



Allometric relationships for selected macrophytes of kettle holes in northeast Germany as a basis for efficient biomass estimation using unmanned aerial systems (UAS)

Marlene Pätzig^{a,*}, Frenze Geiger^{a,b}, Daniel Rasche^{a,c}, Philipp Rauneker^a, Anette Eltner^d

^a Leibniz Centre for Agricultural Landscape Research (ZALF) e.V., 15374, Müncheberg, Germany

^b Institute of Biochemistry and Biology, University of Potsdam, 14469, Potsdam, Germany

^c Section Hydrology, GFZ German Research Centre for Geosciences, 14473, Potsdam, Germany

^d Institute of Photogrammetry and Remote Sensing, Technische Universität Dresden, 01069, Dresden, Germany

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ABSTRACT

Quantifying plant biomass in ecosystems is an essential basis for many ecological questions. A direct estimation of macrophyte biomass proves to be difficult for the large number of kettle holes in Pleistocene landscapes, due to their strong heterogeneities. This study compared a classical non-destructive method for biomass estimation based on allometric relationships built from a larger selection of plant trait variables with regressions only based on plant height and cover of four macrophyte species typical for kettle holes in northeast Germany (i.e. *Carex riparia*, *Phalaris arundinacea*, *Persicaria amphibia*, *Rorippa amphibia*). Their predictive power and potential applicability for remotely sensed biomass estimation using unmanned aerial systems (UAS) was evaluated. The usage of several in-situ measured plant traits of individual plants revealed best macrophyte biomass predictions ($R^2 = 0.84$ to 0.95). Yet, using only plant height and cover to predict biomass still showed a moderate to good correlation ($R^2 = 0.52$ to 0.81). Using *P. arundinacea* as an example, we demonstrated for one kettle hole the potential of calculating plant patch height from digital surface models (DSM) derived from UAS RGB images processed with structure-from-motion (SfM) photogrammetry. After applying a site-specific correction factor for discrepancies between reference field measurements of plant heights and DSM derived plant heights, we were able to calculate *P. arundinacea* biomass of the entire kettle hole based on allometric relationships using plant height and cover. Finally, we briefly discuss how further methodological development can improve UAS-derived plant height as predictor variable for biomass estimation.

1. Introduction

Macrophytes are a key component for biodiversity and biogeochemical processes in natural ponds (Davies et al., 2008; Onandia et al., 2018). They can cover the entire pond bottom or water column and are the main primary producers in these ecosystems (Kazanjan et al., 2018). The biomass of macrophytes is an important factor in ecology and a desirable source of information to address various research questions such as matter and energy turnovers (Carpenter and Lodge, 1986; Rejmánková, 2011). Thus, understanding the spatiotemporal dynamics of macrophyte biomass is a prerequisite to better predict these processes in pond ecosystems. In pond research, only few studies exist that focus on macrophyte biomass or biomass dynamics. Most of them applied destructive biomass estimation methods to assess biomass

information (Fernandez-Alaez and Fernandez-Alaez, 2011; Pineiro-Guerra et al., 2014; Nagengast and Kuczynska-Kippen, 2015; Kazanjan et al., 2018). But biomass harvest is not always possible, especially in sensitive or highly heterogeneous habitats, as it can interfere with long-term surveys, disturb existing associations in macrophyte stands and might remove or harm protected species (Gouraud et al., 2008; Wood et al., 2012). Kettle holes represent such natural ponds with pronounced wet-and-dry cycles that cause a high abiotic and biotic heterogeneity in space and time (Kuczyńska-Kippen and Nagengast, 2006; Lischeid et al., 2018; Reverey et al., 2018). These wetlands (water body < 1 ha) are depressions of glacio-fluvial origin, and frequently found in the agriculturally intensively used Pleistocene landscapes of Europe and North America. For example, the young moraine landscape of northeast Germany is characterised by 150,000–300.000 kettle holes

* Corresponding author.

E-mail addresses: marlene.paetzig@zalf.de (M. Pätzig), frenze.geiger@gmail.com (F. Geiger), daniel.rasche@gfz-potsdam.de (D. Rasche), philipp.rauneker@zalf.de (P. Rauneker), anette.eltner@tu-dresden.de (A. Eltner).

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(Kalettka and Rudat, 2006). Due to their high spatiotemporal heterogeneity, kettle holes are rich in macrophyte species, many of them threatened by extinction (Pätzig et al., 2012; Altenfelder et al., 2014). Currently, the quantification and prediction of kettle hole functions, such as internal biogeochemical processes, is hampered due to insufficient knowledge about macrophyte dynamics (Onandia et al., 2018; Pätzig et al., 2020). In turn, the large number and the high hydrogeomorphological diversity of kettle holes impedes knowledge improvement about spatiotemporal dynamics of macrophytes. Consequently highly variable abiotic and biotic properties between and within pond ecosystems (Kalettka and Rudat, 2006) lead to a lack of research regarding the application and development of efficient survey methods for pond macrophytes and their biomass.

