Leaf Structural and Photosynthetic Characteristics, and Biomass Allocation to Foliage in Relation to Foliar Nitrogen Content and Tree Size in Three *Betula* Species

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Young trees 0·03–1·7 m high of three coexisting *Betula* species were investigated in four sites of varying soil fertility, but all in full daylight, to separate nutrient and plant size controls on leaf dry mass per unit area (M_A), light-saturated foliar photosynthetic electron transport rate (J) and the fraction of plant biomass in foliage (F_L). Because the site effect was generally non-significant in the analyses of variance with foliar nitrogen content per unit dry mass (N_M) as a covariate, N_M was used as an explaining variable of leaf structural and physiological characteristics. Average leaf area (S) and dry mass per leaf scaled positively with N_M and total tree height (H) in all species. Leaf dry mass per unit area also increased with increasing H, but decreased with increasing N_M, whereas the effects were species-specific. Increases in plant size led to a lower and increases in N_M to a greater F_L and total plant foliar area per unit plant biomass (LAR). Thus, the self-shading probably increased with increasing N_M and decreased with increasing H. Nevertheless, the whole-plant average M_A, as well as M_A values of topmost fully exposed leaves, correlated with N_M and H in a similar manner, indicating that scaling of M_A with N_M and H did not necessarily result from the modified degree of within-plant shading. The rate of photosynthetic electron transport per unit dry mass (J_M) scaled positively with N_M, but decreased with increasing H and M_A. Thus, increases in M_A with tree height and decreasing nitrogen content not only resulted in a lower plant foliar area (LAR = F_L/M_A), but also led to lower physiological activity of unit foliar biomass. The leaf parameters (J_M, N_M and M_A) varied threefold, but the whole-plant characteristic F_L varied 20-fold and LAR 30-fold, indicating that the biomass allocation was more plastically adjusted to different plant internal nitrogen contents and to tree height than the foliar variables. Our results demonstrate that: (1) tree height and N_M may independently control foliar structure and physiology, and have an even greater impact on biomass allocation; and (2) the modified within-plant light availabilities alone do not explain the observed patterns. Although there were interspecific differences with respect to the statistical significance of the relationships, all species generally fit common regressions. However, these differences were consistent, and suggested that more competitive species with inherently larger growth rates also more plastically respond to N and H.

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Key words: *Betula nana*, *Betula pendula*, *Betula pubescens*, construction cost, leaf density, leaf dry mass per unit area, leaf size, nitrogen content, photosynthetic electron transport rate, plasticity, tree height.

INTRODUCTION

Leaf dry mass per unit area (M_A) and the fractional investment of the biomass in foliage (F_L) are important plant parameters which are strongly linked to plant growth rates (Evans 1972; Poorter and Remkes, 1990; Körner, 1991; Cornelissen *et al.*, 1996, 1998). The total leaf area per unit plant biomass (LAR) is given by F_L/M_A, and, thus, both a large F_L and a small M_A allow formation of an extensive foliar area, thereby enhancing plant potential for light interception and photosynthesis. Therefore, plant growth rates often vary directly with LAR (Ingestad and McDonald, 1989; Poorter 1990; Cornelissen *et al.*, 1996, 1998; Wang *et al.*, 1998).

Along gradients of nutrient availability, the biomass requirements for light harvesting conflict with those of nutrient uptake and, accordingly, the significance of a large

foliar area is greater at high nutrient availabilities and less at low nutrient availabilities. To cope with a broad variability in soil conditions, plants often possess a large capacity for plastic modification of biomass allocation and total leaf area. Increased nitrogen supply invariably leads to a larger LAR (Karlsson and Nordell, 1987; Küppers *et al.*, 1988; Thompson *et al.*, 1988; Ingestad and McDonald, 1989; Bowler and Press, 1996; Wang *et al.*, 1998). Yet, it is still unclear to what extent these changes result from allocation (F_L) and from alterations in leaf structure (M_A). There are consistent positive relationships between F_L and nutrient availability (Ingestad and Lund, 1979; Karlsson and Nordell, 1987; Ingestad and McDonald, 1989; Wang *et al.*, 1998; Poorter and Nagel, 2000), but the evidence of nutrient availability effects on M_A is contrasting. Strong interspecific negative correlations between M_A and foliage nitrogen content per unit dry mass (Turner, 1994; Reich *et al.*, 1995; Niinemets, 1999) are often found, but the relationships are

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weaker in other studies (Reich and Walters, 1994). In addition, increases in nitrogen availability have resulted in a lower whole-plant average M_A (Jurik *et al*., 1982; Waring *et al*., 1985; Karlsson and Nordell, 1987; Hirose, 1988; Thompson *et al*., 1988; Latham, 1992; Bowler and Press, 1996; Grubb *et al*., 1996; Wang *et al*., 1998; Meziane and Shipley, 1999) but, in some cases, M_A is invariable (Karlsson and Nordell, 1989; Kolb *et al*., 1990; Fredeen *et al*., 1991; Latham, 1992; Ackerly and Bazzaz, 1995; Grubb *et al*., 1996; Sims *et al*., 1998*b*; Rosati *et al*., 2000) or even increases (Latham 1992; Sims *et al*., 1998*a*) with increasing nitrogen availability.

Apart from the possible nutrient effects, M_A scales positively with light availability (Latham, 1992; Ackerly and Bazzaz, 1995; Niinemets, 1998; Niinemets and Kull, 1998; Rosati *et al*., 2000). Given that the increase in foliar area as the result of improved nutrition inevitably leads to a greater within-plant shading (Ackerly and Bazzaz, 1995), a decrease in M_A in plants at higher nutrient availability may be a consequence of lower quantum flux densities within these plants. In addition, M_A is also positively related to plant size (Steele *et al*., 1989; Niinemets, 1997*a*; Niinemets *et al*., 1999). Because the plants at higher nitrogen availability are generally larger, plant size effects on M_A may level off or over-rule the influences of enhanced shading, providing an explanation of insignificant or positive effects of improved N nutrition on M_A.

