Contrasting correlation networks between leaf structure, nitrogen and chlorophyll in herbaceous and woody canopies

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Abstract

We studied acclimation patterns in leaf dry mass per area ($M_A$), nitrogen ($N_A$) and chlorophyll ($\zeta_A$) content per area, and chlorophyll to nitrogen ratio ($\zeta/N$) along vertical light gradients in natural temperate mixed herbaceous canopy and deciduous tree canopy. In the deciduous tree canopy, all leaves are formed at approximately the same time, and the light gradient during the rest of the growing season reflects the differences in light availability during leaf development, whereas in the herbaceous canopy, leaf production continues during most of the growing season and major changes in light conditions occur after leaf maturation. $M_A$ and $N_A$ increased strongly with increasing current light availability ($I_D$) in the tree canopy. In the herbaceous canopy, $M_A$ and $N_A$ were generally unrelated to $I_D$. Depending on species, the correlation between chlorophyll content per leaf area ($\zeta_A$) and $I_D$ was positive, negative, or non-significant. Path analyses revealed two opposite effects of $I_D$ on the amount of leaf chlorophyll. In the tree canopy, increasing $I_D$ enhanced $\zeta_A$ through changes in $M_A$ and $N_A$, whereas the direct effect of light was negative in both canopies. The overall correlation network between foliage structural and chemical traits and the relationships with $I_D$ were significantly stronger in the tree canopy, suggesting limited re-acclimation potential in the mixed herbaceous canopy. Within-species acclimation patterns reflected the patterns within the main functional types. These data demonstrate that the relationships of current light availability vs. leaf dry mass per area, leaf nitrogen and chlorophyll contents, and chlorophyll to nitrogen ratio differ among multi-species herbaceous canopies and deciduous tree canopies due to contrasting canopy development.

Keywords: Chlorophyll content; Functional types; Leaf mass per area; Light acclimation; Nitrogen content; Nitrogen partitioning; Remote sensing

Introduction

Experimental and theoretical studies demonstrate that leaf nitrogen content per area ($N_A$) is strongly linked to the within-canopy light gradient (Anten, 2005; Hikosaka, 2005; Kull, 2002; Kull & Niinemets, 1998; Niinemets, 2007). Given that leaf photosynthetic capacity is strongly correlated to $N_A$, such a deterministic distribution of foliar $N$ within the canopy has important implications for total canopy photosynthetic production (Evans, 1989b; Hirose & Werger, 1987; Terashima, Araya, Miyazawa, Sone, & Yano, 2005). As leaves with higher photosynthetic capacity can use more intensive light,
positive scaling of $N_A$ with light availability maximizes canopy photosynthesis for a given total canopy $N$, and, therefore, this distribution pattern has been postulated to reflect optimal distribution of limiting nutrients in the canopy (Anten, 2005).

In contrast, leaf chlorophyll content per area ($\zeta_A$) does not usually exhibit such a clear relationship, as the ratio of the biochemical nitrogen-rich carbon fixing portion of the photosynthetic machinery and the photochemical portion (containing the chlorophyll and chlorophyll-binding protein complexes) is also strongly light-dependent (Evans, 1989b; Terashima et al., 2005). Many studies have shown that the photochemical part of the photosynthetic apparatus increases relative to the biochemical part at low light to enhance light harvesting and to provide the energy for carbon fixation (see Kull, 2002 for a review). This acclimation pattern is expected to lead to an increased chlorophyll-to-nitrogen ratio in lower light and to a relatively weak overall correlation between $\zeta_A$ and light availability. Existing data show the relationships between $\zeta_A$ and light may be positive, negative or non-significant (Hansen, Friedler, & Rank, 2002; Murchie & Horton, 1997).

