**Annual Aboveground Net Primary Production and Livestock Production**

Aboveground Net Primary Production (ANPP- the amount of aboveground plant biomass accumulated over a given period of time) is a fundamental property of any ecosystem, with consequences for secondary and tertiary productivity and ecosystem function. Variation in primary productivity is driven by abiotic processes, including soil type and nutrient content (LeBauer and Treseder, 2008), and climate and weather patterns (e.g. Knapp and Smith 2001; Sala et al. 2012; Moran et al., 2014; Wilcox et al., 2015), and is further modified by biotic factors such as competition and herbivory (Milchunas and Lauenroth, 1993). Measurements of above-ground net primary productivity (ANPP) on an annual basis will form one of the most important core datasets for the network (Scurlock and Olson 2002), and a historic record of ANPP measurements should already exist for each site.

**Objective:**

Conduct ground-based measurements annual ANPP at relevant spatial and temporal scales for LTAR research components at LTAR rangeland sites. The measured values will reflect primary productivity of all species in perennial-dominated, multi-species plant communities and will account for consumption by domestic livestock.

This protocol outlines methods for measuring annual ANPP to accommodate the wide range of physiognomy (herbaceous, woody, succulent), timing of growth and livestock use across rangeland LTAR sites. Methods increase in complexity, time, and labor demands where temporal variation in plant growth is more complex, specifically if (a) there are multiple growing seasons per year, (b) species or functional groups grow asynchronously, or (c) aboveground live biomass does not decline to zero each year (Austin and Sala 2000). Also, complexity is added when accounting for the plant biomass consumed by domestic livestock, and the feedback effects of such consumption on plant growth rates during the remainder of the growing season or during subsequent growing seasons (McNaughton et al. 1996).

The specific techniques used to measure ANPP may differ for herbaceous and non-herbaceous (woody and succulent) species. For herbaceous species, estimates can be based on destructive harvest of current year aboveground standing biomass and collection of any current year litter. For non-herbaceous species, estimates of ANPP will be based on net changes in aboveground live biomass each year. For woody species, this can be estimated as the net change in woody biomass between years plus leaf production. In woody species for which biomass accumulation in lignified trunks and stems is minimal, woody plant ANPP can be approximated by measuring the annual biomass of leaves and new leaders. For succulents, ANPP can be estimated as the biomass of newly grown sections of aboveground stems or cladodes, provided that tissues grown in the current year can be visually distinguished from those grown in prior years. Typically, the sparse and more irregular distribution of non-herbaceous plants compared to herbaceous plants requires careful consideration of plot size and replication for meaningful estimation of ANPP by non-herbaceous plants.

Our protocol is divided into three sections. First, we address issues of the timing and frequency of sampling to account for temporal variation in plant growth pulses within a given year. Second, we address methods to account for herbivory. Third, we address issues of spatial scale and replication.

**Timing of Sampling:**

We assume for the use of this protocol in rangeland LTAR sites in the continental U.S. that there is a dormant season where live aboveground biomass of herbaceous species approaches zero during part of the year.

ANPP is estimated by the summed net annual increase in dry mass of live aboveground biomass of each plant species in the plant community, after accounting for biomass removed by herbivores during the growing season (Sala and Austin 2000, Knapp and Fahey 2007). ANPP consists of two components 1) green living biomass, 2) senesced biomass produced during the current year, which may be either standing dead, or litter on the soil surface. The key point is that the biomass grew in the current year.

In the simplest case where (a) livestock grazing or other large vertebrate herbivory is absent, and invertebrate/small vertebrate herbivory is judged negligible, and (b) all species grow synchronously, ANPP can be estimated from the peak live aboveground biomass measured at a single point in time each year, using the approaches described below. Table 3.2 in Knapp et al. 2007 provides a detailed summary of approaches to ANPP measurement.

Each site will need to assess the magnitude of asynchrony in plant growth pulses, which can arise from differences among species in phenology, or because soil moisture fluctuations result in multiple growth pulses within a year. These sources of growth asynchrony will determine how many times during the year (and when) measurements of standing biomass are needed to attain an estimate of peak standing biomass for each major species or functional group (e.g. cool season vs. warm season grasses). Where logistical constraints (time, labor) limit the number of times that peak biomass is measured annually, and this can affect the precision of the ANPP estimate, more intensive sampling or alternative methods should be used in one year to determine how the selected method affects precision of the ANPP estimate.

