“Manisa bozaka” or “Counting grass”: Global Grassy Group guide to understanding and measuring the functional and taxonomic composition of ground layer plants

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Method Article

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Abstract

Grassy biomes span more than 40% of the global land surface and are central to people, biodiversity and Earth System functioning. There is however limited standardised measurement of herbaceous taxonomic and functional composition in grassy biomes that inhibits the development of a comparative understanding of grassy biomes among geographic regions. Here, we present a protocol for the measurement of herbaceous richness and composition to motivate for much needed data standardisation in the measurement of grassy biomes. The data collection protocol and associated data management system are designed to have utility for fields of research ranging from phylogenetics and taxonomy to functional, community and ecosystem ecology. The described data collection protocol links to a data management system designed to foster collaboration and equity among biologists and ecologists working on herbaceous plants and grassy biomes.

Introduction

Our aim is to quantify species richness and composition of herbaceous plants in grassy biomes by counting species and functional groups in a series of plots matched to a list of rapid questions about site environments and histories. To date, we have applied our method at 400+ sites in Madagascar, Angola, South Africa, Zambia, Mozambique, Thailand, Cambodia, and Brazil. Originally, our approach was to only count grass species, and we now elaborate our method to all herbaceous species.

Across grassy biomes the composition, structure and biomass of the vegetation vary as a product of biogeography, climate, geology, landscape position, soils, drainage, tree cover, herbivore regimes, fire regimes, and land use (for example, recovery from ploughing). Combined, these environmental and historical factors shape plant community composition and the life history strategies and functional traits of the composing flora and can be complex to disentangle the relative role of each without standardised data spanning environmental gradients.

Despite the grassy ground layer being the defining element of grassy biomes, ground layer data are not always collected even though its constituent grasses and forbs drive the processes central to shaping the dynamics of these ecosystems. Among grassy biomes, there is generally a lack of consistency in data collections that due to the nature of herbaceous plants makes it difficult to compare richness, functional and taxonomic composition among sites of varied size and organisation. Our approach is to motivate standardised data to foster and facilitate new data collections in grassy biomes to answer questions about community assembly related to environmental change.

Figure 1 is an example of three sites proximate to one another where fire treatments have been applied as annual late-season fire, annual early-season fire and fire exclusion in central Zambia. These three sites demonstrate the small scale over which conditions can vary. Appropriate management in the context of fire, animals and human use requires an understanding of the composition, structure and functioning of the ground layer alongside the woody vegetation.

Here we outline our data collection protocol that provides the basic data to assess species composition and structure of grassy ecosystems. Our method has evolved from that originally developed by Vorontsova et al. (2016) and further developed by Solofondranohatra et al. (2020).

For more information about our research: https://globalgrassygroup.github.io/.

Reagents

Equipment

1. General

- Field datasheets and clipboard
- Notebook (waterproof if possible)
- Pen, pencil, permanent marker
- GPS working in WGS84 and recording in decimal degrees
- Camera
- Compass to note bearing of transects
- Either 25 m or 50 m measuring tapes (or 25 m of rope with marks at each 5 m interval)
- 1 m diameter circle made of a resilient material – easiest to use flexible pipe or hose
- 2 m tall measuring staff (e.g. plumber conduit pipes with electrical tape marking height increments) and/or 5 m steel measuring tape
- Densiometer or equivalent for measurement of site-level tree cover
- Shovel/trowel

- Stanley knife
- Ziplock (or sealable) plastic bags for soil samples
- Munsell colour chart (or appropriate app)
- Bottle of water
- Low/short wood or metal stakes to mark sites for repeat monitoring (as appropriate)
- Mallet
- Hand lens

2. Sampling plant specimens for identification, DNA and vouchering

- Plant press with sufficient paper and cardboard for drying of specimens
- Notebook for specifically recording plant collection details
- Large robust bin size plastic bags
- Plant tags for labelling specimens
- Silica gel (for DNA vouchers)
- Any breathable small paper envelopes (for DNA) such as tea bags or coin envelopes
- Large tupperware container or heavy-duty gallon size Ziploc bags for holding DNA samples and silica

3. Site-level tree cover measurement

Within our protocol we make a site level estimate of tree cover at the centre plot (Z0). There are numerous alternatives to estimating overstorey cover and different researchers will have access to a different equipment of varying complexity. We find that in the tropics, equipment breaks easily due to the humidity and temperatures. Hence, we advocate for less technical equipment, and that should also make it more widely accessible. Our preferred option for measuring tree cover is a spherical crown densiometer and these are around £80. It is a small box that fits into a pocket with a metal convex mirror. For explanations on how to use it, please go to: https://youtube.com/watch?v=BavU4qicBXE.