Non-destructive methods for estimation of plant biomass have several advantages over destructive methods (Gouraud et al., 2008; Wood et al., 2012). In the last decades, a variety of non-destructive biomass estimation methods have been increasingly applied in order to enable the monitoring of net aboveground primary production throughout the year (Daoust and Childers, 1998; Smith et al., 2000; Cunliffe et al., 2016). From all available approaches, the creation of 3D-vegetation models using unmanned aerial systems (UAS) has the highest potential to accurately predict biomass for the entire pond ecosystem and allows higher spatial and temporal replication (Dandois and Ellis, 2010; Anderson and Gaston, 2013; Bendig et al., 2015; Cunliffe et al., 2016). 3D-vegetation models provide important variables, such as plant height or volume used in classical allometric relationships for biomass estimation. A combination of plant volume or plant height with plant cover in regression models has shown to be sufficient to account for the majority of biomass variations and to serve as a good biomass predictor (Thursby et al., 2002; Haga et al., 2007; Wood et al., 2012). Recent studies obtaining the vegetation height with UAS photogrammetry showed promising results that seem to be even more accurate than traditional field measurements and allow for upscaling to the landscape level (Dandois and Ellis, 2010; Bendig et al., 2015; Husson et al., 2017; Olsoy et al., 2018). Hence, efficient non-destructive biomass estimation at kettle hole scale should be possible by the application of classical regression models that estimate biomass from height and cover, where plant height is derived from UAS derived 3D-vegetation models.

The objective of this study was first to establish classic allometric relationships based on a range of plant trait variables, such as leaf length, leaf width or culm diameter, for four macrophyte species typical for kettle holes in northeast Germany. In a second step, we aimed at comparing the predictive power of these allometric relationships with the predictive power of regression models only based on plant height and cover data. A comparison between the two regression models allows determining the deviation from real biomass and helps to assess the quality of the regression models. Low deviation of biomass estimates for the regressions models that are based only on cover and plant height data could then replace exhaustive field measurements and would allow the potential applicability for remotely sensed biomass estimation using unmanned aerial systems (UAS). This approach would also justify the usage of previously published regression models with these two variables. To test this in a third step, we exemplarily calculated the plant height and mapped the spatial extent of the species *Phalaris arundinacea* L. for one kettle hole based on 3D models obtained from UAS RGB images processed with structure-from-motion (SfM) photogrammetry to estimate the plant biomass of the entire kettle hole.

2. Methods

2.1. Study area

The study was conducted in the AgroScapeLab Quillow, a landscape laboratory coordinated by the Leibniz Centre for Agricultural Landscape Research ZALF, that covers the catchment of the river Quillow (168 km²), located in the lowlands of northeast

Germany (Fig. S1). Geomorphologically, it is part of the young moraine landscape and characterised by a hummocky landscape. The climate is subhumid with a mean annual temperature of 8.6 °C and mean annual precipitation of 563.8 mm for the period 1992 and 2013 (Weather station at the Dedelow Research Station, ZALF). The Quillow catchment harbours over 1500 kettle holes mainly imbedded in intensive agriculture, primarily used for cultivating crops.

2.2. Selecting study objects

Four macrophyte species typical for kettle holes of the Quillow catchment were selected based on a vegetation data set from 32 kettle holes recorded in 2013 (Thomas Kalettka, unpublished data). *Carex riparia* Curtis and *Phalaris arundinacea* were chosen as examples for tall emergent macrophytes, while *Rorippa amphibia* (L.) Besser (creeping emergent macrophyte) and *Persicaria amphibia* (L.) Delarbre (floating-leaved macrophyte) represent herbaceous species (Fig. S2).

In total, 12 kettle holes were selected (Fig. S1) that cover different hydrogeomorphological types (4 with small areas and shore overflow tendency, 3 with large areas and shore overflow tendency and 5 storage types, according to Kalettka and Rudat, 2006) and provide sufficient cover for harvesting and sustaining the studied macrophyte species. Within each kettle hole, three habitats were selected per species. Habitat selection focussed on the amphibian zone (shore, for details see Fig. 2 in Pätzig et al., 2012), as this zone provides the most typical environmental conditions for kettle holes owing to their high water level dynamics. When water levels receded to zero, we considered the aquatic zone (bottom of kettle holes) instead. We considered only habitats in the terrestrial zone (top shoreline) when the other zones were free of plant stands. In each of the three species specific habitats one sample (0.25 × 0.25 m plots) was taken monthly from May to July 2016 resulting in nine replicates per species and kettle hole. The procedure was not always feasible in case of sparse plant populations, particularly for *P. amphibia*, but the total number of observations ($n = 41\text{--}81$ per species) was always sufficient to fit reliable regression models. Sampling over the main macrophyte growth period allowed us to generate regression models valid for different growth stages.

2.3. Measuring plant trait variables

For the phenometric method, besides dry mass as the dependent variable, eight independently measurable plant traits were recorded for each replicate sample of 0.25 × 0.25 m: target species cover, number of individuals, culm diameter, number of dead leaves, number of living leaves, leaf length, leaf width and height without inflorescence. Most of the variables were selected after Daoust and Childers (1998). These data are openly accessible under DOI 10.4228/ZALF.DK.138 (Pätzig and Geiger, 2019).

In the field, we conducted ocular mapping of macrophyte coverage in percentage and converted the data to a 15-part Londo scale (Londo, 1976) (Table S1). In a second step, we counted the number of individuals. The number of individuals for each target species was defined as the number of culms. Particularly with regard to clonal species, it would have been impossible to determine the number of actual individuals. In a third step, we selected ten individuals per replicate sample and stored them in separate bags to avoid shrivelling of the leaves, which was particularly important for *R. amphibia*. On the same day, the plant material was brought to the laboratory for a quick removal of snails, caterpillars or other sample contaminants, and for the measurement of the remaining plant traits as described below.