We studied biomass allocation and leaf morphology in young trees in four sites of contrasting fertility to: (1) determine the extent to which leaf nitrogen availability controls M_A, foliar photosynthetic characteristics and the fraction of plant biomass in foliage (F_L); and (2) understand whether these effects can be ascribed to modifications in intra-plant shading and/or differences in plant size. We also analysed (3) foliar construction cost in terms of unit glucose investment for a unit foliar biomass production (Vertregt and Penning de Vries, 1987; Poorter, 1994) to determine whether the carbon cost of leaves varies as a function of foliar nitrogen. We expected that in less fertile habitats, where carbon availability limits growth to a lesser extent than in more fertile patches, the content of carbon-rich protective chemicals such as lignin and condensed phenolics increases (Waring *et al*., 1985; Chapin, 1989; Chapin *et al*., 1990) thereby leading to a greater foliar glucose cost (e.g. Niinemets, 1997*b*).

Three species of *Betula* were used for the analyses. *Betula nana* L. is a very shade-intolerant shrub species (height 0·4–1·2 m) with characteristic polycormic growth and with leaves 0·4–2 cm² in area on short, 0·5–1 mm long petioles, such that the leaves are seemingly directly attached to the stem. *B. nana* is widespread in tundra heaths (for further details, see Jonasson, 1981; de Groot *et al*., 1997), and as a relic of the last glaciation is restricted to raised bogs in Estonia (Dierssen, 1977). *B. pubescens* Ehrh. and *B. pendula* Roth. are closely related shade-intolerant tree species with leaves 5–20 cm² in area typically on 0·4–2 cm long petioles. Both species are pioneering trees, but *B. pendula* colonizes drier habitats than *B. pubescens*, which often dominates on wet soils (Atkinson, 1992). In Estonia, the maximum height of *B. pendula* is 35 m and that of *B.*

pubescens 25 m (Masing, 1990). Because all the studied species coexisted in the most nutrient-limited wet habitat, but the competitive potential of *B. nana* was apparently lower at greater nutrient availabilities, we suggested that foliage characteristics adjust less plastically to improved nutrient availability in this than in the other species. The hypothesis that species responsiveness to nutrient availability depends on species growth potentials was posed by Coomes and Grubb (2000), but conclusive data to test the hypothesis are unavailable. Given that species success in nutrient-limited open environments is mainly driven by root characteristics, foliar variables of coexisting species may largely differ in these habitats. In contrast, in nutrient-rich systems, there is strong evidence of a functional convergence of foliage structural and physiological characteristics of coexisting species (Niinemets and Kull, 1994; Ackerly and Reich, 1999).

We used foliar nitrogen content per unit dry mass (N_M) as an estimate of internal plant nutrient availability. Plant nutrient contents depend not only on soil nutrient availability, but also on the efficiency and rate of nutrient uptake, and on nutrient partitioning within plant, as well as on the growth rates of the plant (Chapin *et al*., 1986; Hilbert, 1990; Oren and Sheriff, 1995). Thus, internal N concentrations control nutrient-related modifications in foliage structure and fractional biomass allocation, rather than soil-available N concentrations (Hirose, 1988; Levin *et al*., 1989; Hilbert, 1990; Tan and Hogan, 1998). Total tree height (H) was employed as a second independent variable to account for plant size related allocation and morphology differences.

MATERIALS AND METHODS

Study sites

Betula nana L. (16 plants with average ± s.e. total height, H, of 0·39 ± 0·05 m), *B. pubescens* Ehrh. (43 trees, $H = 0·70 ± 0·08$ m) and *B. pendula* Roth. (six trees, $H = 0·58 ± 0·15$ m) were studied at a nutrient-poor site on *Sphagnum* peat in Männikjärve raised bog, Endla State Nature Reserve, Estonia (58°52′N, 26°15′E). The peat layers were extensive—up to 8 m in the centre of the bog, and in general more than 4 m at the margins (Veber, 1974). The sparse tree layer (200 trees/ha) with an average height of approx. 1–2 m and an average age of 50–100 years was dominated by *Pinus sylvestris* L. and *B. pubescens*. Excavations of the entire root systems at the site demonstrated that the roots mostly occupied the upper peat horizons of 5–15 cm, and did not penetrate the dense peat layers. The average characteristics of the upper peat layers are reported in Table 1. Fertilizer experiments in temperate raised bogs have demonstrated that plant growth in these habitats is strongly curtailed by low soil nutrient availability. The data demonstrate that nitrogen and phosphorus are in especially short supply (Paavilainen, 1980; Damman, 1986; Finér, 1992).

Betula pubescens was also investigated at two adjacent fell sites in the Viimsi peninsula. Both sites were previously dominated by *Picea abies* (L.) Karst. and were on acidic, sandy, podsolic soil with a raw humus horizon (Table 1),

TABLE 1. *Site average (± s.d.)* chemical characteristics of the upper soil horizon and average foliar chemical data*

Site	Soil							Foliage			
	pH (H$_2$O)	pH (KCl)	Soluble P (mg/g)	Total P (mg/g)	Total N (%)	C/N molar ratio	*n*	N (%)	P (%)	P/N molar ratio	*n*
Endla	3·28 ± 0·10[a]	2·64 ± 0·27[a]	0·088 ± 0·022[a]	0·57 ± 0·31[a]	1·36 ± 0·32[a]	42 ± 9[a]	13	1·65 ± 0·32[c]	0·143 ± 0·005[b]	0·039 ± 0·006[a]	62
Viimsi-1	3·35 ± 0·18[a]	2·56 ± 0·24[a]	0·067 ± 0·030[a]	0·78 ± 0·27[a]	1·46 ± 0·54[a]	39 ± 11[a]	8	3·17 ± 0·21[a]	0·166 ± 0·015[b]	0·025 ± 0·002[b]	11
Viimsi-2	3·15 ± 0·13[a]	2·42 ± 0·09[a]	0·072 ± 0·018[a]	0·83 ± 0·15[a]	1·96 ± 0·21[a]	29·2 ± 3·3[ab]	3	3·07 ± 0·18[a]	0·30 ± 0·11[a]	0·045 ± 0·019[ab]	8
Voore[†]	n.d.	3·60 ± 0·28[b]	n.d.	0·98 ± 0·15[a]	0·110 ± 0·015[b]	13·8 ± 3·5[b]	2	2·25 ± 0·46[b]	0·26 ± 0·08[a]	0·055 ± 0·029[ab]	10

* Means followed by the same letter are not significantly different (*P* > 0·05 according to one-way ANOVA followed by Bonferroni test).