Other options for measuring tree cover are:

- Periscope Densiometer: http://www.forestry-suppliers.com/Documents/1450_msds.pdf

- Ceptometer (AccuPAR) – (needs 4AA batteries): https://www.metergroup.com/environment/products/accupar-lp-80-leaf-area-index/

- Fish eye lens camera although this will also need batteries and/or charging. Photos need to be processed at a later date to determine leaf area index. There can be limitations on the time of day to take photos to avoid direct sun within an image. Increasingly, there are phone apps or small fish eye lens that can be attached to cameras.

- Mobile app (Android): https://play.google.com/store/apps/details?id=new4.glama.glamanew4

Procedure

SECTION I: BEFORE DATA COLLECTION

1. Choosing site locations

Both the ground layer and overstory should be homogeneous within the sampled 0.25 ha site. It is worth considering whether the sighting of a location is representative of wider environments in terms of soils, slope, drainage, grazing, land use, browsing, and fire. Environmental conditions will need to be taken into account when establishing sites and relative to the purpose and research questions of the investigators responsible for the data collection at any site(s). As an example, sites could be located proximate to one another where there is a demonstrable difference in any of: 1) fire regime as differences in fire frequency and/or season; 2) the top and bottom of a slope reflecting shifts in drainage and soil water, or 3) land-use histories such as relative to clearing and ploughing. With this in mind, site locations can be stratified to compare differences in environment categorically or as a gradient (e.g. species colonisation with time since ploughing; or composition related to variable tree cover). To capture the environmental dimensions of a site, our protocol encompasses a series of rapid metadata questions to enable standardisation and comparability of sites collected by different researchers with potentially different research questions or environmental gradients in different regions. These questions are around landforms, soil characters, vegetation type, fire regimes, herbivore regimes, and human use.

As part of any work involving vegetation composition and site selection, we advise collaborating with and consulting local communities and stakeholders (such as protected area managers) around site histories and the choice of site locations. It should be the default to engage with people on whose land one might be working. Moreover, this engagement is crucial to understand the appropriate social and cultural

histories of sites. Commonly, broadscale remotely sensed spatial datasets are used to interpret environments such as those related to night lights, tenure, towns, and road networks. These remote data have their value but are not a replacement for discussions with communities and stakeholders around fire, grazing and elements of human use (e.g. plant harvesting for foods, medicines and building materials). Ground layer plants are small, and difficult to remotely observe, just as remotely sensed information about herbivores is also problematic.

The protocol metadata include standard requests for basic geographic information around latitude, longitude, and elevation alongside requests for relevant permit numbers. Our request for permit information is a safeguarding measure to ensure researchers engage with appropriate and relevant authorities for the implementation of sites and any plant collections. Plant material either as vouchers or in silica cannot be accessioned to herbaria in the absence of relevant permits. As researchers working within international herbaria, we work according to the Nagoya Protocol (Convention on Biological Diversity, 2011). We are also aware some sites where data are collected may be of cultural significance, or sites may contain information about rare, endangered or CITES-listed species and sharing such geographic information publicly may be inappropriate if it can facilitate exploitation or cause harm to local communities or cultural heritage. If as a researcher you have concerns about sharing site location information that will later become public, please get in touch with us directly and we can discuss data sharing specific to sites to safeguard information as appropriate. The complete description of site metadata can be found in Appendix 1. Further information related to permits, funding and contributors can be found in Appendices 4 and 5.

2. Layout and permanent marking of sites

Our aim is to characterise ground layer plant functional and taxonomic composition at a given site. Site layout is a 50 m × 50 m cross centred in an area of homogenous vegetation cover and covers a 0.25 ha area (Figure 2). The presence of all ground layer plant species is recorded in twenty-one 1 m diameter circular plots arranged in a cross.

