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ORIGINAL PAPER

Usnea lichen community biomass estimation on volcanic mesas, James Ross Island, Antarctica

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Abstract Ground macrolichens dominated by several species of fruticose *Usnea* spp. with foliose *Leptogium puberulum* constitute an important component of the terrestrial ecosystem of James Ross Island. Long-term monitoring of lichen communities in respect to their reaction to ongoing climatic changes in this part of Antarctica became a research task for scientists in recent years. The non-destructive estimation of lichen biomass provides data necessary for the management and protection of Antarctica. We have developed and tested the methodology of non-destructive estimation of biomass of fruticose *Usnea* species, which predominate in the ice-free tertiary basalt outcrop areas on James Ross Island. In 38 experimental squares (non-destructive measurements), the density and height of lichen thalli were measured and digital photography with ground cover evaluation was performed. Lichen biomass was harvested from 14 experimental squares and analysed for dry mass, chlorophyll *a*, *b* content, and thalli surface area (TSA). Predictive linear models were constructed from available non-destructively measured variables with the aim to maximize predictive accuracy for the destructively measured attributes. A total of 82.3 % of variability in the TSA values was explained (87.5 % for biomass determination). Cross-validated prediction error for lichen TSA estimation was 423 cm^2 (11.5 % of the average TSA). In the case of lichen dry mass determination, cross-validated prediction error was 4.53 g m^{-2} (7.3 % of the average dry mass). This study proves that macrolichens in maritime Antarctica can be monitored non-destructively by simple field methods combining digital photography and measurements of lichen thalli in botanical squares.

Keywords Non-destructive field methods · Lichen biomass estimation · *Usnea* species · Maritime Antarctica · Image analysis

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Introduction

Fruticose *Usnea* species are some of the most widespread macrolichens in the maritime Antarctica (Kappen et al. 1991; Schroeter et al. 1995). They have a circumpolar distribution centred in the vicinity of the Antarctic Peninsula. This group of species (*Usnea antarctica, Usnea subantarctica, Usnea sphacelata*), together with the foliose *Leptogium puberulum*, show a wide ecological amplitude (Schroeter et al. 1995). They predominate in the patchy vegetation of the ice-free area on James Ross Island, mostly on the Tertiary basalt outcrop forming table mountains–mesas, reaching heights of 300–700 m.a.s.l. The most developed communities, growing mainly in the stable parts of sorted polygons (on rock surfaces or pebbles), are present on these mesas. On James Ross Island, the communities on basaltic mesas are very rich in biomass. Øvstedal and Lewis Smith (2001) stated that fruticose lichen taxa such as *Usnea* sp. generally predominate at higher altitudes and in more exposed habitats throughout

maritime Antarctica. Already existing ice-free table mountains (mesas) and other formations within the James Ross Island Volcanic Group (JRIVG) (Smellie et al. 2009) on many islands in this geological formation have enabled the *Usnea* sp. community to become the most abundant lichen in this part of Antarctica. Thus, ecological research of this species is of high importance.

This lichen community can be viewed as a bioindicator in long-term ecological studies due to its reaction to recent temporal changes in maritime Antarctica (Benedict 1991; Kappen et al. 1995). The Antarctic Peninsula has experienced a rapid regional warming trend, much more pronounced than observed in other Antarctic areas (Rivera et al. 2005). Temperature data from the past 45 years show that a rise has occurred. In spite of large interannual variability, this trend is confirmed by ice shelf and glacial retreat (Doran et al. 2002). The northern part of the Antarctic Peninsula (including its associated islands) has a cold moist maritime climate. Changes in temperature and moisture can result, for example, in a different growth rate within one lichen species across Antarctica (Sancho et al. 2007).

Estimation of lichen biomass and diversity is a central part of many ecological investigations at high latitudes. Biomass of lichens or lichen thalli can be estimated either by destructive harvesting of randomly selected squares or non-destructively, usually by correlating biomass with some other measured attributes of the lichens.

The aim of this research work was to develop a special non-destructive method which can be used in the area of maritime Antarctica where *Usnea* species are very common. The method is based on measurements of density and height of lichen thalli as well as digital photography of the experimental plots for further evaluation to estimate *Usnea* sp. lichen biomass. In addition, our method should be tested also in different fruticose and/or foliose lichen communities and its validity compared with already existing non-destructive and destructive methods for estimating lichen biomass used mainly in the Arctic (Jonasson 1988; Luscier et al. 2006; Muukkonen et al. 2006; Moen et al. 2007). However, evaluation of differences in various lichen communities was not the aim of this paper.

