover the same area and be done with relatively the same amount of intensity among years. A GPS in tracking mode can be carried during the meandering walk to create a record of routes taken over time. Previous meandering walk routes can be useful in understanding where species richness data specifically originated, but the botanist is encouraged to intuitively search for plant species instead of attempting to follow former routes.

All species encountered whether native or non-native should be recorded along with general abundance and distribution within each soil moisture type. Example data sheets are in SM 1.

Data field details

<u>OVERALL SITE</u>: Record the name of the overall site as it appears in the Database (Element 4) or to correspond with the definitions in Element 2. Refer to Site Survey Coordinator for details.

<u>PLANTING UNIT</u>: Record the name of the planting unit as it appears in the Database (Element 4) or to correspond with the definitions in Element 2. Refer to Site Survey Coordinator for details.

SEED-MIX AREA: Record the name of the seed-mix area as it appears in the Database (Element 4) or to correspond with the definitions in Element 2. This is the area targeted by the meandering walk. Refer to Site Survey Coordinator for details.

SOIL MOISTURE TYPE: Record "dry," "mesic," or "wet," as defined by the soil drainage class(es) within the search area (SOP 3). If more than one category occurs in each seed-mix area, provide the approximate percent of the area in each moisture category, and search each moisture category separately (Elements 2 and 3, SOP 3). Write a comment if what is indicated in the soil survey appears different from what is found on the ground.

DATE: Record the date of the survey as yyyy/mm/dd.

OBSERVER(S): Record each observer's name.

START TIME: Record the time at the start of observations using a 24-hr clock (e.g., 14:32 = 2:32 pm).

END TIME: Record the time at the end of observations using a 24-hr clock (e.g., 17:02 = 5:02 pm).

TOTAL TIME: Record the total amount of time in hours and minutes spent conducting the meandering walk (end time − start time).

AREA SEARCHED: Record the approximate total area searched in acres. This should coincide with the area of the delineated polygon(s) created in Mapping (Element 3).

SPECIES: During the search, record each native and non-native species encountered using current scientific name. Any abbreviations, codes, or names used should be referenced to a master list of corresponding scientific names and the authority (source of the scientific names, such as USDA Plants, Flora of North America, or ITIS) used. If you cannot identify a plant to species, collect or photograph a specimen for future identification (Appendix E), and note genus, if possible, in comments. If possible, flag an unknown plant species, and return later when diagnostic characteristics are apparent. For plants with enough information to distinguish from other species, keep track of unknowns by coding them discretely until they can be properly identified. Record very small seedlings that cannot be identified with confidence as either "unknown forb seedling" or "unknown grass seedling" (Element 2).

ABUNDANCE: Record the general abundance of each species within each moisture category as "sparse," "occasional," "frequent," or "very common" relative to the size of the site surveyed, according to the definitions below (**Table SOP 1.1**). Though subjective, these indications can be useful, particularly to the site manager through time.

Table SOP 1.1. Definitions of abundance categories for the meandering walk.

Abundance Category	Definition
Sparse	Seldom occurring and usually occurring as an isolated plant; few total individuals observed
Occasional	Scattered plants in low numbers; sometimes occur in small clumps
Frequent	Many plants observed; species is regularly seen but rarely becomes dominant
Very Common	Species occurring in large numbers; can be dominant

DISTRIBUTION: Record the general distribution of each species as "localized" or "widespread." A localized species is found in one or a few discrete spots in the area searched. A widespread species is dispersed broadly throughout the area. While many localized species will also be sparse, a frequent species could possibly be localized (e.g., one solid patch of reed canarygrass). Conversely, individuals of a sparse species might be found in several separate places (i.e., widespread).

COMMENTS: Record any relevant comments.

SOP 2: Nested Frequency Plots

In nested frequency plots, the observer uses a frame on a systematic grid of points to assess the frequency of plant species in a seed-mix area (Elements 2 and 3). The nested frequency plots provide quantitative data for comparison across seed-mix areas or over time, as well as for calculation of diversity and evenness.

Attributes measured

- Species encountered in each plot (frequency of occurrence)
- Smallest subplot in which each species occurred

Equipment and supplies

- Map showing seed-mix area boundary, soil moisture type boundaries if applicable, and sampling point locations (systematic grid); satellite imagery or topography is helpful
- GPS unit preloaded with coordinates of sampling points or appropriate map if not using a GPS
- Survey protocol
- Plot frame
- Datasheets or mobile device equipped for data entry
- Clipboard
- Pencils/pens
- List of species planted, if desired
- List of species observed in prior surveys (if any), if desired
- Field references, such as field guides or cheat sheets (hard copy or digital)
- Hand lens
- Compass
- Plastic bags/cooler and/or plant press for specimen collection
- Camera to photograph unknown plants

Survey timing and schedule

The nested frequency plots should be implemented at least every 3–5 years after the initial establishment phase and based on phenological cues. Conduct nested plot surveys according to phenological timing and frequency determined by the Site Survey Coordinator. See Element 2 for more details.

Sources of error

See Element 2 for a full description of sources of error. A brief list to consider is:

- Inconsistent application of survey methods
- Plant misidentification
- Missing species
- Inaccurately determining which plants are inside each subplot

Establishment of sampling units

Each plot will be located using a GPS unit with preloaded coordinates. Use appropriate GPS protocols to navigate to the point within a 2-m error. If using an alternative non-GPS method of locating points (Element 3), indicate this in the comments field, and make sure your methods are well documented. Follow guidance in Element 3 about where to avoid sampling.

Care should be used when monitoring near the boundary of adjacent seed mixes, because there may be a region of overlap (or a gap in application) of seed mixes in a given planting unit. If seed-mix areas are highly interspersed (e.g., in ridge and swale topography) or if seed mixes are applied in very patchy or complicated ways such that monitoring them separately would be impractical, they may be monitored as one seed-mix area and treated as a single sample unit.

Data collection procedures

Using a handheld GPS, or map and compass, navigate to each sample location. Orient the plot frame consistently for all plots as instructed by the Site Survey Coordinator. For example, facing north, place the plot frame in front of and to the left of the observer with subplot A (the smallest subplot) always farthest to the left and closer to the observer; **Figure SOP 2.1**). Alternatively, a random compass direction can be chosen prior to going to the field. To prevent bias when the sample point has been approached, the orientation of the plot frame must be predetermined.