The arrangement of the 21 plots as a cross gives us a picture of composition over a wider area than would be the case if a single central plot of the equal area was sampled. The most common method for documentation of ground layer species composition is one single plot. However, a single plot does not

capture the turnover of species at a small scale that is common in the ground layer and where a series of plots over a wider area are more likely to capture rare species.

The design focuses on counts in each of the 21 plots to provide a site-level estimate of richness and the rank frequency of constituent species (akin to rank abundance). It is common in grassy ecosystems to work with either aerial or basal tuft cover as measures of abundance. However, estimating species level cover can be time-consuming, prone to observer bias, difficult in large plots, impossible to meaningfully compare in grassy ecosystems with mat-forming (i.e. clonal) and tussock plants, and where some species are very small. Further, studies among regions collect cover in substantively different ways that cannot be reconciled.

Finally, counts done over a series of plots of known area enable area-based rarefaction. For studies where we wish to compare richness and composition with alternate methods or site sizes, this approach offers the opportunity to rescale site size for comparability where richness and composition are commonly collected over 2-10 m^2.

Figure 3 shows species accumulation curves based on our protocol at 13 sites in Madagascar. The approach captures site-level grass species richness well but can be more variable with forbs as some sites are surprisingly diverse. Where we collected forbs, we have had variable patterns in the shape of the species accumulation curves: generally forbs turnover at smaller scales than grasses.

Researchers need to calculate ground layer productivity and biomass in conjunction with composition. We advocate avoiding destructive sampling within the 21 plots that constitute a site. Destructive sampling within the 21 plots can, over time, feedback to influence future community composition. Options for quantifying biomass are provided by SEOSAW (https://seosaw.github.io/manuals.html).

a) Repeat measurements through time

In different contexts it can be more or less difficult to establish sites as permanent because materials used to mark the centre and/or corners of sites can be stolen or disturbed by people or animals. At its

most fundamental, stakes of either metal or wood can be used to mark the centre point of a site and the four corners as endpoints of transects. Each researcher will understand best their local site context to determine the most appropriate way to permanently mark sites for repeat measurement. Stakes along with string/rope/tape measure run along the two crossed transects will adequately relocate sites.

Linking ground layer and overstorey layer data collections in grassy biomes with trees and shrubs is fundamental to the holistic consideration of floras and their dynamics. The best way to do this depends on the size of the site used for monitoring trees/shrubs. There is a trend towards 1 ha size permanent sites for monitoring trees and shrubs but many existing tree plots within grassy biomes are smaller than that. To link this protocol to permanent sampling sites that are < 0.25 ha, it would be best to centre the ground layer layout (Figure 2) within the site and to have it simply extend beyond the edge of the woody vegetation site so that the area sampled for ground layer composition remains 0.25 ha and 21 plots. If a permanent monitoring site for trees/shrubs is 1 ha, or larger, there are two basic options. 1) Replicate within the larger tree/shrub site as appropriate, i.e., a 1 ha tree/shrub site would contain four replicates of the outlined ground layer protocol. Or, 2) centre the ground layer site within the tree/shrub site as a nested design. The appropriate option will be researcher-dependent relative to their time and interests: the GGG is happy to receive and manage data contributions that conform to our overall data design.

3. Getting to know taxonomic and functional composition of a site

To bridge the functional group and taxonomic divide that can be intimidating for researchers with limited plant identification skills, we have compartmentalised our protocol with two options as: 1) broad functional groups requiring no taxonomic information (Appendix 2); or, 2) species lists to document composition (Appendix 3).

Contrary to the convenience of trees/shrubs that are permanently visible and able to be structurally measured at a site, no one sampling time within a year enables quantification of total ground layer taxonomic or functional composition: the time of year when sampling occurs will influence the composition recorded (Figure 4). Further, the ability to identify and record different species will change not only within a year but also from year to year due to responsiveness to antecedent rainfall conditions stimulating germination of different cohorts of annual and perennial herbaceous species. Some plants flower only in narrow time windows and are only observable over a few weeks. For example, a number of small grasses such as *Microchloa* species often flower early only to be overtopped one month later by

taller *Aristida* or *Eragrostis* species, which are consequently overtopped by taller later flowering *Cymbopogon* or *Digitaria* species. Forbs with bulbs such as most orchids and lilies produce fleshy leaves that rapidly decompose after flowering and setting seed. There are often distinct cohorts of species that emerge and flower pre-wet season, as the wet season progresses, early dry season and post-fire. Ground layer biomass production peaks with the peak of the growing season that is generally in the latter part of the wet season. This explains why ground layer plant diversity can be poorly characterised and understood.