[†] Soil chemical data are from Reintam (1970). Foliage chemical data were averages of the current study (*B. pendula*) and those of Kõlli and Kährik (1970) [*Corylus avellana* L., *Fraxinus excelsior* L., *Picea abies* (L.) Karst., *Rhamnus cathartica* L., and *Rubus idaeus* L.].

with the mineral soil layers starting at approx. 20 cm depth. The first fell site (Viimsi-1, 59°32′N, 24°52′E) was 2 years old, and first and second year seedlings (24 trees, $H = 0.134 \pm 0.023$ m) were investigated at this site. The second clearing (Viimsi-2, 59°33′N, 24°51′E) was 5 years old, and 2–4-year-old saplings (20 trees, $H = 0.67 \pm 0.07$ m) were sampled. In both the bog and the fell sites, the light availability at the top of the sampled plants was always more than 90 % of that in a completely open location.

We also included another set of trees of *B. pendula* (five trees, $H = 1.10 \pm 0.21$ m) sampled in Voore, Estonia (58°44′N, 26°45′E; Kull and Niinemets, 1993). The 30–35 m tall, and approx. 100 year-old forest was dominated by *P. abies*, and had a vigorous deciduous shrub understory. The soil was a brown pseudopodzol with a mull type A horizon (Table 1). A thorough description of this study site is provided by Frey (1977). The light conditions above each sapling were assessed by hemispherical photography (Kull and Niinemets, 1993). The trees included in the current study grew in recent clearings or at the edges of the forest, and received at least 85 % of above-canopy irradiance.

Overall, the trees of different species that were sampled in various sites were of similar size. According to an ANOVA (analysis of variance), mean tree height was lower than in other cases for the first and second year seedlings of *B. pubescens* sampled in Viimsi-1 ($P < 0.001$), and was not statistically different between other site–species pairs ($P > 0.05$).

Plant and foliar sampling: foliage morphological parameters

Plant sampling was conducted in July 1999 at Endla, September 1999 at Viimsi and August 1989 in Voore. There were no signs of foliar senescence during plant sampling. In Endla and Viimsi, the trees were excavated carefully to yield a reliable estimate of both below- and above-ground plant biomass. All leaves were harvested, and ten leaves from each plant were randomly taken for foliar area measurements. The remaining leaves, stem, coarse (diameter >1 mm) and fine roots were dried at 75 °C for at least 48 h, and weighed. The sample leaves were scanned by a Hewlett–Packard scanner at a resolution of 1200 d.p.i., converted to 1-bit bitmap, and the area of the sample leaves was measured by self-developed computer software. The sample leaves were also dried and weighed, and the leaf dry mass per unit area (M_A) was calculated. Total foliar area per plant was determined as the ratio of total leaf dry mass to M_A. Leaf area ratio (LAR, $cm^2\ g^{-1}$) was further computed as the ratio of total plant foliar area to total plant dry mass.

Given that the foliar characteristics of the plants excavated in Endla and Viimsi were averages for the entire plants, within-plant shading was likely to be considerable, and may have a critical affect on the correlations. To clarify the contribution of intra-plant shading to the relationships observed, we sampled the uppermost leaves of 35 separate plants in Endla. Because the top leaves were fully exposed, foliar characteristics could be studied without the interfering self-shading effects. Although we are not quantifying the degree of within-plant shading this way, the finding of the same correlations with the leaf characteristics of the

uppermost leaves as with the whole-plant average leaf characteristics indicates that these correlations cannot be attributed to modified within-plant shading alone. In Voore, only the topmost four to seven exposed leaves were sampled. For the top leaves, the circumference of each leaf was digitized, and the area was calculated as described by Niinemets *et al.* (1999).

Foliage and soil chemical analyses

Samples for soil analyses were taken from the A (A_0) horizon, where most of the roots of the sampled seedlings and saplings were found (5–10 cm for Endla, 5–15 cm for Viimsi and 3–20 cm for Voore). A sample for chemical analysis consisted of all leaves per plant (excavated plants in Endla and Viimsi) or of the uppermost completely exposed leaves in Endla (35 separate plants) and Voore (five separate plants).

For most samples, nitrogen and carbon contents were estimated by gas chromatography after combustion of the sample at >1000 °C in oxygen (elemental analyzer CHN-O-Rapid; Foss Heraeus GmbH, Hanau, Germany). Phosphorus contents were determined by inductively coupled plasma emission spectroscopy (Integra XMP; GBC Scientific Instruments, Melbourne, Australia) after digestion of pulverized leaves in 65 % HNO_3. For some samples, standard Kjeldahl digestion was applied, and N content was determined by the indophenol method and P content by the molybdenum blue method (Grimshaw *et al.*, 1989). Extensive comparisons of samples from different sites by various analytical routines indicated that the different methods gave similar results for both N and P with the differences ⩽1 %. Ash content was estimated after the combustion of the sample in a muffle furnace at 500 °C for 3 h, and mineral content (A) calculated taking the fraction of minerals in ash as equal to 0.67 (Vertregt and Penning de Vries, 1987).

For the Endla and Viimsi sites, soil pH (1 g dry soil per 5 ml solution) was measured in distilled water (pH_{H_2O}) and in 1 M KCl solution (pH_{KCl}). Soluble P [ammonium lactate–acetate-soluble phosphorus (AL-method, Swedish standard SS 02 83 10; Egner *et al.*, 1960)] was determined by the molybdenum blue method after extraction with an aqueous solution (pH = 3.75) consisting of lactic (0.1 M) and acetic (0.3 M) acids, and ammonium acetate (0.1 M). The detailed soil characteristics and methods for chemical analyses of soils in the Voore site are reported by Kõlli and Kährik (1970), Reintam (1970) and Kull and Niinemets (1993).