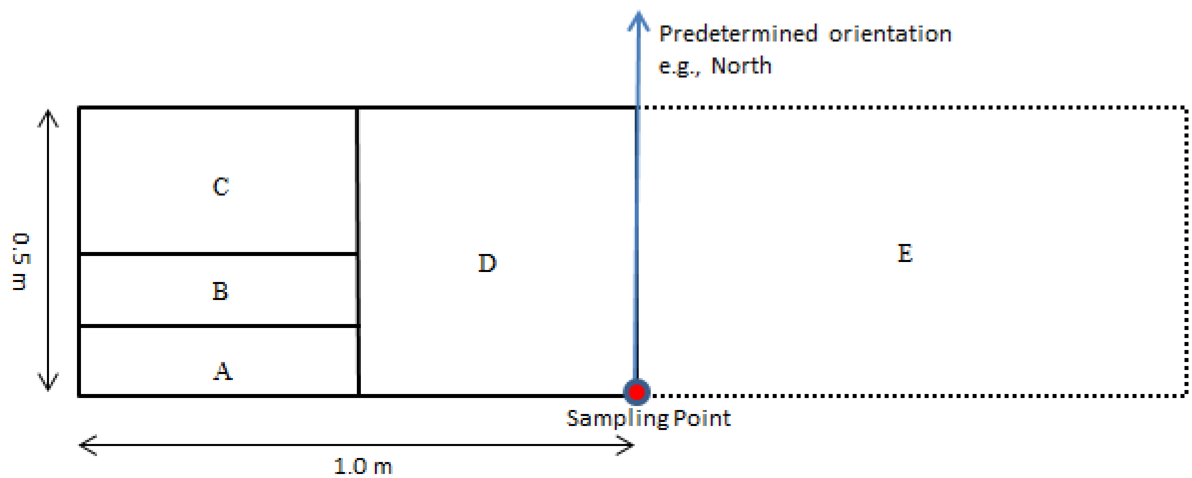


Figure SOP 2.1. Nested plot frame with example predetermined orientation.

Carefully adjust the vegetation so that only plants rooted within each subplot division are recorded. It may be desirable to remove one end of the frame to most easily slide into tall vegetation. Replace the end to allow accurate monitoring. Record all native and non-native species, blooming and non-blooming, in the smallest subplot, and record an A for the appropriate plot on the datasheet or electronic application. Continue inspecting subplots in order from smallest to largest, and record new species along with the subplot in which they first occurred. For any given 1-m^2 plot, a species will only be recorded once and only in the smallest subplot in which it occurred. The final subplot (E) is always the 50 cm x 100 cm area of the plot frame after it is "flipped" on its short axis as indicated in **Figure SOP 2.1**. If using a paper datasheet, use a separate column for each plot; additional datasheets will be necessary for sites requiring more than 10 plots. Also use additional datasheets to accommodate more than 34 species. A simple user guide will be provided for electronic data entry using Survey123 or other application, and when available, it will be located in our monitoring ServCat site.

A sample blank datasheet and an example filled out datasheet are attached to this protocol (SM 1). The key thing to note is that each species is entered only once at each sample plot location, and the smallest subplot within which it is found is entered under the appropriate plot number.

If you find it necessary to subdivide the first subplot further to accommodate highly dominant species (Element 3, Appendix D), assign the new subplot a unique letter and retain letters assigned to other subplots as in **Figure SOP 2.1**. Note the nested plot sizes on the datasheet or electronic application and in the Database. Ensure the same sampling subplots are used for each monitoring event.

Data field details

OVERALL SITE: Record the name of the overall site as it appears in the Database (Element 4) or to correspond with the definitions in Element 2. Refer to Site Survey Coordinator for details.

PLANTING UNIT: Record the name of the planting unit as it appears in the Database (Element 4) or to correspond with the definitions in Element 2. Refer to Site Survey Coordinator for details.

SEED-MIX AREA: Record the name of the seed-mix area as it appears in the Database (Element 4) or to correspond with the definitions in Element 2. This is the area targeted by the nested frequency plots. Refer to Site Survey Coordinator for details.

SOIL MOISTURE TYPE: Record "dry," "mesic," or "wet," as defined by the soil moisture categories within the seed-mix area (SOP 3). If more than one soil moisture type occurs within the seed-mix area, give the percentages of each, based on SOP 3 (sampling is stratified by soil moisture type as described in Elements 2 and 3).

DATE: Record the date of the survey as yyyy/mm/dd.

OBSERVER(S): Record each observer's name.

NESTED PLOT SIZES: Dimensions in cm are given for nested plot sizes 1–5 (**Table 3.2**). If you use other sizes, record the nested plot number and its dimensions.

PLOT NUMBER: Record the plot number. There is room for 10 plots on the datasheet; if more plots are required for a given seed-mix area, you will need additional datasheets.

SOIL MOISTURE TYPE PER POINT: Document the soil moisture type (SOP 3) for each sampling point (plot number). Write a comment if what is indicated in the soil survey appears different from what is found on the ground.

SPECIES: Record each native and non-native species encountered using current scientific name. Any abbreviations, codes, or names used should be referenced to a master list of corresponding scientific names and the authority (source of the scientific names, such as USDA Plants, Flora of North America, or ITIS) used. If you cannot identify a plant to species, collect or photograph a specimen for future identification (Appendix E), and note genus, if possible, in comments. If possible, flag an unknown plant species, and return later when diagnostic characteristics are apparent. For plants with enough information to distinguish from other species, keep track of unknowns by coding them discretely until they can be properly identified. Record very small seedlings that cannot be identified with confidence as either “unknown forb seedling” or “unknown grass seedling” (Element 2).

SMALLEST SUBPLOT: For each species, record the smallest subplot (A, B, C, D, or E) in which that species occurred for each plot, as instructed in Data Collection Procedures. On paper datasheets, if a species did not occur in a given plot, leave the cell blank, or strike through it.

COMMENTS: Record any relevant comments, including presence of a mowed or disturbed area, citing the reason for disturbance if known.

SOP 3: Using the NRCS Web Soil Survey to Identify Soil Drainage Classes Within a Survey Area

This SOP describes the basic steps for finding the soil drainage classes for a particular survey area and reducing them to the three soil moisture categories used in the PRI monitoring protocol.