After measuring cover and the number of individuals in the field, all plant individuals were harvested from the plot area of 0.25 × 0.25 m using a wooden frame. In cases, where *R. amphibia* was found in the water, the frame was positioned on the water surface and only the parts above the water surface were harvested. We only considered individuals inside the frame, whereas individuals rooting outside the

frame were separated manually before clipping. On the same day, the biomass was dried at 60 °C for 48 h in drying chambers. Then, the dry mass was weighed to the nearest 0.01 g with a laboratory scale (Kern EMB 200-2).

In the laboratory, plant height, leaf length and leaf width were measured with centimetre precision by flattening the probes on graph paper. This was done first for complete individuals and in a second step for individual leaves. For the total height without inflorescence, the distance between the cutting point and tip of the tallest leaf was measured. For the measurement of the length and width of the leaves five living leaves were selected (at least 50 % green). In *C. riparia* and *P. arundinacea*, leaf length was defined as the distance from leaf sheath to tip, and for *R. amphibibia* and *P. amphibibia*, only the leaf blade was considered. All widths were measured at half of the leaf length, by folding them in half. To avoid inaccuracy in culm diameters, the arithmetic mean of two measurements was used for *P. arundinacea*, *R. amphibibia* and *P. amphibibia*. For *C. riparia*, culm diameter was measured at the ground leaf rosette in the field before the harvest. In order to obtain one value per variable and per plot for each plant species, we calculated the arithmetic mean of all species-specific individuals before we calculated the arithmetic mean per plot.

2.4. Statistical analyses

To evaluate the relationship between biomass as a dependent variable and the different independent plant trait variables mathematically, multiple linear regression analyses were conducted with the statistical software R version 3.5.1 (R Core Team, 2018).

Prior to statistical analysis, we checked the assumptions for linear models by testing all eight independent variables of the phenometric method for inter-correlation, collinearity, as well as normality and homoscedasticity of the residuals. Inter-correlation was examined using the Pearson correlation coefficient (r) with the function 'corr.test' of the R package 'psych' (Revelle, 2018). In case of significant ($\alpha = 0.05$) r -values equal or higher than 0.90, we removed the variable which had caused the highest measurement effort from further analyses. This was the case for the variable "leaf length" for *C. riparia* and "culm diameter" for *R. amphibibia*. The remaining variables were tested against collinearity with the variance inflation factor (VIF) using the R package 'VIF' (Lin, 2012). In the current study, no variable exceeded $VIF = 10$ and all remained in the following analysis (O'Brien, 2007). The normal distribution of the residuals was tested with one-sample Kolmogorov-Smirnov tests and visual inspection. The residuals' independency was proven by plotting the residuals against each independent variable. Homoscedasticity was examined visually by plotting the residuals against the predicted values. Since the variables did not meet the pre-condition of homoscedasticity all variables were log-transformed. Variables which had a value of 0 were raised by 1 before log-transformation.

For biomass prediction, the linear version (log-log regression model) of a power function was applied, with y = predicted biomass, b_0 = intercept, b_1 = slope, x = value of the independent variable and n = the number of observations (Eq. (1)).

$$\log(y) = b_0 + b_1 * \log(x_1) + b_2 * \log(x_2) + \dots + b_n * \log(x_n) \quad (1)$$

In order to reduce the number of variables and to keep the highest fit for the model we undertook stepwise selection of independent variables by running a final multiple linear regression keeping only variables that proved to be significant ($\alpha = 0.05$) in the first model setup. All multiple linear regression analyses were conducted using standard R function 'lm' that uses least squares optimisation.

The predicted biomass for given plant trait variables in the original units was then calculated using the inverse log function as follows (Eq. (2)).

$$y = \exp^{(b_0 + b_1 * \log(x_1) + b_2 * \log(x_2) + \dots + b_n * \log(x_n))} \quad (2)$$

Finally, we compared the sum of predicted biomass (back transformed into original units) with the sum of measured dry mass per species to calculate the percentage of under or overestimation. To analyse seasonal effects we also tested the influence of shorter sampling periods (each month) on the phenometric regression models using two approaches. After creating a data subset for each month per species, we first fitted a log-log-regression for each month per species and additionally included an interaction term between the other two months and each previously selected plant trait variable. Second, we established log-log-regression to predict biomass for each month per species without including interaction terms for inspection of the model characteristics and comparison of R squares.

2.5. UAS data acquisition

A UAS flight campaign was conducted in September 2018. We used a Goliath 8 multi-rotor platform (Cadmic GmbH) to carry a 24 Megapixel Sony alpha 6000 digital RGB camera with a fixed 16 mm lens. The camera was attached on a gimbal to compensate for pitch and roll movements of the aircraft. Mission planning was done using the ground control software provided by the autopilot manufacturer (Mikrokopter, HiSystems GmbH). Three different flight altitudes (10, 20 and 30 m) were chosen. In total, six individual waypoint tracks were created. First, a flight track with parallel lines and nadir camera orientation was prepared for each height. Then, a circular track was flown ($r = 15, 20$ and 20 m for 10, 20, 30 m height, respectively) with oblique camera orientation looking approximately at the centre of the circle at ground-level. Images are captured at a constant interval of ~ 2 s. By adapting flight speed to each flight height, we achieved forward overlap of 85–90 % and sidelap of 70–85 % for the flight tracks with nadir camera orientation.

