Nested frequency plot SOP

Materials
Plot frame
Map of site showing boundaries and sampling point locations
Compass
GPS pre-loaded with sample points
Clipboard
Pencils
Data sheets
Identification guides and/or keys
Plant press
Hand lens
List of species planted and/or list of species previously found (optional)
Camera to photograph unknown plants

Introduction
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). It avoids observer bias associated with cover estimates. By nesting plots of different sizes, it can be used to assess trends in species that vary substantially in relative abundance. 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% (35% is a good target) provides the greatest statistical power for assessing change over time. This frequency refers to the proportion of plots in which a species is detected within a reconstruction. For example, big bluestem may be dominant at a reconstruction, resulting in detection in every 0.5 x 2 m plot, making it impossible to assess any increase. However, it may be detected less often in a smaller plot size, perhaps 0.25 x 0.125 m. To assess changes in frequency over time of big bluestem in this example, the smaller plot frame size should be used to calculate frequency. When summarizing the data, use the plot frame size that yields approximately 35%.

In addition to monitoring, frequency has also been used to compare plant species occurring in different treatments, such as disturbance types, and within vegetation types (Larson et al. 2001, Larson 2003). Indices, such as species diversity and evenness, also can be calculated from frequency data. The drawback to any protocol that uses plots on transects is that inevitably, some species will be missed. For the most complete list of species occurring at a site, use of both the Nested Plot and the Meandering Walk Protocol is preferable.

Training
Field personnel. Sight-identification of species likely to be encountered (e.g., species planted, regional native and nonnative annuals and "weedy" species), as well as all regional invasive species, is necessary as a starting point. Those using this technique should also have familiarity with plant keys and methods to collect and press specimens for vouchers and identification. The ability to navigate to specific locations using a GPS is required.

Mapping

Prior to the field season, collect the GIS layers for the boundary of the site. If the planting did not take soil type into account, it may also be helpful to use a soil survey layer to separate wet, mesic, and dry prairie types. Soils data is available from the Natural Resources Conservation Service Web Soil Survey (NRCS WSS; https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm). We used soil drainage class as a proxy for wet, mesic, and dry prairie types.

Create a map that includes the site boundaries. Create the systematic grid for the appropriate number of sample points (see Appendix for ArcMap tool) and download the points to a GPS unit. It is helpful to include soil polygons or topography on a satellite imagery base map for use in the field. The map and/or shapefile should be stored with the copy of the datasheets and data.

We chose to sample on a systematic grid to ensure sampling locations were distributed throughout the site to capture variability within the unit. At the same time, a systematic layout allows the observer to walk a straight line from one plot to the next rather than having to wander around to random points.

The number of plots in each seed mix area depends on the size of that particular reconstruction. Between 10 and 40 rectangular plots (0.5 x 2.0 m) are placed in a systematic grid throughout the site. Aim for 3 plots/ha with a minimum of 10 plots and a maximum of 40 plots. 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. The number of plots is based on simulations using test data. The Appendix contains an annotated script in R for creating the systematic grid within ArcMap.

If GIS technology and a GPS unit are not available, the systematic grid will need to be laid out by hand on an appropriate satellite image 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.

Plot frame and data sheet

The plot frame (Figure 1) is made from 0.5 inch PVC pipe, which can be obtained at most hardware stores. The size of the subunits within the plot frame doubles with each larger size. They can be modified, depending on the vegetation characteristics of the site to be monitored, as long as sizes are in multiples of 2 (e.g., the smallest subunit in Figure 1 is 50 cm x 12.5 cm, so the next smaller that could be added is 25 cm x 12.5 cm). The rectangular shape also needs to be retained because species captured varies with plot shape. Keeping in mind the 35% target, a site that is heavily dominated by a single species will need a smaller subunit size than a site that

has greater alpha diversity. 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.