**Accounting for Herbivory:**

For LTAR sites grazed by livestock, measurements of ANPP must account for the amount of biomass consumed each year, and potential effects of such consumption on subsequent plant growth (McNaughton et al. 1996). Peak biomass is best estimated in plots where herbivores are temporarily excluded, but such exclusion eventually causes changes in plant architecture and physiology that can affect growth rates. Thus, the simplest approach is to establish a replicated set of moveable exclosures at a site, and sample peak biomass (or net changes in biomass) within the exclosures, but then move the exclosures whenever grazing begins to affect plant growth rates

We recommend that:

1. For productive grasslands with high grazing intensity (e.g. >50% of ANPP) where post-defoliation regrowth is important, exclosures likely need to be moved (and biomass measured) two or more times per growing season.
2. For grasslands with a single large growth pulse and/or moderate grazing intensity (eg. 20-50% of ANPP) exclosures should at a minimum be moved once per year.
3. For mixed woody/succulent/herbaceous systems, and/or where livestock grazing intensity is low (e.g. < 20% ANPP), exclosures could be moved at less frequent intervals determined by observations of changes in the architecture (canopy shape, plant height) of plants inside vs. outside of exclosures.

*Methods for Measuring Standing Plant Biomass:*

Standing biomass within a temporary herbivore exclosure (herafter referred to as a plot) can be measured at a given point in time either by destructive harvest (herbaceous communities), or indirect, non-destructive methods (any community). Plot size and number needed to represent the fetch area of an EC tower or within a replicate of the common experiment will depend on the amount and scale of spatial heterogeneity within the target sampling area. Examples of sample sizes currently employed at 2 rangeland LTAR sites are reported at the end of this section.

For destructive harvests, aboveground plant biomass is clipped to crown level. We recommend that harvested biomass should be separated by plant species or, at a minimum, by functional groups that differentiate between photosynthetic pathways (C3, C4, CAM) and growth forms (annual vs. perennial; graminoids vs. forbs vs. woody plants). In many grasslands, standing dead biomass can be carried over from the previous year’s ANPP. We recommend each LTAR site develop a standardized protocol for technicians to differentiate between tissues produced in the previous year versus tissues that were produced in the current year but have begun to senesce at the time of harvest (typically identified based on color and degree of weathering). The former should be removed prior to drying and weighing samples. Standing biomass produced in the current year for each species or functional group should be dried at 55C and weighed.

Indirect, non-destructive harvests can be especially valuable in cases where the same plot is measured multiple times over a growing season, and where non-herbaceous species are present. These methods rely on calibrating an inexpensive index of plant biomass such as canopy light penetration with the more expensive harvest method (Knapp et al. 2007). A second method involves passing a set of metal pins or lasers through the plant canopy, and recording interceptions with plant tissues (Frank and McNaughton 1990). Species or groups of species that differ notably in leaf thickness and canopy architecture typically require separate calibrations, but such approaches can achieve r2 for the relationship between canopy interception and functional group biomass on the order of 0.83 – 0.96 (Frank and McNaughton 1990; Augustine 2003). For plant communities where plots often include substantial heterogeneity in plant cover, such as the Jornada Experimental Range, a larger-scale version of canopy interception has been developed (Huenneke et al. 2001). In this method, 1 m2 plots are divided into a grid of 100 10 x 10 cm sections, and cover or projected surface area for each plant or plant part is estimated by counting the grid squares or portions of squares occupied by that plant or part (i.e. canopy interception is recorded with respect to 10 x 10 cm squares rather than interception with a pin or laser). When such indirect methods are used in long-term studies, calibrations should be checked with additional reference harvests in years with extreme or unusual weather conditions (Peters et al. 2012).

*Minimum Requirements*

Because plant community composition and seasonality of growth varies widely among LTAR sites, any of the above methods can be used provided that:

1. Measurements are timed to quantify all major growth periods in a given year, both those associated with variation in conditions facilitating plant growth and variation in phenology among plant species.
2. Moveable exclosures are used to account for effects of livestock grazing, where the frequency of movement accounts for the effect of grazing on plant growth,and standing biomass is measured every time that exclosures are moved.
3. Non-destructive harvest methods are calibrated to account for variation among plant species in leaf thickness and morphology, differences in between plant leaves vs. stems, etc.
4. Selected methods produce an estimate of annual aboveground plant biomass production (kg/ha) that can be separated into key functional groups, and is typically within 10% of the true ANPP.