Our intention was to determine whether the method can offer results as exact and satisfactory as other methods and therefore minimize the destructive impact on Antarctic lichen communities.

The field methods for *Usnea* community biomass estimations were developed with special respect to long-term ecological research focused on the observation of lichen community response to ongoing climate changes.

For the destructive method, we quantified biomass and diversity through the common harvesting and weighing technique. However, clipping and fractionating of the lichens took a long time, as it is a difficult task to be accomplished under Antarctic conditions. To reduce both the time of sampling and disturbance of lichen communities, we combined biomass and diversity estimation with more rapid but less informative measurements of density and height of lichens in experimental squares. At the same time, we conducted an image analysis of ground cover based on digital photographs of experimental plots. If these non-destructive methods provide satisfactory results, considerable time can be saved and lichen community damages can be reduced. Moreover, non-destructive estimations can be done repeatedly on the same surface within one experimental plot with only minor disturbance.

Thirty-eight experimental plots (plots 50 × 50 cm) were set up on three mesas (Berry Hill, Johnson Mesa and Lachman Crags) in the northern part of James Ross Island, NW part of the Weddell Sea, east of the north end of the Antarctic Peninsula (63°48′02″S, 57°52′57″W), where lichen standing crop and diversity were evaluated by both destructive and non-destructive methods.

Materials and methods

Study area

This study was undertaken in the vicinity of the Czech research station J. G. Mendel, James Ross Island (63°48′02″S, 57°52′57″W) during 2 summer months in 2007 and 2008. Three basaltic mesas (Berry Hill, Johnson Mesa and Lachman Crags) rich in *Usnea* species biomass (Figs. 1, 2) were selected for the study. Berry Hill and the northern part of Lachman Crags are almost completely covered with lichens, whereas in the southern part of Lachman Crags and Johnson Mesa only several spots (up to one-third of the mesa area) of *Usnea* species community occurred.

Density and height of *Usnea* thalli and ground cover (non-destructive measurements)

Thirty-eight experimental plots (plots 50 × 50 cm) were set up on the three mesas (Fig. 1), and the density and height of lichen thalli were measured. A measuring square, constructed of two Plexiglas squares 50 × 50 cm in size, was fixed to the corners by an iron pole; the squares were kept at a 5 cm distance from each other. Holes were drilled every 5 centimetres over the Plexiglas square. Before measurements were taken of each experimental plot, the Plexiglas square was carefully levelled using a builder's level (approximate distance of the Plexiglas square from the ground was always from 20 to 40 cm), a calibrated measuring rod was inserted through the Plexiglas square