A sample data sheet is attached to this protocol. The key thing to note is that the species is entered only once at each sample plot location and the smallest subunit within which it is found is entered under the appropriate plot number.
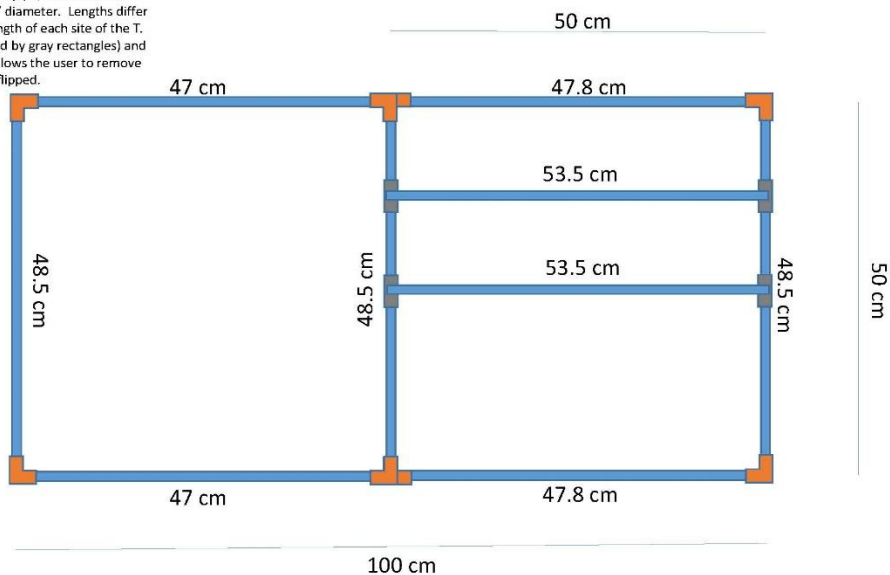

Figure 1. Nested plot frame. Subunit dimensions have been used in tallgrass prairie reconstructions, but in some cases the smallest subunit may need to be halved if any species are found to occur in > 50% of the 12.5 x 50 cm subunit.

Field methods

Methods
Using a hand-held GPS, navigate to the first plot location to be sampled. Select a compass direction at random and orient the plot in the same way with respect to that direction at each sample location. Alternatively, select a compass direction before going to the field and use it for all sampling. The goal is to avoid biasing plot placement.

Carefully adjust the vegetation so that only plants rooted within each subunit division are recorded. Record all native and nonnative species in the smallest subunit and place a 1 in the corresponding box of the data sheet. Continue inspecting subunits in order from smallest to largest, and record new species along with the subunit in which they first occurred on the data sheet. Each species that occurs in a given 1.0 m² plot will only be recorded once by writing the

smallest subplot in which it occurred on the data sheet. The final subunit is always the 50 cm x 100 cm area of the plot frame after it is flipped on its short axis from left to right.

If you cannot identify a plant to species, give the highest level of detail possible or collect a sample for later identification. Ignore very small seedlings that cannot be identified with confidence.

Analysis

If changes in frequency of individual species over time is an objective, initial analyses should focus on determining optimal subunit size (35% frequency is a good target (DeBacker et al. 2011)) for each species of interest. The nested feature of the plots should be retained throughout monitoring so that optimal subunit size can be reassessed each sample period. Optimal subunit size indicates the abundance of species, with a smaller optimal subunit size indicating greater abundance (Curtis and McIntosh 1950). If the initial frequency of a species of interest is between 20 and 50%, it should be possible to track its change in frequency over time, whether it increases or declines.

To determine species richness or other quantitative metrics for the site, use a single subunit size – typically the entire plot so no species are omitted – to calculate frequencies by species.

References
Curtis, J. T., and R. P. McIntosh. 1950. The interrelations of certain analytic and synthetic phytosociological characters. Ecology **31**:434-455.
DeBacker, M. D., J. S. Heywood, and L. W. Morrison. 2011. Optimized Frequency Measures for Monitoring Trends in Tallgrass Prairie. Rangeland Ecology & Management **64**:301-308.
Heywood, J. S., and M. D. DeBacker. 2007. Optimal sampling designs for monitoring plant frequency. Rangeland Ecology & Management **60**:426-434.
Larson, D. L. 2003. Native weeds and exotic plants: relationships to disturbance in mixed-grass prairie. Plant Ecology **169**:317-333.
Larson, D. L., P. J. Anderson, and W. Newton. 2001. Alien plant invasion in mixed-grass prairie: effects of vegetation type and anthropogenic disturbance. Ecological Applications **11**:128-141.

