Examensarbeten **2012:5**
Institutionen för skogens ekologi och skötsel

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Skattning av renlavsbiomassa genom bildanalys

Tomas Jansson

Sveriges Lantbruksuniversitet Jägmästarprogrammet
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Handledare: Urban Bergsten, SLU, Inst för skogens ekologi och skötsel
Examinator: Erik Valinger, SLU, Inst för skogens ekologi och skötsel Umeå 2012

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Handledare / *Supervisor*: Urban Bergsten
SLU, Inst för skogens ekologi och skötsel / *SLU, Dept of Forest Ecology and Management*
Extern handledare / *External supervisor:* Samuel Roturier, INRA, UMR Metafort
Extern handledare / *External supervisor:* Hans Winsa, Sveaskog
Examinator / *Examiner:* Erik Valinger
SLU, Inst för skogens ekologi och skötsel / *SLU, Dept of Forest Ecology and Management*

I denna rapport redovisas ett examensarbete utfört vid Institutionen för skogens ekologi och skötsel, Skogsvetenskapliga fakulteten, SLU. Arbetet har handletts och granskats av handledaren, och godkänts av examinator. För rapportens slutliga innehåll är dock författaren ensam ansvarig.

This report presents an MSc/BSc thesis at the Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU. The work has been supervised and reviewed by the supervisor, and been approved by the examinator. However, the author is the sole responsible for the content.

Abstract

During consultation procedures between forest owners and the Sámi, data on, e.g., reindeer lichen biomass on the current site is needed. Hitherto, the existing methods of measuring lichen cover and biomass has been either objective methods such as the Point Intercept method, which is time consuming, or some sort of subjective visual estimation, which is faster but less accurate. However, both these methods are sensitive to different observers and/or to different inventories. This paper addresses the further development and evaluation of a photographical inventory method that uses colour distribution in images to estimate lichen biomass.

During the autumn of 2011 six different locations, with different grazing pressure and lichen cover, in Norrbotten county were inventoried using both the Point Intercept method and the photographical method, complemented with collection of biomass samples. The sample images were analyzed with respect to lichen and background areas (cover) using WinCAM™. Statistical analyses were used to create equations for estimation of lichen biomass from lichen cover, as well as to compare photographically estimated lichen cover and lichen cover from the Point Intercept method.

Results shows that it is possible to estimate lichen biomass from lichen cover analyzed via image analysis. Results also indicate that different equations should be used for different sites depending degree of lichen cover and ground vegetation.

Sammanfattning

Vid samråd mellan skogsägare och renskötande samer krävs bland annat information om mängden renlavsbiomassa i det bestånd som är aktuellt för skogsbruksåtgärder. Hittills har skattning av lavbiomassa skett genom den objektiva Point Intercept-metoden, vilken är tidsödande, eller genom någon form av subjektiv visuell bedömning, vilken är snabbare men har lägre nogrannhet. Båda dessa metoder är dock känsliga för olika förättningsmän och kan även ge skiftande resultat mellan olika inventeringar. Det här arbetet tar upp vidareutvecklingen och utvärderingen av en fotografisk inventeringsmetod baserad på färgskillnader i bilder.

Under hösten 2011 genomfördes provtagningar på sex olika platser i Norrbottens län med olika betestryck och täckningsgrad av renlav. Både Point Intercept-metoden och den fotografiska metoden användes under provtagningen, dessutom togs lavbiomassaprover. Bilderna analyserades med avseende på lavtäckningsgrad med hjälp av programvaran WinCAM™. Statistiska analyser användes för att skapa funktioner för skattning av lavbiomassa utifrån lavtäckningsgrad, men även för att jämföra lavtäckningsgrader från både Point Intercept-metoden och den fotografiska metoden.

Resultaten visar att det var möjligt att skatta mängden renlavsbiomassa med hjälp av bildanalys, och att biomassafunktioner bör beakta såväl lavtäckningsgrad som betestryck.

Introduction

The Sámi, the indigenous people of northern Fennoscandia, have for centuries been dependent on reindeer (*Rangifer tarandus tarandus*) husbandry and the Sámi still have rights to utilize large areas of Sweden to herd their reindeer (Swedish Reindeer Herding Act 1971:437).

However, the Sámi reindeer herders are not the only ones who utilizes the boreal forest. About 50% of the land area in northern Sweden is classified as productive forest land (Statistical Yearbook of Forestry 2011) and the Sámi herding area in Sweden constitutes about 40% (Kivinen et al. 2010), which means that forest owners and the Sámi to a large extent use the same land.

During winter, reindeer's main source of nutrition consists of terrestrial reindeer-lichens (mainly *Cladonia* spp.) (Heggberget et al. 2002; Holleman et al. 1979). A single reindeer can consume as much as 3 kg of lichen (dry weight) a day (Holleman et al. 1979), and if lichens are abundant they can constitute up to 80 % of a reindeers daily winter diet (Heggberget et al. 2002).

Ground lichens such as reindeer lichens are negatively affected by modern forestry in many ways. Soil scarification, such as harrowing, can affect as much as 50 % of the lichen cover, which is especially negative due to the fact that lichen is slow to recover after disturbances (Eriksson and Raunistola 1990). Fertilization causes the canopy to close and reduces incoming light, which favor mosses at the expense of lichen (Sulyma and Coxson 2001). Overall, an increase in productivity due to modern forestry also supports more nutrition demanding plants (Kellner and Mårshagen 1991). Furthermore the modern forest management may also change density and depth of the snow cover, which can make the ground lichen impossible to reach for the reindeer (Roturier and Roué 2009).

Since neither the forestry industry nor the Sámi can be neglected as land users, consultation procedures between the stakeholders are held prior to any silvicultural measures (Swedish Forestry Act 1979:429). These consultation aims at giving information to reindeer herders about coming silvicultural measures (Sandström and Widmark 2007). However to come up with arrangements that pleases both, information and data on, e.g., amount of lichen at the site in question is needed.

The used methods for estimating the cover of plant communities or plant species differs depending species and tolerable costs for the actual purpose. Subjective methods where lichen cover and height are estimated visually are both fast and cheap but their precision can vary depending on which species/subject that is estimated. More objective methods, such as the Point Intercept method (PI), are based on randomized sample plots where lichen cover and height are thoroughly measured repeatedly. These methods offer higher precision, but are time consuming and hence expensive (Vanha-Majamaa et al 2000; Meese & Tomich 1992; Jonasson 1988). Choosing an inventory method which is both accurate and cheap is therefore important to provide reliable data during the consultation process or further discussion between the stakeholders.

A promising method for measurement of ground cover is based on photographs and previous studies shows good results on some vegetation types (Vanha-Majamaa et al. 2000). This photographical method is normally based on colour distribution and will not distinguish between different lichens with similar colours, but it well distinguishes between objects with clearly deviant colours. However, it is not unlikely that such a method also, apart from cover, could be developed to be used to estimate reindeer lichen biomass as well, and depending on overall accuracy needed and time consumption it may very well be a good alternative to traditional methods.

