Sampling ground layer diversity in tropical grassy biomes

Caroline Lehmann, February 2018

To capture the heterogeneity of the ground layer flora it is advisable to have a collection method that samples over a wide area, as opposed to a single small embedded centre plot (within a woody plant PSP) that may not match the heterogeneity of woody cover and woody diversity within a plot.

Here, we step through three options in terms of ground sampling over 0.25 ha which ideally matches to the permanent plot established for woody plants but that can be used as an independent protocol.

1. Complete diversity inventory along with ground cover and ground layer biomass.
2. Dominant species, ground cover and ground layer biomass.
3. Ground layer biomass and cover.

Plot layout for all three sampling designs

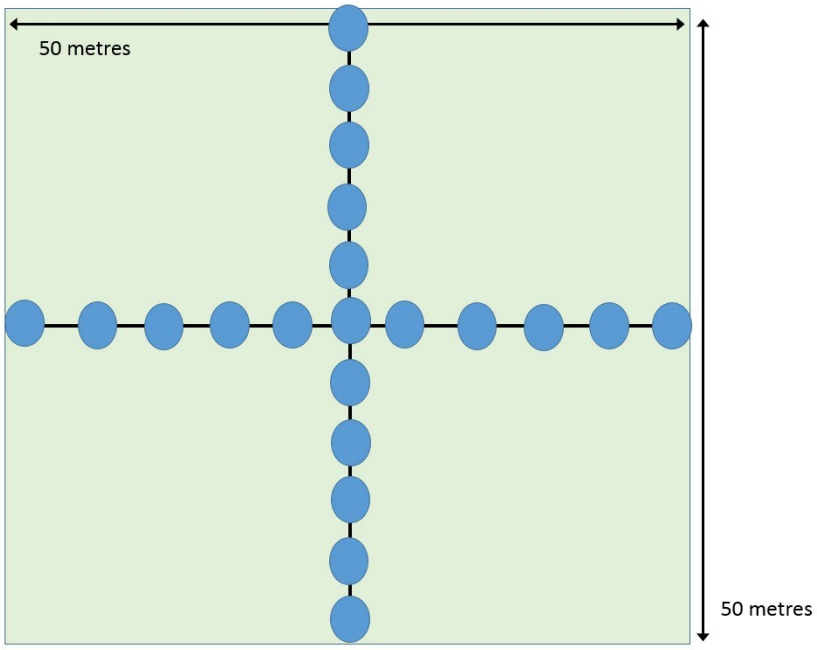


Figure 1: An example of a 50m x 50 m PSP. Not to scale! There is a centre 1m D plot at the centre of the PSP. Roots of the four 25m transects come off the centre plot.

From the centre plot, four transects each 25 metres in length are placed. On flat ground these transects run in cardinal directions (N, S, E, W), and on sloping ground these transects follow the slope and contour.

Requirements for plot establishment and recording

Data recording

- Notebook/data sheets
- GPS
- Compass
- 1m diameter circle made of a resilient material – easiest to use flexible pipe or hose

- Pen/pencil
- Densiometer

Metadata for all methods

- Always take a site photograph
- Latitude and longitude via GPS
- Elevation via GPS, altimeter or later estimated via a digital elevation model
- Slope (Flat, shallow, steep)
- Aspect via a compass
- Drainage, ie. is this a dambo or crest? What is the landscape position?
- Soil colour and texture, using a Munsell soil colour chart.
- General character of the vegetation and an estimation of canopy cover via a densiometer
- Disturbance regime and history – what site info is there on time since fire? Fire frequency? Grazing by domestic and/or wild grazers? Has the site been used for any form of agriculture or harvesting?
- Bedrock - what is the proportion of bedrock exposed? What is the bedrock?

Complete diversity, basal cover and grass biomass

Diversity

The sampling method aims to capture the diversity and relative frequency of Poaceae and associated ground vegetation (ie. monocots and dicots) within a uniform permanent sample plot 0.25ha in size, along with an estimate of bare ground, and grass biomass.