Data sheet
Record the sizes of the subunits used. Don't forget to flip the plot for the final subunit.
Record the smallest subunit within which each species occurred in each plot.

Date:_____ Site ID:_____ Page _____ of _____

Observer:_____ Subunit sizes: (1) _____ (2)_____ (3)_____ (4)_____

(5)_____ (6) _____ (7)_____

Species	1	2	3	4	5	6	7	8	9	10	Comment

Comments: _____
_____

A somewhat different datasheet is on the next page:

Datasheet for Nested Plots Protocol

Record the sizes of the subplots used; use only as many as in the plot. Don't forget to flip the plot frame for the final subplot. Record the smallest subplot within which each species occurred in each plot.

Date:_____ Observer:_____ Overall Site:_____ Planting Site: _____

Seed Mix Area Name: _____ Prairie Type: _____ Page ___ of ___

Subplot 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

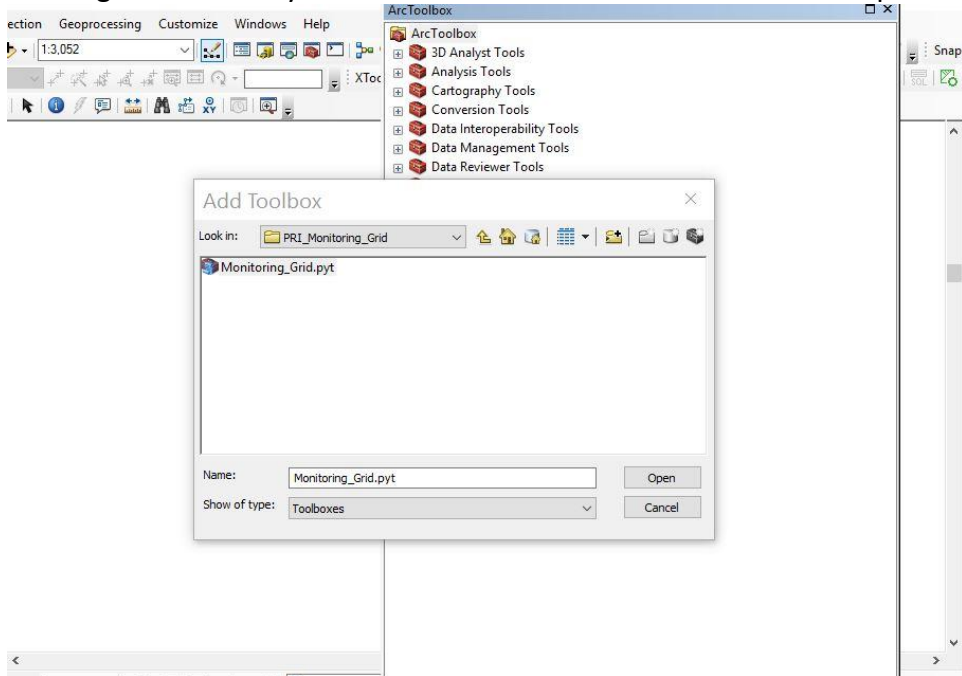| Species	Plot Number (minimum 10, maximum 40)										Comment