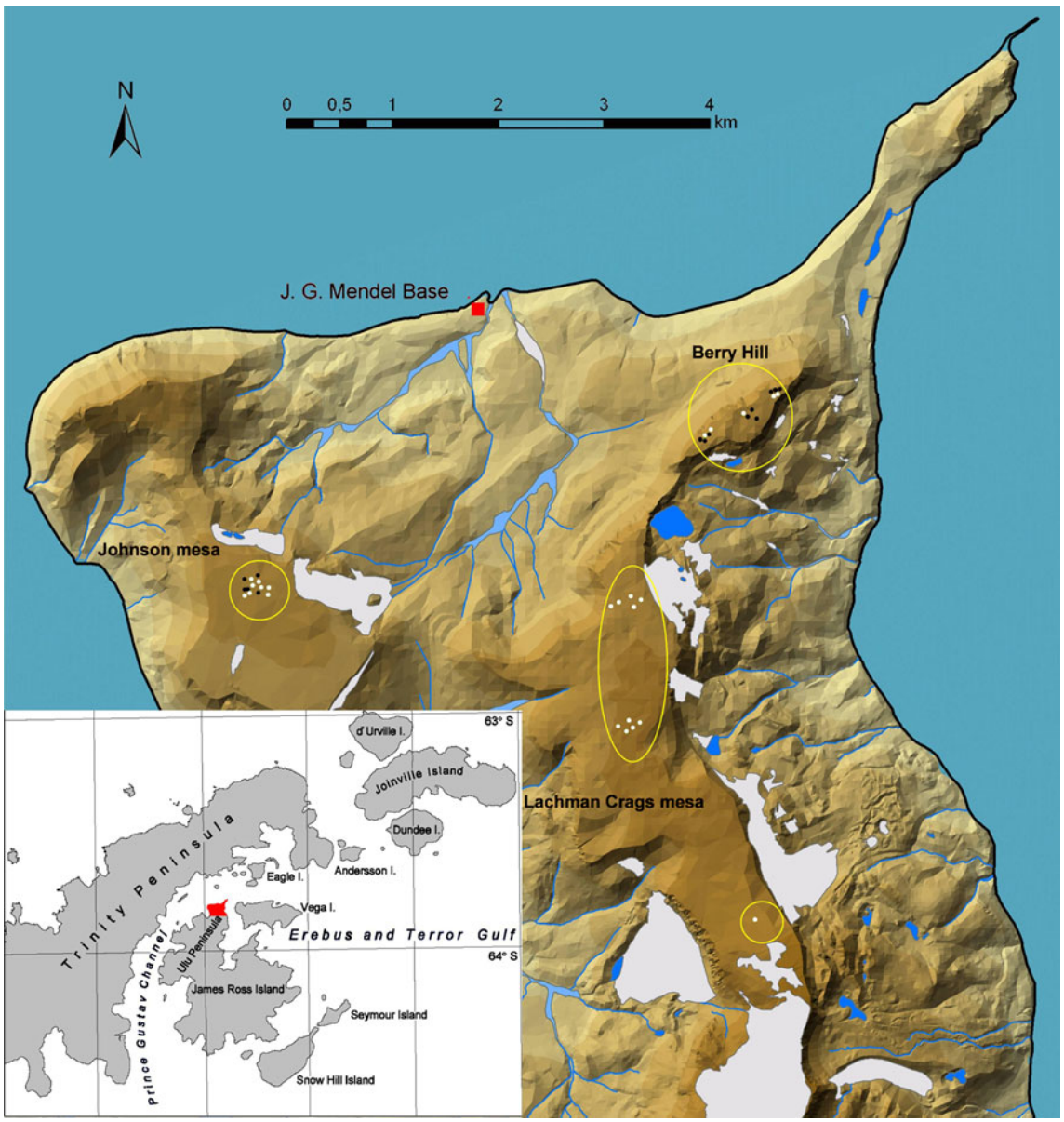

Fig. 1 Situation map—basaltic mesas on James Ross Island, Ulu Peninsula, Antarctica with the indicated experimental plots. *White spots* non-destructive measurement, *black spots* destructive measurement. Modified map of James Ross Island—Northern part, 1: 25,000, Czech Geological Survey, Prague (2009)

holes applied to note the density and measure the height of lichen thalli (DHLT) (*Usnea* and *Leptogium*). This parameter includes the mean number and mean height of *Usnea* species thalli as well as the mean number of *Leptogium*, both per 0, 25 m^2. Ground relief relative altitude values were measured by the same method. First, a digital photograph of the plot was taken (Nikon D70, AF-S NIKKOR lens with focal length 18–70 mm, placed 115–120 cm from the ground, plot shaded from the direct sun, camera fixed on a tripod) and ground cover (GC) evaluation was performed afterwards on a computer using Chips for Windows ver. 4.7 (Chips Development Team 1998). Each experimental plot photograph was analysed in order to separate lichens, stones and other material (matrix) into particular pixel groups (Fig. 3). Accordingly, only the pixel groups referring to *Usnea* species were further analysed for lichen GC. *Leptogium* occurred always in the deepest places in the ground relief and was not properly visible in the digital photographs. The ratio between 1 cm^2 and a corresponding number of pixels was counted for each plot separately. Consequently, the exact lichen GC on each plot was determined.

Fig. 2 *Usnea* species and *L. puberulum* communities in their natural environment on mesas of James Ross Island

Lichen biomass harvest and evaluation (destructive measurements)

Lichen biomass was harvested from 14 experimental plots (50 × 50 cm). The obtained biomass was analysed for dry mass (DM), chlorophyll *a*, *b* content (Chl *a*, Chl *b*—only *Usnea* species were analysed), and lichen thalli surface area (TSA). Only *Usnea* species were used for the thallus surface area estimation: thalli from each plot were scanned (HP ScanJet 3970). The same approach for GC (Chips for Windows ver. 4.7) was used for determination of lichen TSA (ratio between 1 cm^2 and a corresponding number of pixels was counted for each scan separately and only "*Usnea*" pixels were counted for TSA determination). All relevant images from one experimental plot were pooled and summed.

After surface area measurements, lichens were analysed for chlorophyll *a* and *b*. All samples from each experimental plot were freeze dried (lyophilized) for 3 days in order to obtain DM, weighed, cut into small pieces with scissors and then extracted with DMSO following Hansson (1988). A particular fraction of the sample was inserted in a known amount of DMSO in test tubes and warmed up in 50 °C for 15 min (until the lichen was completely colourless). A subsample of 1.25 ml was then taken from each test tube and centrifuged for 10 min at 14,000 rpm (Hettich universal 32R) to remove fine sediments. After the transfer of the supernatant, absorbency was measured by a spectrophotometer (Specort 205, double-beam, Analytik Jena, UK) at 649 and 665 nm in a 1–4 nm spectrophotometer resolution range. Concentration of chl *a* and *b* was calculated using equations by Lichtenthaler (1978) and Porra et al. (1989), respectively.