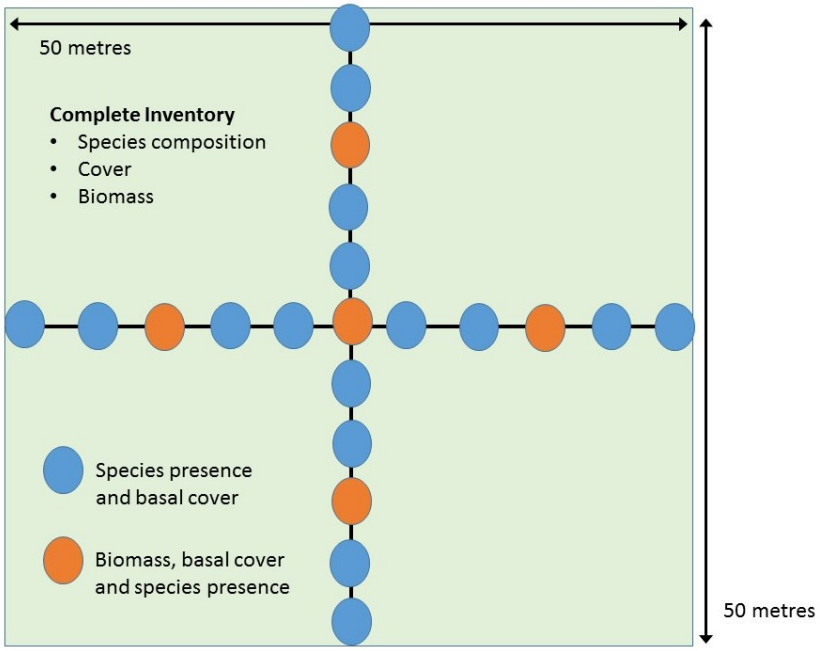


Figure 2: An example of a complete inventory on a 50m x 50 m PSP. Not to scale!

All of species cover (blue and orange circles) at 21 x 1m D plots, and biomass on 5 plots only (orange) that are smaller in size.

Method

1. The first step in the surveying should be to generally familiarise oneself with the diversity of the site by collecting flowering individuals and identifying as many species as possible, and also sorting out what must be identified later. These collections of whole plants can be used in the voucher collections for herbarium specimens, so are not wasted. Once a decent understanding of the grass diversity at the site has been attained, one can then proceed with sampling the plots. This involves identifying and recording all grass and forb species occurring within a plot.
2. At the centre of a 50m x 50m permanent sample plot, a centre circular plot 1 metre in diameter is placed (simply using a circle easily made from flexible hose pipe).
3. Vegetation within the circle plot is examined carefully and a full list of Poaceae and forb species rooted within the circle is made.
4. The percentage cover of bare ground is estimated (based on what is rooted, ie. basal not aerial cover)
5. The 1m diameter circle is then placed at 5m intervals along each transect. At each point, all Poaceae and forb species are listed within the circle and bare ground estimated as per step 3 and 4.
6. Finally, at the orange circles in Figure 2, grasses are clipped for biomass within a smaller area of 0.5m x 0.5m. Grasses are clipped at the base rooting into the soil, and bagged to be dried and weighed as a measure of biomass.

Care should be taken to examine all grasses in the vegetative state, distinguish between these, and find nearby flowering individuals (when possible) to aid identification. A herbarium voucher and silica gel collection should be made of every grass species from a PSP including those in the vegetative state

and those doubtfully distinct from other species. 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. Samples collected as herbarium specimens must be tagged and adequately labelled along with the associated leaf (and seed samples) in silica gel.