Appendix – Creating the Systematic Grid for Nested Frequency Plots

How to utilize the Monitoring Grid toolbox in ArcMap once you have downloaded and extracted the PRI_Monitoring_Grid.zip file from the PRI site.
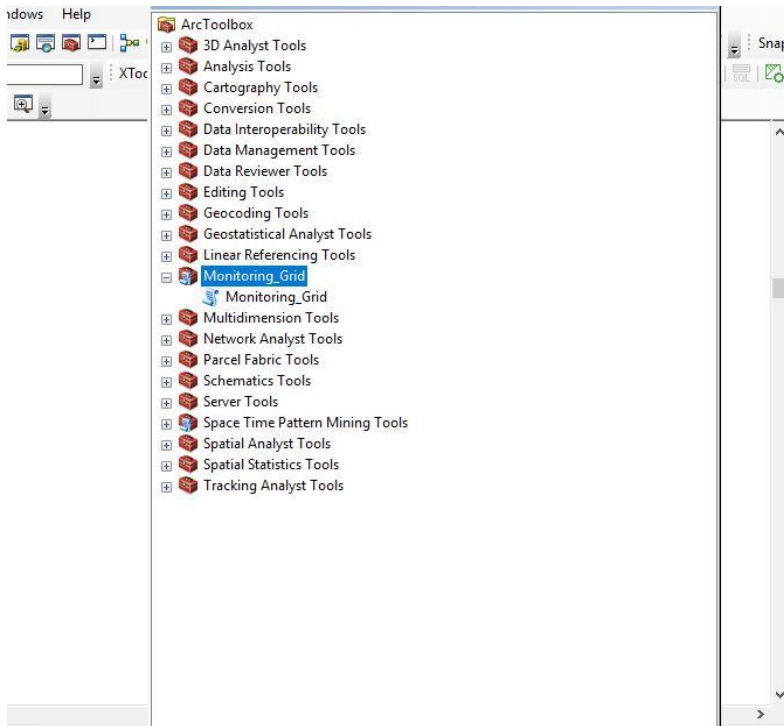
1. Open ArcToolbox
2. Right click in ArcToolbox and select "Add Toolbox"

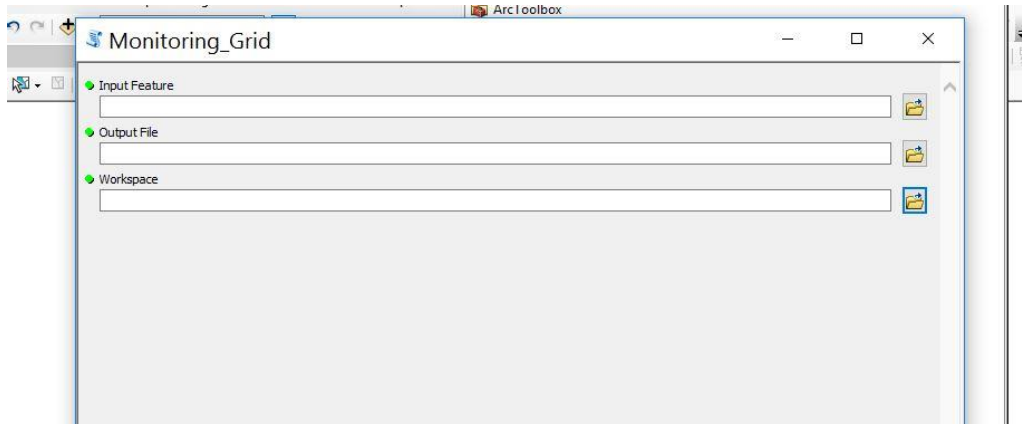3. Navigate to where you saved the extracted files and select and open the Monitoring_Grid.pyt file.

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

5. The **Input Feature** is the shapefile of the polygon of the 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 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. On large survey areas, the tool may create more than 40 survey locations; please be aware and delete unnecessary points.

The process that the script runs to create the monitoring grid is outlined below:

```
            #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")
```

Bee Sampling Protocol –June 2018

Effects of grazing versus fire for prairie management.