In a world with no constraints on sampling, surveys would occur in several seasons throughout the year. But, this is generally not possible and unrealistic as it is time and labour intensive. Further, seasonal site access issues can make access to grassy field sites throughout a wet season difficult or prohibitive.

Within the tropics, **sampling in the late wet season or very early dry season will enable the identification of most grass species** that will have flowered by this time of year, and these are the plants that contribute most to ground layer biomass. Sampling in the late wet season and early dry before substantial curing and senescence of the grasses has occurred will enable species identifications and a robust estimate of cover and biomass if these are to be collected in tandem.

If your research questions relate to quantifying total species richness and composition, repeat sampling at up to four points throughout a year will help capture a closer estimate of this and enable interesting questions about ground layer phenology. These times would roughly correspond to before the rain, mid growing season, end of the season, and post-fire. Take the time to understand your sites, and the sampling time(s) that will be most appropriate for one-off and repeat monitoring (while being pragmatic!) (Figure 5).

a) Plant functional groups

Taxonomic diversity and composition of ground layer plants are less well studied than with trees and shrubs because of the various issues outlined above, and simply that these plants and their distinctive floral characters are small, requiring the use of a hand lens. Full species lists for ground layer sample sites will often only become available after careful identification in herbaria, but in the field it is possible to rapidly assess the presence and frequency of plants with certain functional characteristics. Within

Appendix 2 and Datasheet 2, we have a series of rapid assessment Presence/Absence questions that enable the calculation of rank frequency information of eight functional groups (Appendix 7). In each of the 21 plots, mark which functional groups are present:

- Mat-forming grass
- Tussock grass
- Sedge
- Forb
- Leguminous forb
- Succulent plant
- Spiny plant
- Woody seedling

The relative abundance and frequency of each group varies with environmental conditions. Mat-forming grasses are often associated with grazing. Tussock grasses are often associated with fire. Both grass groups across tropical open ecosystem landscapes are more often than not C_4. Sedges are often associated with high soil moisture and waterlogging and can be either C_3 or C_4. The category of forbs encompasses numerous plant families and can be defined as flowering herbaceous plants of self-supporting growth form, excluding graminoids (grasses (Poaceae), sedges (Cyperaceae), rushes (Juncaceae) and similar groups). We also separately distinguish leguminous forbs (family Leguminosae) which have the capacity to biologically fix nitrogen and in nutrient-poor landscapes can make important contributions to N budgets. Geophytes are important functional components of the ground layer but we do not distinguish them here as a separate group from forbs because they can look superficially similar above ground to forbs (e.g. *Dioscorea* and *Ipomea)* – without excavating roots, it can be difficult to identify them (Figure 6). Succulent species may be indicative of seasonal water stress and these plants generally use CAM photosynthesis. Spines and spiny plants in the ground layer are often indicative of structural herbivore defence. Observation of woody seedling recruitment in grassy biomes can be rare and is worth documenting. Delimitation of a seedling can be done by examining the stem base for scarring and evidence of the previous top-kill by fire or herbivory. Note that some individuals can fit more than one category. For example, a *Euphorbia* can be both spiny and succulent.

b) Measuring grass height

The average grass height should be estimated as the leaf-table height. It is defined as the height equating to the 80^{th} quantile of grass height (the height below which the main bulk of the leaf canopy occurs) (Figure 7). It should be measured in cm with measuring staff.

4. Why voucher specimens and collect DNA?

We request collection of herbarium vouchers and/or plant material in silica of (morpho)species recorded within sites. The majority of ecological plot networks do not request such collections but consequently have no capacity to determine the veracity of species identifications at a later stage. We are cognisant that vouchering is the most time involved component of the protocol for composition but we believe it is fundamental to the long-term utility of data.

All countries hold herbarium collections of their plants, which are libraries of plant specimens open for everyone to use. We recommend colleagues form relationships with their local herbaria, deposit voucher specimens there, and visit the collections to identify their voucher specimens accurately. We will also identify opportunities for DNA sequencing of the vouchers, both for the purposes of identification and for research on genetic diversity.

