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Measurement methods and variability assessment of the Norway spruce total leaf area: implications for remote sensing

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Abstract

Estimation of total leaf area (LAT) is important to express biochemical properties in plant ecology and remote sensing studies. A measurement of LAT is easy in broadleaf species, but it remains challenging in coniferous canopies. We proposed a new geometrical model to estimate Norway spruce LAT and compared its accuracy with other five published methods. Further, we assessed variability of the total to projected leaf area conversion factor (CF) within a crown and examined its implications for remotely sensed estimates of leaf chlorophyll content (Cab). We measured morphological and biochemical properties of three most recent needle age classes in three vertical canopy layers of a 30 and 100-year-old spruce stands. Newly introduced geometrical model and the parallelepiped model predicted spruce LAT with an error >5 % of the average needle LAT, whereas two models based on an elliptic approximation of a needle shape underestimated LAT by up to 60 %. The total to projected leaf area conversion factor varied from 2. 5 for shaded to 3. 9 for sun exposed needles and remained invariant with needle age class and forest stand age. Erroneous estimation of an average crown CF by 0. 2 introduced an error of 2-3 µg cm-2 into the crown averaged Cab content. In our study, this error represents 10-15 % of observed crown averaged Cab range (33-53 µg cm-2). Our results demonstrate the importance of accurate LAT estimates for validation of remotely sensed estimates of Cab content in Norway spruce canopies.

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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Measurement methods and variability assessment of the Norway spruce total leaf area: Implications for remote sensing

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Abstract

Estimation of total leaf area (LA_T) is important to express biochemical properties in plant ecology and remote sensing studies. A measurement of LA_T is easy in broadleaf species, but it remains challenging in coniferous canopies. We proposed a new geometrical model to estimate Norway spruce LA_T and compared its accuracy with other five published methods. Further, we assessed variability of the total to projected leaf area conversion factor (CF) within a crown and examined its implications for remotely sensed estimates of leaf chlorophyll content (C_{ab}). We measured morphological and biochemical properties of three most recent needle age classes in three vertical canopy layers of a 30-year-old and a 100-year-old spruce stands. Our newly introduced geometrical model and the previously published parallelepiped model predicted spruce LA_T with an error less than 5% of the average needle LA_T, whereas two other models based on an elliptic approximation of a needle shape underestimated LA_T by up to 60%. The total to projected leaf area conversion factor varied from 2.5 for shaded to 3.9 for sun exposed needles and remained invariant with needle age class and forest stand age. Erroneous estimation of an average crown CF by 0.2 introduced an error of $2 - 3$ μg cm^{-2} into the crown averaged C_{ab} content. In our study, this error represents 10 - 15% of observed crown averaged C_{ab} range ($33 - 53$ μg cm^{-2}). Our results demonstrate the importance of accurate LA_T estimates for validation of remotely sensed estimates of C_{ab} content in Norway spruce canopies.

Introduction

Measurements and monitoring of forest structural and biochemical properties (e.g. leaf area index, leaf foliar pigment, nitrogen or water content) and physiological processes (e.g. gas exchange, photosynthesis) are important for the understanding of forest carbon sequestration (Luyssaert et al. 2007). Field measurements of forest properties and processes are usually spatially and timely limited, labor demanding particularly in complex canopies such as mixed boreal or tropical forests. Thus, field measurements become impractical for large-scale applications. Emerging remote sensing (RS) imaging spectroscopy (often referred to as hyperspectral RS) has a great potential for regular monitoring of forest properties and processes at larger spatial scales (Kokaly et al. 2009; Rautiainen et al. 2010; Ustin et al. 2004). Currently, imaging spectroscopy data have been used to estimate leaf and canopy biochemical properties such as chlorophyll (Zarco-Tejada et al. 2004; Malenovský et al. 2006; Moorthy et al. 2008), nitrogen (Huber et al. 2008; Schlerf et al. 2010) and water content (Koetz et al. 2004; Clevers et al. 2010), canopy structural properties such as leaf area index (Fernandes et al. 2004), and eco-physiological processes such as mapping of net primary productivity (Ollinger and Smith 2005). Successful calibration and validation of the RS methods, however, depend on accurate and reliable field measurements of canopy biochemical properties that are often expressed at leaf area basis. While estimation of leaf area of broadleaf species is straightforward, it is a challenging task for non-flat coniferous needles. In case of conifers, total leaf area (LA_T) or hemisurface leaf area ($LA_H = \frac{1}{2} LA_T$) seems to be a more appropriate expression for gas exchange or photosynthesis related studies than projected leaf area (LA_P), as stomata are located all around the needle surface (Smith et al. 1991).

71   The LA_T for broadleaf species is computed as two times LA_P, which can easily be

72   measured by planimeters, desktop scanners, or leaf area meters (Beerling and Fry 1990).

73   These techniques can be used to measure LA_P of coniferous species, but they are not suitable

74   for LA_T (or LA_H) measurements due to the three-dimensional shape of needles. Needle LA_T

75   have been determined by a volume displacement method (Johnson 1984) or from absorbance

76   measurements of entire shoots (Serrano et al. 1997), but both methods are used rarely. More

77   frequently, needle LA_T is computed from an approximation of the needle shape by a simple

78   geometrical primitive (Sellin 2000; Niinemets and Kull 1995). Pine needles can be

79   represented as half-cylinders or half-ellipsoids (Svenson and Davies 1992), spruce needles are

80   usually modeled as parallelepipeds or ellipsoids (Sellin 2000). Dimensions of geometrical

81   primitives are based on directly measurable morphometric variables, such as needle length

82   and diameter. For example, Perterer and Körner (1990) proposed a complex model based on 9

83   different measures for spruce and 12 for pine needles, which significantly limits its practical

84   use in forest research. Nevertheless, a detailed and accurate needle geometrical model is

85   fundamental for developing simpler, feasible, but still reliable, methods to estimate LA_T of

86   coniferous species.

87   Once LA_T is accurately determined, a conversion factor (CF) between LA_T and LA_P

88   can be derived and used to estimate LA_T from easily measureable LA_P. Conversion factor is

89   species-specific, but it also varies within a canopy of the same species due to changing

90   irradiance inside a canopy (Niinemets and Kull 1995; Sellin 2000). The characteristic

91   organization of branches and shoots in coniferous canopies produces a heterogeneous

92   radiation regime in a canopy vertical profile (Špunda et al. 1998; Chmura and Tjoelker 2008;

93   Waring 1983). For example lower parts of a young spruce canopy can receive only about 10%

94   of irradiance when compared to the top of the canopy (Kalina et al. 2001). Light availability

95   modulates leaf morphological properties (Hallik et al. 2009; Bond et al. 1999; Niinemets

96   2007); the shaded needles are usually flatter compared to sun exposed needles with more

97 circular or rhomboidal cross-section (Cescatti and Zorer 2003). Usability of CF for LA_T

98 estimation is therefore conditioned by understanding its variability between and within

99 individual tree crowns.