Materials:
GPS unit
Batteries
Stop watch
3.25oz plastic bowls; yellow, white, and blue
Binder clips
Wooden dowels or lengths of bamboo, cut to approximately 0.5m
Dawn dish soap
Gallon jug of water
Aerial insect nets
Ethyl acetate
Eyedroppers
Ethyl acetate kill jars
50mL polypropylene centrifuge tubes
cotton balls
Glassine envelopes
Field notebook
Pencil
Heavy paper
Whirl-Pak® bags
Brine shrimp net
70% ethanol
Cooler
Laser printer and acid-free cardstock for locality labels
Mason jar and metal ring of lid
Fine mesh
Scissors to cut mesh to size
Timer
Hair dryer
Petri dish (and a dry place to store them)
Pins
Foam block

Bee protocols:

We surveyed bees using two methods - passive trapping and active netting. Bowl traps are a highly replicable means of collecting bees over a period of time, with little-to-no observer bias. However, not all bees are attracted to standard bowl traps. Therefore, directed netting at a site by an observer can serve to round-out species lists and provide a more accurate view of species richness when combined with bowl data.

Deploying bowl traps:

Obtain 30 standard 3.25 oz plastic bowls in three colors (white, yellow, and blue). At a randomly selected end of a predetermined transect, deploy bowls in a ladder-like pattern. Place a wooden dowel or length of bamboo approximately 0.5m long into the ground. Use a binder clip to hold the lip of the bowl to the elevated end of the stake. Fill the bowl with a mixture of water and Dawn dish soap. Using this same method, place a second and third bowl set five meters away from the transect in opposite directions, in effect creating a 10m long perpendicular transect. Use one bowl of each color in this set. Pace out twenty meters along the original transect, towards the other end. Place another set of three bowls, using all three colors. Repeat this until 30 bowls are in place. (Note: This modification of a standard bee-bowl transect, in which bowls are placed every five meters, served to create 20-meter gaps through which cattle could pass without encountering bowls.)

Netting bees:

Next, collect bees using an aerial net during a meandering walk of the site. The duration of this walk can vary depending on site size, but should always be recorded so that the collection effort can be calculated later. Wander through the site, looking for flowers. Net bees that are observed on flowers. (Note: We only collected bees when they were on flowers, in an attempt to minimize detectability biases.) Once captured in a net, bees tend to fly upwards. Hold the end of the net up so that bees corral themselves into the end of the net. Insert a 15mL polypropylene centrifuge tube containing an ethyl-acetate soaked wad of cotton into the net and maneuver it underneath a single bee. With some manipulation of the net and the tube, the bee will fall into the tube. Cap the tube while it is still in the net to prevent escape. Using a pencil, record the species of flower the bee was found on, the site name, date and time on a glassine envelope. Place the now quiet bee into this labeled envelope and put the envelope into a charged ethyl-acetate kill jar. Repeat until all bees are removed from the net. Continue the meandering walk, repeating the above procedures when bees are encountered on flowers.

Collecting bowl traps:

Approximately 24 hours later, collect bowls. Unclip bowls from their stakes, and dump the contents of each bowl through a brine shrimp net. (Note: We removed moths and butterflies from our samples, with butterflies set aside and moths discarded.) Collect stakes, bowls, and clips. Once all bowls are removed, or periodically if necessary, transfer the contents of the shrimp net to a single Whirl-Pak®. Label the bag with a permanent marker, noting the site, date, number of bowls collected, the time at which the last bowl was deployed and at which the last bowl was collected. Replicate this information in pencil on a sturdy piece of paper and insert into the bag. Fill bags with sufficient 70% ethanol to fully cover all insects. Seal the Whirl-Pak® by squeezing out as much air as possible, rolling the top of the bag down to the level of the ethanol, bending the wires together and twisting them together. Place samples in the freezer until they can be washed, dried, and pinned.