Despite apparent generalities in the acclimation pattern of the photosynthetic apparatus, there are important differences among species (Evans, 1989a; Murchie & Horton, 1998) and plant functional types (Niinemets, 2007; Terashima et al., 2005) with respect to acclimation of various structural, physiological and chemical traits of leaves, although the species and life-form differences are poorly understood. Such differences in the capacity for light acclimation can be mediated by differences in leaf age at the time of major modifications in light availability. Typically, young developing leaves respond most plastically to altered light environment (Yamashita, Ishida, Kushima, & Tanaka, 2000), and re-acclimation of fully developed mature leaves to altered light climate may be constrained by the inability to modify both the number of cell layers and the limited outer mesophyll cell wall area for additional chloroplasts (Oguchi, Hikosaka, & Hirose, 2005). In deciduous tree canopies, in which a perennial branching framework remains for many years, leaves develop essentially at the same time in the beginning of the growing season and, therefore, leaf light environment is generally constant during the entire leaf lifespan (Niinemets, Kull, & Tenhunen, 2004). In herbaceous canopies, both the leaves and stems are formed constantly during the extension growth of the canopy. This results in a light environment that changes significantly during leaf lifespan (Anten, Miyazawa, Hikosaka, Nagashima, & Hirose, 1998b), suggesting that the capacity for foliage re-acclimation can significantly affect within-canopy profiles of structural and physiological traits in herbaceous species.

Contrasting canopy developmental patterns may influence substantially the way structural and physiological traits are correlated and associated with current leaf light environment in woody and herbaceous canopies. Understanding how general the relationships are between structural and chemical traits, in particular, the relationship between $\zeta_A$ and $N_A$ is important for the application of remote sensing techniques to infer outer canopy surface values and within-canopy profiles of nitrogen and photosynthetic potentials. This information is of key significance to parameterize models of large-scale carbon gain (big leaf models, specifically designed to use remote sensing information for model parameterization, e.g. Dai, Dickinson, & Wang, 2004; Sellers, Berry, Collatz, Field, & Hall, 1992). Using remote sensing, it is relatively straightforward to assess the chlorophyll content of the outer canopy layers by surface reflectance, and with particular assumptions to infer total canopy chlorophyll content (Filella & Peñuelas, 1994; le Maire, François, & Dufrene, 2004). However, whole canopy photosynthetic capacity is usually related more strongly to canopy nitrogen (Evans, 1989b). While $\zeta_A$ and $N_A$ are generally positively correlated (Evans, 1989b), this correlation depends on the variation of the fraction of leaf nitrogen in chlorophyll and light harvesting pigment-binding complexes (expressed as $\zeta/N$ ratio) and corresponding changes in leaf dry mass per unit area ($M_A^\text{N}$; content per area = content per mass times $M_A$). The interaction between the processes controlling $\zeta/N$ ratio and $M_A$ also determines the variation of both the mass-based chlorophyll ($\zeta_M$) and nitrogen contents ($N_M$) within the canopy.

In the current study, we compared the relationships between the leaf structure and chlorophyll and nitrogen contents along vertical light gradients in the herbaceous and woody canopies. The main objective was to determine whether and to what extent distribution patterns of chlorophyll ($\zeta$), nitrogen ($N$), $\zeta/N$ ratio, and leaf structure differ between the stands of two different plant functional types. Specifically, we hypothesized that foliage structural and chemical traits are more strongly linked to current light availability in the tree canopy than in the herbaceous canopy and that this results in different correlation networks between foliage structural and chemical traits.

**Materials and methods**

**Study sites**

The study sites were located in south-eastern Estonia. The first study site was a former agricultural field abandoned five years earlier (58°16′N, 26°18′E). The soil on the top and on the slope (inclination angle ca. 5°) of a
small hillock was strongly eroded calcareous regosol. The soil in the lower parts of the site was a colluvisol. The site supported herbaceous vegetation dominated by Dactylis glomerata L., Festuca pratensis Huds. and Phleum pratense L. Common forbs were Achillea millefolium L., Cirsium arvense (L.) Scop. and Taraxacum officinale Weber ex Wigg. This site is characterized in detail in Aan, Hallik, and Kull (2006).