The overall objective of this study is to compare an improved photographical method to the PI method when estimating lichen cover and biomass in stands with different levels of reindeer lichen biomass. More specifically the objectives are to: (i) evaluate how well photographically estimated lichen cover correlates with actual lichen biomass, (ii) derive equations that estimates lichen biomass using photographically measured lichen cover with or without measurements of lichen height, and (iii) compare the cover and biomass estimates from the PI method and the photographical method.

Material and Methods

Experimental design and sample collection

In the autumn of 2011 a survey of the reindeer lichen was carried out in forest sites with varying lichen mat with respect to lichen height and lichen cover. In this study all the species of the genus *Cladonia* spp. were considered as reindeer lichen, including *Cladonia rangiferina, C. arbuscula, C. stellaris, C. cornuta, C. fimbriata, and C. crispata* (the latter three were sparse), as well as *Stereocaulon paschale.* The lichen mats inventoried were divided into two groups, Grazed (G) and Non-Grazed (N), based on lichen height, and into three cover-classes, High (H), Medium (M) and Low (L) based on the lichen coverage, giving 6 sub-groups: Non-grazed and High cover (NH); Non-grazed and Medium cover (NM); Non-grazed and Low cover (NL); Grazed and High cover (GH), Grazed and Medium cover (GM), and Grazed and Low cover (GL). Lichen cover above 80% was considered as high cover, 25 to 80% as medium cover, and below 25% as low cover. The sites selected for the lichen measurement and sample collection and the degree of lichen cover (visually estimated) were determined in consultation with Hans Winsa from SveaSkog and Lars-Evert Nutti from Sirges reindeer herding community. Both the non-grazed and grazed groups were chosen to represent a gradient from "pure" lichen-heath with high lichen cover (dry soil with small amounts of *Vaccinium* spp., *Empetrum nigrum* and *Calluna vulgaris*) to a moister (dry/mesic) *Vaccinium* spp. type with low lichen cover and a denser schrub layer (representative images of each sub-group are presented in Appendix 1). The sites were all located in Scots pine-stands (*Pinus sylvestris*) of different basal areas and ages. The non-grazed group was collected at different locations surrounding Kallax in Norrbotten County (65°31'N, E 22°7'E and 65°31'N, E 22°1'E) and the grazed ones were collected at locations north of Harads in Norrbotten County (66°14'N, 20° 55'E and 66°10'N, 20°54'E). The collection took place between September 28 and October 10 2011, and a light rain fell more or less continuously over the whole time span. Twenty sample plots (120 in total), were randomly placed along a transect within each site. On smaller sites, it was necessary to turn around and make a new transect parallel to the first one.

At each sample plot a wooden frame (0.50 x 0.50 m; 0.25m^2) with a grid of 25 (0.10 x 0.10 m; 0.01m^2) sub-frames, i.e. 16 intersections, and adjustable legs (set at 19 cm of height) was placed and directed northward (Figure 1). At each of the 16 intersections a metal needle (3 mm in diameter) was lowered with a perpendicular angle until it touched the bottom of the litter. When the lichen thalli were not alive all the way down to the litter, only the living part of the thalli was measured. When the needle hit any lichen, the height of the lichen was recorded at the point of contact. The absence of contact with lichen gave a zero value.

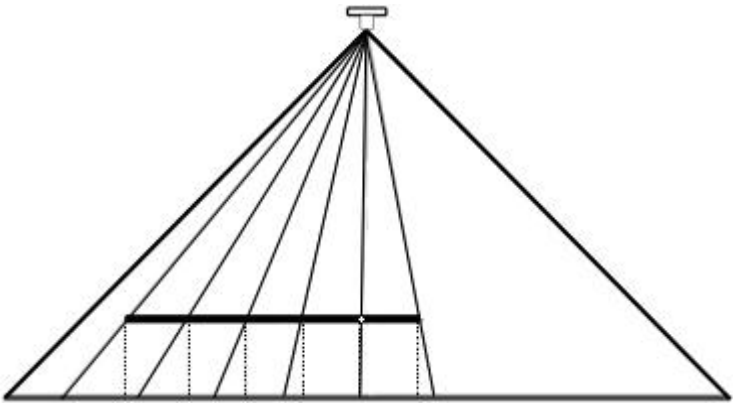


Figure 1. Schematic representation of the camera, the frame (upper thick line) and the ground plane (lower thick line). Captured frame in peripheral lines, central projection shown in thin lines , and orthographic projection in dashed lines. Projection center marked as white dot.

The frame was also used as a scale marker for the photographical part of the sampling. The ground below the frame was lightly cleared from large pieces of debris that could interfere with the image analysis later on. The debris included large mushroom, twigs and branches, bark, large amounts of pine cones, and especially branches with epiphytic lichens such as *Hypogymnia* spp. Excessive amounts of debris on several occasions made it necessary to move the sample plot. The camera (a 10 megapixel Canon EOS 40D Single Lens Reflex (SLR) with a Sigma 18-125/3,5-4,5 DC lens) was mounted on a tripod with a ball joint, adjusted into a perpendicular angle using a spirit-level, and directed in order to fit the frame. Focus was set at maximum focal length and the aperture was set to f8 to avoid focus errors and to gain a deeper depth of field. Furthermore, a remote trigger and mirror lockup was used to avoid any blurring due to camera movement. The pictures were taken at a focal length between 18 to 23 mm (which corresponds to about 61 to 74 degrees of diagonal field of view on the camera body used), and saved in the JPEG file format at the best quality. To ensure a correct, and above all, systematic exposure and white balance, a calibration card (Lastolite EzyBalance Calibration Card) was used to calibrate the camera prior to the exposure. The majority of the pictures were taken on overcast days but when the sun was visible, a *one stop* diffusing screen (Lastolite TriGrip Reflector) was used to erase sharp shadows. Two of the sub-frames (243 in total) were randomly selected as biomass samples at each sample plot. Their positions and the average lichen height (using the same procedure as above) were noted, and a small template (0.10 x 0.10 m) was used to mark the correct position on the ground. The lichen inside each sub-frame was extracted by pulling and/or cutting, and stored in marked plastic bags. Further, in each of the six sub-groups three to five samples of lichen were collected in order to have an estimate of the lichen moisture content. These moisture samples were also stored in plastic bags. During the time of the field work the lichen biomass samples were stored outside (away from sunlight) at a temperature of approximately 10°C.