Processing bowl samples:

Once in a laboratory, bowl traps can be processed. Remove a single sample from the freezer and empty into a Petri dish. Split the sample into multiple dishes if it is too large. Add liquid so that insects can be moved around and slightly disentangled. Using a stereo-microscope and forceps,

separate bees from non-bees, placing non-bees into a separate Petri dish. Pour the bees into a mason jar. Add warm water and a small amount of Dawn dish soap. Place a piece of fine mesh over the mouth of the jar and attach the metal ring component of the jar lid. Cover the mesh with a gloved hand and shake the jar vigorously for one minute. Rinse the soap out through the mesh. This will take multiple rinse cycles. Once insects are no longer soapy, blow a hairdryer through the mesh, rotating the jar until no insects stick to the sides of the jar. Adding small pieces of balled-up paper towel can help fluff bees and remove some excess moisture. Remove metal ring and mesh. Pour all insects from jar into a covered petri dish. Copy collection information from Whirl-Pak® bag onto a slip of paper and include it in the Petri dish. The sample may then be refrozen to be pinned at a later date, or pinned immediately.

Butterfly Sampling Methods

Effects of grazing verses fire for prairie management

June 5, 2016

Julia Leone

Introduction:

Native tallgrass prairie once stretched across much of the Midwestern United States. Now, less than 2.4% of original extent of tallgrass prairie remains (Samson et al. 2004). In Minnesota, we have lost over 98% of the tallgrass prairie and what remains is highly fragmented. Historically, the prairie was maintained through wildfire and bison grazing (Middleton 2013). In its current state, land managers seek to manage the prairie in a way that mimics these historic strategies, using managed burns and, more recently, conservation grazing. The tallgrass prairie of Minnesota is home to many butterfly species which are sensitive to disturbance. Although much has been documented about the effects of fire on northern tallgrass prairie, much less is known about the effects of conservation grazing, particularly for prairie dependent butterflies in Minnesota. The butterfly sampling methods below seek to address the questions of how these different management regimes affect abundance, community composition, species richness, and diversity of butterfly species found in Minnesota's tallgrass prairie.

We will survey butterflies using a modification of the "Pollard Walk" (Pollard 1977; Pollard and Yates 1993). These methods have been widely used to answer questions of butterfly abundance, community composition, species richness, dominance and diversity (Murphy and Weiss 1988; Swengel 1996; Thomas 2005; Swengel and Swengel 2013).

Butterfly surveys will occur during the 2016 and 2017 growing seasons (May-September). Butterfly surveys will be conducted on a subset of 20 randomly-selected remnant prairie polygons out of a total of 75, all with a 10-year known management history. Ten polygons will have been predominantly grazed, 10 predominantly burned.

Butterfly presence and abundance will be assessed in specific survey locations within each polygon, using the same transects used for vegetation surveys and bee bowls. Each of the 20 sites will be surveyed three times per summer, moving from furthest south to furthest north to follow phenology. Start and end time, temperature, wind speed, and % cloud cover will be recorded and surveys will be confined, when possible, between 0930 h and 1830 h when temperatures are above 18°C, sustained winds are less than 17 km/h, and cloud cover is <50%. The observer will walk the transect at a steady pace of approx. 10m/min, identifying and recording each butterfly seen within a 5-meter box to the front (2.5m on either side) (Shepherd and Debinski 2005; Davis et al. 2007; Vogel et al. 2007; Davis et al. 2008; Kadlec et al. 2012). We will take care not to sample any rare or endangered butterflies, such as *Hisperia dacotae* or *Oarisma Poweshiek*.

Transects will be delineated on maps prior to the field season and will run parallel to any elevation gradient; if none exists, a random numbers table will be used to select a compass bearing.

Field Materials:

GPS, spare batteries

Insect nets

Stopwatch (to time walk)

Watch or cell phone (for start and end time)

Wind gauge

Thermometer

Binoculars

CO_2 dispenser

Extra CO_2 cartridges

Clear centrifuge "sleep" tubes (ca. 4cm diameter)

Digital camera/phone for photographing difficult to ID individuals

Kill jars

Ethyl acetate for kill jars

Glassine envelopes

Data sheets

Butterfly identification guides/sheets

Flowering plant identification guides/sheets

Clip boards

Sharpies

Pencils

Duct tape

Flags

Flagging tape

Scissors

First aid kit

Specimen preparation materials:

Pins (size 1, 2, 3)

Pin holder

Label height setter

Glassine paper

Foam block

Butterfly spreading block

Cotton

Scissors

Forceps

Inkjet printer and acid-free cardstock for locality labels

Humidifier

Sampling Procedure:

1) Prior to visiting each field site, a random point generator will be used to create random points within each polygon using ArcMap. Transects will run through a random point, parallel to any elevation gradient, and will equal a total fixed length of 400m. We will start our transects 10 m from the edge of the polygon to minimize edge effects. The number of transects will vary depending on polygon shape and size.