Prior to the flights 9 ground control points (GCPs) were distributed evenly at the periphery of the kettle hole and margin of the water body. Additionally, 20 points within the kettle hole were selected for reference measurements of actual (natural plant height) and full height (straightened plant height) of *P. arundinacea* stands using a folding rule (Fig. 1). A real-time kinematic (RTK) GNSS (Reach RS, Emlid Ltd) was used to record the location of all GCPs and height reference measurements.

2.6. 3D-vegetation model calculation and biomass estimation on kettle hole scale

846 JPG images from all six flights were quality-checked for motion blur and 806 of it subsequently processed in a SfM photogrammetric workflow using Agisoft Photoscan Professional 1.4.2 build 6205 (Agisoft LLC). In a first step, feature points were detected and matched across the images. Afterwards, this information was utilized to estimate the interior (e.g. focal length) and exterior (i.e. location and position of each image capture) camera geometry. Seven GCP coordinates were used in a second step to geo-reference the scene and two were used as check points. After that, a bundle adjustment was performed considering the sparse point cloud (corresponding 3D points of the matched features in an arbitrary coordinate system) and the geo-reference information to optimize the camera geometry. Then, dense matching was performed to derive a dense point cloud (DPC), retrieving a 3D information for almost each image pixel with a reprojection error of 0.679 pix. Further details regarding the SfM workflow are provided in Eltner et al. (2016). The project processing report of the software Photoscan Professional is available in the Supplementary S1. Finally, a digital surface model DSM and an orthomosaic were computed on the basis of the DPC (Fig. 2a & b) and exported in the desired image format Tiff and reference system EPSG: 25833.

Further processing of the data was conducted in the software R. First, all RTK measurements were transformed to a projected coordinate system EPSG: 25833, followed by a resample of the UAS derived raster

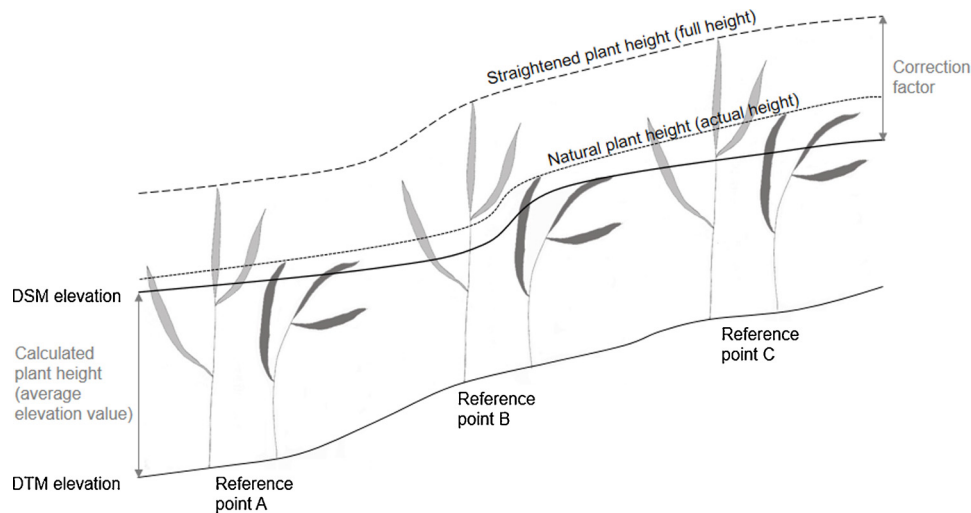


Fig. 1. Straightened plant height (plants displayed in light grey) and calculated plant height (DSM – DTM elevation) of *P. arundinacea* to obtain a site-specific correction factor for biomass estimation. Plants displayed in dark grey indicate natural plant height. DSM = Digital Surface Model, DTM = Digital Terrain Model).

datasets i.e. orthomosaic and DSM. In a next step, we used a pseudo-normalized green-red difference index pseudo-NGRDI calculated from reflectance for red (B_r) and green (B_g) orthomosaic bands (Hunt et al., 2013; Tucker, 1979). This was used as a tool to separate vegetated

(pseudo-NGRDI > 0) from non-vegetated (pseudo-NGRDI ≤ 0) areas (grid cells) (Fig. 2c, Eq. (3)).

