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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.

Keywords

aboveground plant biomass, field spectroscopy, interannual validity, long-term studies, NDVI, precipitation, proxy-biomass relationship, visual cover estimation

Nomenclature

The Plant List (2010)

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 shortgrass 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). 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 semidesert-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 x 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 multispectral radiometer (Cropscan, Inc., Rochester, Minnesota, USA) in each sampling plot. Measurements were taken at 1.8 meters 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 hours 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" (Barton 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).

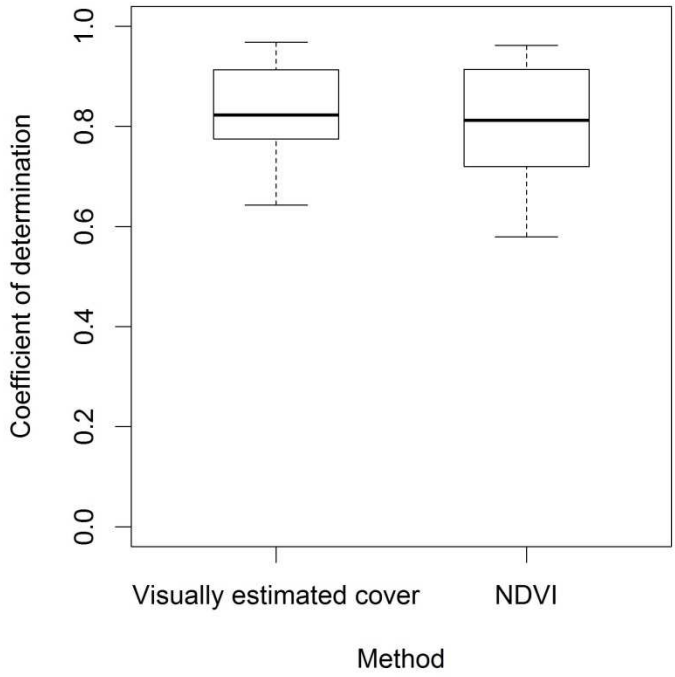

Fig. 1 Coefficients of determination (R^2) of linear regression models between visually estimated cover or NDVI and biomass for ten years (for each regression, n = 10, P < 0.011). For more information about the relationships between harvested biomass and biomass proxies in the different years see Fig. S3.

In the linear mixed models, biomass proxies (visually estimated cover or NDVI) explained much of the variance in aboveground live biomass (Table 1). For each proxy, the proxy-biomass relationship varied between years (see cover*year and NDVI*year in Table 1). Consequently, regressions between biomass proxies and biomass varied considerably among years for both proxies (Fig. 2a,b). At average NDVI of the whole dataset (0.467), modelled biomass ranged from 73 g/m^2 to 192 g/m^2 (ca. 2.6-fold difference). Similarly, modelled

biomass at the mean visually estimated cover (59%) ranged from 90 g/m^2 to 161 g/m^2 (ca. 1.8-fold difference) depending on the year. Possible estimation error when a model fitted to one year was applied to other years ranged from 19.8 g/m^2 to 119.0 g/m^2 for visual cover estimation, and from 24.2 g/m^2 to 274.7 g/m^2 for NDVI (Table S1).

Table 1 Linear mixed models of total aboveground live biomass

Explanatory variables	DF	F	P
cover * year model R^2 = 0.7175			
intercept	1, 71	113.67	<0.001
cover	1, 71	139.41	<0.001
year	9, 71	5.21	<0.001
cover * year	9, 71	3.71	<0.001
NDVI * year model R^2 = 0.7125			
intercept	1, 71	108.36	<0.001
NDVI	1, 71	140.64	<0.001
year	9, 71	4.17	<0.001
NDVI*year	9, 71	4.15	<0.001
cover * precipitation model R^2 = 0.5158			
intercept	1, 87	65.17	<0.001
cover	1, 87	93.84	<0.001
precipitation	1, 87	8.69	0.004
cover * precipitation	1, 87	10.82	0.002
NDVI * precipitation model R^2 = 0.5299			
intercept	1, 87	68.99	<0.001
NDVI	1, 87	95.14	<0.001
precipitation	1, 87	0.07	0.797
NDVI*precipitation	1, 87	10.82	0.002

R^2 shows the adjusted coefficient of determination of the marginal (fixed factor) effect for each model. DF shows the numerator and denominator degrees of freedom, F and P are the statistics and significance levels of the analysis.

When year was replaced by precipitation in the linear mixed models, the cover*precipitation or NDVI*precipitation interaction terms were also significant (Table 1). Thus, cover-biomass or NDVI-biomass relationships were dependent on the amount of precipitation. Regression lines became steeper with increasing precipitation (Fig. 2c,d).