Leaf construction cost

Mineral-free carbon content (C_A, $g\ g^{-1}$) was found as $C_A = C_m/(1 - A)$, where C_m is total leaf carbon content per unit dry mass, and an estimate of leaf construction cost [G, g glucose (g dry mass)$^{-1}$] was computed as (Vertregt and Penning de Vries, 1987; Poorter, 1994; Niinemets, 1997*b*):
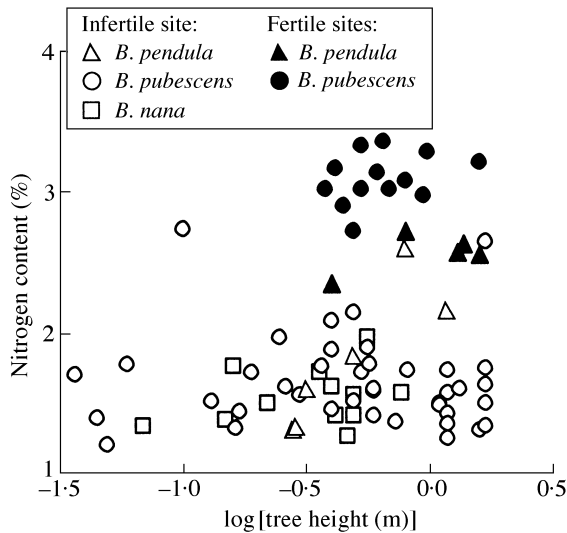
$$G = (5.077 C_A - 1.041)(1 - A). \tag{1}$$

FIG. 1. Scatter plot of foliar nitrogen content per unit dry mass *vs.* total tree height. The data were sampled from the bog (poor site) and from the clear fell sites in Viimsi (*B. pubescens*) and forest edges in Voore (*B. pendula*, Table 1). Average foliar nutrient contents (Table 1) provided the primary criterion to distinguish the sites on the basis of nutrient availability (fertile *vs.* infertile). The same symbols are used for the Viimsi data because (1) the primary difference between the Viimsi sites (Viimsi-1, and Viimsi-2, Table 1) was only the age of the clear fells, and (2) the sites did not differ in measured soil characteristics and foliar nutrient contents. The linear correlation is non-significant ($r = 0.21$, $P > 0.06$ for all data pooled).
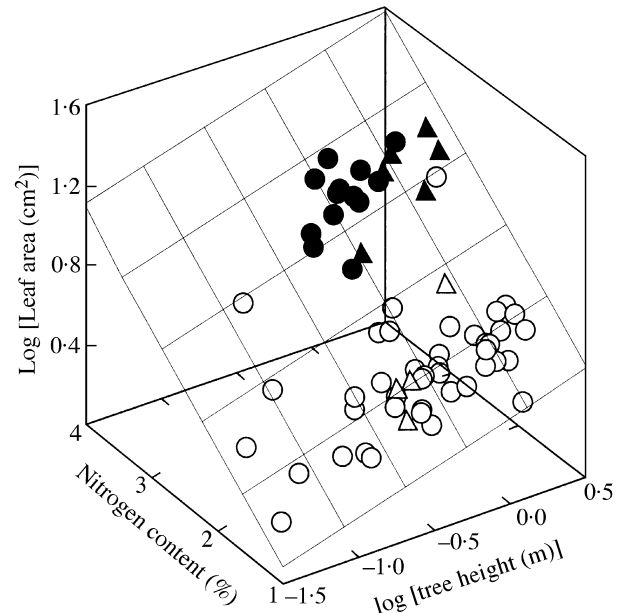

FIG. 2. Dependence of leaf size on foliar nitrogen content and on total tree height in *Betula nana*, *B. pendula* and *B. pubescens*. All data were fitted by a single multiple linear regression: $\log S = -0.190 + 0.358 \log H + 0.447 N_M$ ($r^2 = 0.60$, $P < 0.001$ for both $\log H$ and N_M, $P > 0.1$ for the intercept). Symbols as in Fig. 1.

The primary assumption of eqn (1) is that the source of foliar nitrogen is ammonium or nitrogen in organic form, or that all nitrate is reduced in the leaf (Poorter, 1994; Niinemets, 1997*b*). Given that ammonium and amino acids are the predominate nitrogen source in bogs and raw humus soils (Chapin *et al.*, 1986; Hobbie *et al.*, 2000), we consider this assumption justified.

Estimations of foliage photosynthetic electron transport rate

In Endla (Table 1), chlorophyll fluorescence measurements were conducted in the uppermost leaves with a pulse-amplitude modulated fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). Natural illumination was used for most measurements, but on cloudy days, the internal halogen lamp of the PAM-2000 was used as the source of actinic light. The leaf was held in a beam irradiance of 1200–1600 µmol m^{-2} s^{-1}, which was saturating for photosynthetic electron transport in the studied species. Pulses of white light of 8000 µmol m^{-2} s^{-1} were applied to determine the maximum fluorescence yield of light-adapted sample (F_m') as detailed by Niinemets *et al.* (1998*b*). From these measurements, the effective quantum yield of the light-adapted sample (Φ_{PSII}) was computed as ($F_m' - F$)/F_m', where F is the fluorescence yield in actinic light (Schreiber *et al.*, 1994). Assuming that both Photosystems I and II absorb equal amounts of light, an estimate of the whole chain electron transport rate (J) was computed as described by Genty *et al.* (1989):

$$J = 0.5 \Phi_{PSII} \Theta Q. \qquad (2)$$

where Q is the quantum flux density and Θ is the leaf absorptance. A constant value of 0.84 was used for Θ in the current study. Average ($\pm$ s.d.) leaf temperature was 27.7 ± 3.1 °C during the fluorescence measurements and no attempt was made to standardize the observed values of J to a common temperature.