The second site was a mixed deciduous forest (58°22’N, 27°20’E) on a gleyed pseudopodzolic soil. The site was dominated by Populus tremula L. and Betula pendula Roth. in the upper canopy layer (20–27 m) and by Tilia cordata Mill. in the lower layer (15–20 m). The stand had a leaf area index (LAI) of about 6 m²m⁻². Niinemets, Bilger, Kull, and Tenhunen (1998) provides a detailed description of this site.

Plant sampling and light measurements in the grassland

Ten sample plots of 1 m² were established in the grassland in July 1999. The canopy was divided into three to five 15–25 cm thick layers, depending on the height of the vegetation. Photosynthetically active quantum flux density was measured above and below each canopy layer with a LI-191 line quantum sensor (Li-Cor, Inc., Lincoln, Nebraska, USA). Light was measured before sunrise to characterize the variation in the diffuse component of solar radiation. We define the ratio of the quantum flux readings above a specific layer relative to the readings above the vegetation as the relative diffuse quantum flux density, \( I_D \), and use this index as an estimate of relative light availability. This measure is numerically equivalent to the relative light index obtained in the tree canopy using hemispheric photography (e.g. Gendron, Messier, & Comeau, 1998).

Biomass in each canopy layer was harvested and sorted by species. Leaf area, dry mass, chlorophyll and nitrogen contents were assessed separately for each species in each layer as described in Aan et al. (2006). The species present in grassland plots were A. millefolium L., Agrostis gigantea Roth, Agrostis stolonifera L., Anthriscus sylvestris (L.) Hoffm., Cerastium vulgare Hartm., C. arvense (L.) Scop., D. glomerata L., Elymus repens (L.) Gould, F. pratensis Huds., Festuca rubra L., Helicotrichon pubescens (Huds.) Pilger, P. pratense L., Pilosella officinarum F.W. Schultz et Sch. Bip., Polygala vulgaris L., Potentilla anserina L., Ranunculus acris L., T. officinale Weber ex Wigg, Trifolium pratense L. and Vicia cracca L.

Foliage chemical analyses

Chlorophyll content was determined in 80% aqueous acetone with a S2000-FL spectrometer (Ocean Optics, Dunedin, Florida, USA) using the equations of Porra, Thompson, and Kriedemann (1989). Nitrogen content was measured using a standard Kjeldahl method by Kjeltec Auto 1030 analyzer.

Data analyses

Linear correlation (Pearson pairwise correlation) analyses were conducted to examine the statistical relationships among the studied traits and the fraction of incident light availability (\( I_D \)). Path analysis was further used to investigate the causal relationships between \( I_D \), leaf dry mass per area (\( M_A \)), nitrogen content per leaf area (\( N_A \)) and chlorophyll content per leaf area (\( \zeta_A \)). Separate calculations were made for graminoids, forbs and forest species, and different species were also analyzed separately to test the generality of pooled species models. As the correlative network was qualitatively the same for graminoids and forbs, these species groups were pooled in the final analyses, and we compared the correlative networks between two major groups, grassland and forest species. All statistical analyses were conducted with R.
Table 1. Pearson’s pairwise correlations between light availability expressed as (relative diffuse quantum flux density ($I_D$), leaf dry mass per area ($M_A$), nitrogen content per leaf area ($N_A$), chlorophyll content per leaf area ($\zeta_A$), chlorophyll to nitrogen ratio ($\zeta/N$), nitrogen content per leaf dry mass ($N_M$) and chlorophyll content per leaf dry mass ($\zeta_M$) in temperate deciduous forest and grassland species