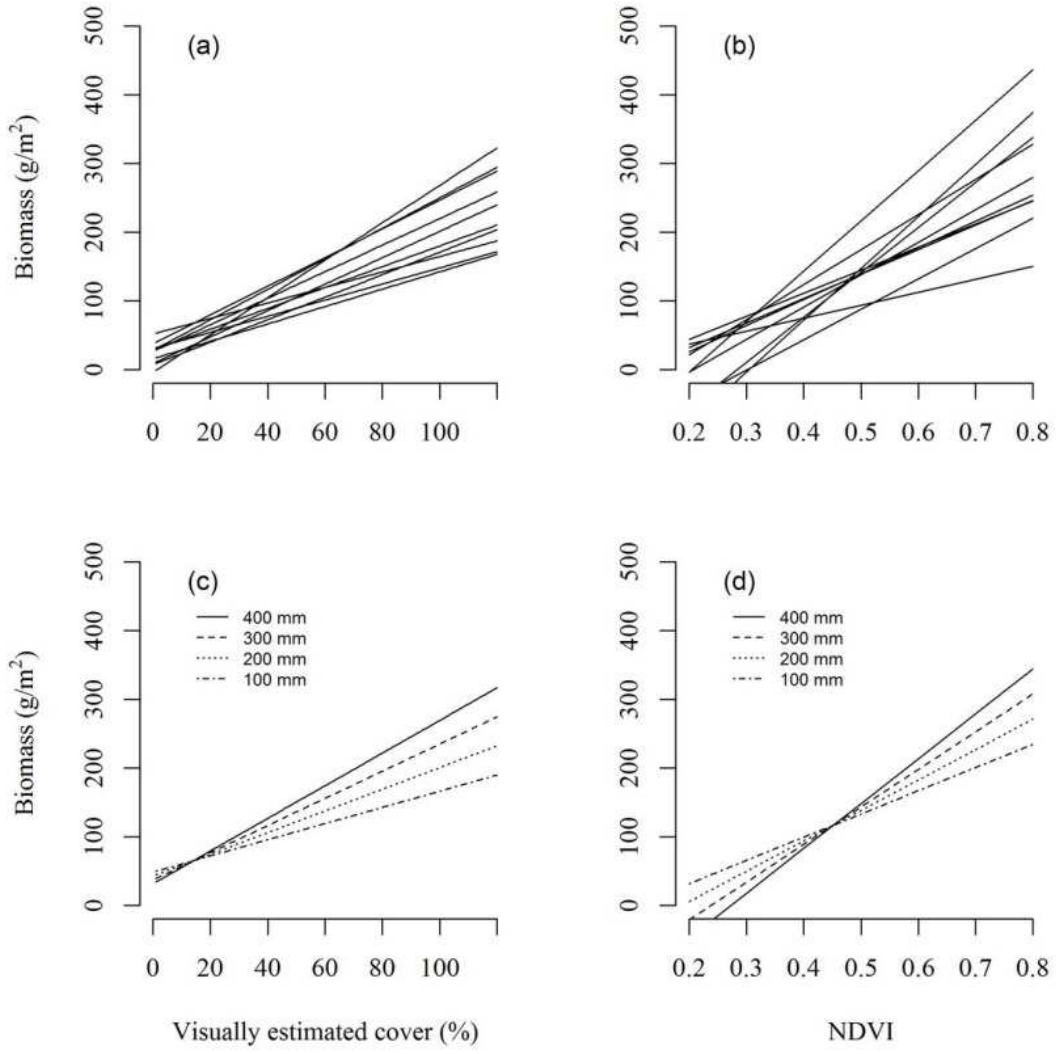

Fig. 2 Regression lines between visually estimated cover (a) or NDVI (b) and aboveground live biomass for each of the ten years; and the effect of precipitation on the linear regression models of visual cover estimation (c) and NDVI (d).

When we performed these same analyses for the cover of different plant functional groups (annuals/perennials, or forbs/graminoids) instead of total vascular cover, the significant cover*year and cover*precipitation interaction terms were maintained in the linear mixed models (Table S2-S5).

4. Discussion

We found that proxy-biomass relationships varied by year for both visual cover estimation and NDVI, which indicates that a single relationship does not hold across multiple years. These findings imply that yearly calibrations may be necessary for reliable biomass estimation, as carried out in some long-term studies (Tielbörger et al., 2014; Zhang and Welker, 1996). Our results do not support the applicability of a proxy-biomass relationship in a subsequent year as found by Wylie et al. (2002), and question the use of a single relationship across multiple years in long-term studies (Brancaleoni et al., 2007; Filella et al., 2004; Lee et al., 2014; Wardle et al., 2016; Wu et al., 2012; Yahdjian and Sala, 2006).

The different proxy-biomass relationships across years may result from changing morphology within each species, or from an altered share of constituent species, i.e. a compositional change. Water availability, for instance, has been shown to affect the specific leaf area (SLA) of plant species (Milla et al., 2008). By changing SLA, water availability can alter the density of biomass within a given area and can therefore cause differences in actual biomass between sites or years with the same biomass proxy value. In addition, as canopy structure depends greatly on plant species morphology, changing species composition between years or experimental treatments may have a profound effect on how biomass proxies translate into biomass (Eisfelder et al., 2017; Shaver et al., 2001). This finding suggests that the labour-intensive approach of calibrating for individual species may be a solution. Indeed, in a comprehensive study of 154 species, Huenneke et al. (2001) found only a minority of cases when the relationship between a biomass proxy ("volume") and biomass for single species changed between years, seasons, or sites. Calibrating for major species groups (Brancaleoni et al., 2007) instead of individual species, however, may not be enough, as we found significant year and precipitation effect also for the species groups tested.