Statistical analysis

The maximum height of the excavated plants was only 1.7 m, but the height range of the studied plants was more than 50-fold, and significant effects of tree height were found on both foliar morphological and plant allocation parameters. Because tree height and foliar nitrogen content were not strongly correlated (Fig. 1), the effects of total tree height and N could be studied independently. To improve the normality of the residuals, tree height, average leaf area, average dry mass and leaf area ratio were transformed to logarithms before the statistical analysis was performed. Simple and multiple linear regression analyses were employed to examine the dependencies of foliar and plant characteristics on total tree height and foliar nitrogen content. The species and site effects were investigated either by analyses of variance (Site and Species as main effects) when $\log H$ and N effects were non-significant or by analyses of covariance ($\log H$ and N as covariates), and the means were separated by the Bonferroni test. Preliminary analysis indicated that the Site as well as Site $\times$ N and Site $\times$ $\log H$ interactions were generally non-significant
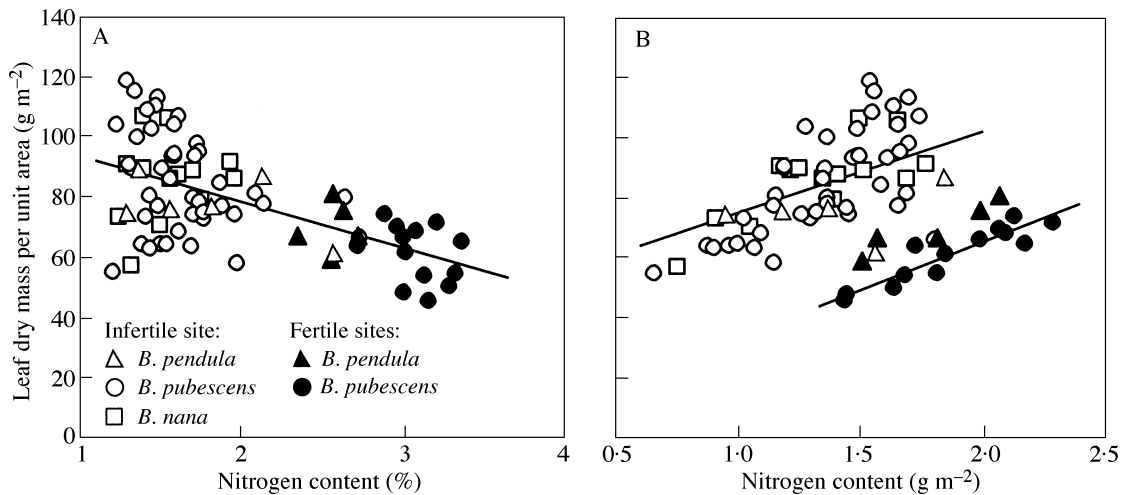
FIG. 3. Correlations between leaf dry mass per unit area (M_A) and leaf nitrogen content per unit dry mass (A) and N per unit area (B). In A, all data were fitted by a single linear regression ($r^2 = 0.33$, $P < 0.001$). In B, separate regressions were fitted for all sample points from the infertile site (upper regression line, $r^2 = 0.31$, $P < 0.001$) and for *B. pubescens* from the fertile site (lower regression line, $r^2 = 0.85$, $P < 0.001$). Symbols as in Fig. 1.
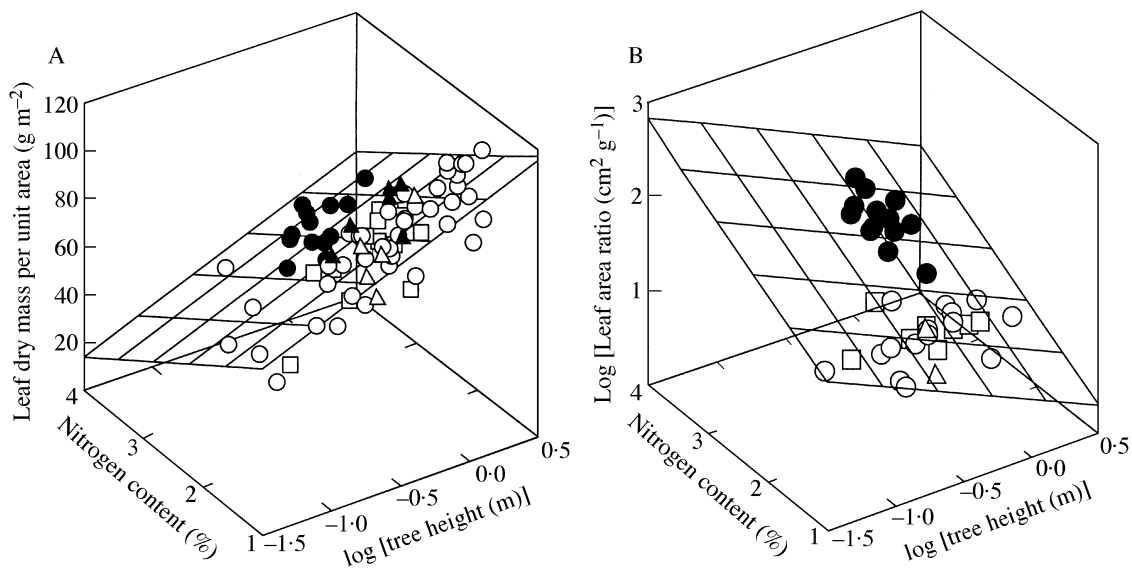

FIG. 4. Effects of leaf nitrogen content and total tree height on M_A (A) and on leaf area ratio (B). The regression surfaces were fitted through all data: $M_A = 123.4 + 23.2\log H - 18.8 N_M$ ($r^2 = 0.64$) for A, and $\log(\text{LAR}) = 0.622 - 0.340\log H + 0.277 N_M$ for B ($r^2 = 0.49$). All regression coefficients were significant at $P < 0.001$. Symbols as in Fig. 1.

($P > 0.05$), indicating that Species, log$H$ and N were the primary determinants of foliar characteristics. Given the low explaining power, the Site effects as well as the third-order interactions were suppressed in the final ANOVA model.