100 Taking the advantage of high resolution digital photography and computer image

101 processing techniques, the first objective of this study was to propose an accurate geometrical

102 model to estimate LA_T of Norway spruce needles and compare it with five previously

103 published LA_T estimating methods. The second objective was to investigate variability of the

104 total to projected leaf area conversion factor (CF) taking into account three sources of

105 variability: i) needle position within a crown vertical profile, ii) needle age, and iii) canopy

106 structure due to different forest stand age. Finally, the third objective was to quantify the

107 influence of biased LA_T measurements on the estimation accuracy of crown averaged

108 biochemical properties, which are being used for calibration and validation of remote sensing

109 derived products.

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111

112 **Material and methods**

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114 **Study area and needle sampling**

115 Morphological and biochemical properties of Norway spruce needles were analysed for

116 needle samples collected at the Bílý Kříž experimental research site (Moravian-Silesian

117 Beskydy Mts. at the eastern part of the Czech Republic bordering with Slovakia; 18.53°E,

118 49.50°N, mean altitude of 880 m a.s.l.). The microclimatological conditions of the site are

119 described in Urban et al. (2007).

120 Two montane Norway spruce (*Picea abies* (L.) Karst) forest stands of different age

121 and structure were selected for this experiment: a 30-year-old regular plantation (further

122 referred to as the "immature" stand), and an about 100-year-old stand (further referred to as

123 the "mature" stand), both growing on a moderate slope (13°) with S-SE orientation. In 2006,

124 the average tree height was 12.5 m in the immature and 40 m in the mature stand, the average

125 diameter at breast height was 14 cm and 53 cm, respectively, and the canopy density was

126 about 1400 trees ha^{-1} (the stand area ~ 7.5 ha) and 160 trees ha^{-1} (the stand area ~ 2.5 ha),

127 respectively.

128 Ten immature and twenty mature representative trees were selected for the needle

129 sampling. Double amount of mature trees was chosen under expectation of higher variability

130 in needle morphological and biochemical properties due to a larger structural heterogeneity of

131 the older mature stand. One branch was collected from the upper (sun exposed zone - E),

132 middle (transition zone - T), and bottom (sun shaded zone - S) canopy layer in order to

133 capture varying irradiation conditions inside the canopies. From each branch the last three

134 needle age classes were sampled resulting in 9 needle samples per tree. About 5-7

135 representative and visually healthy shoots (i.e. the annual growth segments) per needle age

136 class were selected, and about 30 individual needles were sampled from the central part of

137 each shoot. Each needle sample was divided into three subsets with about 10 needles each: the

138 first subset was used for estimation of needle LA_T and LA_T/LA_P conversion factor, the second

139 subset for needle water content and specific leaf area, and the third subset for photosynthetic

140 pigment analysis.

141 Posterior statistical assessment of the optimal sample size using the Power t-test

142 (Erdfelder et al. 1996) indicated a minimum sample size of 21 trees to assess the total

143 variance of CF, which was exceeded with total of 30 trees sampled.

144

145 **Estimation of needle LA_T and CF**

146 The needles from the first subset for LA_T estimation were kept deep-frozen until the

147 laboratory processing. The entire needle subset was first scanned on a desktop scanner to

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148 measure LA_P and then five needles were randomly selected for further processing (preceding

149 analysis indicated that 5 needles is sufficient to obtain LA_T representative for the entire

150 subset; results not shown). Five needles were scanned on a desktop double-lamp scanner to

151 determine their individual LA_P and length along curvature (L). Then three cross-sections

152 (approximately 100 μm thick) were taken from the base, middle and top part of a needle using

153 a hand microtome. Micrographs of cross-sections were acquired with the Canon EOS 450D

154 digital camera, which was mounted on the Novex BT PL microscope. The micrographs were

155 captured uncompressed with maximum possible resolution of 12 MPix to ensure high

156 precision of image analysis. Perimeter and length of both, the major (D_1) and the minor (D_2)

157 diameter, were measured automatically for each cross-section using a self-developed image

158 analysis procedure using GNU (General Public License) Image Manipulation Program

159 (GIMP, v. 2.6).

160   The total leaf area of spruce needles was estimated using six methods. We proposed a

161 new geometrical model for LA_T estimation of spruce needles (method I), which was based on

162 the model of Perterer and Körner (1990). Our model approximated the spruce needle shape to

163 three geometric primitives: two adjacent circular cone frustums, and a cone cap representing a

164 tapered needle's top (Fig. 1). The total leaf area of a needle was calculated according to the

165 following equation:

166 $$LA_TI = \frac{P_B+P_M}{2} . L'_{B-M} + \frac{P_M+P_T}{2} . L'_{M-T} + \frac{P_T.L'_T}{2}, \tag{1}$$

167 where P_B, P_M, and P_T are the measured perimeters of three cross-sections placed at the base

168 ($_B$), middle ($_M$) and top ($_T$) of a needle, respectively. L'_{B-M} is a slant height of the cone

169 frustum between the base and the middle cross-sections calculated from the measured cone

170 height L_{B-M} as $L'_{B-M} = \sqrt{L^2_{B-M} + (P_B/2\pi - P_M/2\pi)^2}$. L'_{M-T} is a slant height of the cone

171 frustum between the middle and the top cross-section calculated analogous to L'_{B-M}. L'_T is a

172 slant height of a cone cup calculated as $L'_T = \sqrt{L^2_T + (P_T/2\pi)^2}$. We assumed that

7

173    $L_T = 1.5$ mm, $L_{B\text{-}M} = L_{M\text{-}T}$ and the sum of the three lengths was equal to the total needle length

174   measured along the curvature of the needle central axis (L) as illustrated at Fig. 1b.

175   The other five methods for estimation of needle LA_T were previously published in

176   scientific literature. More details on methods II-V can be found in Sellin (2000) and on

177   method VI in Pokorný (2002). Here we provide only the final formulas and the variables are

178   explained at Fig. 1:

179   Method II - a needle side approximated to a parallelepiped:

180    $LA_T II = 2.L.\sqrt{D_1^2 + D_2^2}$ (2)

181   Method III - a needle side approximated to an ellipse:

182    $LA_T III = \dfrac{\pi.L.\sqrt{D_1^2 + D_2^2}}{2}$ (3)

183   Method IV - a needle approximated to an ellipsoid:

184    $LA_T IV = \pi.\sqrt[3]{D_1^2.D_2^2.L^2}$ (4)

185   Method V - a needle side approximated to a rectangle with tapering ends to a half-ellipse:

186    $LA_T V = \dfrac{4.r.L.\sqrt{D_1^2 + D_2^2} + (1-r).\pi.L.\sqrt{D_1^2 + D_2^2}}{2}$ (5)

187   (r is the relative length of the rectangular part of a needle and it was equal to 0.75)

188   Method VI - CF derived as the ratio of the middle cross-section perimeter and major diameter:

189    $LA_T VI = \dfrac{P_M}{D_1}.LA_P$ (6)

190   Six LA_T estimating methods (eq. 1 - 6) were compared at the individual needle level

191   against the same reference($\overline{LA_T}$). The reference total leaf area was calculated for 21 needles,

192   which were selected across the entire sample pool to capture the variability of a needle shape.