$$\text{pseudo-NGRDI} = (B_g - B_r) / (B_g + B_r) \quad (3)$$

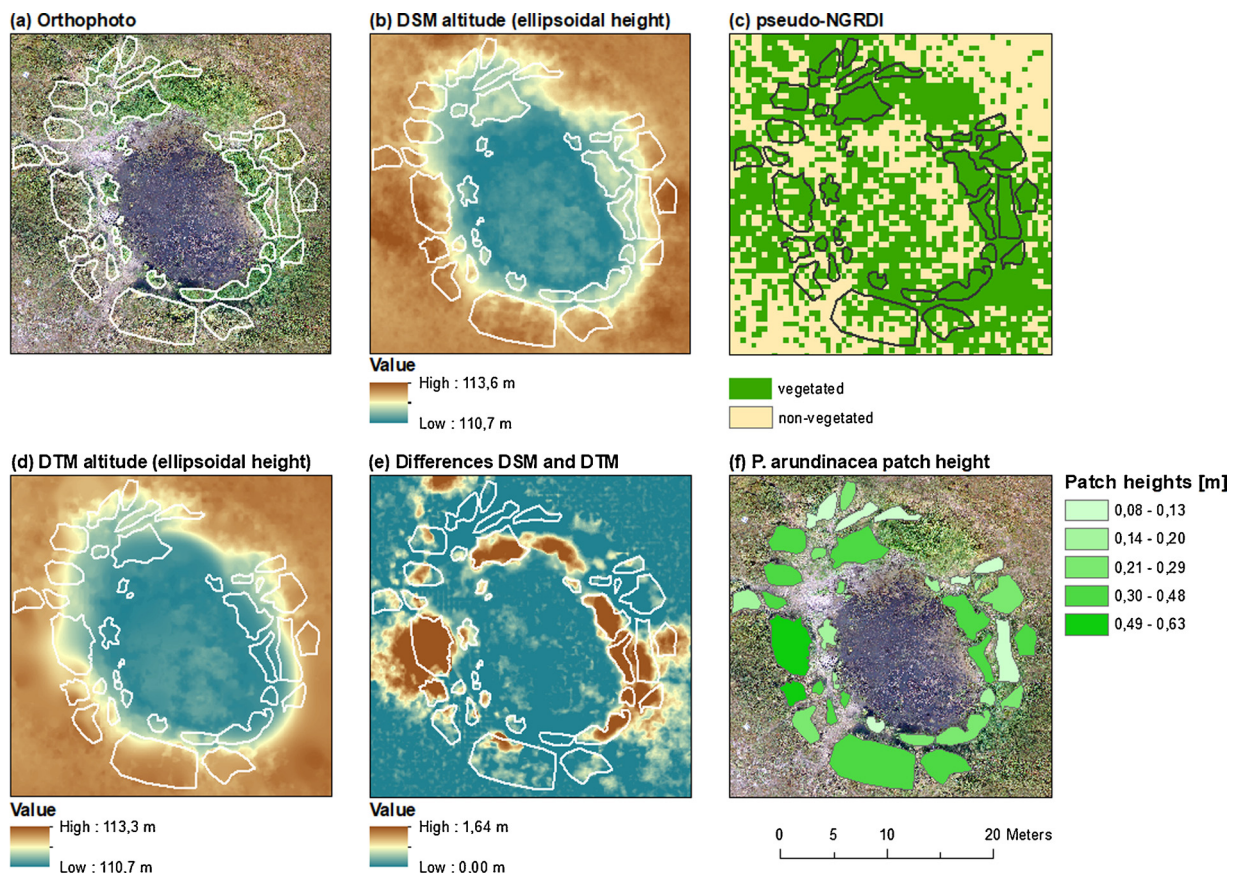


Fig. 2. Derivation of *P. arundinacea* stand heights from an UAS based orthophoto and digital surface model (DSM). (a) Orthophoto and *P. arundinacea* stands (white outlined areas) based on field mapping. (b) Structure from motion derived DSM using a UAS with blue pixels indicating lower and red pixels indicating higher elevations. (c) Pseudo-normalized green-red difference index (pseudo-NGRDI) with green areas indicating vegetation. (d) Derived digital terrain model (DTM) with blue pixels indicating lower and red pixels indicating higher elevations. DTM has the same spatial resolution as the DSM. (e) Subtraction of DTM and DSM: DTM has higher elevations than DSM in red areas while blue pixels indicate lower DTM elevations. (f) Average *P. arundinacea* stand height per patch: darker green indicates larger mean stand height. White or grey, respectively, outlines represent *P. arundinacea* stands identified in the field and were drawn in a UAS image taken one day before the actual survey. DSM = Digital Surface Model, DTM = Digital Terrain Model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

In a next step, non-vegetated pixels from the digital surface model (DSM) were used to create an interpolated digital terrain model (DTM), representing terrain elevations without vegetation (Fig. 2d). For the interpolation between DSM elevations of centroids of non-vegetated orthophoto grid cells and thus, the calculation of a gridded DTM, 20 % of the spatial points were randomly extracted from the data set and stored for calibration of the interpolation algorithm. In this study, the inverse distance weighting approach (IDW) was used for spatial interpolation of point elevations. In the IDW algorithm, observations at points closer to the location of prediction receive a higher weight where the rate of weight decrease with distance is determined by the β -value. To obtain the best interpolation result, the IDW was performed for the remaining 80 % of the point dataset with β -values ranging from 1 to 10 in intervals of 0.1. The root mean square error (RMSE) was calculated between the interpolated grid cell values and the corresponding point elevation values extracted for calibration in the previous step. The β -value producing the smallest RMSE (β -value = 5; RMSE = 0.05 m) was then used for the final interpolation of the DTM with the entire dataset of non-vegetated points. Before computing a canopy height model as basis for biomass estimation, the DSM has to be subtracted from the newly generated DTM (Fig. 2e). Negative values in the resulting DTM-DSM difference raster indicate a DSM elevation being lower than the DTM elevation resulting in erroneous results as the DTM should not be higher than the DSM. Therefore, pixels of the DTM with negative values in the DTM-DSM difference raster received the respective value of the DSM (see also Dandois and Ellis, 2013; Cunliffe et al., 2016).

Finally, the average elevation value (calculated plant height) per *P. arundinacea* patch was calculated from the DSM and DTM (Figs. 1 & 2 f). Reference measurements of straightened plant heights of individual plants in the field are used to calculate an average correction factor between straightened reference measurements and calculated plant heights derived from the UAS survey (Fig. 1). The correction factor accounts for a manifold bias including erroneous values of the predicted DTM as well as differences between straightened and natural plant heights (on average 6.9 %) (Fig. S3). Multiple regressions derived from analyses described in the previous sections based on plant height and cover (Table 1) were used to calculate the biomass of *P. arundinacea* on kettle hole scale. Estimates were calculated with and without applying the site-specific correction factor considering a cover of 95 % mapped in the field for each *P. arundinacea* patch on the same date of the UAS flight campaign.