Index Herbariorum (NYBG, n.d.) is a searchable database of global herbaria: Index Herbariorum - The William & Lynda Steere Herbarium and here you can find contacts to a herbarium in the region and country where you plan to work.

a) We request vouchers for multiple reasons:

- As good as ecologists think they are at identifying plants, even specialists very often get identifications wrong. More than 50% of tropical plant specimens (both woody and herbaceous), on average, are likely incorrectly named (Goodwin et al., 2015). Without a reference collection, naming

cannot be cross-checked or updated at a later stage rendering species concepts essentially void when seeking to compare composition among sites and regions;

- Building herbarium records of specimens that will be shared with wider research communities is central to progressing biodiversity science whether it is through GBIF to examine macroecological patterns or for taxonomy and naming of understudied plant groups among regions;

- A species is our fundamental study unit of which we connect layers of information integrating evolution and functional traits and hence taxonomically verified information is vital. For example, material in silica can be sequenced to contribute to anything from population genomics to phylogenetics. Just as specimens provide a future basis for functional trait research;

- Ensuring appropriate and relevant links/collaborations with local botanists and herbaria to build regional collections. In many tropical countries of the Global South, botany is drastically under-resourced hampering the development of in-country collections and expertise in the midst of the biodiversity crisis. Historical inequalities due to colonial exploitation see privileged institutions such as Edinburgh and Kew maintain internationally significant collections that enable researchers of those organisations to often be better placed to understand regional plant diversity than the researchers in the country of plant material origin. Integrating collaboration with local and regional institutions is essential to good practice and developing regional collections.

b) Our approach to plant collection:

- We make two duplicate vouchers to be submitted to two different herbaria. A) One specimen remains in-country with the collaborating herbarium and botanist, B) One specimen is lodged with a major herbarium (e.g., SANBI, Kew, Edinburgh), and where it is more likely the material will be scanned and placed online in the foreseeable future for accessibility. This does require collaboration with herbaria and professional botanists.

- A herbarium voucher and silica gel collection are made of every herbaceous species including those in the vegetative state.

- Samples collected as herbarium specimens must be tagged and adequately labelled along with the associated leaf (and seed samples) in silica gel. A good approach to this is to put individual samples into labelled breathable bags (teabags work well), and put all of these into a Tupperware or ziplock bag with silica gel in it. Samples collected in silica can be kept in perpetuity once a sample has been dried out in the silica gel. It is important to collect samples in silica given the rapid degradation of DNA. Moreover, vouchers should be pressed and preserved in silica already at the site to minimise errors.

- We do not voucher CITES-listed species. In grassy biomes, vouchering of ground layer plants invariably requires destructive harvesting of a whole plant. CITES-listed species can not easily have material moved across borders. In this case we use photographs for identification. CITES-listed species such as orchids, cacti and other succulents such as aloes are generally well studied and a high-resolution photo is generally enough for identification. If a researcher is interested in using this protocol specifically to understand CITES or endangered species we would be happy to discuss directly the best application of the vouchering process.

Learning plant collection itself is a skill and is why collaboration between ecologists and botanists is vital to progressing our understanding of the biodiversity and ecology of these ecosystems.

Here are links to videos about plant collection: https://globalgrassygroup.github.io/resources.

5. Soil description and sampling

At a site, we make a basic in situ description of soils. There is an optional request for a physical sample of soil to be kept for soil analyses at a later date. Within grassy biomes, soil characters will play a significant role in community composition and life-history strategies.

At a site, we sample soils from 0-5 cm depth. We collect soils from the centre plot (Z0) and the end plot of each transect (A25, B25, C25, D25). These are then bulked. Use this bulk sample to assess:

- Munsell colour
- Texture using the approach in Figure 8

We make site-level categorical characterisation of the drainage, estimated soil depth, soil organic content and any other relevant notes on the soils, e.g. large fragments of charcoal, or termite activity.

If a soil sample is collected for subsequent analysis, the soil should be air-dried prior to storage, and no more than 500 g of the bulk sample is required.

Be aware that permits for the collection of soils and soil exports are generally different to those for plant collections.