<table>
<thead>
<tr>
<th>Grouping</th>
<th>$n$</th>
<th>Pairwise correlation coefficients and statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$I_D$</td>
<td>$I_D$</td>
</tr>
<tr>
<td>Tree species pooled</td>
<td>64</td>
<td>0.83***</td>
</tr>
<tr>
<td><em>Betula pendula</em></td>
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<td>0.92***</td>
</tr>
<tr>
<td><em>Populus tremula</em></td>
<td>19</td>
<td>0.89***</td>
</tr>
<tr>
<td><em>Tilia cordata</em></td>
<td>20</td>
<td>0.91***</td>
</tr>
<tr>
<td>Grassland species pooled</td>
<td>121</td>
<td>ns</td>
</tr>
<tr>
<td><em>Graminoids</em> pooled</td>
<td>81</td>
<td>ns</td>
</tr>
<tr>
<td><em>Forbs</em> pooled</td>
<td>40</td>
<td>ns</td>
</tr>
<tr>
<td><em>Graminoids</em>:</td>
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<td></td>
</tr>
<tr>
<td>Dactylis glomerata</td>
<td>32</td>
<td>ns</td>
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<tr>
<td>Festuca pratensis</td>
<td>12</td>
<td>ns</td>
</tr>
<tr>
<td>Phleum pratense</td>
<td>33</td>
<td>0.56***</td>
</tr>
<tr>
<td><em>Forbs</em>:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achillea millefolium</td>
<td>9</td>
<td>0.78*</td>
</tr>
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<td>Cirsium arvense</td>
<td>9</td>
<td>0.72*</td>
</tr>
<tr>
<td>Taraxacum officinale</td>
<td>13</td>
<td>ns</td>
</tr>
</tbody>
</table>

Correlation coefficients are calculated for all studied tree species pooled and for each species separately; for pooled samples of all studied herbaceous species, all graminoid and forb species, and separately for three most abundant graminoid and forb species. Number of analyzed samples ($n$) is shown and statistical significance is denoted as:

ns $p > 0.05$.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$. 

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Version 2.1.0. All statistical effects were considered significant for $p<0.05$.

$M_A$ and $N_A$, like several other variables in our study (Table 1), share the same basis of expression (area). While the same basis of expression does not imply that the correlation between the variables reflects a necessary mathematical relationship, i.e. “spurious” correlation (Prairie & Bird, 1989), the measurement error in the shared component can lead to over-estimation of the correlation between the variables. In our study, measurement errors in area and dry mass were less than 1%, and, hence, we suggest that the implication of such effects is negligible.

**Results**

**Light-dependent changes in leaf dry mass ($M_A$) and nitrogen content ($N_A$) per unit area**

The light conditions were similar between the tree ($I_D$ ranging from 1 above the canopy to 0.1 measured above the lowest layer) and herbaceous ($I_D$ ranging from 1 to 0.2) canopies. The relationship between $M_A$ and $I_D$ differed between trees and herbaceous plants. In trees, strong positive correlations between $M_A$ and $I_D$ were found both for all three species pooled (pairwise correlation coefficient, $r = 0.83$, $p < 0.001$) and within each species ($r$ ranging from 0.89 to 0.92, $p < 0.001$; Fig. 1A, Table 1). In grassland, the correlations between $I_D$ and $M_A$ were not significant ($p > 0.05$) neither when all species were pooled, nor when the forbs and graminoids were analyzed separately (Table 1). However, within species, $I_D$ and $M_A$ correlate significantly for some graminoid and forb species (Table 1).

The relationships between $I_D$ and $N_A$ were qualitatively identical with $I_D$ vs. $M_A$ relations (Table 1, Fig. 1). The correlation between $M_A$ and $I_D$ varied from insignificant ($p > 0.05$) to strongly positive ($p < 0.001$) within tree species (Table 1). When all grassland species were pooled, the correlation between $M_A$ and $I_D$ was significant but weak, and within-species correlations were not significant (Table 1).