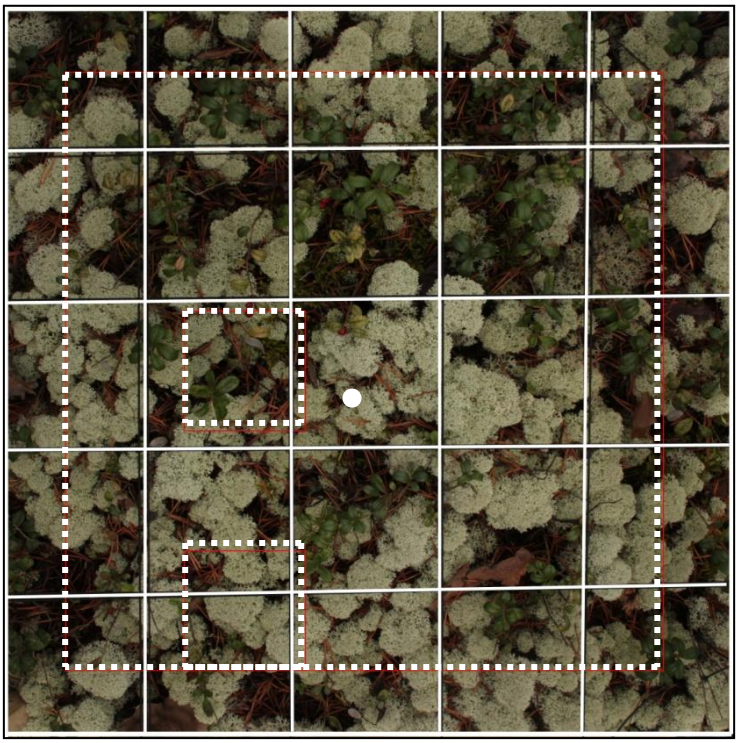


Figure 2. Solid lines indicate the central projection of the wooden frame and sub frames. Dashed lines indicate the orthographic projection of the frame and two of the sub frames. The dot indicates projection center.

Laboratory work

In the laboratory the moisture samples were weighed fresh (0.0001 g) in moist condition, and then dried in an oven at 80°C for 24 hours. The dried samples were then placed in a desiccator to cool for a couple of minutes, and then weighed again. The moisture content was calculated on the dry weight basis.

The biomass samples were thoroughly cleaned in fresh conditions, and the decomposed part was removed from the living part of the thalli on the basis of lichen discoloration with necrosis. The cleaned lichen were then dried, cooled and weighed using the same procedure as above. During the time of cleaning and weighing of the lichen biomass samples, the samples were stored in a refrigerated room at 4°C.

Image analysis

Since the wooden frame stood on legs and a camera uses central projection, the images acquired in the field had to be corrected due to a projection error (Figure 1 and Figure 2) that results in scale errors. There was also a positioning error due to the fact that the frame center and projection center did not coincide. By using the software Photoshop® (PS) (Adobe Systems 2007) the horizontal and vertical size of the frame and displacement of the projection center in relation to the frame center were measured in pixels, and then converted to cm. Using known factors (angular field of view, frame height and size, and picture size) the vertical distance between the camera and the frame was calculated via simple trigonometric functions. This distance was then used together with the projection center displacement to calculate the actual size of the ground plane. Each image was cropped in PS at the inside of the frame border, and scaled to its actual ground plane size. A digital grid of 25 frames (a representation of the wooden frame with sub-frames) was then placed on top of the cropped image and set to a scale of 0.50 x 0.50 m (to correct the scale error). Previous calculations also gave the digital grids position (i.e. the positioning error). The digital grid could then be orthographically (i.e. the true position and size of the frame) projected on the image below. The outside border and the two sub-frames that were randomly selected for each sample plot were then

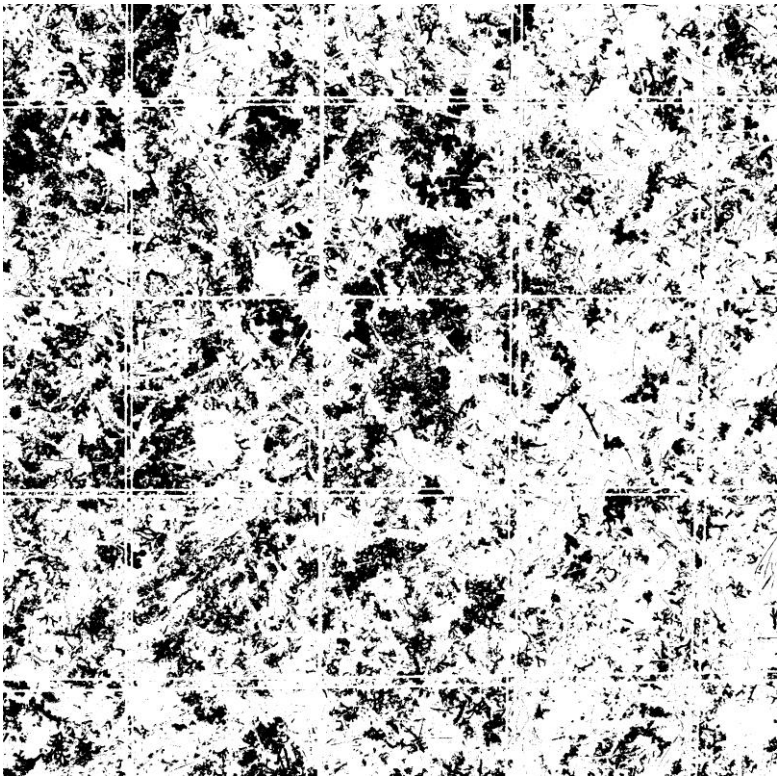


Figure 3. Lichen image sample divided into *lichen* (black) and *background* (white), using WinCAM™.

marked upon the image (Figure 2). The digital frame could then be removed, and the image was saved as JPEG at maximum quality. This correction procedure had to be done separately for each image.

The images were then to be analyzed with respect to lichen cover (Figure 3). This was done using WinCAM ™ (WC) (Regent Instruments 2007). A color calibration file was made for each group (or when necessary), and then the lichen areas- and background areas were measured (in percent), using the corrected images, in the whole frames as well as in the two randomly selected sub-frames.

Statistical analyses

Two different sets of regression analyses were done to analyze the data. The first *biomass regression* was done using lichen cover and lichen height of the 0.10 x 0.10 m *sub-frames* (values from WinCAM/WC) to explain lichen biomass (d.w.). Simple (*cover*) and multiple (*cover***height*) linear regression analysis were tested for all sub-groups combined, for all sub-groups separately, for both grazing groups (Grazed and Non-grazed), and for the three cover-classes (High, Medium, and Low). When necessary, combinations of logarithmic, squared and weighted transformation were used to transform the data prior to the analysis (Table 1).

Table 1. Final input parameters for the biomass regressions. Multiple regressions within parenthesis. (* failed to meet the requirements for normal distribution)

	Response	Predictors		Weighted
	y	x_1	x_2	
All	log weight (log weight)	log cover (log cover)	- (log height)	-
NH	weight	cover	-	-
NM	weight (log weight)	log cover (log cover)	- (log height)	- (1/weight)
NL	log weight	log cover	-	-
GH	log weight (log weight)	log cover (log cover)	- (log height)	-
GM	log weight	log cover	-	-
GL	log weight	log cover	-	-
G_x	log weight	log cover	-	-
N_x	*	*	*	*
H_x	log weight (log weight)	log cover (log cover)	- (log height)	-
M_x	log weight (log weight)	log cover (log cover)	- (log height)	-
L_x	log weight	log cover	-	-

The second set of regression analyses, the *coverage regression*, was done to compare the cover estimates by Point Intercept method (PI) and by image analysis with WC at the *whole frame* level. The regression analysis was also used to derive equations to translate between PI and WC results. The lichen cover from the PI was calculated by dividing the number of lichen hits with the total number (16) of intersections. WC cover was selected as response and PI cover as predictor. Squared, logarithmic and weighted transformations were used to transform the data when necessary (Table 2).