Requirements for sampling of plant specimens for identification and DNA

- Plant press with sufficient paper and cardboard for drying of specimens
- Large plastic bags
- Plant tags
- Silica gel
- Tea bags
- Large Tupperware container

Dominant species, ground cover and ground layer biomass

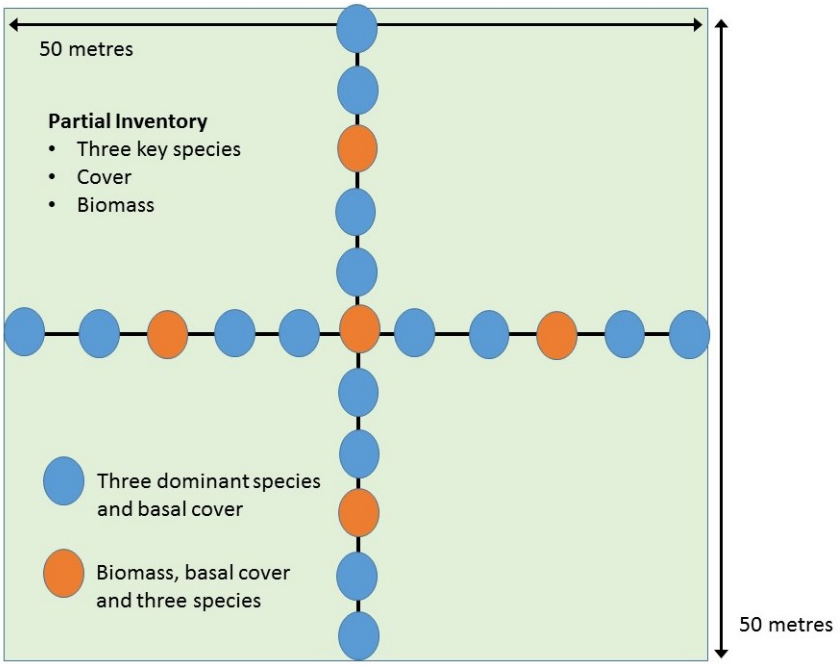


Figure 3: An example of a limited inventory on a 50m x 50 m PSP. Not to scale!

Three dominant species, basal cover (blue and orange circles) at 21 x 1m D plots, and biomass on 5 plots only (orange) that are smaller in size.

In this method, we are sampling for the three most dominant species and basal cover (at each blue circle) and biomass (at the orange circles).

Method

1. At the centre of a 50m x 50m permanent sample plot, a centre circular plot 1 metre in diameter is placed (simply using a circle easily made from flexible hose pipe).
2. Examine the vegetation within the circle plot and list only the three most common grass species within the plot.
3. The percentage cover of bare ground is estimated (based on what is rooted, ie. basal not aerial cover)
4. The 1m diameter circle is then placed at 5m intervals along each transect. At each point, all Poaceae and forb species are listed within the circle and bare ground estimated as per step 3 and 4.
5. Finally, at the orange circles in Figure 3, grasses are clipped for biomass within a smaller area of 0.5m x 0.5m. Grasses are clipped at the base rooting into the soil, and bagged to be dried and weighed as a measure of biomass.

Ground layer biomass and cover

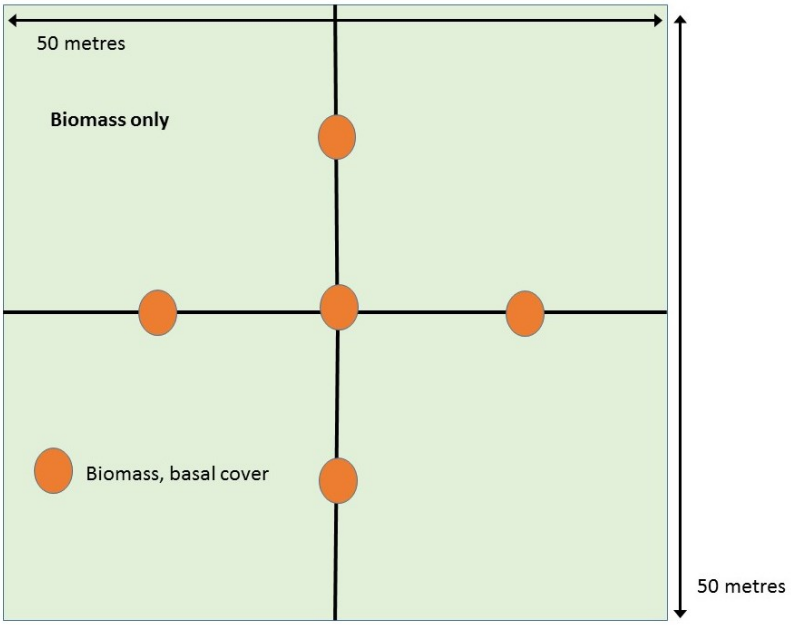


Figure 4: Biomass only on a 50m x 50 m PSP. Not to scale!