193   We took 9 to 15 cross-sections per needle, depending on its length, and calculated $\overline{LA_T}$ using

194   the same principle as presented for method I (eq. 1), but instead of two we integrated surface

195   area of up to 14 cone frustums.

196   Finally, the conversion factor between total (method I) and projected (scanned) leaf

197   area was calculated as a simple ratio: $CF = LA_T / LA_P$. We applied a three-way analysis of

198   variance (ANOVA) at the significance level $\alpha = 0.01$ and with a prior normality test to analyse

199   CF variability between and within spruce crowns considering three potential sources of CF

200   variability: i) needle position within a crown vertical profile, ii) needle age, and iii) forest

201   stand age.

202

203   **Fig. 1 about here**

204

205   **Measurement of needle biochemical properties and upscaling to a crown level**

206   The second and the third needle subset were used to analyse the following needle biochemical

207   properties: specific leaf area (SLA), water (C_w), chlorophyll a+b (C_{ab}) and carotenoid (C_{xc})

208   content. Needles for SLA and C_w content determination were weighted immediately after

209   clipping, stored in paper bags, dried in an oven at 60°C for 48 hours, and weighted again after

210   drying. Needles for photosynthetic pigments (C_{ab} and C_{xc}) were kept in deep freeze and dark

211   until being processed in a laboratory. Pigments were extracted according to the method of

212   Porra et al. (1989) using the dimethylformamide solvent and the pigment concentration was

213   determined spectrophotometrically according to the equations of Wellburn (1994). Following

214   the terminology proposed by Datt (1998), we define the constituent concentration as a mass

215   fraction per unit dry leaf mass (in mg.g^{-1}), and the constituent content as a mass fraction per

216   unit leaf area (in mg.cm^{-2}). Eq. 7 shows the conversion between concentration and content of

217   a constituent X (C_w, C_{ab} or C_{xc}) using the specific leaf area (SLA_H) that is defined as the ratio

218   of hemisurface leaf area (cm^2) to the corresponding dry mass weight (g):

219    $X\,[\mathrm{mg\,cm^{-2}}] = \dfrac{X[\mathrm{mg\,g^{-1}}]}{SLA_H[\mathrm{cm^2 g^{-1}}]}$ (7)

220   The constituent content was upscaled to the crown level by averaging leaf level

221   biochemical contents, obtained per tree from nine needle samples (i.e. three crown vertical

222   layers and three needle age classes). The mean values per crown, which is hereafter referred

223   as "crown averaged content", is often used in remote sensing studies (e.g. Zarco-Tejada et al.

224   2004; Huber et al. 2008; Schlerf et al. 2010). To evaluate the influence of LA_T estimation on

225   crown averaged biochemical content of SLA, C_w, C_{ab} and C_{xc}, we calculated crown averaged

226   biochemical content using six LA_T estimating methods (eq. 1 – 6). SLA_H computed from six

227   different LA_T values served as the basis for conversion of needle biochemical concentrations

228   to contents (eq. 7).

229   Finally, we considered a theoretical case, where needle biochemical concentration

230   (mg g^{-1}) and corresponding LA_P is known, but a sample LA_H is unknown. Missing LA_H was

231   then calculated as $LA_P*CF*0.5$, where LA_P is the measured projected leaf area of a sample

232   and CF is a theoretical value of the total to projected leaf area conversion factor. The

233   theoretical CF varied within a physically meaningful range from 2 (flat needles) to 4 (square

234   shaped needles) with steps of 0.2, while assuming a constant theoretical CF value for entire

235   crown vertical profile. Once again, we computed crown averaged biochemical content (X_{CF})

236   as a simple average of nine needle biochemical contents, but this time using the theoretical CF

237   to estimate a sample LA_H. X_{CF} was then compared with crown averaged biochemical content

238   (X_{REF}), based on the LA_T estimating method I (eq. 1), using the root mean square error

239   (RMSE):

240    $$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(X_{i,CF} - X_{i,REF}\right)^2} ,$$ (8)

241   where n is the number of trees.

242

243

244   **Results**

245

Accuracy of LA$_T$ estimating methods

The accuracy of six LA$_T$ estimating methods was assessed at the level of individual needles (in total 21 needles selected from the entire sample pool) by comparing all methods against one reference (Fig. 2). The reference total leaf area ($\overline{LA_T}$) was calculated from 9 – 15 cross-sections taken along the needle length using similar principle as presented in eq. 1. The average total area of a single needle was 52.2 (±9.5) mm^2. Detailed cross-sections analysis showed that the minor diameter of needle cross-sections is almost invariant along the entire needle length, whereas the major diameter decreases towards the needle ends (Fig. 3). The coefficient of determination (R^2) between the LA$_T$ estimating methods and the reference was generally high, varying between 0.79 and 0.99. Methods I and II provided accurate estimates of LA$_T$, R^2 was higher than 0.98 and RMSE was equal to 2.4 and 1.6 mm^2, respectively. In both cases, the relative RMSE was smaller than 5% of the average needle LA$_T$. Method III, which modeled needle side as an ellipse, and method IV, which modeled needle as an ellipsoid, systematically underestimated LA$_T$, with RMSE of 12.1 and 31.4 mm^2, respectively. Relative RMSE was up to 60% of the average needle LA$_T$.

Fig. 2 and Fig. 3 about here

Variability of total to projected leaf area conversion factor for method I

The sample specific conversion factor (CF) computed between LA$_T$ (method I, eq. 1) and scanned LA$_P$ varied from 2.5 to 3.8 (95th percentile). We examined three sources of CF variability: i) needle position within a crown vertical profile, ii) needle age, and iii) forest stand age (Fig. 4 and Table 1). CF of the sun exposed needles was higher than the CF of transition and shaded needles with a statistical significance $p \leq 0.01$. The mean values of CF were 3.47 (sun exposed needles), 3.18 (transition), and 2.84 (shaded needles) for the immature canopy and 3.44, 2.90, and 2.85, respectively, for the mature canopy. We did not

272   find any statistically significant differences among three investigated needle age classes. The

273   CF was nearly invariant between the immature and mature stand, except the transition canopy

274   level, where the CF of mature trees was compared to immature trees lower.