The statistical analyses were done using Minitab 16 (Minitab Inc. 2010)

Significance level was chosen as $p \leq 0.05$.

Sub-group	Response *y*	Predictor *x*	Weighted
NH	PI cover	WC cover^2	-
NM	PI cover	WC cover	1/PI cover
NL	PI cover	log WC cover	-
GH	PI cover	log WC cover	1/PI cover
GM	PI cover	log WC cover	-
GL	PI cover	log WC cover	-

Table 2. Final input parameters for the linear cover regressions.

Results

Biomass, coverage and moisture content of lichens

Mean lichen biomass for the sub-groups varied between 0.39 and 3.16 g per 0.01m^2 (390 and 3160 kg per hectare), with a distinct gradient from NH to GL. Mean lichen cover ranged between 5.5 and 50 %, and mean height ranged from about 20 to 57 mm. The moisture content of the lichen samples ranged between 86 to 214 % of lichen dry weight, the moisture content average for all sub-groups was 169.3 %. NL, GM and GL had the highest moisture contents averaging between about 212 and 214 % of lichen dry weight, and NM the lowest at 86 %. GH and GM had the largest internal variations of moisture content with 116 to 249.1 % and 145 to 257.4 % respectively. NM and GL had the lowest internal moisture contents with 47.1 to 116.9 % and 187.1 to 228.4 % respectively (Table 3).

Table 3. Mean weight, mean cover, mean height and mean moisture content for reindeer lichen of each sub-group. ±SE values within parenthesis.

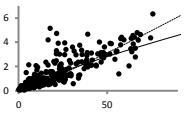
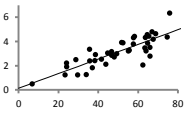
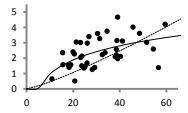
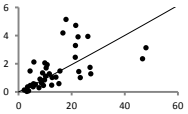
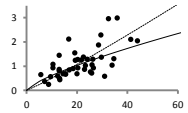
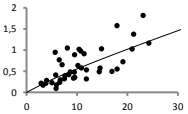
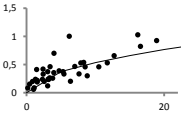
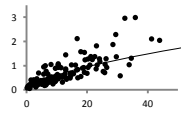
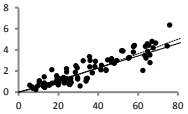
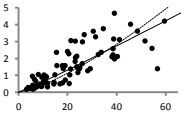
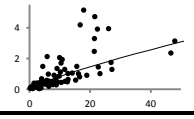
Sub-group	Mean weight (g)	Mean cover (%)	Mean height (mm)	Mean moisture content (%)	Min. moisture content (%)	Max. moisture content (%)
NH	3.16 (0.19)	50 (2.6)	56.5 (1.8)	135.6 (21.4)	81.1	175.9
NM	2.39 (0.15)	31.3 (1.9)	38.0 (2.1)	86.0 (11.3)	47.1	116.9
NL	1.57 (0.21)	14.3 (1.7)	47.0 (1.2)	214.0 (17.0)	189.6	246.6
GH	1.15 (0.09)	21.2 (1.4)	20.1 (1.5)	191.7 (27.5)	116.0	249.1
GM	0.66 (0.06)	11.01 (0.9)	33.0 (2.0)	212.4 (27.5)	145.0	257.4
GL	0.39 (0.04)	5.5 (0.8)	50.4 (1.8)	213.3 (13.1)	187.1	228.4
Total	1.60 (0.08)	22.4 (1.2)	40.6 (1.1)	169.3 (13.2)	47.1	257.4

Biomass regression

The regression analysis for groups, sub-groups and cover-classes gave adjusted R^2 values between 44.4% and 81.2%, were NM had the lowest value and H the highest. The regression equation lines showed good fits for sub-group NH, coverage-classes H and M, and group G (Table 4 and Figure 4). The residuals for all regressions were equally distributed around zero.

Using height as an additional predictor gave significant effects ($p \leq 0.05$) in five cases, and in those cases the differences in R^2 (adj) value were only about 2 to 4 percentage units compared to simple regression without height. The value for NM, on the other hand, rose from 26.8% to 44.4%. The differences towards a lower significance level ($p \leq 0.10$) were slight. Only one relationship (NH) was strictly linear, and a couple of them were close to linear. All regressions had p-values <0.005 (Table 4). Estimated weights (calculated from the regression equations with the highest R^2 (adj) values in Table 4) showed varying results. Overall, accuracy tended to decline with increasing weights (i.e. increasing lichen cover), which was most apparent in low cover-classes. NH and H, however, showed the most promising results in those aspects, with no such tendencies regardless the extent of lichen cover (Figure 4).

Table 4. Adjusted R^2 values and derived equations from the final regression models. Plots with regression equation, dry weight of reindeer lichen (original data, g 0.01m^{-2}) on Y axis, and lichen cover (original data, % 0.01m^{-2}) on X axis. Multiple regressions within parenthesis.

	N	*p*	StDev	R^2 (adj)	Simple regression equation Weight (W) Cover (C)	Multiple regression equation Weight (W) Cover (C) Height (H)	Plot Simple regression (solid) Multiple regression (dashed)
All	242 (237)	<0005 (<0.005)	0.221 (0.210)	73.5% (76.1%)	$W = 10^{-0.956} * C^{0.827}$	$W = 10^{-1.44} * C^{0.827} * H^{0.311}$	
NH	40	<0.005	0.665	68.5%	W = 0.139 + 0.0604 C	-	
NM	40 (40)	<0.005 (<0.005)	0.835 (0.135)	26.8% (44.4%)	W = -2.08 + 3.05 * logC	$W = 10^{-1.55} * C^{0.657} * H^{0.555}$	
NL	40	<0.005	0.300	54.7%	$W = 10^{-1.08} * C^{1.05}$	-	
GH	43 (43)	<0.005 (<0.005)	0.177 (0.167)	42.6% (48.5%)	$W = 10^{-0.963} * C^{0.750}$	$W = 10^{-1.39} * C^{0.818} * H^{0.274}$	
GM	40	<0.005	0.206	49.7%	$W = 10^{-1.14} * C^{0.882}$	-	
GL	39	<0.005	0.181	60.2%	$W = 10^{-0.782} * C^{0.512}$	-	
G_x	120	<0.005	0.187	68.1%	$W = 10^{-0.856} * C^{0.642}$	-	
H_x	83 (83)	<0.005 (<0.005)	0.154 (0.138)	76.9% (81.2%)	$W = 10^{-1.26} * C^{1.01}$	$W = 10^{-1.43} * C^{0.855} * H^{0,264}$	
M_x	78 (78)	<0.005 (<0.005)	0.196 (0.189)	73,5% (75.3%)	$W = 10^{-1.26} * C^{1.06}$	$W = 10^{-1.70} * C^{1.02} * H^{0.325}$	
L_x	79	<0.005	0.275	63.5%	$W = 10^{-0.885} * C^{0.808}$	-	