Because of differences in species abundance and dispersal patterns at the various sites, different numbers of plants were available for each species–site pair. In particular, the number of plants of *B. pubescens* was considerably larger than that of other species. This lack of balance may have potentially altered interspecific comparisons and the conclusions with respect to the significance of the pooled regressions. To test for these possibilities, we constructed another dataset by randomly removing the data of *B.*

pubescens to give a total of 16 observations for this species. Although this procedure altered the statistical significance of various differences and relationships, all multiple correlations were qualitatively the same for this and for the entire dataset, indicating that the pooled regressions were not affected by the lack of balance in the data.

RESULTS

Differences in the soil nutrient availability between the sites

The measured soil characteristics between the bog (Endla) and the fell sites (Viimsi-1 and Viimsi-2) were not

TABLE 2. *Species-specific regressions between foliar structural characteristics, and foliar nitrogen content and total tree height*

Species	Dependent variable*	Nitrogen content (N_M, %)					Tree height [log(H, m)]					Multiple regression†	
		Intercept	P	Slope	r^2	P	Intercept	P	Slope	r^2	P	r^2	P
B. nana	logS	−1·24	0·005	0·681	0·42	0·02	0·139	0·06	0·687	0·70	0·001	0·83	0·001
B. pendula	logS	−0·607	0·05	0·704	0·87	0·001	1·10	0·001	1·02	0·62	0·005	0·87	0·001‡
B. pubescens	logS	0·0469	0·4	0·317	0·69	0·001	0·822	0·001	0·191	0·12	0·001	0·83	0·001
B. nana	logM	−16·3	0·001	1·41	0·41	0·02	−13·5	0·001	1·24	0·50	0·005	0·65	0·001
B. pendula	logM	−15·1	0·001	1·71	0·83	0·001	−10·9	0·001	2·41	0·56	0·01	0·83	0·002‡
B. pubescens	logM	−13·9	0·001	0·951	0·84	0·001	−11·7	0·001	0·0687	0·00	0·6	0·86	0·001
B. nana	M_A	68·9	0·02	10·7	0·04	0·5	96·7	0·001	26·3	0·34	0·02	0·40	0·02§
B. pendula	M_A	85·4	0·001	−6·05	0·12	0·3	71·9	0·001	−2·83	0·01	0·7	0·32	0·2
B. pubescens	M_A	112·5	0·001	−16·8	0·38	0·001	81·5	0·001	26·1	0·38	0·001	0·70	0·001
B. nana	log(LAR)	1·13	0·05	0·143	0·04	0·6	1·19	0·001	−0·210	0·16	0·2	0·36	0·3
B. pubescens	log(LAR)	0·835	0·001	0·230	0·37	0·001	1·32	0·001	−0·479	0·38	0·001	0·56	0·001
B. nana	F_L	−0·0567	0·7	0·159	0·20	0·2	0·140	0·02	−0·0461	0·04	0·5	0·27	0·4
B. pubescens	F_L	0·00884	0·8	0·0749	0·34	0·001	0·138	0·001	−0·233	0·46	0·001	0·46	0·001

* logS = logarithm of average leaf size (cm²), logM = logarithm of average leaf dry mass (g), M_A = leaf dry mass per area (g m⁻²), log(LAR) = logarithm of leaf area ratio (cm² g⁻¹), F_L = fraction of foliar biomass in leaves (g g⁻¹), F_L = LAR × M_A.
† Both nitrogen content and logH included as explaining variables.
‡ LogH was not statistically significant in the multiple regression ($P > 0·4$).
§ N_M was not statistically significant in the multiple regression ($P > 0·9$).

statistically different. However, the upper soil horizons at the Voore site were significantly less acidic, with lower C/N molar ratio compared with the other sites (Table 1). Nevertheless, foliar nitrogen and phosphorus contents (Table 1) suggested that the nutrient availability was significantly larger at the fell sites than in the bog, possibly because of intensive soil mineralization following felling, or because the seedlings and saplings had access to deeper soil mineral horizons at these sites.

We observed a large within-site range in foliar nutrient contents, especially in the bog where foliar N contents ranged from 1·21 % to 2·74 %. Non-quantitative observations suggested that these differences were tightly related to the microheterogeneity in topography (e.g. the hummock–hollow gradient characteristic of the bogs, Titus and Wagner, 1984), and accordingly, in the water table level. In general, leaves with greater nutrient contents were sampled at higher locations, where the soil mineralization rates were likely to be higher.

According to Wassen *et al*. (1995), foliar P/N molar ratios >0·032 indicate primary N deficiency, and P/N ratios <0·02 indicate P deficiency in peat and raw humus soils. Given that the lowest P/N ratio observed in our study was 0·025 (Table 1), we conclude that the vegetation was primarily N-limited in all sites.

Average leaf area and mass in relation to species, tree size and foliar nitrogen content

Average leaf area was positively correlated with foliar nitrogen content per unit dry mass (N_M) in all species (Table 2, $r^2 = 0·49$, $P < 0·001$ for all data pooled). Taller trees also had larger leaves such that total tree height and S were positively related (Table 2, $r^2 = 0·08$, $P < 0·002$ for all

data pooled). Given that logH and N_M were essentially independent (Fig. 1), inclusion of both explaining variables into a multiple regression generally resulted in a higher fraction of explained variance than the separate regressions (Table 2, Fig. 2).

First ignoring the possible interactions, both the one-way analyses of variance (Table 3) and covariance (common slope model with logH and N_M as covariates) suggested that at a common height and foliar nitrogen content, *B. nana* had the smallest leaves, *B. pubescens* intermediate, and *B. pendula* the largest leaves ($P < 0·001$; Table 3). Thus, interspecific variability was an important determinant of leaf size. However, further analysis of the species effects by a separate slope model indicated that the interaction term Species × N was also significant ($P < 0·001$, separate slope analysis of co-variance), as well as the interaction term Species × logH ($P < 0·05$). These significant interactions were indicative of lower responsiveness of leaf area in *B. pubescens* to N_M, and logH (lower slope values, Table 2).