Precipitation is the most important limiting factor of productivity and aboveground herbaceous biomass in semi-arid grasslands (Mowll et al., 2015), but its effect on the relationship between biomass proxies and biomass has not been documented yet. We showed that precipitation affects this relationship for the aboveground live biomass of all vascular species and of the studied species groups. This finding implies that applying a relationship obtained under a certain precipitation regime for data collected under a different precipitation regime may be problematic. It can be particularly misleading in precipitation manipulation experiments (Beier et al., 2012; Kröel-Dulay et al., 2015; Wu et al., 2011), where differently treated plots receive different amounts of precipitation. As we found that regression lines become steeper with increasing amount of precipitation for both methods, establishing a

relationship in control plots (or outside the plots) and then applying the same relationship for drought or irrigated plots (Evans and Burke, 2013; Köchy and Wilson, 2004; Kongstad et al., 2012; Tielbörger et al., 2014) may introduce systematic error into biomass estimations.

The two non-destructive biomass estimation methods, visual cover estimation and NDVI, performed similarly well in estimating aboveground plant biomass in our study system (R^2 around 0.8), and provided consistent results regarding the effect of sampling year or precipitation. The consistency of our results across the two methods suggests that the effects of sampling year and precipitation are robust against changing biomass proxy. The similarly high performance of the two methods indicates that both are suitable for estimating above-ground plant biomass, and method choice can be based on additional things, such as specific research question, the structure of the studied vegetation, and the available workforce (Catchpole and Wheeler, 1992).

Given the potential systematic error in proxy-based biomass estimations we demonstrated above, one could conclude that we should report proxies and not convert them into biomass However, we argue that emphasis should rather be placed on increasing the reliability of biomass estimations. Ecosystem responses reported by using biomass proxies (e.g. cover, NDVI) only, and not biomass itself, may be valid at the level of each individual study, but it makes the synthesis of multiple studies difficult and problematic. A recent meta-analysis of vegetation responses to warming in arctic ecosystems (Elmendorf et al., 2012) included studies that report biomass, cover, frequency, or point-frame hits, and referred to these metrics collectively as “abundance”. However, this aggregate variable may easily be biased by the different sensitivities of these various proxies to treatments. Therefore, the importance of improved biomass estimations should be emphasized in future studies.

To better guide future research efforts, we have synthesized the following recommendations. 1. Whenever possible, direct measurements by biomass harvesting should be preferred over biomass estimation by proxy (Fay et al., 2011; Sherry et al., 2008). 2. Where direct harvesting is not possible, calibration should be repeated in each year of the study (Byrne et al., 2013; Kongstad et al., 2012; Tielbörger et al., 2014; Zhang and Welker, 1996). 3. In precipitation manipulation experiments, measuring multiple indicators of vegetation abundance in addition to cover, such as height (Byrne et al., 2013; Filella et al., 2004; Kongstad et al., 2012; Tielbörger et al., 2014) and leaf blade length (Williamson et al., 1987) may improve the calibration. 4. In addition, where multiple calibrations from years with different amounts of precipitation are available, the data can be used to parameterize an improved model. This step could lead to a higher accuracy of biomass estimation in treatments with different

precipitation amounts. 5. In experiments where compositional change may occur, separate calibrations for major species (Huenneke et al., 2001; Reichmann et al., 2013) may be important to account for changing species composition (Shaver et al., 2001). 6. Finally, when an experiment is shut down, a final biomass harvest should be done to evaluate the accuracy of non-destructive biomass estimates used during the course of the experiment.

5. Conclusions

We found that the relationship between biomass proxies and actual live herbaceous biomass varied between years, and was affected by interannual precipitation variability. We also illustrated that two very different methods – visual cover estimation and field spectroscopy – yielded very similar results. Our findings 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. These findings highlight the need for testing the domain of validity of proxy-biomass relationships in such studies, and, if necessary, for a refinement of biomass estimation methods.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 RMSE values (g/m^2) for visually estimated cover (A) and NDVI (B) as biomass proxies

Fig. S1 Relationships between sampling year (as explanatory variable) and annual mean values of biomass, visual cover estimation and NDVI (as dependent variable)

Fig. S2 Time dependence of the strength of precipitation - biomass relationship

Fig. S3 Relationships between visual cover estimation or NDVI (as explanatory variables) and aboveground live biomass (as dependent variable) in different years

Table S2 Linear mixed models of aboveground live biomass of graminoid (grasses and sedges) species

Table S3 Linear mixed models of aboveground live biomass of forb species

Table S4 Linear mixed models of aboveground live biomass of annual species

Table S5 Linear mixed models of aboveground live biomass of perennial species

Table S6 Main characteristics of the sampling years

Table S7 List of vascular plant species occurring in the sampling plots (2003-2012)