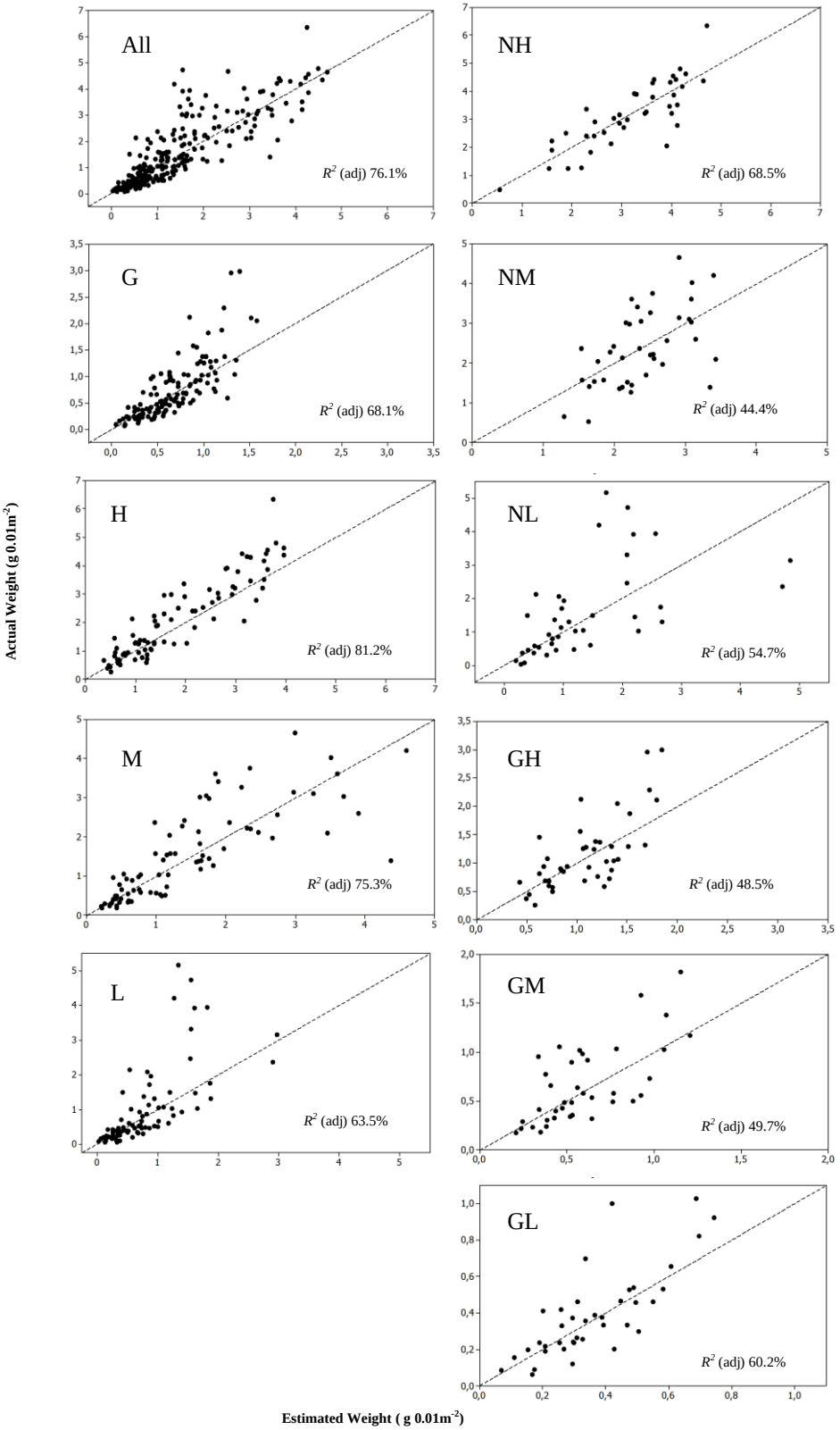


Figure 4. Scattered plots of groups, sub groups and cover classes, showing actual dry weight (g 0.01m^{-2}) of reindeer lichen versus estimated dry weight (g 0.01m^{-2}). Estimated weights were calculated using respective regression equation with the highest R^2 (adj) value in Table 4.

Cover regression

Compared to PI, WC underestimated lichen cover. Since cover estimated by WC were consistently lower than the cover estimates by PI there was a tendency to a systematic difference (Figure 5). The mean lichen covers measured with PI and WC were 58 and 20% respectively. Largest differences were found in GH and GM were PI averaged 79 and 68%, and WC 20 and 11% respectively. The lowest differences between the two methods were found in NL were PI averaged 17% and WC 6%.

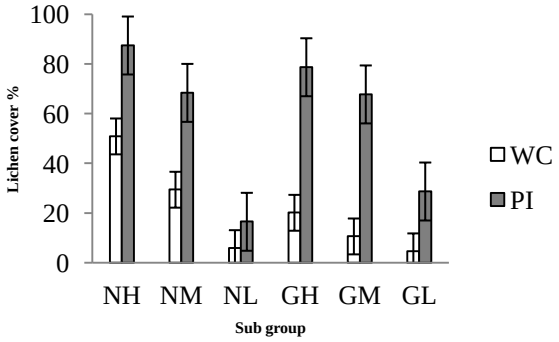


Figure 5. Mean lichen cover (%) and ±SE values for WinCAM™ (WC) and Point Intercept method (PI) for the different sub groups.

Regressions of WC versus PI gave variying results. R^2 (adj) values had a quite wide range between 25.4 and 81.1%. The low cover-classes (NL and GL) seemed to have the best fits with R^2 (adj) values of 62.4 and 81.1% respectively. Remaining sub-groups had R^2 (adj) values ranging between 25.4 to 38.6%. In both NL and GM one observation was deleted (marked as white dots), as they both had to much leverage on remaning observations. (Figure 6).