Statistical analyses

Predictive linear models were constructed from the available non-destructively measured variables in order to maximize predictive accuracy for the destructively measured attributes (Thallus surface area—TSA, DM). For each candidate predictor (density and height of lichen thalli—DHLT, GC), multiple parametric transformations were considered to maximize the linearity of its relation to a particular response variable: no transformation, log transformation, square transformation and square-root transformation. Final models were chosen from the set of candidate predictors (with pre-selected transformations) using the stepwise selection procedure based on the AIC value (Sakamoto et al. 1986). In order to estimate model reliability correctly, adjusted R^2 values were estimated, as well as the standard error of prediction, based on a jackknife method (Efron and Tibshirani 1993). Effects of individual predictors in selected linear models were visualized (Figs. 4, 5) with the effect plots, using the package "effects" (Fox 2003). All analyses were performed in R system version 2.8 (R Development Core Team 2008).

Results

Usnea species thalli surface area (TSA) estimation

The parameters used for construction of linear regression models are summarized in Table 1.

The final model was:

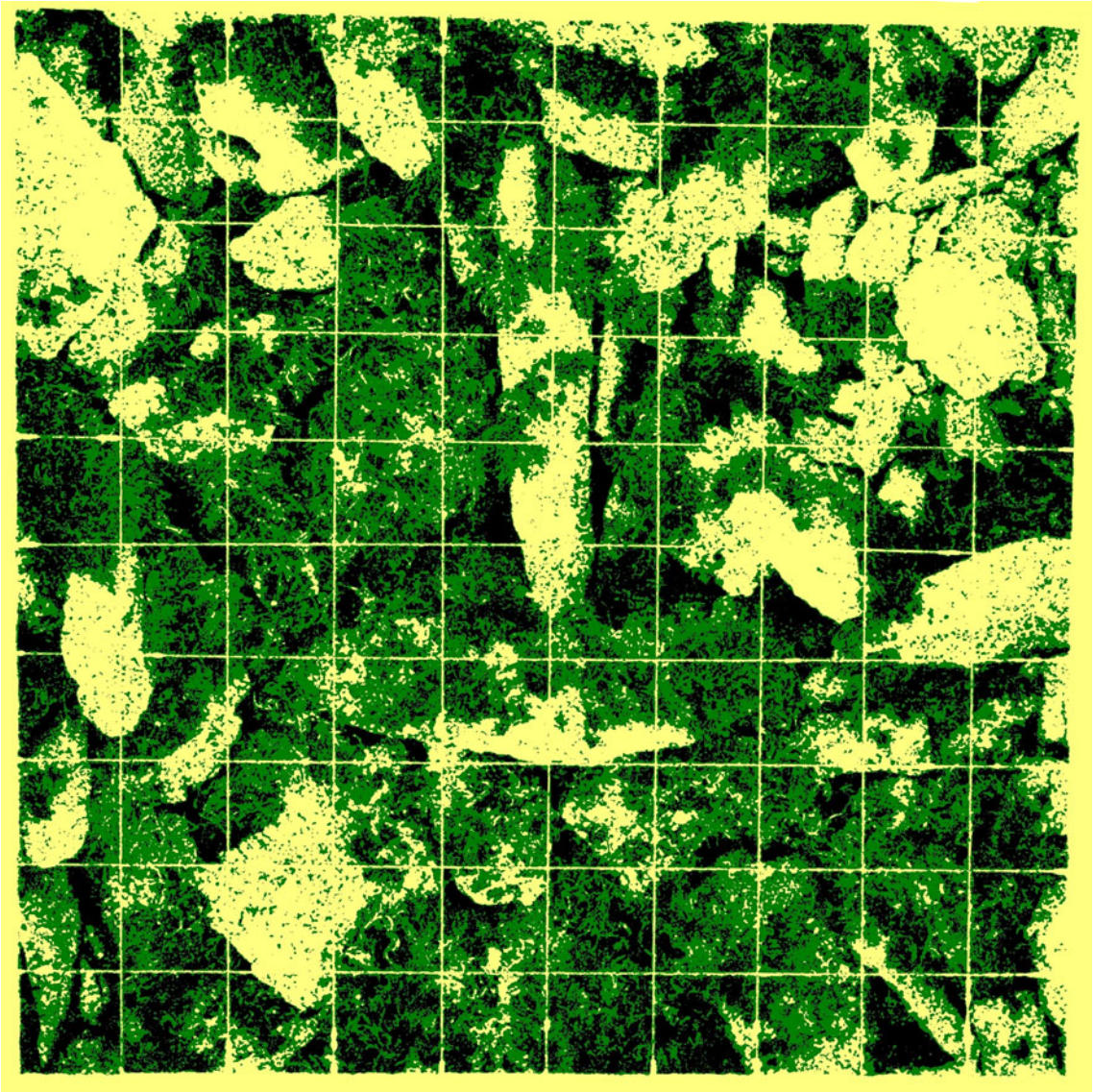

Fig. 3 Image obtained by chips for Windows 4.7 program with lichens, stones and matrix separated into different pixel groups

$$\mathrm{TSA}\left(\mathrm{cm}^2\right) = (\mathrm{N.\,lepto})^2 + (\mathrm{GC}) + \mathit{Usnea}.\,\mathrm{avg} + (\mathit{Usnea}\,\mathrm{avg})^2 + \log(\mathrm{N.}\,\mathit{Usnea})$$

The individual parameters of the fitted model are summarized in Table 2. Adjusted R^2 of this model was 0.82 ($F_{5,8} = 13.1$; $p = 0.001$).

The statistical models, in which digital photography (GC) together with lichen thalli measurements were used, gave the most accurate results for TSA estimation. A total of 82.3 % of the variability in the values was explained. The correlation between fitted and real TSA values was 0.944 and the prediction error was 237 cm^2 on (50 × 50) cm. A more realistic cross-validated prediction error (based on a jackknife method) was 423 cm^2 (approx. 11.5 % of the average TSA).

Thalli surface area (TSA) estimation determined only from the digital photographs of an experimental plot was much worse; only 22.7 % of the variability in surface values was explained (fall from 82.3 %), with cross-validated prediction error of 675.34 cm^2 (18 % of the average TSA; $F_{1,12} = 4.819$; $p = 0.049$).

Usnea species dry mass (DM) estimation

When predicting the *Usnea* species DM, the final model included the following predictors:

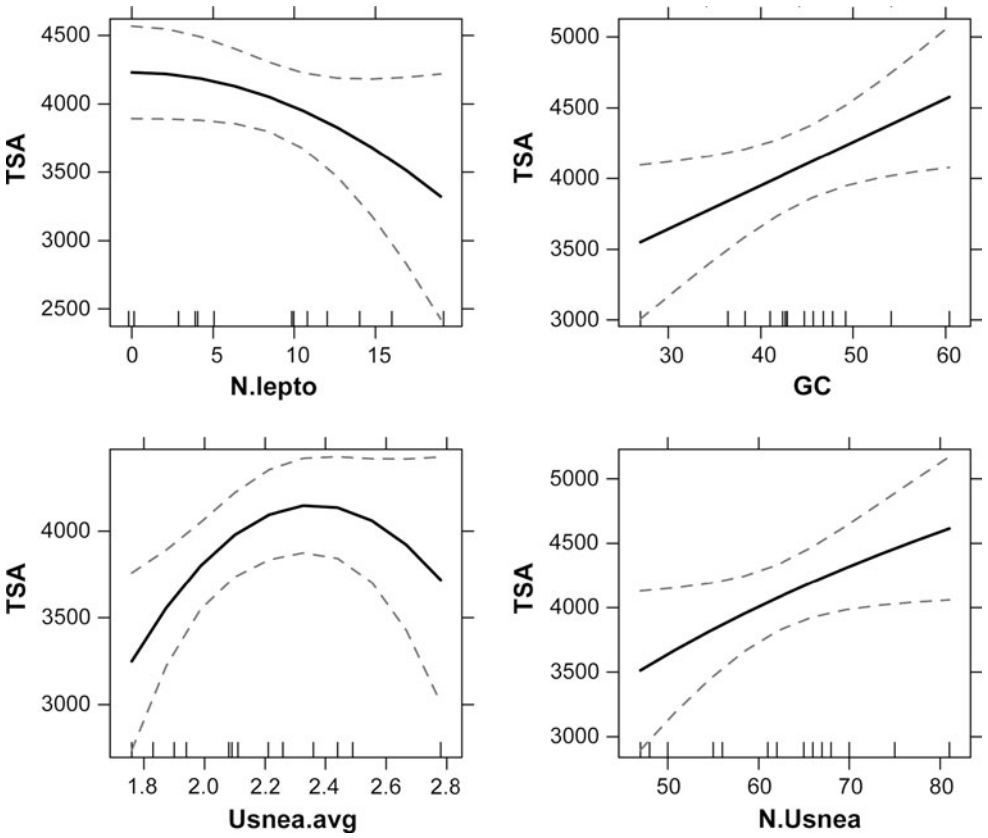

Fig. 4 Effect plot of individual model terms for linear model predicting thallus surface area (cm^2). *Solid lines* show partial effects expressed by the model regression coefficient and (optional) predictor transformation, *dashed lines* represent 95 % confidence regions. *Vertical segments* emanating upwards from the *horizontal axis* represent reiterated observation values for particular predictor

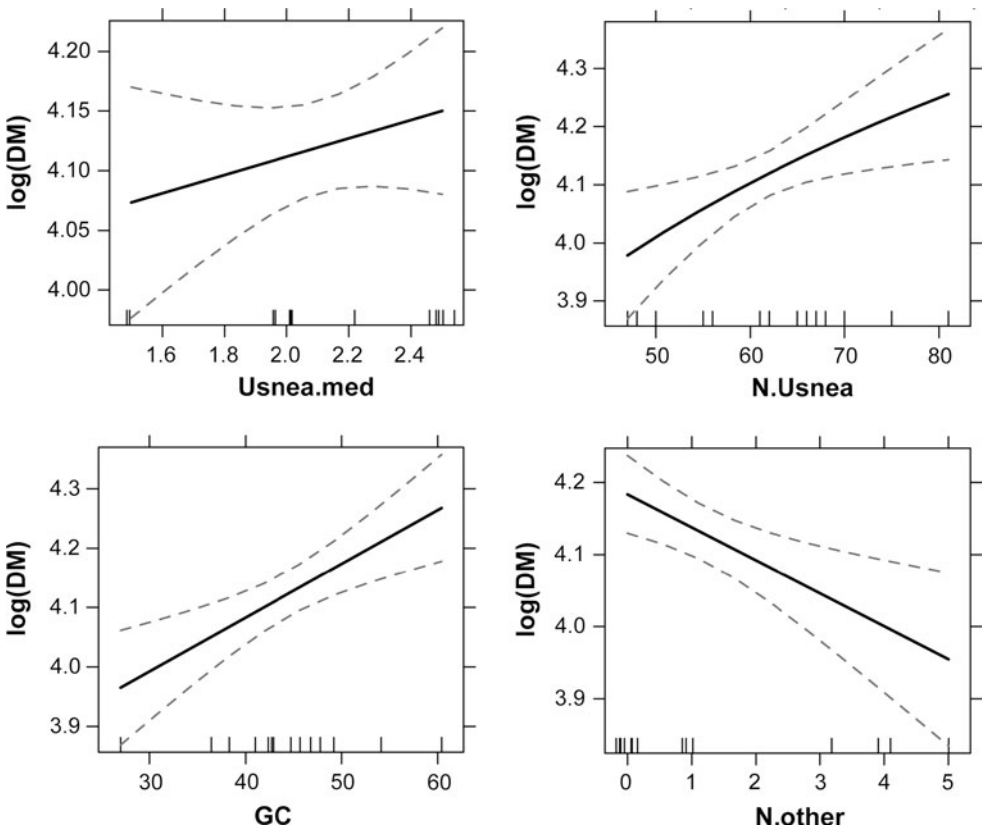

Fig. 5 Effect plot of individual model terms for linear model predicting log of dry mass. *Solid lines* show partial effects expressed by the model regression coefficient and (optional) predictor transformation, *dashed lines* represent 95 % confidence regions. *Vertical segments* emanating upwards from the *horizontal axis* represent reiterated observation values for particular predictor

Table 1 Measured parameters used in fitted regression models

Parameters	Chl *a* (μg g)	Chl *b* (μg g)	DM (g m)^2	TSA (cm^2)	GC (%)	N. *Usn.*	N. *Lep.*	N. other	*Usn.* avg. (cm)	*Usn.* med. (cm)	*Usn.* SD (cm)	Relief. SD (cm)
Minimum	240.0	357.3	44.54	2,525	18.10	30.00	0.00	0.00	1.72	1.50	0.51	2.69
1st quartile	270.4	401.2	57.47	3,310	36.56	46.25	0.00	0.00	1.99	2.00	0.66	3.16
Median	297.0	445.2	60.03	3,844	42.52	62.00	0.00	3.50	2.19	2.00	0.71	3.42
Mean	295.4	453.4	62.13	3,766	41.87	57.42	3.32	8.42	2.18	2.14	0.73	3.64
3rd quartile	313.9	486.7	69.39	4,276	48.07	69.00	4.00	17.50	2.37	2.50	0.80	3.90
Maximum	382.4	579.4	79.66	4,854	63.41	81.00	19.00	32.00	2.78	2.50	1.03	5.92