275   Finally, the CF was closely related to the ratio of the middle cross-section perimeter

276   (P_M) and its major diameter (D_{1M}), with R^2 equal to 0.73, and thus CF could be reasonably

277   modeled as:

278    $$CF = 0.47\left(\frac{P_M}{D_{1M}}\right) + 1.31 \tag{9}$$

279

280   **Fig. 4 about here**

281

282   **Impact of LA_T on upscaling of foliar biochemistry from leaf to crown level**

283   At the needle level, biochemical concentration and content varied with needle age and canopy

284   vertical position as summarized in Table 1. Pigment concentration (i.e. normalized by the dry

285   mass) increased with increasing needle age and shadowing, whereas content (i.e. normalized

286   by LA_H according to eq. 7) increased only with needle age and remained nearly invariant

287   among canopy vertical layers. Needle water content did not vary with needle age, but the

288   typically sun shaded needles had lower C_w content than exposed needles. Specific leaf area,

289   the ratio between needle LA_H and the dry mass, was the most variable needle property. It

290   varied between 30 and 140 cm^2 g^{-1} and it decreased with needle age and increased with

291   increasing shadowing inside the canopy.

292   At the crown level, we first examined the influence of different LA_T estimating

293   methods on the crown averaged biochemical content (Fig. 5). Second, we examined whether

294   the crown averaged biochemical content is sensitive towards biased LA_T estimates due to

295   variable CF (Fig. 6). Although we analysed all biochemical properties, for brevity, we present

296   results only for the chlorophyll content, because it is one of the most frequently studied

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297   vegetation property by remote sensing (Ustin et al. 2009; le Maire et al. 2004) and all

298   biochemical properties showed similar response to different LA_T estimating methods.

299   An average crown averaged C_{ab} was equal to 39 µg cm^{-2} for the immature and

300   42 µg cm^{-2} for the mature spruce trees and it varied between 33 and 53 µg cm^{-2} (values based

301   on LA_T estimations using our adjusted geometrical model, i.e. method I, hereafter used as the

302   reference crown averaged C_{ab}). Fig. 5 shows how different LA_T estimating methods yielded

303   different crown averaged C_{ab} values. Methods II, V and VI, which estimated LA_T similar to

304   our geometrical model (method I) produced crown averaged C_{ab} within the similar range (33 -

305   53 µg cm^{-2}). Methods III and IV, which underestimated LA_T, overestimated crown averaged

306   C_{ab} up to 1.5 times. For illustration purposes we also show that the crown averaged C_{ab}

307   normalized by LA_P is about 50% higher than C_{ab} normalized by LA_T (cf. the first and the last

308   box of Fig. 5).

309   The small case study with the theoretical CF, which varied between two and four with

310   steps of 0.2, demonstrated how crown averaged C_{ab} content is sensitive to potentially biased

311   LA_T estimates. Crown averaged chlorophyll content was exponentially increasing with

312   decreasing theoretical CF (Fig. 6b). The smallest RMSE (eq. 8) between the reference crown

313   averaged C_{ab} and C_{ab} estimated using the theoretical CF was found for the value equal to 3.0

314   (Fig. 6a). The smallest RMSE agreed well with the mean measured conversion factor for the

315   immature (CF = 3.2) and the mature (CF = 3.1) spruce crowns. Large errors up to 25 µg cm^{-2}

316   in crown averaged C_{ab} were observed for the lower values of the theoretical CF (closer to flat

317   needles). A bias of 0.2 from the true conversion factor introduced an error of 2 - 3 µg cm^{-2} in

318   crown averaged C_{ab} estimates.

319

320   **Table 1, Fig. 5 and Fig. 6 about here**

321

322

Discussion

Accuracy of LA_T estimating methods

Modeling a spruce needle using our new geometrical model (method I), parallelepipeds as a proxy of needle sides (method II), or parallelepipeds with half-elliptic tapering (method V) resulted in LA_T estimates closely comparable with the true reference $\overline{LA_T}$ (Fig. 2). Only methods I and II estimated LA_T of a single needle with relative RMSE less than 5% of the average LA_T. According to Frey (1981 in Niinemets 1997), method II systematically underestimated real Norway spruce LA_T by 5 - 8%, but we did not observe any systematic underestimation. Contrary to the accurate LA_T estimates by methods I and II, method III (needle sides modeled as an ellipse) and method IV (a needle modeled as an ellipsoid) systematically underestimated needle LA_T by 23% and 60%, respectively. Similar result was reported by Sellin (2000), who modeled a needle shape as an ellipse, which underestimated spruce needle LA_P by up to 20%. Our results suggest that an elliptic approximation is not suitable for modeling Norway spruce needles, because it introduces unrealistic tapering starting already at the middle part of a needle. Analysis of needle cross-sections for computing the reference total leaf area ($\overline{LA_T}$) confirmed that the major and the minor cross-section diameters are nearly invariant for 75% of the needle length and only the major diameter decreases towards needles' ends (Fig. 3). Thus modeling Norway spruce needle shape as suggest by methods I, II and IV seems to be more suitable for LA_T estimation.

A reliable predictor of the total to projected leaf area CF seems to be the ratio between the middle cross-section perimeter and its diameter ($R^2 = 0.73$). The same approach uses method VI (Pokorný 2002) to estimate LA_T from LA_P measurements, which was in reasonably good agreement with the reference $\overline{LA_T}$, as well as, with our geometrical model Method VI underestimated a single needle LA_T by less than 15%.

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Variability of total to projected leaf area conversion factor

The values of total to projected leaf area CF varied from 2.5 to 3.8 (Fig. 4), which is in agreement with previously published CF values for various Norway spruce canopies. A CF between 2.3 and 3.1 (mean of 2.4) was observed for a 15-year-old experimental plantation in the Czech Republic (Pokorný 2002), 2.3 - 3.7 (median around 2.5) was observed for current-year needles of 12 to 32-year-old trees in Germany (Niinemets 1997), 3.0 - 3.3 (mean of 3.1) was observed for a 30-year-old forest in Estonia (Sellin 2000), or slightly higher CF values in the range of 3.0 – 4.0 (mean of 3.6) were observed for current-year needles of a 40-year-old plantation in Northern Sweden (Stenberg et al. 1999). Our results showed, nevertheless, higher CF values than the average CF presented by Pokorný (2002), who studied the same immature Norway spruce stand in 1999. He reported an average CF of 2.6, whereas mean of our CF measurements was equal to 3.2. The discrepancy can be attributed to different methodologies and possibly also to higher light availability due to increasing canopy openness with the time induced by natural disturbances and managed thinning.

The majority of studies measuring the CF of Norway spruce were carried out on trees with an age less than 40 years and the results from those studies indicate that CF increases with increasing canopy age. In our study we sampled trees in their mature age (around 100 years old) and we found that the average CF was almost equal to the one measured in the 30-year-old spruce canopy. Our results indicated that CF values of entirely sun exposed and shaded needles were not significantly different in both stands. Some differences in CF were observed in the transition canopy vertical layer (Fig. 4), which can be characterized by more variable irradiation conditions. The needles from the transition zone of the mature canopy tended to have CF similar to shaded needles. This indicates that the transition needles of the mature trees were actually sampled deeper in the canopy, i.e. from locations with less available light, than the transition needles of the immature stand. A more accurate physical

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374   based delineation of canopy sampling positions, e.g. using measurements of incident

375   radiation, might solve this mismatch and assure inter-comparability of needle samples

376   collected from different forest stands.