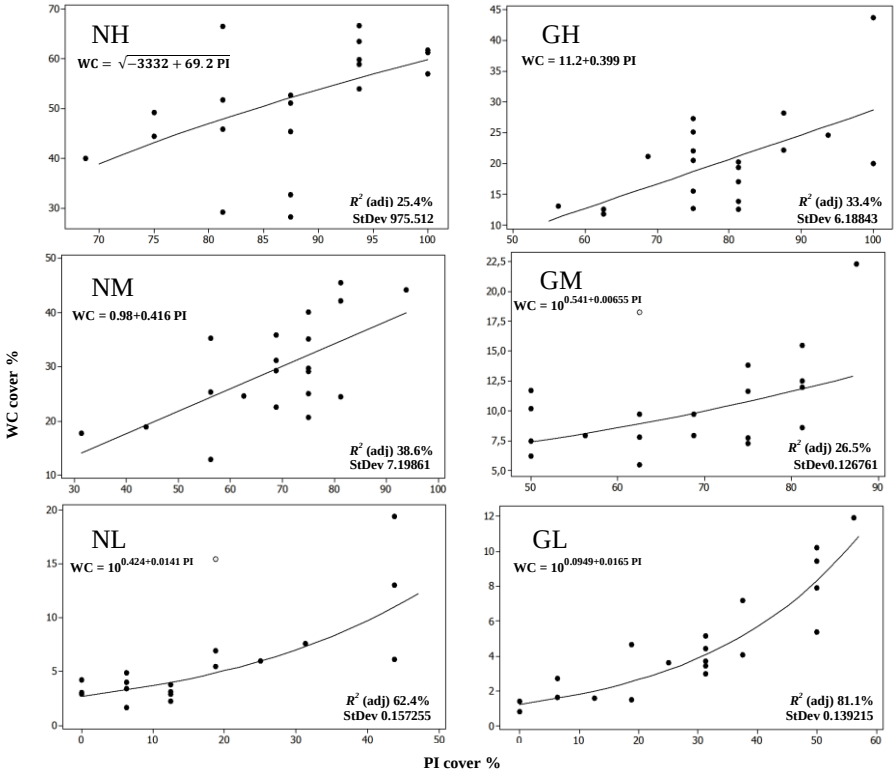


Figure 6. Lichen cover estimation by WinCAM (WC) against estimation by Point Intercept method (PI) scattered plots per sub-group, with regression (Table 2) equations lines, standard deviation, adjusted R^2, and regression equation. Whole frame data. Removed observations marked as hollow dots.

Discussion

Overall discussion

The regressions for NH, H, M, and G showed the best fits. The other regressions showed lower fits with R^2 (adj) values, still above 40%.

The estimations of biomass showed similar variation in the Grazed (G) groups, i.e. GH, GM and GL, and a slight underestimation at higher degrees of lichen cover. The variation in the Non-grazed group (N) was higher, with a good prediction in NH and a poorer prediction in NL. The same tendency can also be seen between the different cover-classes (H, M and L). It seems that prediction of reindeer lichen biomass on different sites with different cover and vegetation is difficult, and the use of different estimating equations is recommended rather than the use of one overall equation. Still, the prediction for the groups, cover-classes and sub-groups with higher lichen cover seems to be more reliable than for those with lower lichen cover (Figure 4). The residuals for all biomass regressions above were equally distributed around zero, suggesting that the equations should give good estimations if a sufficient number of samples are collected.

Using height as an extra predictor only gave small improvements on R^2 (adj) values. The R^2 (adj) value for NM, however, rose significantly when using height (Table 4). The NM sub-group was sampled when the sun was barely visible just above the tree tops which gave the pictures a light-gradient. Although no major problems were encountered during image analysis, there is still a probability that this gradient led to varying precision in cover estimates. Another possible explanation is the fact that this sub-group had by far the lowest moisture content and the least internal variation of moisture content (Table 3). When sampling the NM sub-group it did not rain at all and it had been less previous rain compared to when the other sub-groups were sampled. This was probably the cause of the low moisture content of this sub-group. Pech (1989) claims that reindeer lichen probably is the fastest drying surface material in Canada (actually: surface fuel), which should be an applicable statement in Sweden as well. Furthermore, a study by Van Wagner (1969) showed that reindeer lichen can have moisture contents as high as 400% (when soaked in water), and lose most of its free moisture within no more than seven hours. If the low moisture content in NM was the only cause of this difference (between simple and multiple regression) one could make the assumption that lichen moisture content is an essential factor to control when using this inventory method, and especially if using the estimation equations presented in this paper.

Moisture content in the sub-groups (Table 3) varied between 86 to 214% of lichen dry weight, which is in accordance with previous studies. According to Heatwole (1966), for several *Cladonia* species even at 100% relative humidity, the moisture content of lichen does not exceed 70%. Given the weather conditions during the sampling in this study, a moisture content of 86% for NM and 135.6 to 214% for the others seems to be reliable figures.

In a study by Moen et.al (2007) results derived from the PI method (adjusted to volume) gave R^2 values between 88 and 95% when regressed against lichen dry weight, and similar results can be seen in a study by Jonasson (1988), where number of intercepts were regressed against biomass of, e.g., leaves and stems of blueberry, and cowberry. Results (R^2 (adj) values) from

these studies are far better than in this study, but Jonasson (1988) does point out that the analysis of $1m^2$ sample frame with 200 intersections averaged about 90 minutes. The methodology in this study may have a lower precision, but requires considerably less time in comparison. In this study, to photograph one sample took about 1 minute. To that the time for image analysis should be added: Initial creation of the colour calibration files needed for the image analysis of the first sub-group took about 30 minutes. Creation of the colour calibration files for the following sub-groups demanded less time since some of the calibration files could be re-used or just slightly adjusted. Although most of the six sub-groups needed "their own" calibration files, and the total time for creating the calibration files were hence about 2 hours. The image analysis itself averaged about 30 seconds per image.

One big advantage of this photographical method is that new inventories (batches of images) can be processed very fast, given that a suitable colour calibration file is already available in a ready- or near-ready form. Another key advantage is that the method should be insensitive to different observers (photographers) (Vanha-Majamaa et al 2000; Meese and Tomich 1992). The arguments above do require a proper, and consistent, camera calibration during sampling (see below Notes on the Methodology). Learning of the photographical methodology, as well as the methodology for the image analysis and creation of the colour calibration files, should also be fast.

When studying the results from the *cover regression* (Figure 5) one could immediately point out that WC clearly underestimated the cover compared to the PI method. However, the question is which method gives the most reliable results? Based on the author's personal observations WC could underestimate the cover to some extent due to the fact that the edges of the lichen on the picture often becomes shadowed or discolored, which causes the image analysis software (WC) to discard these pixles. Adjusting the calibration file to deal with these extreme borders resulted in that too many non-lichen pixels were counted as lichen. This problem occurred most frequently in the GH and GM sub-groups which were heavily grazed and mechanically damaged. However, there were always some other objects in the picture that were counted as lichen by mistake, which could make up for some of the underestimation. The PI method on the other hand, could very well be overestimating the degree of cover. Warren Wilson (1963) points out that cover inventory methods that use some sort of needle to count object contacts are theoretically correct, but are likely to overestimate cover in the real world. This is due to the fact that the needle is supposed to act as a theoretical *point* with no area of contact. But in a real situation the needle has a non-negligible area. He points out that the larger the diameter of the needle is and/or the smaller the objects to measure are, the more the overestimation of the cover will be. When studying the sample images (see Appendix 1) one can easily see that even the most dense lichen thalli has a large number of small internal "gaps", and taken into consideration that the metal needle used in this study had a diameter of three millimeter it is reasonable to assume that overestimation most likely occur. This could very well be the reason why the regressions of WC versus PI on the low-cover sub-groups (NL and GL) showed much better fit than the others, since the amount of lichen, and hence internal gaps, only constitutes a much lower portion of the entire sample frame. Overestimation of PI towards WC is supported in Vanha-Majamaa et al. (2000). In their study PI cover was compared to the cover derived from manually delineated raster images, and showed an

overestimation in comparison. The same study also compared automatic image analysis via sophisticated algorithms, which gave an underestimation of cover towards the manually delineated images.