2) Download GPS points for transects within each polygon onto GPS unit from computer or laptop prior to going into the field.

3) Locate the first transect within the polygon. Ends of transects will be marked with flags and numbered. Each numbered transect corresponds with a GPS location.

4) Charge kill jars with ethyl acetate to prepare to collect any difficult to identify/voucher specimens.

5) Record start time, wind speed, temperature, and percent cloud cover. Record end time when finished surveying transect.

6) Each 400m transect will be surveyed once per site visit. The observer will walk the transect at a steady pace of 10m/min. All butterflies seen 5m ahead and 2.5m on either side of the observer will be identified and recorded. The clock should be stopped to process and record individuals. Butterflies can be netted if difficult to identify on the wing and, in rare circumstances, collected to identify in the lab (see below). Butterflies netted for identification will be released after identification.

7) If a butterfly cannot be identified by netting and examining in the field, it can be collected, placed in a sleep tube, given a light pulse of CO_2 to knock it out, and then identified or photographed for later identification. Butterflies should be removed from sleep tube as soon as they have ceased moving to prevent any harm and identified or photographed in hand. Recovery takes 30 seconds to a few minutes, after which butterfly will be released.

8) If a butterfly cannot be identified by netting and examining in the field or by the CO_2 method described above, we will collect the specimen for identification in our lab at a later date. Each butterfly will be placed in its own glassine envelope with unique number (initials + polygon ID + transect number), time of capture and % open sky, and the envelope placed in a kill jar charged with ethyl acetate. We will collect at least one voucher specimen of each butterfly species encountered across all sites to assemble a voucher reference collection.

9) Vouchered butterflies will be spread, pinned, identified, and databased at the University of Minnesota Insect Collection.

10) After transect walk is complete, a time-constrained opportunistic walk will be conducted to look for other butterflies not seen along transect. Record starting and ending time. Time will vary based on polygon size, shape, habitat quality, etc.

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Effects of Grazing Verses Fire for Prairie Management Study - **2017 Butterfly Transect Datasheet**

Date:_____

Location: _____

Transect length: _____

Prairie type: _____

Transect ID: _____

Management: _____

Observer: _____

Avg Wind speed:_____km/h

Temperature:_____ C

Cloud Cover: _____ %

Start time:_____ h

End time:_____ h

No.	Time	Butterfly Species	#	Activ.	Dist (m)	Status	PT	Comments
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								

Management: G=Grazed, B=Burned **Prairie type:** D=Dry, M=Mesic, W=Wet **Dist**: Est. distance from observer **PT** = Prairie type

Activity: N = nectaring, R = resting, I = interacting, F = flying, FL = flushing, M = mating, OV = ovipositing, O = other

Status: CR=captured and released, P=photographed, C=CO2 method, V=vouchered, GS=good sight, S=sight, PS=Poor sight

Effects of Grazing Verses Fire for Prairie Management Study - **2017 Butterfly Opportunistic Walk Datasheet**

Avg Wind speed:_____km/h

Temperature:_____ C

Cloud Cover: _____ %

Date:_____   **Observer: _____**

Location: _____   **Management: _____**

Start time:_____ h

End time:_____ h

No.	Time	Butterfly Species	#	Activ.	Dist (m)	Status	Comments
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							

Management: G=Grazed, B=Burned

Activity: N = nectaring, R = resting, I = interacting, F = flying, FL = flushing, M = mating, OV = ovipositing, O = other

Status: CR=captured and released, P=photographed, C=CO2 method, V=vouchered, GS=good sight, S=sight, PS=Poor sight