377   Decreasing trend of CF within the vertical canopy profile is attributed to the

378   decreasing light availability, especially in the lower parts of the canopy. Lower CF values

379   together with more horizontally oriented foliage result in larger foliar surface, which is

380   improving the light harvesting capacity of shaded branches. Similar trend of decreasing CF

381   with decreasing light availability has been reported for Central European Norway spruce by

382   Niinemets (1997) and by Niinemets and Kull (1995), and also for Silver fir dominated stands

383   by Cescatti and Zorer (2003). Contrasting result, i.e. no significant trend between CF and light

384   availability, was reported by Palmroth et al. (2002) for spruces growing in central Sweeden.

385   This independency was attributed to the narrower crown habitus and typically more open

386   canopies, which ensure more equal distribution of light within the crown vertical profile.

387   Furthermore, suppressed shade-tolerant Silver firs (Cescatti and Zorer 2003) and shade-

388   intolerant Scots pines (Niinemets 2010) did not exhibit any clear trend either. This indicates

389   that local ecological factors and tree social position plays important role in foliage adaptations

390   towards varying irradiance intensities.

391   Finally, we did not observe any significant difference in CF of three recent needle age

392   classes, which is in agreement with results published previously by Sellin (2000).

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Impact of LA_T on upscaling of foliar biochemistry from leaf to crown level

395   Leaf biochemical properties, such as chlorophyll and water, are in quantitative remote sensing

396   studies often expressed per leaf area (Jacquemoud et al. 1996). Consequently, biochemical

397   properties of non-flat spruce needles with a quadratic cross-section can significantly differ if

398   being expressed against the projected or the total leaf area (Niinemets 2010 and Fig. 5 in this

399   study). As demonstrated in Fig. 4, CF is decreasing with decreasing light availability inside

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the spruce canopies. Assuming a single, average CF value around 3.0 for the entire canopy profile, CF causes an overestimation of LA_T for shaded and an underestimation of LA_T for sun exposed needles. This error is further propagated into the measurements of biochemical content at the leaf level, as well as, into crown averaged values. Based on Fig. 6a, erroneous estimation of the mean crown CF by 0.2 introduces already an error of 2 - 3 $\mu g\, cm^{-2}$ in crown averaged chlorophyll content. Considering that observed range of crown averaged C_{ab} was only 20 $\mu g\, cm^{-2}$ (i.e. it varied between 33 and 53 $\mu g\, cm^{-2}$), the error represents 10 - 15% of the observed range. However, the variability of observed crown averaged C_{ab} was small as it can vary between 20 and 100 $\mu g\, cm^{-2}$ (Malenovský et al. 2006). The maximum RMSE due to erroneous estimation of the CF was up to 25 $\mu g\, cm^{-2}$. This error is even higher than the accuracy of common remote sensing methods estimating crown averaged chlorophyll content in coniferous canopies, which is usually around 10 $\mu g\, cm^{-2}$ (Malenovský et al. 2006; Zarco-Tejada et al. 2004; Moorthy et al. 2008).

High attention should also be paid to the method upscaling leaf biochemical properties to the crowns and canopies. In this study, we used a simple mean of leaf measurements, as this is the most frequent approach used in remote sensing studies investigating forest biochemical properties (e.g. Zarco-Tejada et al. 2004; Huber et al. 2008; Schlerf et al. 2010). Nevertheless, more sophisticated upscaling schemes, which would for example account for real distribution of leaf biomass within crown vertical profile, can likely provide more representative crown integrates. In Lukeš et al. (2009), we combined the vertical distribution of leaf biomass and an apparent extinction of photosynthetically active radiation in a scaling scheme producing more realistic ground truth for validation of remotely sensed chlorophyll content of spruce crowns. In this respect, upscaling studies should consider to employ the rapidly developing methods of terrestrial and airborne laser scanning, which are able to map 3D foliage distribution of not only individual tree crowns (van der Zande et al. 2006), but also of structurally more complex forest stands (Morsdorf et al. 2010).

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Conclusions

Two out of six evaluated LA_T estimating methods, our newly proposed geometrical model based on three needle cross-sections (method I) and the parallelepiped model (method II), predicted Norway spruce needle LA_T with an error less than 5% of the average needle LA_T. Considering the overall feasibility of both methods, we can conclude that the parallelepiped model seems to be more suitable for an operational LA_T estimation in eco-physiology and applied remote sensing research, because it requires less inputs than the new geometrical model. Methods III and IV, which suggest an elliptic approximation of a needle shape, underestimated LA_T by up to 60%, and thus are not suitable for prediction of spruce needle LA_T. The conversion factor between total and projected leaf area (CF) was estimated with a reasonable accuracy ($R^2 = 0.73$) using the ratio between the needle perimeter and the major diameter of a cross-section taken from the middle of a needle. CF varied from 2.5 (shaded needles) to 3.8 (sun exposed needles). The variability of CF was mainly driven by the position of needles in the vertical canopy profile, or in other words by the decreasing irradiation in the lower canopy layers. Influence of the needle and the stand age on the CF variability was insignificant. Therefore, for future field measurements of CF we recommend sampling needles irrespective of their age (i.e. a mixed sample of several needle age classes), but taking into account several canopy vertical layers.

Since leaf area normalized biochemical properties (e.g. leaf chlorophyll and water content) of forest canopies can be estimated using the airborne and satellite imaging spectroscopy methods, representative and accurate field measurements are required for calibration and validation of the remote sensing methods. We demonstrated that crown

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451   averaged chlorophyll (C_{ab}) content normalized by LA_P is about 50% higher than LA_T

452   normalized C_{ab} content. Moreover, inaccurately estimated LA_T due to biased CF can introduce

453   an error into crown averaged chlorophyll content reaching up to 25 µg cm^{-2}. If we consider a

454   possible range of crown averaged C_{ab} between 20 and 100 µg cm^{-2}, the error can represent up

455   to 30% of the total C_{ab} range, which can seriously affect the reliability of remote sensing

456   methods.

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Tables

605 **Table 1** Morphological and biochemical properties of Norway spruce needles obtained from

606 sampling of 10 immature and 20 mature trees from three canopy vertical layers (exposed,

607 transition and shaded) and three most recent needle age classes (1 ~ most recent, 2 ~ last year,

608 3 ~ two-year-old needles). The values are presented as mean ± standard deviation.