Chl *a,* Chl *b*, DM, GC, TSA, *N-Usnea* (number of *Usnea* thalli per experimental plot − 0.25 m^2), *N-Leptogium* (number of *Leptogium* thalli per experimental plot), *N*-other (number of other lichens thalli per experimental plot), *Usnea avg*. average height of *Usnea* thalli per experimental plot, *Usnea med* median height of *Usnea* thalli per experimental plot, *Usnea SD* standard deviation of height of *Usnea* thalli within each plot, relief. *SD* standard deviation of the ground relief values within each plot

Table 2 Summary of parameter estimates of the model for total thalli surface area of *Usnea* species

Parameter names	Estimate	SE	*t* value	*p*
(Intercept)	−5,686.330	3,693.529	−1.540	0.1622
I (N. *Lepto*^2)	−2.515	1.300	−1.934	0.0891
GC	30.692	11.798	2.601	0.0316
Poly [(*Usnea* avg.) 2]1	657.960	342.985	1.918	0.0913
Poly [(*Usnea* avg.) 2]2	−806.306	313.971	−2.568	0.0332
Log (N. *Usnea*)	2,024.300	840.082	2.410	0.0425

Intercept, *I* (N. *Lepto* ^2) second power of number of *Leptogium* per experimental square, GC, *poly* [(*Usnea* avg.) 2]1 second power polynom of average *Usnea* thalli surface area, *poly* [*Usnea* avg.) 2]2 second power polynom of average *Usnea* thalli surface area, *log* (N. *Usnea*) logarithmic transformation of *Usnea* thalli number per experimental plot

Table 3 Summary of parameter estimates of the model for the total aboveground dry mass weight of *Usnea* species

Parameter names	Estimate	SE	*t* value	*p*
(Intercept)	1.520422	0.797919	1.905	0.08910
Usnea. med	0.076513	0.065004	1.177	0.26936
Log (N. *Usnea*)	0.508289	0.169331	3.002	0.01491
GC	0.009059	0.002242	4.040	0.00293
N. other	0.045748	0.013387	−3.417	0.00766

Intercept, *Usnea med* median height of *Usnea* thalli, *log* (N. *Usnea*) logarithmic transformation of *Usnea* thalli number per experimental plot, *GC*, *N. other* number of other lichens per experimental plot

$$\log \mathrm{DM}\left(\mathrm{g\,m^{-2}}\right) = \mathit{Usnea}.\,\mathrm{med} + \log\left(\mathrm{N}.\,\mathit{Usnea}\right) + \mathrm{GC} + \mathrm{N}.\,\mathrm{other}$$

The fitted parameters of this model are summarized in Table 3. The adjusted R^2 of this model was 0.87 ($F_{4,9} = 23.7$; $p < 0.001$).

The same statistical method was used for lichen biomass (DM) determination and again both digital photography GC and lichen thalli measurements had to be included in the calculation. In this case, 87.5 % of the variability was explained, with the correlation between the fitted and real values being 0.956. Prediction error was 1.05 g m^{-2} while the more realistic cross-validated prediction error was 4.53 g m^{-2} (approx. 7.3 % of the average DM).

When using a model only with the digital photography GC predictor, the cross-validated prediction error was 10.39 g m^{-2} (approx. 16.7 % of the average DMW), and adjusted R^2 was 0.20 ($F_{1,12} = 4.27$; $p = 0.061$) (Table 4).

Discussion

When using the non-destructive methods of terrestrial plant standing crop estimation, biomass is usually predicted from

Table 4 Summary of parameter estimates of the model for the Chl *b* concentration

Parameter names	Estimate	SE	*t* value	*p*
(Intercept)	109.89	83.7	1.313	0.21378
Log (relief. SD)	270.03	65.1	4.141	0.00134