- Section A lists steps to visualize the information using the Natural Resources Conservation Service (NRCS) soil survey website.
  - When you use the online tools, you can import a shapefile for one seed-mix area, or you can digitize the outline of the seed-mix area on a basemap.
  - If you wish to do all your GIS work in ArcMap (for example, you have a layer that outlines each of your seed-mix areas), skip Section A, and download the soils map for your county as described in Section B.
  - If you already have a soils layer for your area with drainage class as one of the soil polygon properties, you can skip directly to Section C.
- Section B gives short guidance for downloading the map created in Section A for a particular area of interest (AOI; i.e., an individual seed-mix area in this case). Alternatively, you can download the soils map for your county and do all the GIS work in ArcMap.
- Section C gives a brief description of steps that might be taken in ArcMap to process the information.

Keep in mind that the NRCS soil survey was mapped at a certain scale depending on when and where it was mapped. Therefore, boundaries between soil map polygons are approximations. When you examine the soil survey for a seed-mix area, you may get a message that "you have zoomed in beyond the scale at which the soil map for this area is intended to be used." We recognize that the extent and location of mapped soil units within each seed-mix area are approximations. Nonetheless, the NRCS soil survey is a standard tool we can use to better understand how soil moisture type influences the outcomes of plantings.

A) Visualizing soil moisture categories online

1) Go to NRCS Soil Survey page: https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm
2) Click the green circular "Start WSS" button, or click "Start Web Soil Survey" blue text in right sidebar.
3) Click the "Area of Interest (AOI)" tab to define the location for your map.
   a) You can import a shapefile with one seed-mix area or follow the steps 3b-3d to define a seed-mix area using the NRCS web tools.
   b) Zoom in on the map of the US, or use the State/County dropdowns on the left, and click "View" to zoom in; continue to zoom until your survey area more or less fills the view.
   c) Use the polygon AOI tool or the rectangle AOI tool to outline your area of interest (i.e., seed-mix area that you plan to monitor).
   d) If you wish to remove streams, roads, etc. from the map, click the "Legend" tab at top left of the map, and uncheck the appropriate layer(s).
   e) To define a new AOI for a subsequent map, click "Clear AOI," then redo the above steps.

f) Note that you may download the soil survey for your county (Section B); then you would have the layer for your whole extent and could use a local copy of ArcMap to create individual maps for each seed-mix area.

4) The following steps assume you have created an AOI for your seed-mix area.
5) Click the "Soil Map" tab to see the outlines of the soil polygons within your AOI.
    a) You can change the layout on your screen by choosing one of the two buttons just above the map and to the right—either normal map layout or full width map layout. With normal layout, the table with the soils descriptions is to the left of the map. With full width layout, it is below the map.
    b) You can click on a soil name to see a description of that soil (map unit).
6) Click the "Soil Data Explorer" tab to visualize properties of the soils within your AOI, specifically drainage class.
    a) Click on the "Soil Properties and Qualities" sub-tab.
    b) Expand "Properties and Qualities Ratings" on the left sidebar (if not already expanded).
    c) Further expand "Soil Qualities and Features."
    d) Click "Drainage Class."
        i) The Map and Table checkboxes should both be automatically checked. Dominant Condition should be automatically chosen as the aggregation method.
        ii) Click the "View Rating" button to see your AOI with the soil polygons color coded by drainage class.
        iii) Click the "Legend" tab at the upper left of the map to see which color represents which drainage class, and find the corresponding PRI moisture type (i.e., dry, mesic, or wet; **Figure SOP 3.1**). Note that the basemap can distort the colors in the polygons.
        iv) Scroll down the page to see a table summary by map unit.
            (1) If your AOI outlines a seed-mix area, you can use the percentages given in the table to determine how many plots need to be in each soil moisture category for that seed-mix area.
            (2) To get this information into ArcMap, see steps B and C.
        v) **Figure SOP 3.2** depicts an example map generated from the NRCS Web Soil Survey site and composed of all mesic soils, so stratification is not needed for the monitoring protocol.
        vi) **Figure SOP 3.3** depicts a map with both wet and mesic soils. Monitoring for this seed-mix area would be stratified by soil moisture type.
7) To download a shapefile of the soils map for your AOI, go to B).
8) Click "Shopping Cart (Free)" tab to download a report with a printed soils map of your area of interest, if desired.

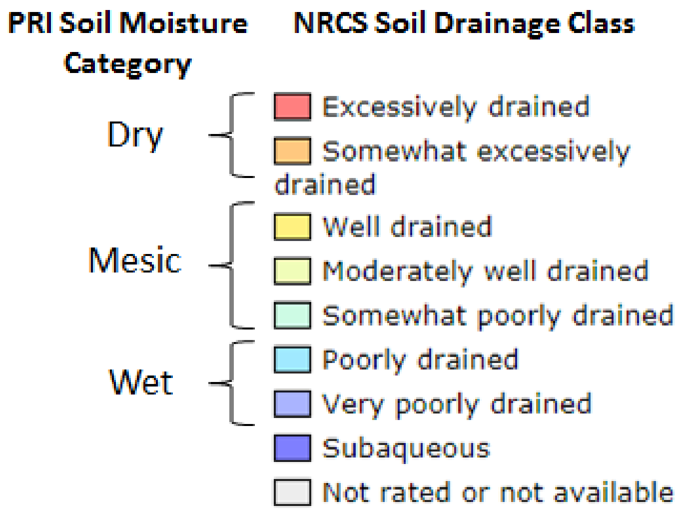


Figure SOP 3.1. Correspondence of soil drainage classes from the NRCS Web Soil Survey ("Soil Rating Polygons" in legend with colored boxes) to soil moisture categories used in this protocol.