Biomass is clipped on 5 plots only (orange) that are 50cm x 50cm in size. Basal cover is also collected.

In this method, we are sampling only for biomass and basal cover (at the orange circles).

Method

1. After laying out the plot, and marking the five points for biomass, the percentage cover of bare ground is estimated (based on what is rooted, ie. basal not aerial cover)
2. Second, at the orange circles in Figure 4, grasses are clipped for biomass within a smaller area of 0.5m x 0.5m (clipping over a 1m D area can be quite unmanageable and easy to generate errors in the area). Grasses are clipped at the base rooting into the soil, and bagged to be dried and weighed as a measure of biomass.

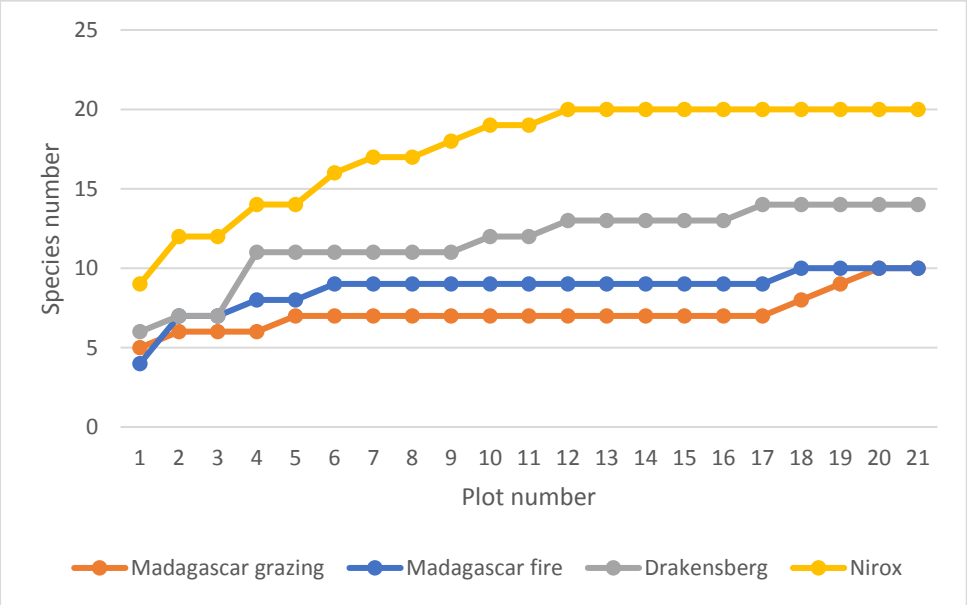
How is this a permanent plot?

Ideally, a woody plant PSP matches this, and where the four corners or centre point are well established. One can use the four corners and end points of transects to relocate the centre point. Alternatively, a centre point could be buried and relocated via a metal detector. Whatever the method, it must be aligned with the method used to re-locate the PSP for woody plants, and ideally, the PSP is established and re-surveyed for the entire flora not just the woody plants, and the flora is treated as a whole.

This method will be sensitive to compositional change and changing basal cover – better than a point-intercept method (like the Levi bridge), it will pick up compositional shifts in rarer species as they only need to be counted within the circle as opposed to a "rarer" species meaningful proportion of cover before being intercepted.

Why this size?

We want to ensure we sample the diversity at a given location. We can check this by looking at species accumulation curves. Figure 2 shows species accumulation curves based on the outlined method for four sites in two regions (Madagascar and South Africa).



