

# EVALUATION OF THE ARTICLE “ESTIMATING HERBACEOUS BIOMASS OF GRASSLAND VEGETATION USING THE REFERENCE UNIT METHOD” BY BOYDA, ET AL. 2015

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## ABSTRACT

An evaluation of the scientific article “Estimating Herbaceous Biomass of Grassland Vegetation Using the Reference Unit Method” by Boyda, et al. 2015 (Prairie Naturalist) is relevant because authors state that herbaceous biomass can be accurately and precisely estimated throughout the entire Buffalo Gap National Grasslands (BGNG) and beyond with the Reference Unit Method. The authors failed to provide easy to follow methods, a complete data set with all results, a study site map while only providing partial data and analyses for prairie dog colonies or areas adjacent to prairie dog colonies. The authors did not provide an improvement in protocol and methodology of the weight estimated method (double sampling-estimating and clipping) described by Pechanec and Pickford (1937) and its application on rangelands to estimate above ground biomass for many ecological types. Evaluations were very limited to few plant species: western wheatgrass (*Pascopyrum smithii*), purple three-awn (*Aristida purpurea*), needle-and-thread (*Hesperostipa comata*), and green needle grass (*Nassella viridula*). Other comparisons were groups of plants with unidentified species and may produce questionable results with different species mixes when applied to other grasslands or locations on the BGNG. The protocols developed with double sampling (clipping with oven dry weights and corrected to visual estimates) is still the standard with no improvement by Boyda et al. (2015). The article by Boyda et al (2015) may provide erroneous results with application of the Reference Unit Method and is not recommended for estimating herbaceous biomass.

**Key words:** estimating biomass, double sampling, grasslands, plant biomass.

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## DISCUSSION

Authors of the article by Boyda, E. D., J. L. Butler and L. Xu. 2015 Prairie Naturalist suggests that by applying the Reference Unit Method to estimate herbaceous biomass of grassland vegetation can provide more accurate and precise estimates of plant biomass with plant species and functional groups that involve combining species with similar origin (native or introduced), life form and duration. The protocol and procedures presented are not an improvement in methodology to estimate herbaceous biomass, especially not the standard double sampling method. The weight estimated

method (double sampling-estimating and clipping) described by Pechanec and Pickford (1937) and its application on rangelands to estimate above ground biomass for many ecological types is still the standard with no improvement. Since the inception of the double sampling method, it has been the standard to estimate plant biomass on the grasslands for years (Wilm et al. 1944, NAS-NRC 1962, Francis et al. 1979, Cook and Stubbendieck 1986, Robin et al. 1983, Bonham 2013). The article by Boyda et al. 2015 did not provide an improvement of the double sampling procedure by using the Reference Unit Method.

The authors failed to present the double sampling equation used for analyses in this publication which made methodology more difficult to follow. Equations listed in Table 1 are not provided in the methods such as;

$\tilde{Y}_e = y_r + b(x_e - \bar{x}_r)$ , as given in Cook and Stubbendieck, 1986, Society for Range Management, p 246; Equations 42 and 43.

$\tilde{Y}_e$  = estimated corrected yield.

$y_r$  = ratio estimate (actual/estimated).

$b$  = regression coefficient.

$x_e$  = ocular estimated weight of a plot.

$\bar{x}_r$  = average of ocularly estimated weights of plots in regression sample.

According to the field methods which are not clear; it was a ratio estimated as defined on page 75 of the publication (*The biomass of each individual species and functional group was estimated within each plot as a ratio of the reference unit: estimated unit in increments of 0.1*). That would be " $y_r$ " in the equation above. However, the authors then weighted the " $y_r$ " with the " $b$ ", a regression coefficient. The authors did not provide any numerical results of statistical analyses for these equations for testing the intercepts and coefficients in Table 1. The " $b$ " coefficient did not add anything to the estimated weight and could have been deleted. All equations in Table 1 were apparently solved with the ratio of reference/actual combined as stated on page 75. Their equation as given in Table 1 contains " $x$ " which is the ratio as stated. It is difficult to believe that a ratio of two different plot areas (references in sample areas) can be estimated at 0.1 of unit (gram?) increments.

The authors applied equations in Table 1 with the " $b$ " coefficient (slope) to convince the reader that they estimated the constant, and the mean clipped value was a fraction of the estimate to the nearest one tenth of  $g/0.25\text{ m}^2$ , which only adds confusion to the analyses. Therefore, all results and conclusions are questionable.

The first objective to evaluate the Reference Unit Method on a broad spatial scale was not accomplished because of their limited homogenous site selection procedures within two ecological soil sites,

Clayey and Loam. The authors describe the experimental design and selection of nine prairie dog colonies to provide sampling and placement of four transects on each colony, colony edge and off colony. Off colony sites were located adjacent to the prairie dog colonies, resulting in very similar vegetation.