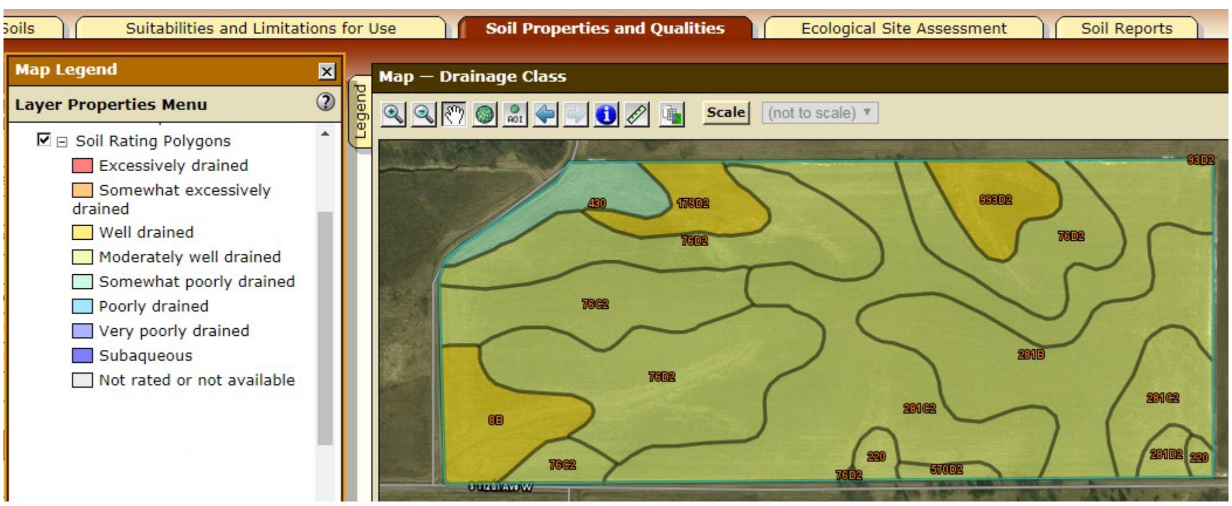
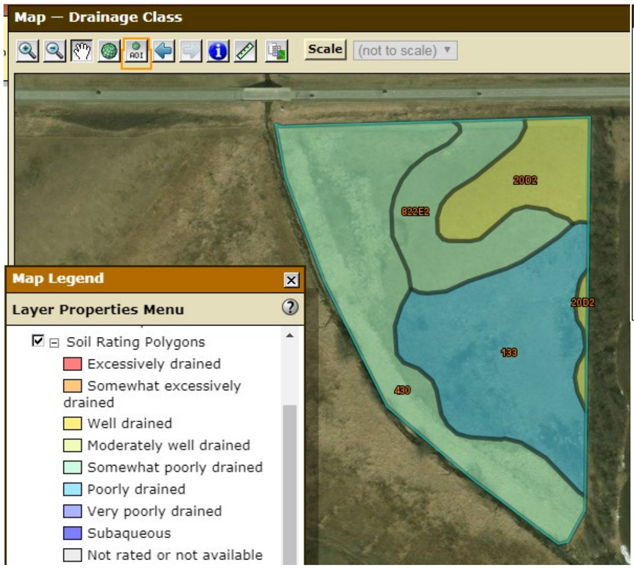


Figure SOP 3.2. An example of a soils map that was generated using the NRCS Web Soil Survey, with only one soil moisture category. The three soil drainage classes that occur in this seed-mix area are all categorized as mesic, so this site would be monitored without stratification.



Tables — Drainage Class — Summary By Map Unit

Summary by Map Unit — Jasper County, Iowa (IA099)

Map unit symbol	Map unit name	Rating	Acres in AOI	Percent of AOI
20D2	Killduff silty clay loam, 9 to 14 percent slopes, eroded	Moderately well drained	3.4	13.6%
133	Colo silty clay loam, 0 to 2 percent slopes, occasionally flooded	Poorly drained	8.2	33.0%
430	Ackmore silt loam, 0 to 2 percent slopes	Somewhat poorly drained	9.3	37.4%
822E2	Lamoni silty clay loam, 14 to 18 percent slopes, moderately eroded	Somewhat poorly drained	4.0	16.0%
Totals for Area of Interest			**24.8**	**100.0%**

Figure SOP 3.3. An example of a soils map that was generated using the NRCS Web Soil Survey, with more than one soil moisture category. The poorly drained soil (which comprises 33% of the total area) is categorized as wet, while the other soils are mesic. For this seed-mix area, monitoring would be stratified by soil moisture category. Species would be documented separately in the wet polygon for the meandering walk, and 33% of the nested frequency plots would be placed in the wet polygon while 67% of plots would be placed throughout the remainder of the planting. The area is 10 ha (24.8 acres) and would have 30 plots (3 plots/ha), 10 in wet and 20 in mesic.

B) Downloading soils data from NRCS Web Soil Survey

1) These steps assume that you followed Section A through Step 7 (and would like to download a soils map for your AOI) or that you want to download a county-wide soils map.
2) Click "Download Soils Data" tab to download a folder containing shapefiles and soils data.
3) You have three options for downloading data:
   a) Your AOI (SSURGO)—this is a detailed soil survey for your previously defined area of interest.
   b) Soil Survey Area (SSURGO)—this is a detailed soil survey that can be downloaded by county.
   c) U.S. General Soil Map (STATSGO2)—this is a general soil survey that can be downloaded by state – however, it is not at a level of detail appropriate for a parcel-level view and should not be used for this protocol.
4) Click "Create Download Link" button to generate a link to a zip file with your data (link appears at the bottom of the page). When downloading a county map (3b), the link is automatically generated.
5) Once extracted from the zip file, the downloaded folder contains a number of items, including a "readme.txt" help file.
   a) Shapefiles are in the "spatial" folder.
      i) All spatial data are provided in Esri Shapefile format in WGS84 geographic coordinates. To be able to calculate area the data need to be transformed into a projected coordinate system such as UTM for the zone appropriate for your area

(this can be done in ArcMap using "ArcToolbox--Data Management Tools--Projections and Transformations").

ii) Codes used in layer names:
   (1) soilmu = soil map unit
   (2) soilsf = soil special feature
   (3) _p_ = point
   (4) _l_ = line
   (5) _a_ = polygon
   (6) soilsa_a_ = soil survey area boundary
   (7) aoi_a_aoi = boundary of the area of interest

iii) The primary shapefile of interest for these purposes (the one that you need to work with in ArcMap) is "soilmu_a_*****", the polygon layer with the soil map units.

iv) A soil map unit is a collection of areas (i.e., polygons) defined and named the same in terms of their soil components (e.g., Tama silty clay loam 2 to 5% slopes). See NRCS documentation for more detail.

b) Data tables are in the "tabular" folder.
   i) Double click on the Access database template in the main folder, and follow the instructions to import the data tables into a usable format.
   ii) The "muaggatt" table contains information about soil drainage class.

c) Consult the "readme.txt" file for additional guidance in downloading and using the NRCS soils data.