An overview of the used material and key processing parameters according to James et al. (2019) is available in Table S2. The key steps conducted to exemplary estimate biomass of the species *Phalaris arundinacea* on kettle hole-scale based on a UAS derived 3D vegetation model is available in Fig. S4.

3. Results and discussion

3.1. Phenometric non-destructive biomass estimation from field and laboratory work

The regression models established based on the phenometric method explained between 84 % for *C. riparia* and 95 % for *R. amphibia* of the variation in the aboveground plant biomass (Table 1, Fig. 3). The predicted biomass differed marginally from the actual biomass, and the maximum average deviation was an underestimation of 2.44 % (*R. amphibia*) followed by *P. arundinacea* and *C. riparia* with an average deviation of 2.27 or 2.25 %, respectively. The lowest underestimation was calculated for *P. amphibia* with 0.59 %. The regression models based on the entire data set had higher or similar R squares compared to the regressions for single month per species, except for June for *R. amphibia*. Single-month regression models showed low deviation in intercept and slope (Fig. S5), which we also validated by including an interaction term between the different plant trait variables and the respective month. For *P. arundinacea* and *P. amphibia*, no interaction

Table 1
Intercept and slope of predictor variables including standard errors for logarithmic biomass [g per m²] estimation using the Phenometric-Method. For each species, three different sets of variables were used.

Species	Number of sample plots	Set	Intercept	Cover of target species	Average total height without inflorescence	Average culm diameter	Average Leaf Width	Average Leaf Length	Average number of living leaves	Average number of dead leaves + 1	Number of individuals	Adjusted R ² (*Normal R ²)
<i>C. riparia</i>	81	Set with the highest R ²	1.32 ± 0.49	0.13 ± 0.06	0.49 ± 0.12	-	1.68 ± 0.16	-	-	-	0.89 ± 0.09	0.84
		Cover & height	1.05 ± 0.69	0.39 ± 0.09	0.80 ± 0.17	-	-	-	-	-	-	0.52
		Height only	0.68 ± 0.76	-	1.21 ± 0.16	-	-	-	-	-	-	0.41*
<i>P. arundinacea</i>	66	Set with the highest R ²	-1.01 ± 0.80	0.22 ± 0.07	1.08 ± 0.15	0.55 ± 0.14	-	-	-	0.32 ± 0.08	0.61 ± 0.09	0.86
		Cover & height	-1.64 ± 0.60	0.59 ± 0.07	1.30 ± 0.12	-	-	-	-	-	-	0.74
		Height only	0.48 ± 0.76	-	1.35 ± 0.17	-	-	-	-	-	-	0.48*
<i>R. amphibia</i>	41	Set with the highest R ²	-1.42 ± 0.39	-	1.70 ± 0.10	-	0.62 ± 0.20	-0.83 ± 0.28	-	0.34 ± 0.10	0.68 ± 0.07	0.95
		Cover & height	-0.82 ± 0.51	0.41 ± 0.10	1.32 ± 0.11	-	-	-	-	-	-	0.81
		Height only	0.70 ± 0.42	-	1.31 ± 0.13	-	-	-	-	-	-	0.73*
<i>P. amphibia</i>	42	Set with the highest R ²	-0.98 ± 0.79	0.13 ± 0.05	0.98 ± 0.16	0.99 ± 0.39	-	-	0.60 ± 0.19	-	0.83 ± 0.10	0.90
		Cover & height	0.01 ± 0.75	0.42 ± 0.05	0.90 ± 0.22	-	-	-	-	-	-	0.72
		Height only	-0.6 ± 1.18	-	1.38 ± 0.33	-	-	-	-	-	-	0.3*

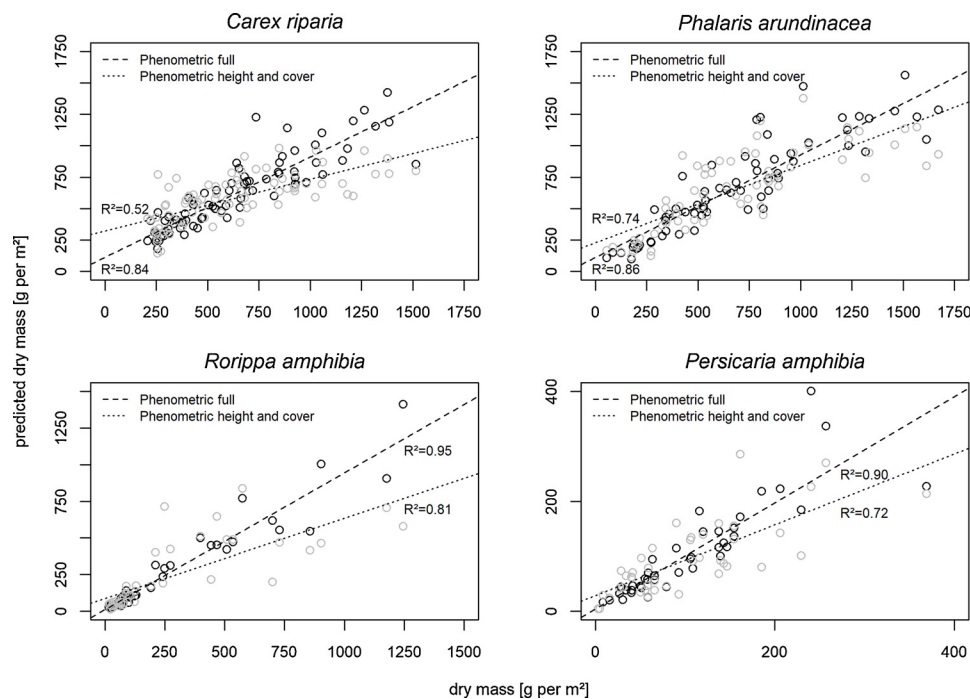


Fig. 3. Comparison of best regression models obtained using the Phenometric-Method with all significant variables vs. cover and average height without inflorescence as predictor variables only.