The species ranking was also identical in terms of average leaf dry mass (Table 3). As with the leaf area, the responsiveness of leaf dry mass to N_M and logH was lower in *B. pubescens* than in the other species ($P < 0·03$ for Species × N and $P < 0·05$ for Species × logH interaction).

Effects of tree size and nitrogen content on leaf dry mass per unit area (M_A)

Increases in the area of individual leaves with N_M were larger than increases in the dry mass, such that M_A scaled negatively with nitrogen content per unit dry mass (Fig. 3A, Table 2). Similarly, lower responsiveness of leaf area than leaf mass to logH led to a positive relationship between M_A and logH (Fig. 4A, Table 2). The scaling of M_A with N_M was

TABLE 3. *Averages (± s.e.) of foliage structural and chemical characteristics and foliar construction cost: results of one-way analyses of variance**

Species (site)	Leaf size (cm^2)[†]	M_A (g m^{-2})	LAR (cm^2 g^{-1})[†]	N (%)	C (%)	G (g glu g^{-1})
B. nana (Endla)	0·90 ± 0·19[d]	84·1 ± 3·1[a]	21·3 ± 2·7[c]	1·56 ± 0·06[c]	49·66 ± 0·19[b]	1·500 ± 0·010[b]
B. pendula (Endla)	5·1 ± 1·8[bc]	74·8 ± 3·1[a]	11·7 ± 3·1[c]	1·89 ± 0·20[c]	49·33 ± 0·33[b]	1·485 ± 0·017[b]
B. pendula (Voore)	16·8 ± 2·4[a]	69·6 ± 3·4[ab]	n.d.	2·57 ± 0·06[b]	n.d.	n.d.
B. pubescens (Endla)	4·72 ± 0·44[b]	84·8 ± 2·5[a]	18·7 ± 2·5[c]	1·65 ± 0·05[c]	48·69 ± 0·20[b]	1·451 ± 0·010[b]
B. pubescens (Viimsi-1)	6·3 ± 0·6[b]	49·4 ± 1·2[c]	92·6 ± 4·9[a]	3·17 ± 0·12[a]	54·7 ± 0·7[a]	1·762 ± 0·034[a]
B. pubescens (Viimsi-2)	12·2 ± 1·0[a]	56·7 ± 2·1[bc]	35·8 ± 2·7[b]	3·08 ± 0·05[a]	54·14 ± 0·15[a]	1·739 ± 0·008[a]

M_A = leaf dry mass per unit area, LAR = leaf area ratio (total leaf area per unit total plant mass), G = leaf construction cost (eqn. 1), n.d. = not determined.

* Each individual species-site pair was used as a factor level. Means followed by the same letter are not significantly different at $P < 0·05$.

[†] The variable was transformed to a logarithm before statistical comparisons.

significant only in *B. pubescens*, and scaling with log$H$ in *B. nana* and *B. pubescens* (Table 2). Nevertheless, the species effect ($P > 0·6$) as well as Species × N ($P > 0·4$) and Species × log$H$ ($P > 0·8$) interactions were non-significant, indicating that despite the data scatter in *B. nana* and *B. pendula*, they adhered the same overall trend (Fig. 4A).

All plants included in the study were exposed to more than 85 % of the light in a completely open location. However, the values of M_A depicted in Figs 3A and 4A were obtained either as an average of all leaves per plant or as the average of uppermost exposed leaves. To test the possibility that the negative scaling of M_A with N_M resulted from greater self-shading because of a larger foliar area in plants with better N nutrition, we also computed the regressions separately for the whole plant average M_A and for the uppermost M_A values. In both sets of data, M_A was negatively correlated with N_M and positively with log$H$. For all species pooled, $r^2 = 0·55$ ($P < 0·001$ for both independent variables) for the uppermost leaves, and $r^2 = 0·61$ ($P < 0·001$) for the whole plant averages. Thus, increased self-shading within the canopy did not provide the only explanation for the strong negative relationships between M_A and N_M (Table 2, Fig. 4A).

Leaf nitrogen content per unit area ($N_A = N_M \cdot M_A$) and M_A were positively related (Fig. 3B). However, because of the differences in N_M and a negative relationship between M_A and N_M (Table 2, Figs 3A and 4A), the intercept of the M_A *vs.* N_A relationship was larger in the leaves with lower N_M ($P < 0·001$ for the site effect, Fig. 3B). Despite N_M being independent of tree height (Fig. 1), positive effects of log$H$ on M_A (Fig. 4A) also led to a positive correlation between N_A and log$H$ ($r^2 = 0·43$, $P < 0·001$).

Dependence of leaf area ratio (LAR) and fraction of plant biomass in leaves (F_L) on tree size and nitrogen content

Leaf area ratio (total foliar area per total plant dry mass) was negatively related to log$H$ and positively to N_M in *B. pubescens*, but not in *B. nana* (Table 2, Fig. 4B). However, covariance analysis demonstrated that the values of LAR of *B. nana* overlapped with those observed in *B. pubescens*. Both interaction terms, Species × log$H$ and Species × N, were non-significant ($P > 0·6$ for both) according to the

separate slope ANCOVA model, and the main effect, Species, was insignificant according to the common slope model ($P > 0·08$). Thus, despite the very different plant architecture, the LAR values of both species were similar.

The fraction of plant biomass in foliage (F_L) was also negatively related to log$H$ and positively to N_M (Table 2, $r^2 = 0·38$, $P < 0·001$ for all data pooled). Again, the interaction terms were non-significant in the separate slope model ($P > 0·7$ for both) and the species effect was non-significant according to the following common slope analysis ($P > 0·1$).