C) Using ArcMap to incorporate soils data

1) Make sure the soils layer and any other layers you need are in the same projection. Add a basemap if desired.
   a) As indicated above (B.4.a.i.), if a spatial data layer is in geographic coordinates, it needs to be transformed into projected coordinates.
2) Add a column to the attribute table that contains the area for each soil polygon.
   a) Open the attribute table for your projected layer. The field Shape_Area is in square meters. Using "Table Options--Add field," create a new field named Hectares (or Acres, if preferred; however hectares are used to calculate the number of plots needed), and choose type "float" (has decimal places, takes up less room than "double").
   b) A new empty column will be appended.
   c) Right click on the heading, and choose "Calculate Geometry."
      i) Choose the desired units to be calculated. Uncheck the box "Calculate selected records only." Click "OK."
      ii) It seems that the calculation cannot be done on a joined table, so do this prior to joining to the soil moisture table, or unjoin the tables, perform the calculation, and then rejoin.
3) You can add the "muaggatt" table in the downloaded Access database to ArcMap; however, the database is read only, making it difficult to create and edit the needed new field that assigns drainage class to the appropriate soil moisture category (dry, mesic, wet).

4) This is one workaround for getting only the necessary information into ArcMap (there may be other, more efficient ways to achieve this).
   a) We are interested in table “muaggatt” and its field “drclassdcd,” or drainage class-dominant condition, which gives the most common drainage class by area for each soil map unit. The procedure below will work for either of these cases:
      i) If you downloaded data for an entire county, the tables contain records for all soils found in the county.
      ii) If you downloaded data for an area of interest, the tables contain records only for soils found in that delineated area. (However, if you generate a report, the acreages are for the entire county.)
   b) Open the “muaggatt.txt” file in Excel as a delimited file. We only really need the “drclassdcd” column (16th column, P) and the “mukey” column (last column, AN); so delete the other columns. However, you may find it informative to keep the map unit name (“muname,” 2nd column) or other information.
      i) To view the other column names, open the table in Access (step B.3.b.).
      ii) Insert a row for the column headers, and type them in. Use names with 10 or fewer characters.
   c) Using Excel, assign values to a new column (give it an appropriate heading such as SoilMoist) using a nested IF command that references the appropriate cell. Example formula:
      i) =IF(B2="Excessively drained","Dry",IF(B2="Somewhat excessively drained","Dry",IF(B2="Well drained","Mesic",IF(B2="Moderately well drained","Mesic",IF(B2="Somewhat poorly drained","Mesic",IF(B2="Poorly drained","Wet",IF(B2="Very poorly drained","Wet","Null")))))))
      ii) In this case, the formula was going into line 2 of the SoilMoist column, and the drainage class was in column B; the formula was then copied and pasted to the other cells in the column.
      iii) In general, if drclassdcd is not one of the listed values, it is a permanently-flooded or other non-soil feature.
   d) The “mukey” column in the Excel file needs to be formatted as text in order to match the field type of “MUKEY” in the soils layer.
   e) Save as an Excel workbook, and import the table into ArcMap. Join to the soil map layer on MUKEY/mukey.
      i) When you join, it is apparently more efficient for future performance to “Keep only matching records.”
      ii) Prior to joining the layers, perform an area calculation as described in step 2.
5) In “Properties” you can create labels for the polygons and/or change the symbology (i.e., color differently) according to soil moisture category to visualize the three categories within your area of interest.
   a) You can also use labels or color to visualize the distribution of the original seven NRCS drainage classes.
6) If desired, you can dissolve the soil polygon boundaries of adjacent like polygons. However, maintaining those boundaries can be informative when using the map in the field.

7) Optionally, to include additional soils information, follow these steps.
    a) Open the Access database template (B.3.b.), and add it to the map. You only need the "muaggatt" table to create maps based on soil drainage class, but you may include other tables if you are interested in that information. See the readme.txt help files in the downloaded zip file for more guidance.
    b) Right click on the "soilmu_a_*****" layer, and create a join to any of the tables in the database using the variable "MUKEY."
8) Compute the total area within each moisture category in your AOI. Summarize each seed-mix area separately.
    a) Open the attribute table of the projected layer for your AOI, which now has hectares (or acres) and soil moisture category for each soil polygon.
        i) If the summary below fails (the join seems to interfere with the summarization), export the attribute table to a text file, add it to the map, and proceed with these steps.
    b) Right click on the soil moisture heading, and choose "Summarize."
    c) Expand Hectares in 2, and choose "Sum" to get total hectares for each soil moisture category.
    d) Save, and add to the map. If the save fails, you may have to change the file type.
9) For a given seed-mix area, find the number of plots needed in each soil moisture category.
    a) To get the proportion of each moisture category, take the hectares for that category, and divide by the total hectares of the seed-mix area.
    b) To determine how many plots should be in each category, take the above proportion, and multiply by the total number of plots (3 plots/ha but no fewer than 10 and no more than 40; see Elements 2 and 3). Round to the nearest integer.

Note: Instructions for the NRCS soil survey website are accurate as of 7/12/2019.

SOP 4: Creating the Systematic Grid for Nested Frequency Plots

We describe two methods to lay out a sampling grid, stratified by soil moisture regime. The first is a beta version of a tool developed by PRI that works best if you have a single polygon in the layer. It only generates points within your polygon. The second consists of guidance about using the Fishnet, Buffer, and Clip tools in ArcGIS to generate an appropriate grid of points. Both methods may require that you make some adjustments and rerun the tools because there are too many or too few points or points are too close to boundaries.

You should make your own calculation for how many sampling points you need, based on the total area of the seed-mix area (3 plots/ha but no fewer than 10 and no more than 40; Element 2). You should also calculate how many points you need relative to the area of each soil moisture category (Elements 2 and 3, SOP 3).

When the output of the grid tool is at or just above what you require, you can discard one or more points, as necessary. If the grid is almost acceptable except that one or a few points are too close to a boundary, it is acceptable to move the point slightly away from the boundary. This should be done prior to going out to the field to prevent inadvertent bias in placement.

The same sampling points should be used on subsequent monitoring events at a seed-mix area. Whichever method is used, the last step should be to uniquely number or label each point for consistency among surveys.

