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Original Articles

Estimating aboveground herbaceous plant biomass via proxies: The confounding effects of sampling year and precipitation

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ABSTRACT

Direct measurements of aboveground plant biomass are often not feasible, thus various biomass proxies are in use. To obtain biomass estimates, these proxies are calibrated against actual biomass, and the resulting proxy-biomass relationship is often used across multiple years and experimental treatments within a study. We investigated how the proxy-biomass relationship varied across years and considered interannual precipitation variability as a contributing factor.

We sampled a perennial grassland for ten consecutive years (2003–2012) in central Hungary and estimated vegetation cover and Normalized Difference Vegetation Index (NDVI); two frequently used biomass proxies representing two contrasting methods. Aboveground live herbaceous plant biomass was harvested from each plot after sampling, and regression models were used to assess the relationship between biomass proxies and actual aboveground biomass.

We found that cover and NDVI were equally effective at estimating biomass. However, the relationship between either biomass proxy and actual biomass varied amongst years, and this was related to the amount of precipitation. In wetter years, proxy-biomass relationships were steeper than in drier years.

These results indicate that using the same proxy-biomass relationship across different years or precipitation regimes may not be valid and may introduce systematic error into biomass estimations in long-term studies or precipitation manipulation experiments.

1. Introduction

Aboveground plant biomass and aboveground net primary productivity (ANPP) derived from biomass data are amongst the most important properties of ecosystems (Eisfelder et al., 2017; Knapp et al., 2015; McNaughton et al., 1989). The direct way for assessing aboveground biomass in grasslands is the harvest method, when the aboveground plant parts are cut, divided into fractions, dried, and weighed (Singh et al., 1975). However, direct measurement of biomass is very time intensive and is not feasible in studies where plot size is limited and regular biomass removal is not part of system dynamics. Therefore, non-destructive biomass estimation methods are widely applied in ecosystem research (Paruelo et al., 1997), especially in long-term field experiments (Kongstad et al., 2012; Tielbörger et al., 2014). The values obtained from these non-destructive methods are either reported as proxies for aboveground biomass, or are converted to aboveground biomass via allometric equations obtained through calibration (Byrne et al., 2011; Sala and Austin, 2000; Singh et al., 1975).

The relationship between biomass proxies and actual biomass (called as "proxy-biomass relationship" hereinafter) may change within an ecosystem, but this is often overlooked in multi-year studies. Williamson (1987) found that the proxy-biomass relationship in short-grass prairie varied markedly between years and even seasons. Despite this, long-term studies often only calibrate proxies to aboveground biomass once (Filella et al., 2004; Wang et al., 2012; Wardle et al., 2016; Wu et al., 2012; Yahdjian and Sala, 2006), and the resulting relationship is used for multiple years. Even when calibrations are performed yearly, they are generally only conducted under control or ambient conditions (Byrne et al., 2013; Evans and Burke, 2013; Köchy and Wilson, 2004; Kongstad et al., 2012; Tielbörger et al., 2014).

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However, these relationships may be inappropriate for estimating biomass across all treatments, since experimental treatments may change the relationship between biomass proxies and actual biomass. For example, both fertilization (Shaver et al., 2001) and grazing (Frank and McNaughton, 1990) have been shown to alter the proxy-biomass relationship due to changes in plant community composition (Sala and Austin, 2000; Shaver et al., 2001). These findings raise the question whether the same relationship can be used across multiple years or across different treatments.

The objective of this study was to evaluate the interannual validity of the relationship between biomass proxies and actual live herbaceous biomass for two biomass estimation methods: visual cover estimation and Normalized Difference Vegetation Index (NDVI) measured by field spectroscopy. Furthermore, since these non-destructive methods are often used in long-term experiments where precipitation is manipulated, in a second step we wanted to test if the proxy-biomass relationship is affected by the amount of precipitation in the different years. Specifically, we asked three questions: (1) How do the two non-destructive biomass estimation methods, visual cover estimation and NDVI, differ in accuracy? (2) How does the proxy-biomass relationship differ among years? (3) How is the proxy-biomass relationship related to the precipitation of different years?

2. Materials and methods

2.1. Study site and sampling design

The study site was located in the Kiskunság National Park (Central Hungary), in Orgovány (N 46° 47′, E 19° 28′). The climate of the study area is temperate continental. Mean annual precipitation is around 500 mm; mean monthly temperature ranges from −2 °C in January to 21 °C in July (Kovács-Láng et al., 2000). The parent material is wind-blown calcareous sand, resulting in a very poor sandy soil (sand content is over 95%) with extremely low (< 1%) humus content. The natural vegetation is forest-steppe, where grassland patches range from semi-desert-like grasslands (dominated by *Festuca vaginata* Waldst. & Kit. ex Willd., and *Stipa pennata* L.) to steppe-like grasslands (dominated by *Poa angustifolia* L., *Stipa capillata* L., and *Scirpoides holoschoenus* (L.) Soják). These grasslands are completely unmanaged, and only moderately grazed by wild herbivores (roe deers, hares, and invertebrates).