The authors state that this allowed them to evaluate along a "considerable gradient of plant community composition and abundance". However, only nine carefully selected prairie dog colonies with conditions that the prairie dog colony had to be greater than 12 ha in size, near roads, restricted to two soil types (Clayey and Loamy), with consistent soil texture characteristics were evaluated. Sites selected for sampling were in relatively flat terrain and excluded the nearby undulating topography. This failure is observed with results of a single plant species (western wheatgrass) and a combination of buffalograss (*Bouteloua dactyloides*) and blue grama (*Bouteloua gracilis*) defined as (SHORT) for their claims of multiple species assessment with the Reference Unit Method. Other evaluations were groups with unidentified plant species. The article lacked a map of nine prairie dog town locations to demonstrate broad spatial scale, the edge sites were still within the colony, and off colony sites were within 200 m, providing variability of prairie dog activity, but little or no variability of the Buffalo Gap National Grasslands (BGNG) (Boyda et al. 2013).

The second objective was to examine multi-species reference calibrations. Unfortunately, western wheatgrass (PASSMI, *Pascopyrum smithii*) was the only plant species to be identified individually and included in their results (Table 4). The other "visually dominant" species like purple three-awn (ARIPUR, *Aristida purpurea*), needle-and-thread (HESCOM, *Hesperostipa comata*), and green needlegrass (NASVIR, *Nassella viridula*), which represent a significant part of the vegetation net primary production depending upon range condition on the BGNG, were so infrequent on or near

these prairie dog colonies that creation of regression equations with the Reference Unit Method failed to address these plant species. The “SHORT” functional group which is a combination of two species (buffalo grass and blue grama) had results presented, so at best they can claim multi-species (3 plant species) were evaluated. Plant species that were combined into the other groups were never identified from the list of 481 plant species on the (BGNG) (Kostel 2006). Therefore, their objective to examine the multi-species reference calibrations and abundance was not achieved.

Objective three was to evaluate season-long calibration equations. This study claims that Reference Unit Method can be used over the growing season which was at most a 12-week sample period. Season long grazing on the BGNG is approximately 24 weeks. Evaluation of the Reference Unit Method did not assess early vegetative plant growth, mid-growth or mature (dry vegetation) to determine the feasibility of the method.

The analyses as presented in Methods have a total sample size of 108 transects across three treatment effects related to prairie dogs (interior, edge, and adjacent off-colony) over two ecological sites. Only 70 transects were used as the main data set and 38 transects for validation with statistical procedures. However, sample sizes in tables and figures are confusing and do not add up to either 70 or 38 transects used for analyses. Nevertheless, 19 transects are considered as outliers in Table 1, but some outlier transects were apparently reused for analyses of functional groups. Sample size for various analyses and precise methods were not clearly defined nor presented.

The INFLUENCE option used in the regression analysis is based on statistics developed by Belsley et al. (1980) and measures the influence that each observation has on the parameter estimates (SAS 1988). Influential data identified in the study may not always indicate true outliers, or variation within the sample procedures, biological variation, or observer variation. Removing all data identified as influential increases the

regression model precision and accuracy. It also fails to provide a robust procedure for estimating herbaceous biomass and fails to evaluate the reference unit method by limiting the range of data (See Figure 1, majority of data near origin).

The INFLUENCE option provided authors a method to carefully remove all data that reduced best model results with no explanation. There appears to be more than 19 transects deleted as outliers throughout the analyses. In addition, the validation data set was calibrated using previously created regression equations and while doing this calibration of the validation data set, the observation weights are changed, or set to a value of zero, which cause select observations to be excluded from the analysis (SAS 1988). Therefore, by both calibrating the validation dataset and weighting the data greatly improves linear regression performance, however no true comparison of estimated weight is made with actual weights.

The fourth objective (Validation) includes comparisons among different observers and are displayed partially in the tables and figures. Regression plots displayed in Figure 1 clearly demonstrate why differences among observers were low, giving the perceived appearance of great accuracy and precision. A sample size of 38 transects for validation was defined in the methods but ranged from 13 to 15 transects (Table 2). Validation of functional groups for each observer between calibrated biomass estimations and actual biomass showed that a total of 13 t-tests were different from estimated vs actual among observers at  $P < 0.15$  (Table 2). Based on 9 functional groups and TOTAL in Table 2, (three observers ( $n=29$ )), the results showed a 45% error rate or 14% error rate for each observer after estimates had been corrected and estimates were made to a tenth of a gram. On an individual bases, observer 1, with experience with this method was correct 90% while observer 2 and 3 failed 60% and 67% of the time respectively, using functional groups ( $P < 0.15$ ). Other inconsistencies with no explanations were

Observers 1 and 2 are included in analyses for Table 2 with functional group PFI, while Observer 3 is absent from Table 2, but is then used for the same PFI functional group in Table 3, then in Figure 1 all three observers are used for the regression lines with only  $n=16$  or less and all values were less than 1.5 grams. Results are not positive when comparing calibrated biomass with actual biomass with 60% of the functional groups being different ( $p=0.10$ ) (Table 4).

The reference unit method evaluated by Boyda et al. 2015 cannot be repeated as presented and explained within the publication. Field sampling and statistical analysis sections are very confusing as to repeatability, in addition the statistical gyrations required are too cumbersome for efficient fieldwork. Improved accuracy and precision by the Reference Unit Method with an improvement of the double sampling procedure would be welcomed in science and rangeland management. However, this study falls short of achieving and improving the double sampling protocol defined as the Reference Unit Method for both science and management.

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