PRI Monitoring Grid Tool

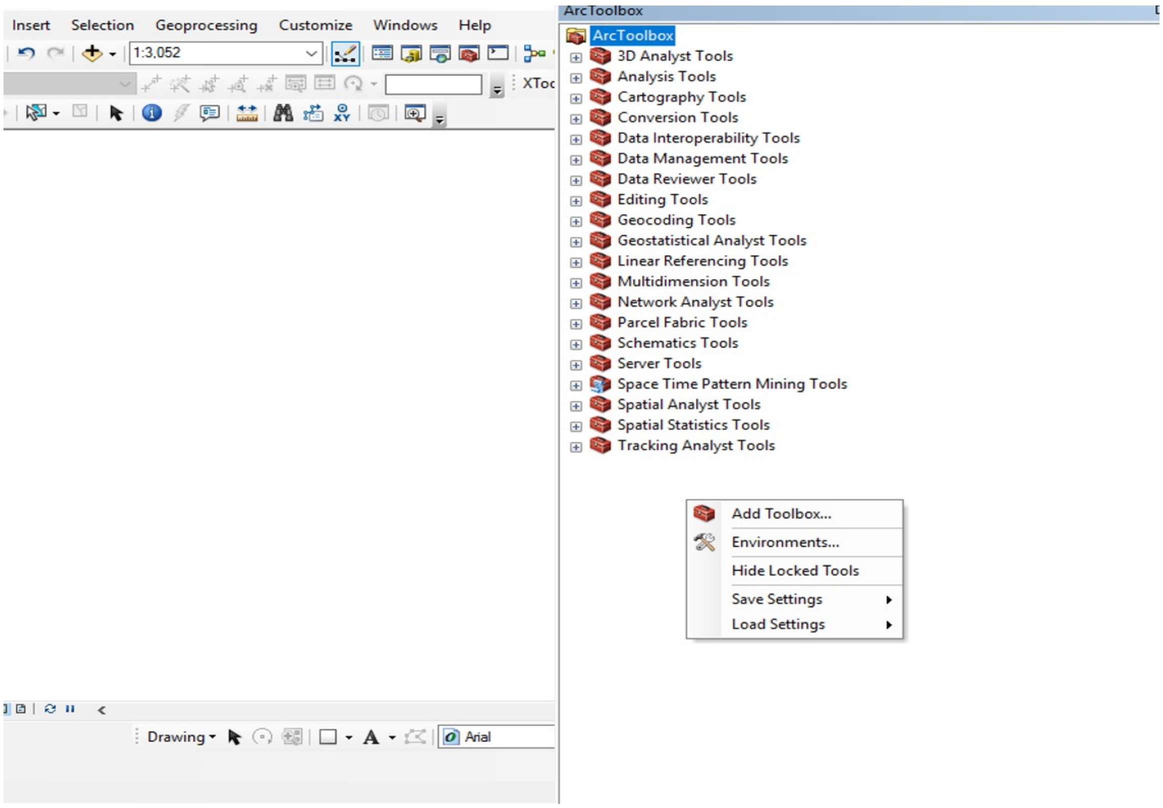
PRI has developed an ArcMap tool to facilitate creating a grid of points for a seed-mix area. The beta version of the tool is currently available on our ServCat site and described here. We are working on an improved version.

This internally developed tool finds the area of the polygon in square meters and calculates the number of sampling points needed. The spacing of the points is determined by dividing the area by the number of points and taking the square root of the result. This will be an approximation based on a square survey area.

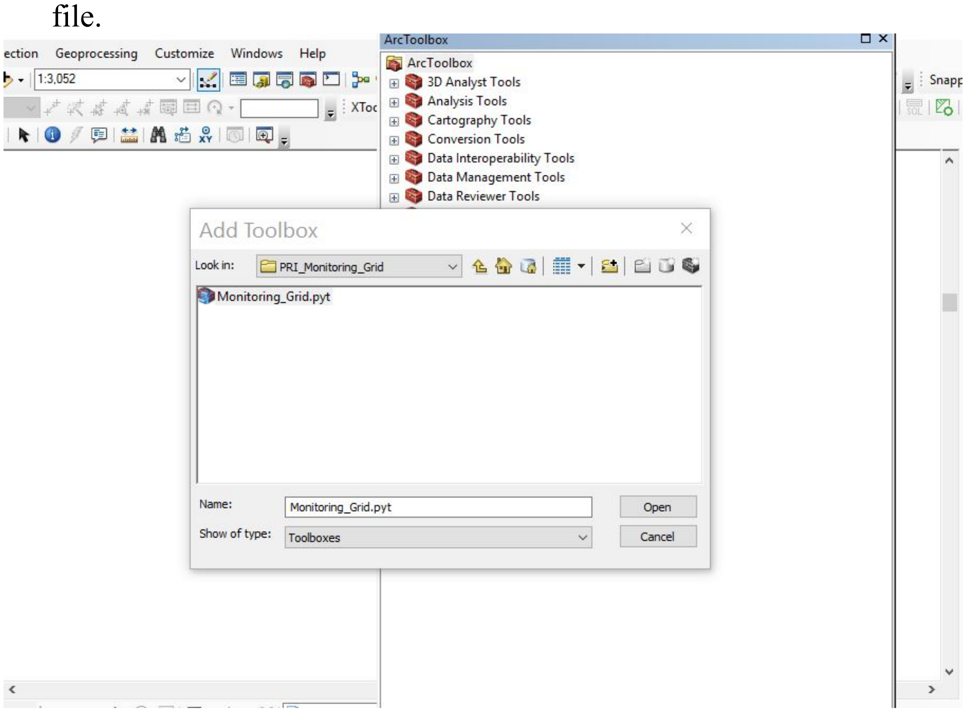
The beta version of the tool works best with one polygon in the shapefile and a relatively rectangular or convex-shaped seed-mix area. If your seed-mix area is very oddly shaped, you may have better results using the second method (below). The input feature must be in a projected coordinate system. If you are working with a derived layer (e.g., one that has been projected or dissolved), you may have to export the layer as a shapefile before being able to use it in the tool.