Intercept, *log* (*relief. SD*) logarithms of standard deviation of the ground relief values within each square

the height and density of plant thalli, and/or with plant GC. GC or height of thalli is used more often to predict the biomass of low-growing species such as low shrubs or herbs. However, they can be applied to fruticose and, exceptionally, foliose lichens (Alaback 1986; Dunford et al. 2006; Moen et al. 2007). Estimates based on the height of the thallus are generally poor while those based on cover have to be treated with caution, because of the overlap of plant components (Jonasson 1988). There is no single variable that can be used with equally high predictive power among a broad spectrum of plant species or growth forms. For these reasons, we have developed and tested the methodology of non-destructive estimation of biomass of fruticose *Usnea* species, which predominate in the ice-free Tertiary basalt outcrop areas on James Ross Island, Antarctica. The reasons why we did not directly apply the method developed for estimating lichen biomass in the Arctic (Jonasson 1988; Luscier et al. 2006; Muukkonen et al. 2006; Moen et al. 2007) are: (1) fruticose lichens are morphologically diverse and (2) there is a lack of vascular plants on James Ross Island. Both of these reasons are highly specific to each type of lichen community. Without development and/or at least testing of the significance of non-destructive estimation methods of the lichen's diversity, standing crop methods cannot be applied.

At present, with the ongoing changes of climate recorded in polar regions, the non-destructive estimation methods of ground lichen biomass can be used in ecosystem and carbon-cycle modelling (Smith 1990; Cornelissen et al. 2001; Callaghan et al. 2004a, b). The highest rise of temperature in the Southern hemisphere, 2.5 °C during the last 50 years, was recorded on the sub-Antarctic islands and the Antarctic Peninsula with neighbouring islands (Vaughan et al. 2003). James Ross Island in the maritime Antarctica is one of the localities where these climate changes have been demonstrated.

In our study, 82.3 % of the variability in the TSA values was explained and the correlation between fitted and real TSA values was 0.944 with an accuracy of TSA estimation (50 × 50) cm = ±237 cm^2. In the case of biomass determination (DM), 87.5 % of the variability was explained and the correlation between fitted and real values was 0.956. The tests clearly show that *Usnea* TSA and its DM can be precisely estimated by non-destructive methods (density and height of thalli measurements and GC—digital photography evaluation in 50 × 50 cm experimental squares). Various photographic techniques have been successfully used for estimating cover in single-layer vegetation (Dietz and Steinlein 1996; Luscier et al. 2006), including lichen-rich vegetation (Gaare and Tömmervik 2000). However, evaluation of GC by digital photography has certain limitations. *Leptogium* occurred in the deepest places in the ground relief and was not properly visible in the digital photographs. Only *Usnea* species were considered in GC analyses. To the best of our knowledge, such an evaluation combination (GC and DHLT) has not been performed for the *Usnea* species and *L. puberulum* community in the Antarctic until now. However, non-destructive lichen biomass estimations were developed for the Arctic, because the ground lichens constitute a vital part of the reindeer diet. Non-destructive estimation of lichen biomass is therefore crucial in providing objective data for the management of lichen resources (Muukkonen et al. 2006).

The studied *Usnea* species and *L. puberulum,* which predominate on the Tertiary basalt outcrops on James Ross Island, differ in their photobiont composition. Whereas *Usnea* species (only *Usnea* species were analysed for chlorophyll content) contain a trebouxioid photobiont (green eukaryotic algae from the genus *Trebouxia*, order *Pleurastrales*) with chlorophyll *a* and *b*, *L. puberulum* is a typical cyanobiont with *Nostoc* containing chlorophyll *a* only (Øvstedal and Lewis Smith 2001). The content of chlorophyll in lichen thalli has been analysed by several authors (Lange et al. 1986; Demming-Adams et al. 1990; Schroeter et al. 1995; Kappen 2000). These studies have shown that the shaded parts of a lichen community contain a higher number of photobiont cells with a higher content of chlorophyll, while sunny parts have lower levels. This was the main reason why it was not possible to estimate Chl *a* concentration from the measured values in our statistical test. The content of chlorophyll is directly related to light conditions of a particular experimental site. Moreover, in our analyses, the Chl *b* concentration was dependent on the experimental plot micro-relief. *L. puberulum* frequently occurs in the deepest, and probably the most humid, parts of rock and/or pebble surfaces. This proves that the occurrence of the foliose cyanobiont (*Nostoc*) lichen *L. puberulum* is dependent on the experimental plot micro-relief. However, it can also be concluded that chlorophyll *a* and *b* contents cannot be used for non-destructive estimation of *Usnea* standing crop on the Tertiary basalt outcrops on James Ross Island. In this study, it has been shown that the macrolichen *Usnea* species community can be precisely monitored by non-destructive measurements (combination of estimation of density and height of lichen

thalli and subsequent GC evaluation with the help of digital photography both in experimental squares). The combination of these simple field methods is proposed for long-term monitoring of lichen communities in respect to their reaction to ongoing climate changes that is occurring in this part of Antarctica.

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