**Variations in chlorophyll content per area ($\zeta_A$) with light, leaf structure and nitrogen**

The correlation between $I_D$ and $\zeta_A$ was slightly positive or insignificant for the tree species (Table 1, Fig. 1C) and negative or insignificant for the herbaceous species (Table 1). The correlation between $M_A$ and $\zeta_A$ varied from insignificant ($p > 0.05$) to strongly positive ($p < 0.001$) within tree species (Table 1). When all grassland species were pooled, the correlation between $M_A$ and $\zeta_A$ was significant but weak, and within-species correlations were not significant (Table 1).

Despite large variations in $M_A$, the tree canopy was characterized by a relatively constant $\zeta_A$, whereas $\zeta_A$ was more variable in the grassland (Fig. 2B, Table 2). The coefficient of variation (CV), calculated for all
species and canopy layers pooled, of $\zeta_A$ was 82% for the grassland and 16% for the tree canopy (Table 2). In the grassland, within-group variation was less, indicating that part of the variation in $\zeta_A$ in the grassland canopy was due to differences in $\zeta_A$ between forbs and graminoids (generally higher $\zeta_A$ in forbs, Fig. 2B).

As the number of species was higher in the herbaceous canopy, we also assessed the variation of $\zeta_A$ for the most common graminoid species. The variation coefficient of $\zeta_A$ was 33% ($F. pratensis$), 50% ($D. glomerata$) and 54% ($P. pratense$). For the main forb species CV was 33% ($T. officinale$), 44% ($A. millefolium$) and 88% ($C. arvense$). Among the tree species, the CV of $\zeta_A$ ranged from 9% ($P. tremula$) to 15% ($B. pendula$) with $T. cordata$ being intermediate (11%). These data collectively indicate that the reduced variability of $\zeta_A$ in the forest canopy resulted not from fewer dominating

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**Table 2.** Variability of nitrogen and chlorophyll contents per area ($N_A$ and $\zeta_A$) and dry mass ($N_M$ and $\zeta_M$) and leaf dry mass per unit area ($M_A$) for all data pooled, within functional types and within species

<table>
<thead>
<tr>
<th>Grouping</th>
<th>n</th>
<th>$N_A$</th>
<th>$N_M$</th>
<th>$\zeta_A$</th>
<th>$\zeta_M$</th>
<th>$M_A$</th>
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<tr>
<td>All data pooled</td>
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<td>46</td>
<td>33</td>
<td>56</td>
<td>69</td>
<td>40</td>
</tr>
<tr>
<td>Average within species a</td>
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<td>16</td>
<td>16</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Trees</td>
<td>64</td>
<td>33</td>
<td>22</td>
<td>16</td>
<td>52</td>
<td>45</td>
</tr>
<tr>
<td>Average within species a</td>
<td>23</td>
<td>8</td>
<td>12</td>
<td>19</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Grassland data</td>
<td>121</td>
<td>47</td>
<td>27</td>
<td>74</td>
<td>72</td>
<td>35</td>
</tr>
<tr>
<td>Average within species a</td>
<td>26</td>
<td>21</td>
<td>50</td>
<td>50</td>
<td>18</td>
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<tr>
<td>Graminoids</td>
<td>81</td>
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<td>28</td>
<td>48</td>
<td>50</td>
<td>27</td>
</tr>
<tr>
<td>Average within species a</td>
<td>28</td>
<td>45</td>
<td>45</td>
<td>48</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>Forbs</td>
<td>40</td>
<td>38</td>
<td>22</td>
<td>56</td>
<td>67</td>
<td>36</td>
</tr>
<tr>
<td>Average within species a</td>
<td>24</td>
<td>18</td>
<td>55</td>
<td>53</td>
<td>53</td>
<td>22</td>
</tr>
</tbody>
</table>

The number of analyzed samples (n) is shown for pooled data. See Table 1 for species-specific values of n.

*Average of species-specific values of coefficient of variation. Only the most abundant species (see Table 1) were used for calculating the species-specific values.

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**Fig. 2.** Relationships between (A) nitrogen content per leaf area and (B) chlorophyll content per leaf area with leaf dry mass per area for trees, graminoids and forbs. The correlation coefficients for the three functional types are provided in Table 1.