SECTION II: PROTOCOL 1 - TAXONOMIC AND/OR FUNCTIONAL COMPOSITION SITE SURVEY

1. The first step is to familiarise oneself with the diversity of plants at the site by collecting flowering individuals, identifying as many species as possible, getting local names from local experts working with you, and giving descriptive names to species that cannot be identified (i.e. identify morphospecies). These collections of whole plants can be used in the voucher collections for herbarium specimens, and so are not wasted. Once a decent understanding of herbaceous diversity at the site has been attained, then proceed with sampling the site.

a. If you are only collecting functional composition ensure to be able to differentiate between: 1) mat-forming grass, 2) tussock grass, 3) sedge, 4) forb, 5) leguminous forb, 6) succulent, 7) spiny plant and 8) a woody seedling (Appendix 7).

b. If you are collecting taxonomic composition, collect all species visible and identifiable at the site. Care should be taken to examine all plants that are not flowering, distinguish between these, and find nearby flowering individuals where possible to aid identification and for the herbarium voucher. *If no flowering individuals can be found then you should still collect herbarium vouchers.*

2. At the centre of the site, place a centre circular plot 1 m in diameter. If the 1 m plot overlaps a tree or rock that is too large to place the circular plot over then relocate the plot so the tree/rock is just outside the circle. However, trees and rocks that are smaller than 1 m in diameter should still be included in the plots (make sure to estimate their cover as noted below in 6j and 6k).

3. From the centre of your 0.25 ha site, lay out two 50 m transects perpendicular to each other that cross at the centre plot. By default, we use N, S, E, W. Although this may vary if sites are located on steep slopes or along forest edges.

4. Compile site metadata using *Datasheet 1* linked to the descriptions of these listed in *Appendix 1*. These are all information and measures that are a once-off for the site. These include: noting the plant groups recorded, location, landform and geology, a site photo, soil sample and soil characterisation, and descriptions of herbivores, fire, tree cover and land use. The list is designed to ensure comparability among sites.

5. From the centre plot, record the bearing for each of the four transect lines using a compass and record this on *Datasheet 2*.

6. Using *Datasheet 2*, now start data collection for each plot starting with the centre plot (Z0). *Datasheet 2* is a rapid assessment largely framed around Y/N questions to quantitatively calculate grazing intensity, and structural and functional characters of the vegetation. And, at each plot, this can be done in approximately 1-2 minutes. Indicate:

a. Is dung present in the circular plot? Y/N

b. If there is dung present, can the animal species of the dung(s) be added?

c. Is charring or ash visible in the circular plot? Y/N i.e. is there evidence of recent fire?

d. Is a tree/shrub canopy above the circular plot? Y/N

e. Is there evidence of grazing within the circular plot? Y/N i.e. are grasses chewed on?

f. What is the average grass height? Measured in cm with measuring staff. This measure is not the maximum height including inflorescences but leaf table height (Figure 7), which equates to approximately the 80th percentile of the grass height.

g. What is the average litter depth? Measured in cm with measuring staff.

h. Within the circular plot, what is the % cover of the bare ground?

i. Within the circular plot, what is the % cover of litter?

j. Within the circular plot, what is the % cover of rock?

k. Within the circular plot, what is the % cover of tree stems rooted in the ground?

l. Within the circular plot, what is the % cover of ground layer plant cover? Measured as basal tuft cover, to ensure the five cover values (h, i, j, k, l) sum up to 100%.

7. Continuing with *Datasheet 2*, we collect data on the presence or absence of broad functional groups described in Appendix 7. Carefully examine vegetation rooted within the circular plot. If you are familiar with the functional groups, this can take < 1 minute per plot. At each plot, indicate with a Y/N the presence of:

a. Mat-forming grass (Poaceae)

b. Tussock forming grass (Poaceae)

c. Sedge (Cyperaceae)

d. Forbs that do not fix N

e. Nitrogen-fixing forb (Leguminosae)

f. Spiny plants

g. Succulent plants

h. Woody plant seedlings

NOTE: If you only intend to complete FUNCTIONAL GROUP COMPOSITION at a site, the protocol is a matter of now repeating Step 6 and 7 at each of the 21 circular plots. You will not collect any data using Datasheet 3 that is for the listing of plant composition. Based on the above, with a team of 2-3 people, a site can be completed within 1 hour for functional composition.