between the month and the respective variables were significant on a significance level of $p = 0.05$. For *C. riparia*, only the interaction term between a single month and the number of individuals was significant, and for *R. amphibia* only the interaction term between a single month and the number of dead leaves was significant. Thus, we report and use the regression built for the entire data set per species.

The selected variables in our regression models have also been found to be good predictors for non-destructive biomass estimations using the phenometric method in other studies (Daoust and Childers, 1998; Gouraud et al., 2008; Miao et al., 2008). Especially, the inclusion of leaf-related variables, i.e. average leaf width and length as well as average number of living and dead leaves, increases the predictive power of the allometric relationships. However, these leaf-related variables did not raise the coefficient of determination higher than $R^2 = 0.95$ (Daoust and Childers, 1998). The fits probably lagged behind those of other studies, because other leaf-related variables such as “sum of leaf length to base”, “average leaf length to base” or “average width at the mid-point” were not included (Daoust and Childers, 1998). The variables were excluded, as they are difficult to acquire and only added a small part to the explained biomass variation in Miao et al. (2008). The consideration of other complex variables, such as volumetric measurements or “Leaf Area Index”, may have resulted in a better model fit for the target species in this study. Again, the acquisition is immensely time-consuming and most studies recommend using only independent variables that show a linear relation to biomass for predictions with multiple linear regressions (deLeeuw et al., 1996; Miao et al., 2008; Wood et al., 2012). In this study, plant height was always selected in the models that considered all acquired variables and thus was the most important variable to predict biomass, similar to findings of other studies (deLeeuw et al., 1996; Miao et al., 2008; Wood et al., 2012).

3.2. Biomass estimation based on cover and plant height in the field

The high explanatory power of plant height for biomass estimation that we found in this study, is of crucial importance for the establishment of allometric relationships that are based on 3D vegetation models

derived from UAS images. However, we achieved low coefficients of determination when height without inflorescence was the only variable used to predict biomass per square meter (Table 1). This was especially true for *C. riparia*, *P. arundinacea* and *P. amphibia* where height explained only 30–48 % of variation. This indicates the necessity of a second predictor for estimating biomass of the species investigated. When we used height (without inflorescence) and cover of the target species to predict biomass, the regressions explained between 52 % for *C. riparia* and 81 % for *R. amphibia* of the variation in aboveground plant biomass (Table 1, Fig. 3). The lowest underestimation of 5.3 % was determined for *P. arundinacea*, whereas *R. amphibia* has the highest underestimation of biomass, of 13.8 %. *C. riparia* and *P. amphibia* revealed underestimations of 7.1 % and 10.4 %. The lower explanatory power compared to the regression models that used up to six plant trait variables can simply be attributed to the reduced number of variables, which cannot describe the differences in plant traits with sufficient accuracy. Compared to existing allometric relationships that are based on height and cover the predictive power was in a similar range. For example, a coefficient of determination was $R^2 = 0.68$ for mean macrophyte biomass estimated from height data obtained with echosounding (Haga et al., 2007). For submerged macrophytes the R^2 ranged between 0.54 and 0.96 in a shallow river (Wood et al., 2012), and explained variance was 96 % for *Phragmites australis* (Cav.) Steud (Thursby et al., 2002). Hence, allometric relationships based on cover and height are helpful tools for quick biomass assessments rather than solely using plant height. Both variables can be gathered in the field (direct measuring or using UAS) easily even on kettle hole scale, which allows for the areal estimation of biomass from UAS images derived 3D-vegetation models.

3.3. Exemplary biomass estimation of *Phalaris arundinacea* on kettle hole scale using UAS

Applying the regression model based on height and 95 % cover for *P. arundinacea* on kettle hole scale, dry mass was estimated to a total of 45.4 kg referring to $248.1 \text{ g}^{-1} \text{ m}^{-2}$. However, plant height values measured at reference points in the field differed substantially from

height values derived from subtracting DSM and DTM at reference point locations. On average, straightened plant heights measured in the field were 2.4 times larger than heights derived from digital surface and terrain model at reference locations.

The strong deviation in plant height can mainly be attributed to the creation procedure of the DTM. As pixels of bare soils are unevenly distributed in and around the kettle hole studied (Fig. 2c), irregularly positioned elevation points are used for interpolation, which results in erroneous elevations of the DTM compared to the DSM. This becomes visible in Fig. 2e, where areas with higher DTM elevations can be observed. Since, the majority of kettle holes is characterised by dense living or dead vegetation cover also in wintertime, the generation of DTMs based on UAS-RGB images seems to be largely hindered compared to other habitats with larger areas of bare soil, such as dryland ecosystems (Cunliffe et al., 2016; Olsoy et al., 2018). For these ecosystems it has been shown that plant height estimates derived from UAS based point clouds provided a good fit to field-based measurements at the patch scale ($R^2 = 0.51\text{--}0.75$) (Olsoy et al., 2018).