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**Fig. 3.** Path diagrams relating light availability ($I_D$), leaf dry mass per area ($M_A$), nitrogen content per leaf area ($N_A$) and chlorophyll content per leaf area ($\zeta_A$) for herbaceous species pooled (A) and tree species pooled (B). Path coefficients were calculated based on standardized values and non-significant paths, marked as dashed line, were excluded. Asterisks denote significance: ***$p<0.001$; **$p<0.01$; *$p<0.05$ and n.s. $p>0.05$. 

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species, but rather reflected lower variability within each individual species.

Alternatively, the greater number of ramets per unit ground area in the grassland canopy may also account for greater variability in $\zeta_A$. However, within-species variations in $N_A$ and $M_A$ were similar between tree and herbaceous species (Table 2), suggesting that the greater variability of $\zeta_A$ in herbs was not due to greater numbers of individuals in the grassland canopy.

In accordance with simple correlations, path analyses showed a direct positive effect of $N_A$ on $\zeta_A$ for both forest and grassland species (Fig. 3). Although simple correlations showed a positive effect of $M_A$ on $\zeta_A$ as well (Table 1, Fig. 2B), the increase in $\zeta_A$ was remarkably smaller, because plants enhanced foliage chlorophyll content per dry mass in low light ($\zeta_A = M_A \zeta_M$). Path analysis further indicated that the positive correlation between $M_A$ on $\zeta_A$ was indirect (Fig. 3). Light had an indirect positive effect on $\zeta_A$ via $M_A$ and $N_A$, but the direct effect of light on $\zeta_A$ was negative (Fig. 3).

**Variations in chlorophyll content per dry mass ($\zeta_M$) and chlorophyll to $N$ ratio**

Strong negative correlations between $I_D$ and $\zeta_M$ were observed for the trees, both for all species pooled ($r = -0.63, p<0.001$) and individual species ($p<0.001$, Table 1). For pooled grassland species, the correlation was significant but low ($r = -0.27, p<0.01$). For the majority of individual grassland species, this relationship was not significant ($p>0.05$, Table 1).

Herbaceous plants exhibited strong positive correlation between $\zeta_M$ and $\zeta_A$ for both pooled ($p<0.001$) and individual species ($p<0.01$, Table 1). This correlation for trees was neither significant for pooled nor for individual species (Table 1).

The $\zeta/N$ ratio and $\zeta_M$ were positively associated within all functional groups and within species ($p<0.001$, Table 1), indicating that $\zeta_M$ scaled positively with the fractional nitrogen investment in light harvesting. Overall, nitrogen content per dry mass ($N_M$) was much less variable than $\zeta_M$ (Table 2). In trees, $I_D$ was negatively associated with $\zeta/N$ both when all species were pooled and within each species ($p<0.001$, Table 1). This relationship was weaker or non-significant in the grassland (Table 1).

**Discussion**

**Light acclimation of leaf dry mass per unit area ($M_A$)**

Leaf dry mass per unit area is a key foliar structural trait that describes the degree of stacking of photosynthetic biomass per unit leaf area, and is determined mainly by light conditions during leaf development (e.g. Niinemets, 2007; Niinemets et al., 2004; Uemura, Ishida, Nakano, Terashima, Tanabe et al., 2000). In temperate deciduous trees, all leaves form at about the same time and remain for the entire growing season. Thus, leaves in different canopy positions experience similar light environment throughout their lifespan, accounting for the strong positive correlations of $M_A$ with light availability (Fig. 1).