8. Using *Datasheet 3*, SPECIES COMPOSITION: Carefully examine ground layer vegetation rooted within the circular plot. List all species present and mark the presence of that species simply with a '1' in the column linked to that plot. See the worked example of the data entry template. At this point, please also indicate whether you are recording only certain plant groups (e.g., grasses, forbs, or all plant groups) and indicate this in the metadata *Datasheet 1* against the variable 'SampledVeg'. Plant species that cannot be

identified should be given descriptive morphospecies names that can be used along with the herbarium vouchers, to be identified later. When recording species composition and collecting vouchers, the entire site sampling of 21 circular plots can be completed within 2-4 hours depending on the number of people and experience in plant identification, and the plant diversity at the site.

In total, 21 circular plots will be sampled for presence/absence, resulting in a frequency measure of each species at a site.

9. If you are intending to submit these data to the Global Grassy Group database, please now complete *Datasheet 4* which contains additional permit/funding information, and *Datasheet 5* which lists data contributors, their role in the project and data collection alongside their contact details.

10. Data can be submitted to the GGG database manager (email address can be found at https://globalgrassygroup.github.io/contact/).

Troubleshooting

Time Taken

Anticipated Results

References

Convention on Biological Diversity (2011) *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity*. Geneva, Switzerland: Secretariat of the Convention on Biological Diversity.

Goodwin, Z.A., Harris, D.J., Filer, D., Wood, J.R.I. and Scotland, R.W. (2015) 'Widespread mistaken identity in tropical plant collections', *Current Biology*, 25(22), pp. R1066-R1067.

GROW Observatory (2019) 'Carrying out the soil texture test', *GROW Observatory*. Available at: https://knowledge.growobservatory.org/index.html@p=478.html (Accessed: 10/05/2022).

New York Botanical Garden [NYBG] (n.d.) 'Index Herbariorum', *The New York Botanical Garden.* Available: http://sweetgum.nybg.org/science/ih/ (Accessed: 10/05/2022).

Solofondranohatra, C.L., Vorontsova, M.S., Hempson, G.P., Hackel, J., Cable, S., Vololoniaina, J. and Lehmann, C.E.R. (2020) 'Fire and grazing determined grasslands of central Madagascar represent ancient assemblages', *Proceedings of the Royal Society B: Biological Sciences*, 287(1927), 20200598.

Vorontsova, M.S., Besnard, G., Forest, F., Malakasi, P., Moat, J., Clayton, W.D., Ficinski, P., Savva, G.M., Nanjarisoa, O.P., Razanatsoa, J., Randriatsara, F.O., Kimeu, J.M., Luke, W.R.Q., Kayombo, C. and Linder, H.P. (2016) 'Madagascar's grasses and grasslands: anthropogenic or natural?', *Proceedings of the Royal Society B: Biological Sciences*, 283(1823), 20152262.

Wigley, B.J., Charles-Dominique, T., Hempson, G.P., Stevens, N., te Beest, M., Archibald, S., Bond, W.J., Bunney, K., Coetsee, C., Donaldson, J., Fidelis, A., Gao, X., Gignoux, J., Lehmann, C., Massad, T.J., Midgley, J.J., Millan, M., Schwilk, D., Siebert, F., Solofondranohatra, A.C., Staver, A., Zhou, Y., Kruger, L.M. (2020) 'A handbook for the standardised sampling of plant functional traits in disturbance-prone ecosystems, with a focus on open ecosystems', *Australian Journal of Botany*, 68, pp. 473-531.

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Figures

Figure 1

Three sites within 20 m of each other in Kitwe, Zambia. The herbaceous structure and composition vary with tree cover and fire regime.