How to use the PRI Monitoring Grid toolbox

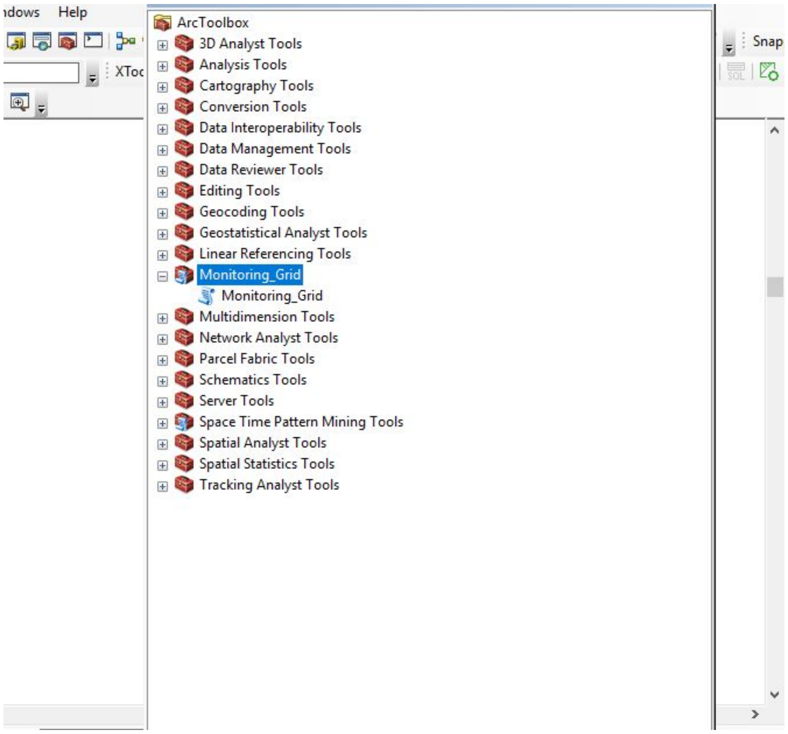
1. Download and extract the PRI_MonitoringGrid_ArcMapTool_BetaVersion.zip file from the PRI ServCat site
2. Open ArcToolbox
3. Right click in ArcToolbox, and select “Add Toolbox”



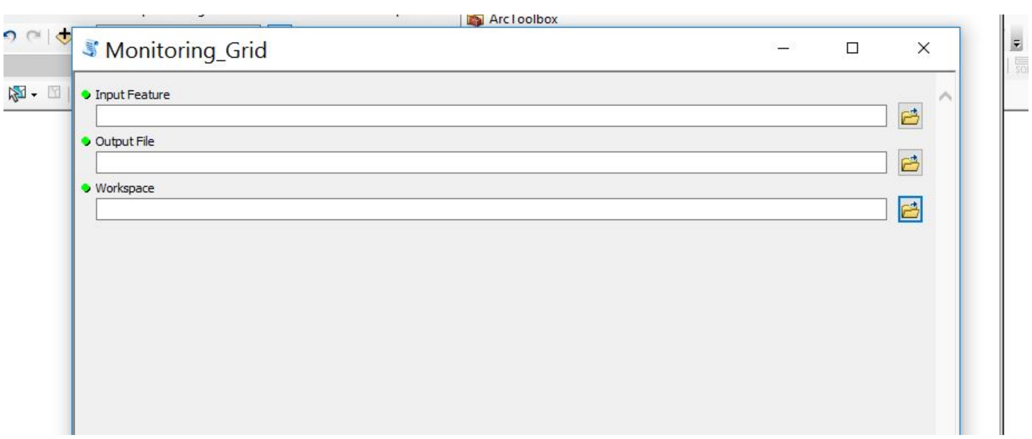
4. Navigate to where you saved the extracted files, and select and open the Monitoring_Grid.pyt file.



5. Once the Monitoring_Grid folder is added to your toolbox, expand and select the script contained within.



6. Run the tool. The **Input Feature** is the shapefile of the polygon of the seed-mix area that you want to monitor. This tool is most efficient if the survey area polygon is the only polygon contained within the shapefile. Please contact Ben Walker (benjamin_walker@fws.gov) if you have many survey areas that you want to process in a single event. The **Output File** is the systematic grid that the tool will create; navigate to an appropriate directory, and provide a name to the file. **Workspace** is the folder location where the script will temporarily work to create the necessary files and subsequently delete them.



7. On large survey areas, the tool may create more than 40 survey locations; please be aware of this, and delete unnecessary points.
8. Finally, give each monitoring point a unique number or label.

Essential script for creating monitoring grid

```
#Copy the shapefile so we aren't editing it#
arcpy.CopyFeatures_management(inputfile1, "point_cr.shp")

#Add a Square Meter Field, and calculate the value#
arcpy.AddField_management("point_cr.shp","area_sqm","Double")
expression1 = "{0}".format("!SHAPE.area@SQUAREMETERS!")
        arcpy.CalculateField_management("point_cr.shp", "area_sqm",
"!SHAPE.area@SQUAREMETERS!", "PYTHON", )

#Define layers for next step#
outraster = "ratemp.tif"
field = "area_sqm"

#Pull area value from polygon#
value = arcpy.da.SearchCursor("point_cr.shp", ("area_sqm",)).next()[0]

#Set the sideboards for the number of sampling points#
size1 = sqrt(value/40)
size2 = sqrt(value/(math.ceil(value/10000)*3))
size3 = sqrt(value/10)

#Convert our layer to a raster based on the sideboards#
if int(value) >= 130000:
arcpy.PolygonToRaster_conversion("point_cr.shp", field, outraster, "", "", size1)
        elif int(value) in range(30000, 130000):
        arcpy.PolygonToRaster_conversion("point_cr.shp", field, outraster, "", "", size2)
                else: arcpy.PolygonToRaster_conversion("point_cr.shp", field, outraster, "", "",
size3)
#Convert back to a point shapefile based on each cell's centroid#
arcpy.RasterToPoint_conversion(outraster, outfile2, "Value")

#Cleanup intermediate files#
arcpy.Delete_management("point_cr.shp","")
        arcpy.Delete_management("ratemp.tif","")
        arcpy.AddMessage ("POINTS SUCCESSFULLY CREATED")
```

An alternate method using the Fishnet tool

Hyslop (2012) gives good instructions for using the Fishnet tool in ArcMap iteratively to create a grid of sampling points. However, it may take a lot of fiddling to get the target number of points positioned reasonably away from boundaries. Some of the adjustments you can make are adding or subtracting a certain distance (e.g., 10 m) from the Top, Bottom, Left and/or Right of the template extent, which has the effect of changing the fishnet origin. You can also adjust the cell size width and height. Generally, we recommend a square grid, but if a rectangular grid fits your needs better, that is acceptable.