In contrast, the foliage of herbaceous species develops at high light availabilities in the beginning of the growing season, and becomes gradually shaded as plants grow taller. Thus, the light conditions during leaf development are not likely similar to conditions where the leaf grows throughout most of its lifespan (Niinemets, 2007; Terashima et al., 2005). The extent of re-acclimation of mature leaves depends strongly on species and leaf age at the time of the change in light availability (Frak, Le Roux, Millard, Dreyer, Jaouen et al., 2001; Oguchi, Hikosaka, & Hirose, 2003). In our study, except for a few species, $M_A$ was unrelated to current light conditions in the herbaceous canopy (Table 1), suggesting that modification of light availability during leaf lifespan and limited foliage re-acclimation potential curbed structural adjustment to current light availability.

**Light acclimation in leaf nitrogen content per area ($N_A$)**

As canopy photosynthesis is dominated by the upper leaves, which intercept most of the light, nitrogen redistribution between leaves can make a dramatic contribution to the potential photosynthesis of the canopy (Evans, 1993). However, whether the within-canopy gradient in $N_A$ is achieved mainly by active retranslocation of nitrogen to higher irradiance or by stacking of nitrogen per unit leaf area due to leaf thickening at higher light is still under debate. $N_A$ is a function of $M_A$ and leaf nitrogen content per dry mass ($N_M$). Accordingly, if $N_M$ remains constant – as is common in many tree species (e.g. Niinemets, 2007) – the gradient in $N_A$ can result solely from light-dependent modifications in $M_A$. Indeed, for the tree species, we found that the influence of light availability on $N_A$ was indirect, mediated through changes in $M_A$ (Fig. 3). Although $N_A$ also scaled positively with $M_A$ in the grassland species, the overall effect of light on both these traits was weak (Table 1), suggesting a “non-optimal” distribution of resources.

In addition to light, nitrogen re-allocation is also driven by leaf ageing processes, but light environment can influence greatly the leaf aging process (Hidema, Makino, Mac, & Ojima, 1991). Experiments have shown...
that shading the whole plant delays leaf senescence, while shading individual leaves accelerates senescence via re-translocation of nitrogen (see Hikosaka, 2005; Niinemets, 2007 for reviews). Thus, light-driven changes in \( N_A \) as observed in several monotypic herbaceous canopies can reflect primarily ageing-dependent reduction in \( N_M \) in shaded leaves (e.g. Hirose & Werger, 1987). However, in our study, \( N_M \) scaled positively with light in only one forb species (Table 1), and the control of \( N_A \) by \( M_A \) was more significant than by \( N_M \), meaning that nitrogen re-translocation from shaded leaves was not detectable in given species nor at the whole canopy scale. In the natural multi-species grassland, individual species are generally either dominant or subordinate, thus, the contribution of subordinate and dominant individuals within a given species was likely minor to limited nitrogen redistribution. Apparently, tolerance of low light conditions rather than active gaining in height and \( N \) turnover was part of subordinate species survival strategy in this multi-species grassland. This can further explain why the competition for light was symmetric in this mixed-species grassland (Aan et al., 2006), in contrast to evidence from monospecific stands, in which the competition for light is generally strongly asymmetric (Anten & Hirose 1998a, 2001).

Although graminoids and forbs are often thought to respond differently to environmental conditions (Collins & Pinder, 1990), in our study, the responses of foliage structural and chemical variables to the within-canopy light gradient were qualitatively the same for these functional types. While sedges can respond differently than grasses to environmental gradients (Pons, van Rijnberk, Scheurwater, & van der Werf, 1993), sedges were not present in the herbaceous community in our study.

**Light acclimation of leaf chlorophyll content (\( \zeta \)) and \( \zeta/N \) ratio**

Acclimation to high light can result in two contrasting effects on leaf chlorophyll content per area (\( \zeta_A \)). Increased irradiance can lead to greater \( \zeta_A \) via increased number of chloroplasts and greater number of mesophyll cell layers when leaf-level quantitative changes dominate, or result in a reduction of \( \zeta_A \), when chloroplast-level photoprotection dominates (Kull, 2002). Correlations between \( \zeta_A \) and light in our data were positive, negative or non-significant (Table 1), suggesting that the relative magnitude of the opposite trends differed among species and functional types.