	Age class	Exposed	Immature Transition	Shaded	Exposed	Mature Transition	Shaded
Needle length [mm]	1	15.9±1.2	16.3±2.6	13.3±2.0	15.8±2.2	17.7±2.0	17.1±2.5
	2	16.5±1.1	15.3±1.7	14.8±1.4	18.1±2.2	16.6±2.5	16.0±2.8
	3	18.8±1.8	16.9±2.3	17.1±2.4	17.3±2.5	18.4±2.7	17.6±2.9
Major diam. D_1 [mm]	1	1.19±0.06	1.16±0.07	1.07±0.10	1.29±0.14	1.13±0.07	1.10±0.09
	2	1.23±0.09	1.18±0.04	1.15±0.05	1.38±0.10	1.12±0.08	1.07±0.08
	3	1.26±0.07	1.22±0.04	1.16±0.06	1.25±0.13	1.18±0.10	1.11±0.09
Minor diam. D_2 [mm]	1	1.04±0.12	0.67±0.13	0.61±0.25	1.10±0.07	0.64±0.08	0.54±0.06
	2	1.08±0.09	0.84±0.15	0.61±0.17	1.18±0.13	0.66±0.10	0.64±0.11
	3	1.03±0.15	0.88±0.14	0.67±0.19	1.19±0.09	0.59±0.05	0.55±0.06
LA_T / LA_P CF [-]	1	3.46±0.23	3.09±0.25	2.83±0.27	3.51±0.18	2.89±0.15	2.85±0.24
	2	3.55±0.09	3.25±0.29	2.92±0.37	3.40±0.19	2.94±0.28	2.87±0.19
	3	3.41±0.21	3.30±0.23	2.77±0.24	3.43±0.20	2.89±0.19	2.85±0.17
C_{ab} conc. [mg g^{-1}]	1	1.47±0.28	2.33±0.65	3.29±0.96	1.86±0.35	2.68±0.52	2.77±0.40
	2	2.06±0.32	2.63±0.48	3.11±0.58	2.36±0.49	3.15±050	3.30±0.48
	3	2.21±0.45	2.57±0.33	3.38±0.54	2.23±0.47	3.46±0.51	3.76±0.52
C_{ab} content [μg cm^{-2}]	1	23.7±4.5	32.3±12.3	38.7±6.6	32.2±6.8	32.7±3.3	30.1±4.0
	2	40.4±6.2	41.3±8.4	48.2±9.0	48.8±9.6	43.8±7.4	41.0±5.8
	3	43.0±8.4	43.4±5.5	50.4±13.6	46.6±12.5	49.2±8.2	45.2±5.6
C_{xc} conc.	1	0.26±0.0	0.36±0.12	0.46±0.15	0.30±0.0	0.36±0.06	0.37±0.0

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		5			5		5
[mg g^{-1}]	2	0.35±0.06	0.41±0.07	0.46±0.09	0.38±0.07	0.44±0.07	0.46±0.07
	3	0.37±0.08	0.42±0.05	0.51±0.06	0.38±0.07	0.50±0.07	0.54±0.07
C_{xc} content	1	4.1±0.8	5.0±2.3	4.3±1.0	5.2±0.9	4.4±0.4	4.1±0.5
[µg cm^{-2}]	2	6.9±1.1	6.4±1.1	5.6±1.6	7.8±1.5	6.2±1.1	5.8±0.9
	3	7.3±1.4	7.1±0.8	7.3±1.9	7.8±1.9	7.1±1.3	6.5±0.9
C_w conc.	1	0.59±0.02	0.62±0.02	0.63±0.02	0.55±0.02	0.61±0.02	0.61±0.03
[g g^{-1}]	2	0.56±0.02	0.59±0.02	0.61±0.03	0.53±0.02	0.58±0.05	0.57±0.02
	3	0.56±0.01	0.58±0.01	0.61±0.03	0.50±0.03	0.56±0.03	0.57±0.03
C_w content	1	23.8±1.6	22.8±3.2	16.7±2.4	22.0±3.1	20.2±5.8	17.9±4.7
[mg cm^{-2}]	2	25.1±1.9	23.0±3.4	19.3±3.1	23.5±3.0	19.0±3.5	16.7±3.0
	3	25.4±2.7	23.3±1.9	22.0±5.5	21.2±3.2	18.1±2.6	16.4±1.7
SLA_H	1	61.8±3.3	74.2±10.6	105.8±24.9	58.7±9.4	82.4±15.3	91.0±17.3
[cm^2 g^{-1}]	2	51.3±5.1	64.5±8.5	82.7±17.5	48.6±5.2	73.1±12.7	81.6±13.1
	3	51.5±4.7	59.6±6.5	75.6±23.2	48.7±5.1	71.1±10.2	83.8±10.8

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610 **Figures**
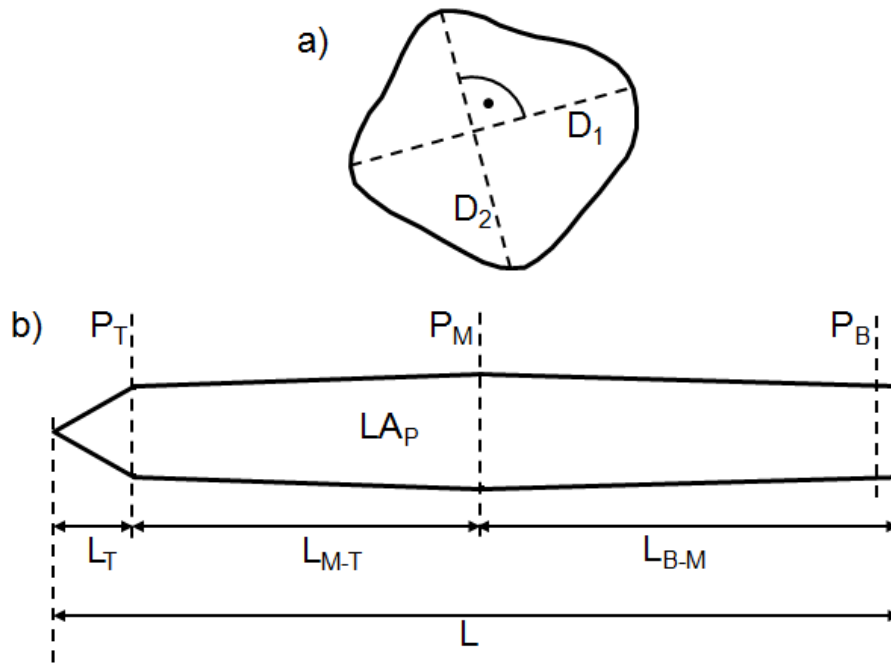
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Fig. 1 Geometrical model developed in this study to calculate total leaf area of Norway spruce needles and its inputs: a) needle cross-section and its major (D_1) and minor (D_2) diameter, b) schematic position of three cross-sections (P_T, P_M, P_B are perimeters of a cross-section at the top, middle, and bottom part of a needle, respectively; L_{B-M}, L_{M-T} are lengths of segments between two cross-sections assuming that $L_{B-M} = L_{M-T}$; L_T is height of cone cap, which was approximately 1.5mm; L is total needle length measured along the curvature of the needle central axis; LA_P is projected leaf area of a needle).