Methodological concern

As reported in Material and Methods, the photographical sampling led to scale errors in the images. The correction procedure of this problem was theoretically sound, but it did assume that: (i) the camera, the frame and the ground were all perfectly parallel to each other and (ii) there was no internal topography within the frame. As done in this study the sampling frame and the ground was parallel to each other, but the camera was leveled with a spirit level which resulted in that some images still has a small varying scale error which affects the cover estimates of WC. As for the matter of internal topography (which results in higher cover in high parts and lower cover in low parts) this cannot be easily corrected. However this matter is probably negligible, since the majority of the sample plots had a very low internal topography. When using wide angle lenses a certain amount of distortion will occur in the pictures, which often tend to get worse towards the borders. All of the pictures in this trial had a small amount of distortion towards the image borders, but during the correction procedure most of it was cropped away. Hence, distortion is probably to be considered as a negligible problem in this study. When photographically sampling the sub-group NM the sun stood just above the tree tops, and created a light gradient on the pictures. Although no major problems arose during the image analysis, there is still a risk that this phenomenon led to variation in cover estimate precision of that sub-group. In a similar fashion, there was frost in the vegetation at the site where the sub-group GM was sampled. The colour of the frost is similar to the colour of reindeer lichen, which affected the image cover analysis somewhat.

The extraction of the biomass samples from the ground proved to be difficult in some cases. When the borders of the small 0.10 x 0.10 m template (see Material and Methods) crossed the exact center of a lichen thalli, the correct way to extract the sample would have been to split the lichen thalli and discard the half outside the frame. Since this is impossible to do in practice these lichen thalli were often extracted whole, and hence some of the biomass samples contain lichen from an area a little bit bigger than $0.01 m^2$ which results in a slight overestimation of lichen per unit of area. When cleaning the lichen biomass samples the decomposed parts were removed on the basis on discoloration with necrosis (see Material and Methods). The cleaning of the lichen biomass samples took several weeks and hence it is possible that decay of the lichen could have affected the separation point. However, sub-groups were cleaned one at a time which means that the decay should not have a systematic effect on variation inside the sub-groups. The exceptions from this argument could be the GH and GM sub-groups which were particularly time consuming, and taking a couple of days to clean each one. Another, more severe, issue when cleaning the two sub-groups above is the fact that these sub-groups were made up of a high number of small lichen thalli which grew on sandy sites with mechanical damage from both reindeer and machines. The biomass samples extracted from these sites therefore had some sand mixed into them, which was hard to clean away and may have lead to slight overestimations. Since all of the sample plots in these groups were homogeneous it is likely that this error affected each sample to the same degree, and did not affect the internal variation.

Problems during the image analysis consisted of creating a "perfect" colour calibration file. This problem was caused by the fact that extreme borders of lichen thalli often is shaded and/or discolored. A color calibration file that is calibrated towards the colours of "healthy" lichen will neglect these areas (pixels) that have a deviant colour. Adjusting the color calibration file to deal with this will only result in that other, non-lichen, pixels will be counted as lichen. Hence, most of the cover estimates by image analysis are assumed to be slightly underestimated (even though some non-lichen areas always were counted as lichen as mentioned earlier). Lawrence et al. (1996) suggests that different smoothing techniques should be applied to deal with groups of deviant pixels when estimating cover via image analysis. However, their study analyzed larger and more homogenous areas than the lichen analyzed here, and it is doubtful that smoothing techniques would work satisfactory when analyzing reindeer lichen which has a large proportion of "edges".

Suggestions for further use of image analysis

If using this inventory method, *consistency* is the key word. Consistency when collecting data (photographs) will ease the amount of work later on, and of course also improve the accuracy. In this work a tripod was used, but it was used at different heights for each picture which led to an unnecessary correcting procedure during the image analysis. A customized tripod with a centered camera mount, a constant height above ground, and a permanently mounted frame (with a suitable area) beneath will keep the camera, the frame and the ground perfectly parallel to each other, which would favor consistency during the photographical part. The frame can be placed at any height above ground as long as it is kept at this height. Placing the frame above ground level will require scale correction to calculate the real (orthographically projected) frame area, but if the set up above is used the correction will be the same for all images.

Care should also be taken to place the whole *frame-tripod-carriage* parallel to the ground plane and on a spot where the internal topography is low, to avoid different scales in different parts of the picture. If the set-up described above is used to sample in a steep slope, it will lean and risk tipping over. One solution to this is to adjust the tripod legs but this will interfere with the camera, the frame and the ground being parallel to each other, resulting in unnecessary correction procedures later on. The best solution in such conditions would be to prevent the set-up from tipping over, for instance via extra legs. Short focal lengths (as ≈ 20 mm, or ≈ 67 degrees, used in this trial) will create more distortion than longer ones. On the other hand a longer focal length requires a longer distance between the camera and the ground or a smaller frame, which can be tedious. Care should also be taken when setting aperture. If using a lens with a longer focal length and a larger aperture the depth of field could turn out to be insufficient if sampling higher vegetation such as non-grazed lichen, resulting in blurring of the upper and/or bottom parts of the lichen. The exposure parameters and white balance should be calibrated prior to each exposure, and the exposure must be done immediately after calibration to avoid changes in light in-between. Doing so will create pictures that are consistent with respect to colour information, which results in that only a few colour calibration files will be needed. When calibrating the camera the use of a proper calibration card is highly recommended since this will make the colour data in the images consistent. Consistent colour data will make the colour calibration files working with images (with no or few adjustments)

from different inventories, reducing both costs and time needed. If using the biomass estimation equations calculated here, care should be taken to do the sampling on lichen mats with similar moisture content. The moisture content of the lichen could be measured by drying and weighing of lichen samples collected, or it could be measured with some sort of portable device. Near Infra Red (NIR) is a technique that is widely used to measure moisture content in different substrates, and accuracy on, for instance, peat is about 4 percentage points (O'Mahony 1998)

There is no need to use a SLR (as used here). Any compact camera with tripod mount and with the possibility to manually set the exposure and white balance would meet the requirements. A compact camera also has an apparently longer depth of field, which is preferable. To erase sharp shadows and decrease harsh contrasts some sort of diffusing screen is recommended. In this trial a *one stop* diffusing screen was used, which worked fine when the sun stood high on the sky. When dusk approached and the sun stood just above the tree line, the diffusing screen did not work satisfactory. It did erase the shadows, but also created a light-gradient which interfered somewhat with the image analysis. A stronger diffusing screen or just a solid sheet to block the light would probably have worked better. If using a customized tripod set-up as above, the amount of time needed just to take one picture (with camera calibration) should not exceed 20 seconds after a couple of hours training. As for the image analysis the initial creation of colour calibrations files is the main time consuming part, but once a number of calibration files have been made a large number of images can be processed in a short time. These calibration files can of course be re-used in other inventories (if care is taken to calibrate the camera as above) and multiplied with small changes, which would make the time consumption lessen considerably. The software (WinCAM™) did, unfortunately, not present the opportunity to manually adjust orphan- and/or groups of pixels that were neglected or wrongly counted as lichen. But this is most likely a feature that is (or will be) present in newer versions or in other similar software. Such a feature would present the opportunity to, in a smooth way, correct portions of the image without the need to adjust the whole calibration file.