Mature leaves have limited ability to modify leaf anatomy, in particular, the number of mesophyll cell layers, to respond to changed irradiance, but in contrast, these leaves have a significant capacity to adjust foliage chlorophyll contents per unit mass and the fraction of leaf nitrogen in light harvesting (\( \zeta/N \) ratio) (Brooks, Sprugel, & Hinckley, 1996; Oguchi et al., 2003). Given the fixedness of \( M_A \) in re-acclimated leaves and modifications in \( \zeta/N \), re-acclimation can result in large variation of \( \zeta_A \) at given \( M_A \). Indeed, transfer experiments have shown that the variation of \( \zeta_A \) increases after modification of leaf light environment (Frak et al., 2001; Naidu & DeLucia, 1998; Oguchi et al., 2003). We found both increased variation in \( \zeta_A \) and a lack of correlation between \( M_A \) and light availability in the herbaceous canopy. This suggests that in the herbaceous canopy, mature leaves had undergone certain re-acclimation to changed light conditions. However, the correlation of \( \zeta/N \) with light availability was weak (Table 1), suggesting that the re-acclimation was limited in the herbaceous canopy. This is in marked contrast with strong \( \zeta/N \) vs. light correlations in tree canopy (Table 1), where the light gradient was essentially invariable after full leaf expansion and the variation of \( \zeta_A \) was small (Table 2). Previously, it has been found that in tree canopies, both chlorophyll content per dry mass (\( \zeta_M \)) and \( \zeta/N \) are determined mainly by light conditions during leaf development with minor changes occurring during the growing season (Niinemets et al., 2004).

**Conclusions and consequences**

Although the canopies of different plant functional types are currently believed to perform in the same manner (e.g. Sellers et al., 1992; Dai et al., 2004), the results of our study demonstrate that the strength of the correlative relationships of structural and chemical traits with current light availability differs between forest and herbaceous canopies (Fig. 3). These results have important implications for using remote sensing techniques to estimate canopy physiological capacities from remotely assessed leaf traits. Correlations of leaf reflectance at certain wavebands with \( \zeta_A \) are commonly used to estimate foliage chlorophyll contents (Le Maire et al., 2004; Sims & Gamon, 2002). The correlations between \( \zeta_A \) and \( N_A \) are further employed to estimate \( N_A \) from remotely assessed \( \zeta_A \) and predict foliage photosynthetic capacities (Schepers, Blackmer, & Francis, 1998; Weih & Rönnberg-Wästjung, 2007). As remote sensing techniques generally provide the reflectance of the outer surface of vegetation, further assumptions are necessary to predict the variation in foliage photosynthetic potentials and chlorophyll within the canopy (e.g. de Pury & Farquhar, 1997; Friend, 2001). The assumption of an “optimal” distribution of nitrogen or arbitrary correlations between nitrogen and current leaf light environment would be the most feasible approach to predict the profiles of nitrogen from the estimates obtained by remote sensing of outer canopy layers.
(e.g. Dai et al., 2004; Friend, 2001). However, as our study demonstrates, modified light environments during vegetation expansion can greatly alter the correlations between light and nitrogen, and light and chlorophyll. Such a (limited) re-acclimation further alters and breaks down the correlative relationships between foliage structural and chemical traits. In particular, foliage re-acclimation in multi-species grassland canopies was associated with larger variability of $\zeta_A$ at any light and $N_A$, implying inherent difficulties in using $\zeta_A$ to predict foliage nitrogen contents in such canopies.

Overall, the results of this study suggest that light availability during leaf formation, re-acclimation to modified light environments and leaf aging and senescence are the primary drivers determining the correlation network between foliage chemical and structural traits within the canopy. These results collectively underscore the importance of gaining more advanced insight into the functioning of multi-species grassland canopies, where light significantly changes during leaf lifespan, but nitrogen reallocation as the result of re-acclimation and senescence is limited.

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**References**