**Spatial Scale and Replication:**

ANPP data will be used in at least two major ways 1) to quantify inter-annual variation in productivity across LTAR sites, and 2) quantify within-site differences between treatments, such as between Aspirational and Business as Usual practices or between livestock stocking rate levels or other experimental treatments. The choice of how many ANPP samples to collect, the size of sampling plots, and their distribution on the landscape will vary with a number of factors, including vegetation structure and spatial heterogeneity, and the identity and magnitude of spatial gradients that may influence ANPP on the study site. For ANPP measurements associated with the eddy covariance towers, the spatial extent of sampling needs to represent the major sources of spatial variation within the flux tower fetch. Stratification of sampling should be employed where it will increase precision and sampling efficiency.

We suggest at a minimum:

1. Moveable grazing exclosures be distributed systematically across the fetch area, with a minimum of 15 exclosures at relatively homogenous sites.
2. Where the destructive harvest method is used, biomass should be clipped within one quadrat per exclosure. Quadrats could vary from 0.1 to 1 m2 depending upon plant size and density; elongated (e.g. 20 x 50 cm or 20 x 100 cm) quadrats are recommended to best accommodate small-scale heterogeneity in plant and gap distributions.
3. Where larger plants are present (particularly woody plants) larger exclosures and quadrats may be necessary, and ideally will encompass multiple woody plants plus their interspaces.

*Coordination with other biological measurements:*

Forage production: Where biomass is estimated by species, and each species can be assigned a forage value, then ANPP methods will also provide an estimate of forage production. If species vary temporally (e.g. among years) or among plant parts in their forage value, then sorting of ANPP could be conducted according to forage value in order to provide annual estimates of both forage production and ANPP.

Plant nutrient content: Biomass harvests for ANPP can be used to provide tissues for plant nutrient content sampling (see associated protocol); additional sorting by plant part may be required.

Diversity: Plant species diversity will need to be measured at same fetch area as the ANPP exclosures, but will most likely require larger sampling effort (more and larger plots), and use methods targeted at sampling rare species.

*Additional potential measurements linked with ANPP:*

1. Plant-based measurement of herbivore consumption: Additional biomass harvests conducted in paired quadrats outside the grazing exclosure could be used to measure livestock consumption rates in addition to ANPP (McNaughton et al. 2006). Depending on the grazing management regime and plant growth/senescence patterns, this may require additional paired measurements of plant biomass in/out of grazing exclosures at other times of the year besides peak biomass, e.g. at the end of the grazing season.

1. Light levels: At the time of biomass estimation (e.g. immediately prior to clipping), light availability could be measured using a light meter capable of integrated measures of PAR. We recommend following the same protocol as the Nutrient Network, i.e. readings must be taken on a cloudless days as close to solar noon as possible (11 am to 2-m), with two light measurements at ground level and one above the canopy. Light availability can be calculated as the ratio of PAR below and above the canopy. If you use a point sensor, record the mean of at least 10 readings in different locations (this is done automatically with the linear sensors).

**Livestock Production**

Estimates of ANPP for cropland LTAR sites will include an estimate of food (e.g. grain) yield as a component of the ANPP measurement, but this is not true for rangeland LTAR sites. In addition to measuring ANPP, the rangeland sites will measure food production in terms of the net increase in livestock biomass on their sites on an annual basis. Under most circumstances, this will be done by measuring the net change in weight per animal per year on a known area, or by weighing calves produced each year on a known area. Each LTAR site with livestock should provide an annual measurement of livestock production on a kg/ha and kg/animal basis.

Table 1. Examples of sampling designs used to estimate annual ANPP for non-LTAR experiments at two rangeland LTAR sites. This table is provided only as an example of sample sizes and methods used to estimate ANPP at these sites, not as a recommended protocol. Measurements at CPER are for relatively homogeneous grassland sites, with ANPP measured in the presence of grazing using 1 m3 moveable grazing exclosures. Measurements at JRN are in highly heterogeneous mixed herbaceous/shrub communities, with ANPP measured in the absence of grazing (i.e. all 49 plots in a single large, permanent exclosure).

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| LTAR | Effect of Interest | Scale of a Site | # Sites | # Temporary Exclosures or Plots per Site | Plot size | # Times Standing Biomass Estimated per Year |
| CPER | Topography | ~1 ha | 3 | 15 | 0.25 m2 | 1 |
|  | Grazing Intensity | 130 ha | 3 | 60 | 0.1 m2 | 1 |
|  | Grazing Exclusion | ~4 ha | 6 | 24 | 0.1 m2 | 1 |
| JRN | Ecosystem types | ~1 ha | 15 | 49 | 1 m2 | 3 |

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