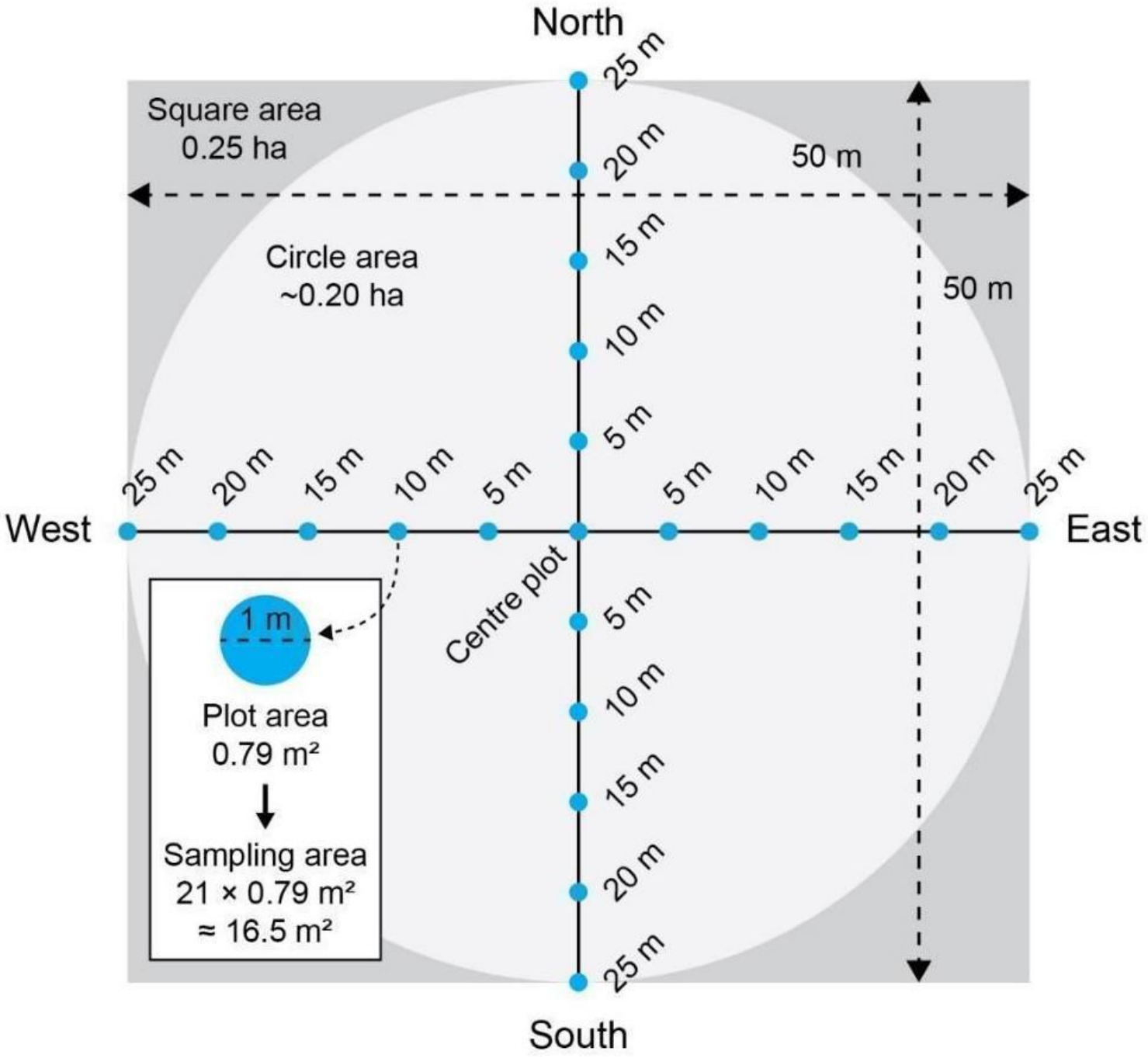

Figure 2

An example of a 50 m x 50 m layout. Not to scale! There is a centre 1 m diameter plot at the centre of the site. Roots of the four 25 m transects come off the centre plot. The resulting area sampled is 16.5 m^2 over a 0.25 ha area. This method is more spatially representative, quicker to sample than one large plot, and enables understanding of species accumulation.

Figure 3

Species accumulation curves based on the outlined method for 13 sites in Madagascar.

Figure 4

Herbaceous plants vary in their flowering times. Some will not be visible at all when not flowering, others will be harder to identify. The aim is just to record what is visible at the time of sampling.

Figure 5

Data collection in the field – specimen collection and species identification.

Figure 6

Diversity of underground storage organs (USO) structures that characterise geophytes. Geophytes are associated with seasonality and regular disturbance forming a wide diversity of long-lived belowground storage organs. Source: Wigley et al. (2020).

Figure 7

Measuring height of (a) tussock and (b) mat-forming grasses. Adapted from Wigley et al. (2020).

Figure 8

Suggested finger test for soil texture. First, wet the soil and knead to break down all aggregates. Source: GROW Observatory (2019).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Appendices.docx
- GGGfieldsheets.xlsx
- GGGsubmit.xlsx