Adapted from the protocol developed in Vorontsova et al. (2016)

Vorontsova, M. S., et al. (2016). Madagascar's grasses and grasslands: anthropogenic or natural? Proc. R. Soc. B, The Royal Society.

How do I voucher the species at a site?

A full collection involves the following:

- Collecting a minimum of two (one for the local herbaria and one for export as a duplicate, generally Kew for our purposes) and up to six fertile specimens (of the complete flowering plant including roots) of each species recorded in the sampling plots. The decision on how many specimens to collect is based on the historical sampling intensity in a region – more specimens should be collected in under-collected regions.
    - Additional collections can be sent to Paris, Edinburgh Missouri etc.
- If no fertile specimens are found, a sterile collection can be made [max two, but one is sufficient].
- We need to ensure that enough metadata/environmental data is collected to allow for completion of the herbarium specimen labels – the current data collection protocols attached to this sampling method is adequate.
- DNA vouchers are required for each species at a site.
    - Minimum of two silica collections per species per site (one stays with the local collector, and the other to Kew)
    - Preferably 2 – 3 new leaves (i.e. freshly developing) should be collected
    - Place these leaves in an appropriately labelled tea bag and add it to the Tupperware container with silica
    - It is ESSENTIAL the DNA specimen matches the voucher collection and species ID

For a speedy collection, 1) DNA vouchers must be collected for every species as per above, and 2) any grass species whose identity isn't 100% certain should be collected as per above.

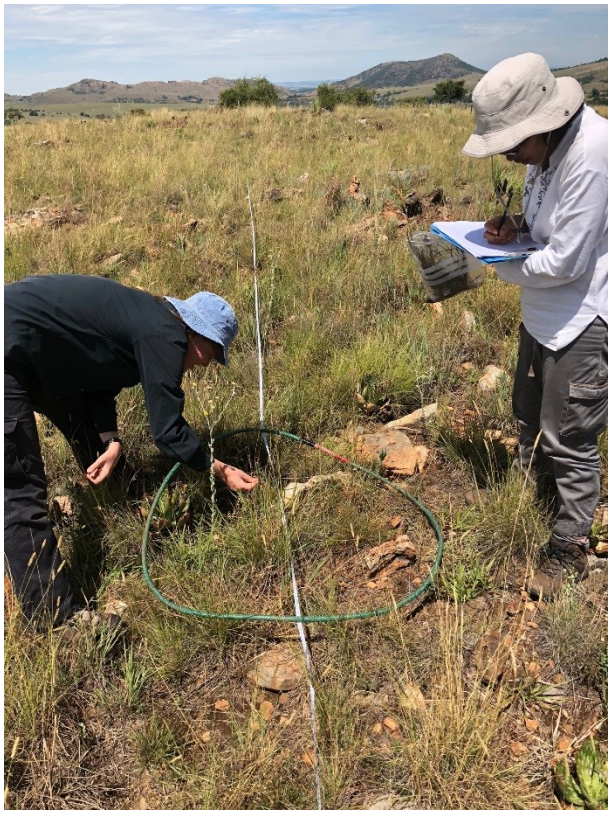
What does a herbarium specimen look like?

Images such as below can give you an idea of what should be collected – diagnostic traits on grass species are the inflorescences (flowering culms), but architecture, leaf arrangement and life form (e.g., is the tussock tightly clustered, or is it laterally spreading) are also helpful, especially when trying to cross-references identifications with plant specimens that aren't flowering.

Specimens should always be lodged with the national herbarium in the country where we are working and where plants were collected, rather than just exporting specimens to major regional (SANBI and Nairobi) or international herbariums (Kew, Paris, Leiden, Edinburgh). Most herbariums do not accept specimens that do not have flowering culms as it make identification impossible.

What does this all look like in the field?

Above, Maria, Cedrique, Jess and Sally are looking at a plot on a transect to identify grass species.



Above, Maria identifies grass species while Cedrique records the data.