This method may give approximately the correct number of points in each soil moisture category when using the entire seed-mix area as the polygon layer. However, you can also try creating a separate layer for each soil moisture category within your seed-mix area. In this case, the grids would probably not line up with each other, but you may have better success getting the correct number of points in each soil moisture type.

Finally, these instructions explain how to use the "Clip" tool to keep only points within the polygon of interest. You may need to discard one or two points if you cannot obtain the exact number of points you require. The instructions also explain a process for giving each point a unique label.

References

Hyslop MD. 2012. Generate a regular grid of sampling points. Michigan Technological University. Available: http://gis.mtu.edu/wp-content/uploads/2012/06/Regular_Sampling_Tutorial.pdf (August 2018)

Supplemental Materials (SM)

SM 1: Sample Datasheets

List of datasheets:

1. Meandering Walk – stratified by soil moisture type, 2 pages
2. Meandering Walk – without soil moisture types (for sites that contain only one type), 2 pages
3. Nested Frequency Plots
4. Nested Frequency Plots – with example data entered

Datasheet for Meandering Walk – *Stratified by Soil Moisture Type*

Overall site: __________________ **Planting unit:** _________________ **Seed-mix area name**: ____________________

Soil moisture types: ____________________ **Date**: ____________________ **Observer(s)**: __________________

Start time: ____________ **End time**: ____________ **Total time**: _____________ **Area searched (acres)**: __________

Record species encountered, a general abundance category for each species in each soil moisture type based on the number of individuals encountered on the walk, and whether the individuals were localized or widespread.

Species Name	DRY		MESIC		WET	
	Abundance (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)

Meandering Walk – Stratified **Seed-mix area name**: __________________ **Date**: ________________ **p.__ of __**

Species Name	**DRY**		**MESIC**		**WET**	
	Abundance (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)

Comments:

Datasheet for Meandering Walk – *one soil moisture type*

Overall site: __________________ **Planting unit:** _________________ **Seed-mix area name**: ____________________

Soil moisture type: ____________________ **Date**: ____________________ **Observer(s)**: __________________

Start time: ___________ **End time**: ___________ **Total time**: _____________ **Area searched (acres)**: _________

Record species encountered, a general abundance category for each species based on the number of individuals encountered on the walk, and whether the individuals were localized or widespread.

Species Name	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)	**Species Name**	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)

Meandering Walk Datasheet **Seed-mix area name**: ___________________ **Date**: _______________ **p.__ of __**

Species Name	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)	**Species Name**	**Abundance** (Sparse, Occasional, Frequent, Very Common)	**Distribution** (Localized, Widespread)

Comments:

Datasheet for Nested Frequency Plots

Record the sizes of the (cumulative) nested plots (**Table 3.2**) used if different from standard plot frame (Element 3, Appendix D). Fill in the soil moisture type for each plot (SOP 3). Record the smallest subplot (A–E) within which each species occurred in each plot (**Figure 3.1**). Do not forget to “flip” the plot frame for the final subplot.

Date:______________ Observer:_________________ Overall Site:______________ Planting Site: _____________________

Seed-mix area Name: _________________________ Soil Moisture type(s): _________________ Page ___ of ___

Nested plot sizes (cm)—*Write in dimensions if you did not use these*: (1) 12.5 x 50 (2) 25 x 50 (3) 50 x 50 (4) 50 x 100 (5) 50 x 200

	Plot Number (minimum 10, maximum 40)										**Comments**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Soil Moisture Type ⇨ (Dry, Mesic, Wet)											
⇩ Species											

PARTIALLY FILLED IN EXAMPLE (FOR 5 PLOTS)

Started with alphabetical list of species planted (a couple of these were not observed in the plots)—added additional species at the end as they were encountered—3 more datasheets will be necessary to accommodate plots 11–20, 21–30 & 31–40

Datasheet for Nested Frequency Plots

Record the sizes of the (cumulative) nested plots (**Table 3.2**) used if different from standard plot frame (Element 3, Appendix D). Fill in the soil moisture type for each plot (SOP 3). Record the smallest subplot (A–E) within which each species occurred in each plot (**Figure 3.1**). Do not forget to "flip" the plot frame for the final subplot.

Date: 06/20/2018 Observer: A McColpin Overall Site: MySpot Planting Unit: UnitA

Seed-mix area Name: A-Upland Soil Moisture type(s): Dry-63%/Mesic-37% Page 1 of 1

Nested plot sizes (cm)—*Write in dimensions if you did not use these*: (1) 12.5 x 50 (2) 25 x 50 (3) 50 x 50 (4) 50 x 100 (5) 50 x 200

	Plot Number (minimum 10, maximum 40)										**Comments**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Soil Moisture Type ⇨ (Dry, Mesic, Wet)	Dry	Dry	Mesic	Mesic	Dry						
⇩ Species											
(Planted Species)											
Andropog. gerar.	A	--	D	D	A						
Antennaria negl.	C	E	--	--	--						
Boutel. curtipen.	A	B	--	A	D						
Coreops. palmata	D	--	B	E	E						
Dalea candida	--	B	--	E	C						
Dalea purpurea	--	--	--	--	--						
Echinac. pallida	B	--	B	B	E						
Elymus canade.	--	A	--	E	C						
Eryngium yucc.	--	E	A	--	A						
Helianthus occid.	--	--	--	--	--						
Heliopsis helian.	A	E	C	E	D						
Lespedez. capit.	--	D	E	C	--						
Liatris aspera	--	C	D	--	B						
Ratibida pinna.	--	B	A	--	B						
Rudbeckia hirta	A	A	--	D	B						
Schiza. scopari.	D	C	E	A	C						
Solidago nemor.	--	E	--	C	A						
Sporobolus heter.	E	B	D	--	E						
(Additional Spp.)											
Setaria viridis	A	A	B	E	--						
Trifolium repens	B	--	--	--	D						
Brassica nigra	E	B	--	--	C						
Taraxacum off.	--	C	A	--	D						
Oxalis stricta	--	--	--	B	--						

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