Besides the difficulties with the generation of the DTM from UAS RGB images, vegetation is difficult to reconstruct from UAS imagery due to the strong change of appearance of plants in images that change their perspective only slightly. The general habitus of the plant species with rarely completely straightened leaves under field conditions and the windy conditions during image acquisition resulted in a flattened photogrammetrically derived DSM. The wind conditions are a crucial factor in wind-exposed young moraine landscapes that needs to be incorporated in the methodological development for UAS-based plant biomass estimation of kettle holes.

Bending of plant individuals hampered a direct application of the regression model, because the model relies on straightened plant heights measured in the lab. Therefore, field reference measurements of plant heights with individual plants manually straightened are essential to find adapted regression models to estimate biomass with a UAS-based DSM and DTM. We used the calculated difference between straightened plant heights in the field and plant heights from subtracting DSM and DTM at 20 reference measurements points for correcting the patch heights of *P. arundinacea*. Applying a mean height correction factor of 2.36 to all *P. arundinacea* patches with 95 % cover resulted in a much higher total dry mass of 130.2 kg compared to 45.4 kg without correction factor, referring to $712.1 \text{ g}^{-1} \text{ m}^{-2}$. Five percent differences in plant cover (90 or 100 %, respectively) changed biomass estimates about 3 %. These estimates lie within the large range of biomass measurements observed for *P. arundinacea* in earlier studies that depended among other factors on moisture, light, nutrient statuses and interspecific competition (Cizkova et al., 2015; Nielsen, 2008; Pocienė et al., 2013). The different environmental conditions that influence the biomass development of *P. arundinacea* and the prevalent wind conditions during image acquisition do not allow for the usage of our height correction factor in future studies. It requires local and date-specific reference height measurements for each desired target species to obtain accurate results.

Similar to the procedure of Cunliffe et al. (2016), who calculated volume from UAS derived 3D-vegetation models and applied published regressions to estimate biomass, the present study demonstrated the high potential of 3D-vegetation models for exhaustive mapping of plant heights in kettle holes. Also Bendig et al. (2015) demonstrated the general robustness of height models compared to vegetation indices for biomass estimation. Areal information on plant height can then be used in allometric regressions based on height and cover to estimate biomass on kettle hole scale. This procedure is particularly relevant when attempting to use published allometric regressions. Although 3D reconstruction from UAS-based RGB imagery has the advantage of being less cost intensive, we suggest optimizing our workflow by the use of additional techniques, such as UAS-based LiDAR scanning. LiDAR should be applied to obtain DTMs as they can penetrate vegetation due to multi-pulse capabilities as a basis for spatial and temporal estimation

of plant biomass development, either with a DSM derived from UAS-RGB images or first returns from multi-pulsed LiDAR. Future studies that compare and test different combinations of DSMs and DTMs derived photogrammetrically and based on LiDAR measurements would be beneficial.

A more direct way for estimating plant biomass of kettle holes would be the establishment of regressions that directly use plant heights calculated from a 3D-model as an independent variable. Again, this would require considering the effect of wind on the relationship between biomass and photogrammetrically derived DSM. Such approach does not allow the usage of published allometric regressions.

In order to develop the UAS-based SfM photogrammetric approach towards a practical tool for biomass estimation of kettle holes or other ecosystems with dense vegetation further improvements are necessary. These include the aforementioned aspects and the determination of optimal flight height and image quantity (per area) required to obtain sufficient overlap and redundancy for the calculation of 3D-vegetation models. These improvements should ideally consider the fast changing appearance of vegetation during image acquisition through wind movement. Developing standard processing procedures as brought forward by Cunliffe and Anderson (2019) are highly beneficial as they increase the comparability between sampling dates, locations and studies.

In summary, this study has demonstrated the possibility to sufficiently predict biomass of grass-like and herbaceous species in kettle holes with multiple regression models based on height and cover measurements. Results were compared to regression models containing a range of variables. Regression models solely based on height and cover of the target species showed lower predictive power than the phenometric model built on more plant variables, but their moderate to good explanatory powers still make them useful for biomass observation on high-temporal resolution and for biomass estimation on a larger spatial scale. The usage of the regression model of *P. arundinacea* with plant height and cover as independent variables in combination with a UAS-based SfM photogrammetric approach showed a high potential for an efficient and accurate biomass assessment on kettle hole scale. Still, there remains further potential to improve our UAS-based workflow for biomass estimation. This is of particular importance, because biomass estimation and monitoring will remain in the focus of ecological pond research.

Authors' statement

MP conceptualized the study supported by FG and AE. MP led the project and was responsible for the use and re-use of research data. Methodologies to establish allometric regressions were equally designed by MP and FG. PR and MP equally developed the design for image acquisition using unmanned aerial systems. Data collection in the field was led by FG and PR supported by MP and DR. PR provided all hardware and software for acquisition and processing of images collected with unmanned aerial systems. FG, PR and DR analysed the data supported by MP. DR did the programming for the geospatial analysis. MP visualized the data. All authors discussed the results. MP and FG led the writing of the original draft. MP led the writing and editing of the review. All authors contributed critically to the drafts and gave final approval for publication.

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Appendix A. Supplementary data

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