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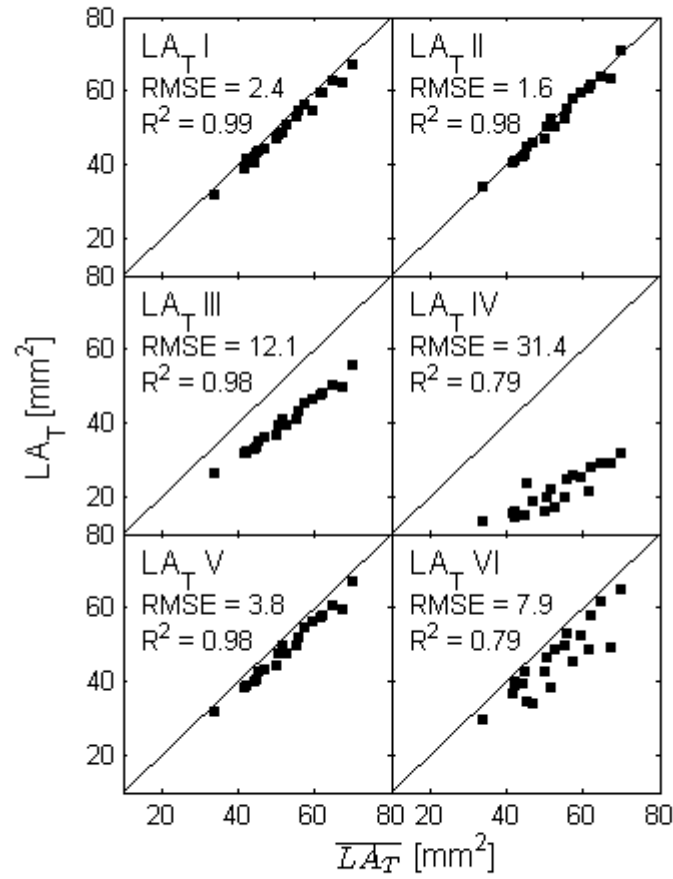
Fig. 2 Comparison of six methods for estimation of total leaf area (eq. 1 – 6) for individual

spruce needles (n = 21) with the reference method ($\overline{\mathbf{LA_T}}$). Root mean square error (RMSE)

between each method and the reference, and coefficient of determination (R^2) are indicated
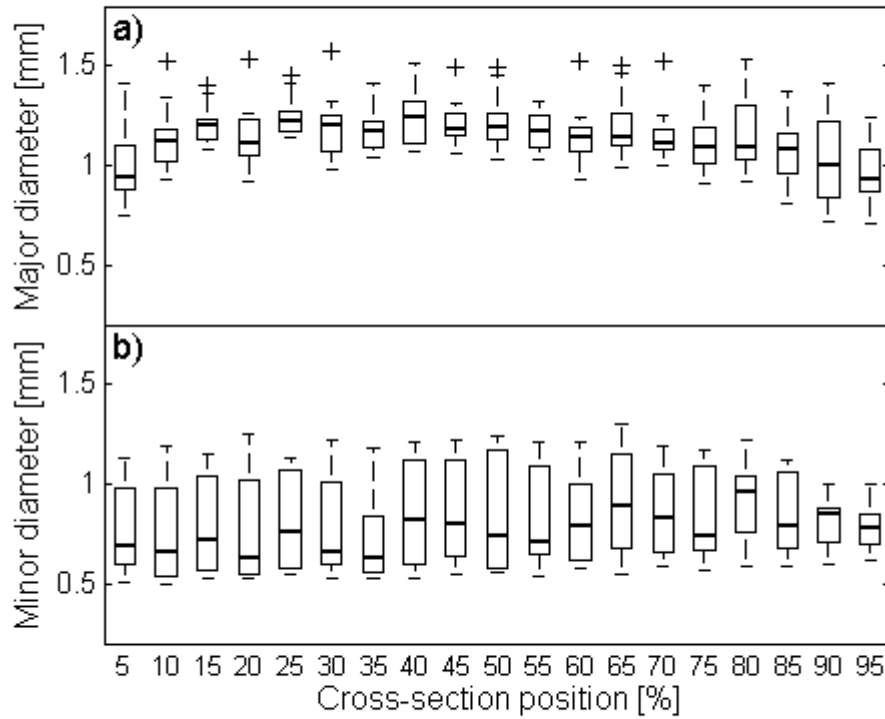
for each method.

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Fig. 3 Changes on needle cross-section major diameter (a) and minor diameter (b) along the needle length. The position of needle cross-section (x-axis) is expressed in relative units, where 0% refers to the needle base and 100% to the needle top. Legend: central line in a box represents median, box height represents 50% of the data (interquartile range), whiskers represent the minimum and the maximum values, and crosses represent outliers (when an observed value exceeded 1.5 times the interquartile range).
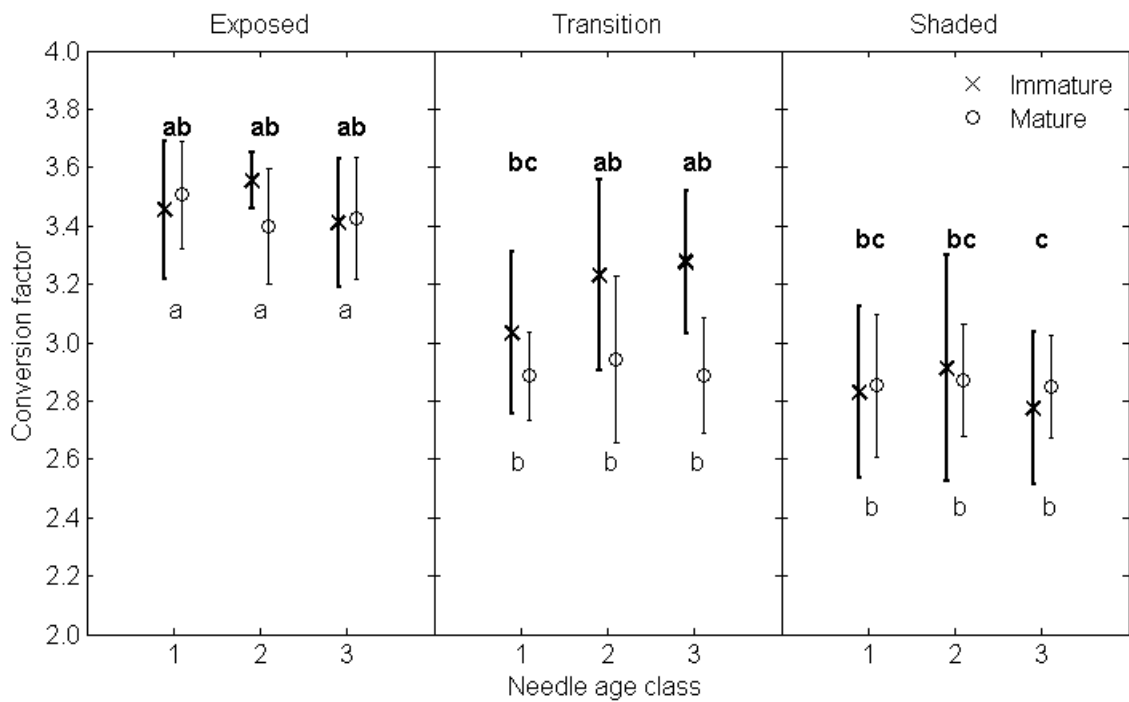
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Fig. 4 Total to projected leaf area conversion factor (CF) of two experimental Norway spruce