To study the relationship between biomass proxies obtained from non-destructive sampling and harvested biomass, we chose ten homogeneous grassland patches (ca. 5 m in diameter) that covered the variation in grassland productivity within a 1-km^2 area. This resulted in a relatively wide range of biomass values which made it easier to estimate relationship between biomass proxies and actual biomass. We sampled one, randomly located 0.5 m × 0.5 m plot in each patch in each year between 2003 and 2012. Patches were permanent, but plots within patches were not permanent to avoid the effect of disturbance due to repeated sampling. In each plot, in each year, we estimated two biomass proxies in a single day in mid-June at peak aboveground green biomass (Table S6): plant cover through visual cover estimation and NDVI calculated from field spectroscopy data.

2.2. Sampling methods

In cover estimation, canopy cover of each vascular species was visually estimated. We typically used values of 25%, 30%, 35% etc. above 20% cover, full numbers between 2% and 20%, and estimated to one decimal digit when cover was below 2%, i.e. 1.5% or 0.4%, in accordance with findings that finer resolution is needed at the ends of the scale (Hahn and Scheuring, 2003). Sampling was performed by the same person (G. Ónodi) throughout the study according to previous recommendations (Sykes et al., 1983). We calculated total canopy cover of all vascular plant species by summing up all species' covers (referred to as 'cover' hereinafter). In addition, we also calculated the cover of species groups based on lifeform (graminoids and forbs) and life span (annuals and perennials) (Table S7). Most of our species were too rare (less than six occurrences) to conduct species-level analysis.

NDVI data were obtained by measuring incoming and reflected light intensity at eight wavebands using a portable Cropscan MSR87 multi-spectral radiometer (Cropscan, Inc., Rochester, Minnesota, USA) in each sampling plot. Measurements were taken at 1.8 m height above the center of each sampling plot, thus capturing the entirety of each sampling unit, with some additional area with the same vegetation. We calculated NDVI (Tucker, 1979) values based on reflectance measured by the red (R; centered at 660 nm, bandwidth 10 nm) and near-infrared (NIR; centered at 810 nm, bandwidth 10 nm) channels of the instrument:

$$NDVI = (NIR_{810} - R_{660})/(NIR_{810} + R_{660})$$

NDVI is correlated with the amount of green vegetation (Tucker and Sellers, 1986), and can be used as a proxy of aboveground live biomass in temperate perennial grasslands (Briggs et al., 1997; Paruelo et al., 1997).

Aboveground vascular plant biomass was harvested in each sampling plot immediately after completing non-destructive sampling. We sorted biomass by species, and separated live materials from the standing dead and litter components. Only live material was considered in our analyses. Biomass samples were dried at 60 °C for 48 h and then weighed.

2.3. Data analysis

The relationships between biomass proxies and harvested biomass were tested by linear regression models for each year separately (Faraway, 2005) in accordance with numerous similar methodological studies (Redjadj et al., 2012; Röttgermann et al., 2000). Goodness of the fitted calibration lines was measures by coefficient of determination (R^2) and root mean squared error (RMSE). The coefficients of determination (R^2) of the regression models of the two proxies obtained for each of the ten years were compared using pairwise *t*-tests (in this comparison RMSE would give the same results). The coefficients of determination met the assumptions of normality for the paired *t*-test. RMSE was also calculated for cases when calibration lines fitted in each year were applied in other years.

Linear mixed models were built for biomass as dependent variables and biomass proxy (visually estimated cover or NDVI), year (as categorical variable), and their interaction as fixed explanatory variables and plot as random factor (Zuur et al., 2009). In order to explore the effect of precipitation on the relationship between proxies and biomass, we substituted precipitation for year in a second set of linear mixed models. Here, we considered cumulative precipitation during the 60 days preceding biomass harvest (Table S6), because a preliminary analysis showed that this period had the highest correlation with biomass in our study system (Fig. S2). All analyses were carried out in R (R Core Team, 2013), for mixed models the "nlme" (Pinheiro et al., 2016), for R^2 calculation the "MuMIn" (Bartoń, 2016) packages were used.

3. Results

We found positive relationships between biomass proxies (visually estimated cover or NDVI) and aboveground live biomass for both methods in each year (Fig. S3). Across the ten-year period, the two methods did not differ in the coefficient of determination (R^2) of the regressions (Fig. 1) (mean $R^2_{cover} = 0.8252$, mean $R^2_{NDVI} = 0.8042$, paired *t*-test $t = 0.9117$, $df = 9$, $P = 0.3857$). Annual mean values of biomass and biomass proxies showed no linear trend through time (Fig. S1).

In the linear mixed models, biomass proxies (visually estimated