Conclusion

The photographical inventory method further developed and evaluated here was originally intended to result in an easy, accurate, and fast inventory method which could be used by both reindeer herder's and forest managers prior to consultations. The photographical inventory method seems to deliver moderate to good predictions of lichen biomass. Major advantages compared to the Point Intercept method are low time consumption (especially in the field), small or negligible differences between different observers and inventories and a low difficulty level and hence a short training of the observer. Note that the biomass estimation equations and equations for translation between WC and PI in this study were based upon lichen cover estimation at a certain interval of lichen moisture content, and may not work satisfactory at other levels of moisture content. Furthermore, since the moisture content of the lichen may be an important factor, further studies is recommended to increase the practical use of this method.

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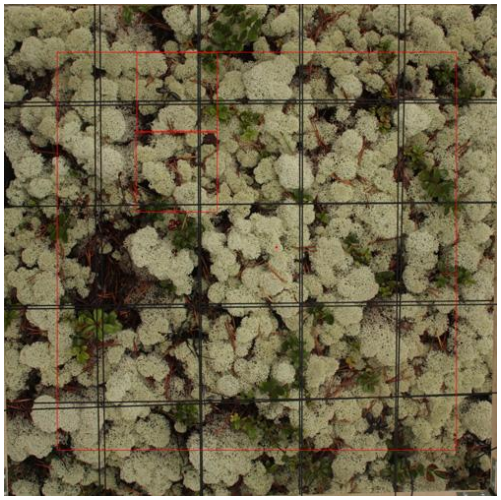
Appendix 1

Representative pictures of each sub-group.

Sub-group HH



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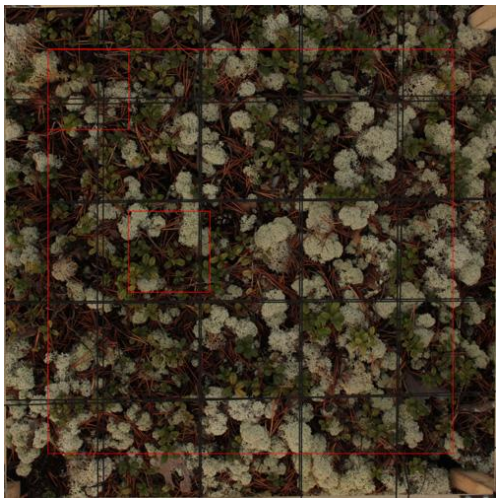

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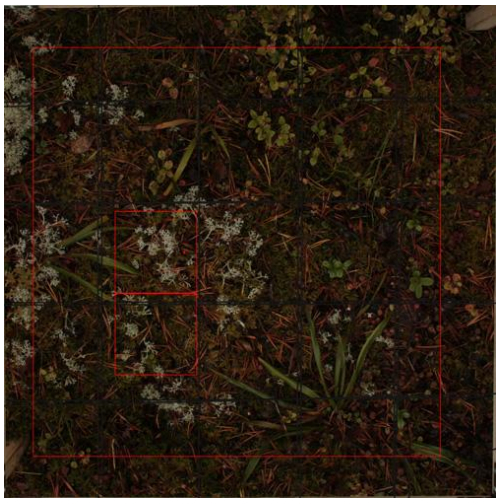
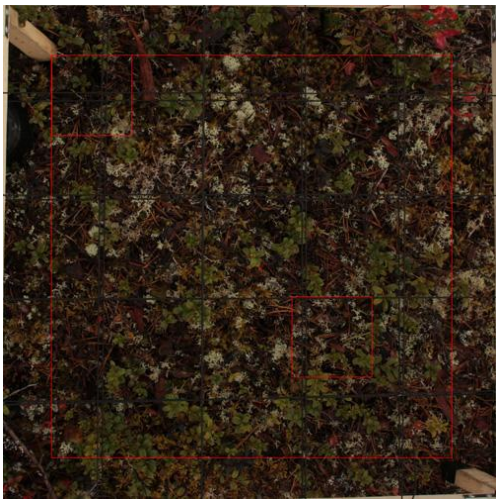


Sub-group LH

Sub-group LM



Sub-group LL

SENASTE UTGIVNA NUMMER

2011:5 Författare: Josefin Lundberg
Var finns rehabiliteringsskogen? Hur preferens och upplevelse av skogsmiljö kan användas för att återfinna rehabiliteringsskogen på landskapsnivå

2011:6 Författare: Fredrik Hedlund
Dimensionsavverkningens inverkan på natur och kulturvärden i fjällnära naturskog – en jämförelse av två områden inom Harrejaur naturreservat i Norrbotten

2011:7 Författare: Linda Nilsson
Skogar med höga sociala värden inom Sundsvalls kommun – olika intressenters attityd till den tätortsnära skogen och dess skötsel

2011:8 Författare: Charlotte Nauclér
Kan urskog vara kulturlandskap? – En tvärvetenskaplig studie av kulturspår och naturvärden i Eggelatsområdet

2011:9 Författare: Anton Larsson
Val av markbehandlingsmetod inom Sveaskogs innehav i norra Sverige

2011:10 Författare: Hanna Lundin
Lika oriktigt, som det är att ensidigt hålla på blädning lika förnuftsvidrigt är det att endast vilja förorda trakthuggning" – Tidiga kalhyggen i Norrland

2011:11 Författare: Ida Karlsson
Brunnsröjning med kedjeröjsåg – effekter på kvarvarande bestånd

2011:12 Författare: Elsa Järvholm
Högskärmar och kalhyggesfritt skogsbruk på bördig mark i Medelpad

2011:13 Författare: Susanne Wiik
Kalkbarrskogar i Jämtland – vad karakteriserar de områden där kalkberoende mykorrhiza-svampar förekommer?

2011:14 Författare: Andreas Nilsson
Krymper barrmassaved vid lagring? – En fallstudie i SCA:s Tövasystem

2011:15 Författare: Steve Fahlgren
Kärnvedsbildning i tall (*Pinus sylvestris* L.) – Startålder samt årlig tillväxt i Västerbotten

2011:16 Författare: Kerstin Frid
Kan hamlingen fortleva som tradition? – en studie över hamlingens historia och framtid i Bråbygden med omnejd

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2012:1 Författare: Liisa Sars
Röjningsformens effekt på den yttre kvalitén hos björkstammar när beståndet närmar sig första gallring

2012:2 Författare: Daniel Timblad
Kvalitet och skador i tallungskog efter röjning vid olika stubbhöjder

2012:3 Författare: Aron Sandling
Epiphytic lichen flora in a boreal forest chronosequence

2012:4 Författare: Elsa Bengtsson
Leaf area index in *Vittelaria Paradoxa* parklands in Burkina Faso estimated by light interception and leaf sampling

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