stands, immature (×) and mature (○). The symbols represent mean values per needle category

(combination of three needle age classes and three canopy vertical layers – sun exposed,

transition and shaded). The whiskers represent the two-sided standard deviation. Bold fonts

refer to the immature spruce trees, while regular fonts refer to the investigated mature trees.

Equal letters above and below the data points indicate statistically insignificant difference at *p*

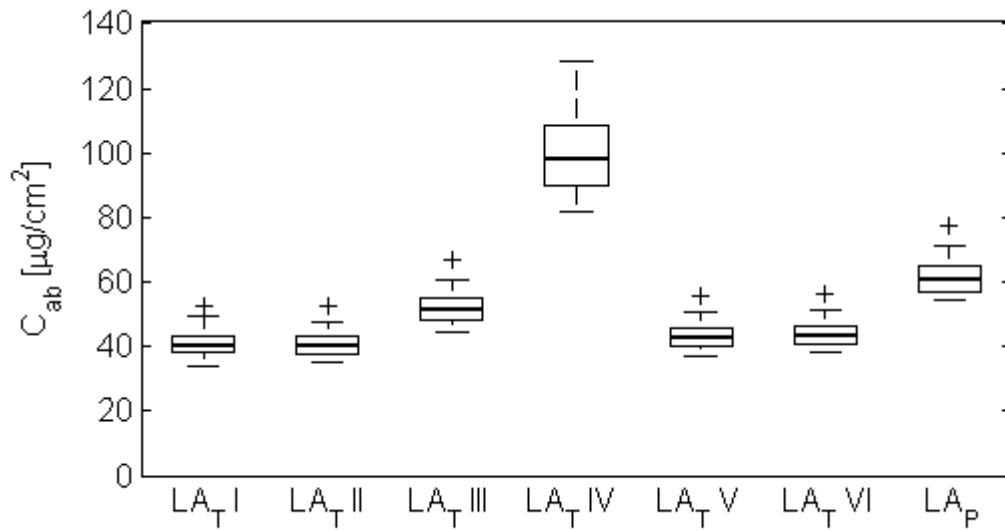≤ 0.01, based on three-way ANOVA (Tukey's post hoc test).

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Fig. 5 Summary statistics of crown averaged chlorophyll content for 30 Norway spruce trees.

Six methods to estimate total leaf area (LA$_T$) and directly measured projected leaf area (LA$_P$)

were used to express measured chlorophyll per leaf area. Detailed explanation of the box plot
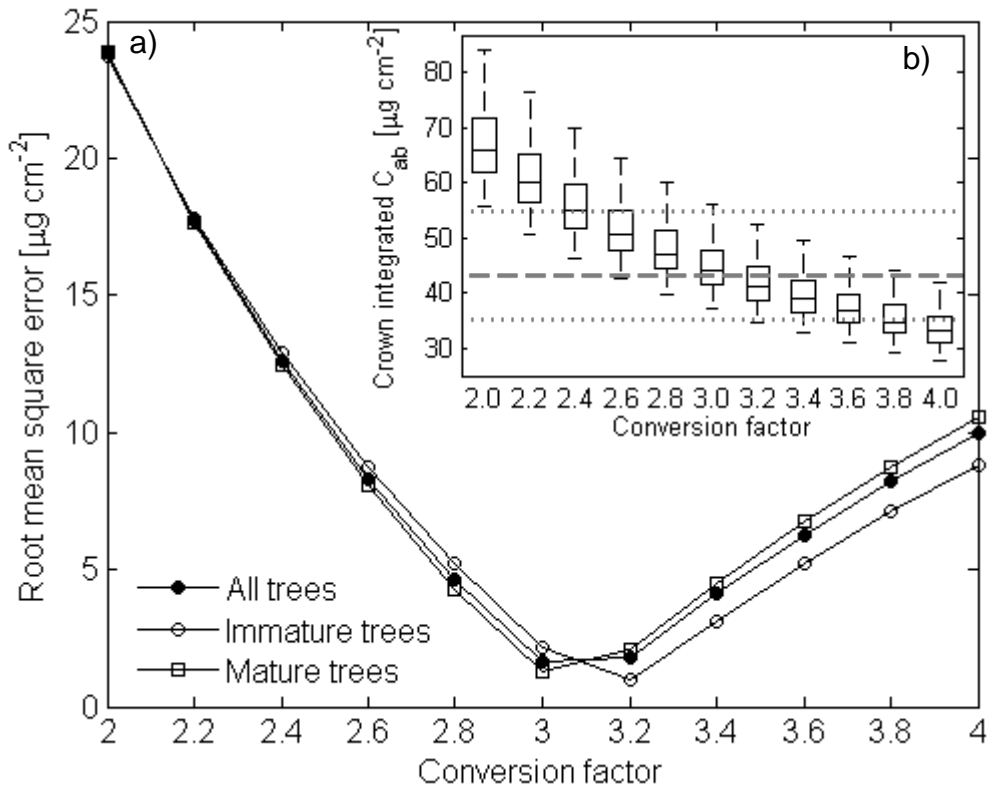
graph can be found in caption of Fig. 3.

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Fig. 6 (a) RMSE (eq. 8) between crown averaged leaf chlorophyll (C_{ab}) content normalized by LA_H calculated using sample specific CF (based on the LA_T estimating method I) and theoretical CF (i.e. single value for entire crown vertical profile, which varies between 2 and 4 with steps of 0.2). The mean RMSE values were calculated for 30 Norway spruce crowns (black dots), i.e. 10 immature (squares), and 20 mature (circles). The inserted figure (b) demonstrates how crown averaged C_{ab} decreases with increasing theoretical CF. The grey dashed line represents the median and the dotted grey lines indicate min. – max. range of the crown averaged content based on our LA_T estimating method I. Detailed explanation of the box plot graph can be found in caption of Fig. 3.