Given that LAR is dependent on both M_A and F_L (LAR = F_L/M_A; Fig. 5), the plasticity in both of its components with respect to N_M and log$H$ controls the variation of LAR along gradients of nutrient availability and with tree size. To determine the extent to which the N_M- and log$H$-related variability in LAR can be ascribed to variation in F_L and M_A, we calculated the expected values of F_L and M_A using the minimum and maximum measured values of log$H$ and N_M in interspecific regressions (Fig. 4A). For the observed range of log$H$ (−1·50 … 0·23), the expected value of F_L varied 6·7-fold, and that of M_A only 1·9-fold. For the observed range in N_M (1·21 … 3·36), the expected value of F_L varied 1·9-fold, and that of M_A 1·3-fold. Thus, we conclude that F_L responded more plastically than foliar structure to both log$H$ and N_M, and was, accordingly, a more important determinant of LAR.

Foliar photosynthetic characteristics and construction cost vs. M_A, nitrogen and tree height

The light-saturated rate of photosynthetic electron transport per unit dry mass (J_M) was positively related to foliar nitrogen content (Fig. 6A), whereas the regressions were significant for each individual species ($P < 0·05$), as well as for all data pooled. However, J_M scaled negatively with M_A (Fig. 6B). The rate of photosynthetic electron transport per unit area (J_A) correlated also positively with N_A (Fig. 6C), but because of the negative correlation between J_M and M_A, J_A ($J_A = J_M \cdot M_A$) was independent of M_A (Fig. 6D). A multiple regression analysis of J_M *vs.* M_A and N_M ($r^2 = 0·73$) indicated that the negative scaling of J_M did not solely result from N_M effects on M_A (Fig. 3A), but both M_A ($P < 0·001$)
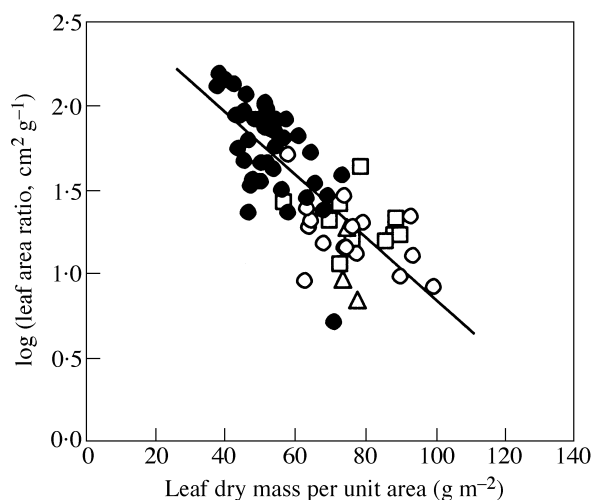
FIG. 5. Leaf area ratio in relation to leaf dry mass per unit area. The regression was fitted through all data. Symbols as in Fig. 1.

and N_M ($P < 0.001$) were significant determinants of J_M. The ratio of J_M to N_M was negatively related to M_A ($r^2 = 0.30$, $P < 0.001$), further showing that the nitrogen use efficiency in photosynthesis declines with increasing M_A.

Multiple linear regression of J_M *vs.* N_M and log$H$ indicated that, at common N_M, J_M was lower in larger trees ($r^2 = 0.68$, $P < 0.01$ for log$H$), possibly because of a larger M_A (cf. Figs 4A and 6B). However, J_A did not depend significantly on log$H$ ($P > 0.1$) in the multiple regression of J_A *vs.* N_A and log$H$. None of the correlations highlighted differed between species ($P > 0.5$ according to common-slope ANCOVA analyses).

Foliar construction cost (G) tended to be higher in sites with greater nutrient availability (Table 3) and there was a positive relationship between G and N_M for all data pooled ($r^2 = 0.63$, $P < 0.001$), but not with log$H$ ($P > 0.4$).

DISCUSSION

Changes in leaf size with nitrogen status

Strong positive relationships between average leaf area and foliar nitrogen content were found in all species (Fig. 2, Table 2). According to laboratory studies, enhanced nitrogen availability increases both the rate of leaf expansion as well as cell division (MacAdam *et al.*, 1989; Taylor *et al.*, 1993; Roggatz *et al.*, 1999) and leads to larger individual leaves (Karlsson and Nordell, 1989; Trápani and Hall, 1996; Sims *et al.*, 1998*a*). Whole plant experiments further indicate that, in addition to larger leaves, the number of leaves may also increase with increasing N availability (Ackerly and Bazzaz, 1995; Grubb *et al.*, 1996). Although the same total foliar area may be formed by a fewer larger leaves or by a larger number of smaller leaves, we suggest that the plant strategy to increase the area of individual leaves with increasing nitrogen availability has distinctive ecological advantages over the strategy to increase leaf number only. First, height growth becomes an increasingly

more important competitive attribute as the nutrient limitations decrease, because enhanced nutrient supply leads to augmented light competition (Tilman, 1986; Gleeson and Tilman, 1992). As the interspecific correlations suggest, the branching requirements are less for larger leaves (Givnish, 1984; King, 1991, 1998). Thus, larger leaves allow a greater fractional investment of plant biomass in height growth relative to the lateral growth (King, 1991). In addition, simulation studies demonstrate that with increasing length of leaf blades and petioles, the degree of foliar self-shading by other leaves and stem decreases, improving the light interception efficiency of unit foliar area (Takenaka, 1994). All the species of *Betula* studied here possess short petioles, and increases in leaf lamina size should significantly improve the light interception efficiency in these species.

Given this ecological reasoning, interspecific differences in leaf size (Table 3) may partly explain the contrasting competitive ability of the studied species (*B. nana* < *B. pubescens* < *B. pendula*) in nutrient-rich early-successional habitats. Especially in *B. nana*, the extremely small leaf size and lack of petioles leads to a large fraction of foliar area that is shaded by the stem. We suggest that this, in combination with genetically constrained height growth potential, is the primary factor limiting the competitive potential of this relic of the last glaciation period in nutrient-rich habitats.

Leaf dry mass per unit area in relation to foliar nitrogen

In agreement with the contrasting patterns of nitrogen content *vs.* M_A relationships of previous studies, M_A *vs.* N_M relations were weaker than leaf size *vs.* N_M dependencies (Table 2). Nevertheless, there was a statistically robust negative correlation between N_M and M_A in *B. pubescens* (Table 2). Although the M_A of both *B. nana* and *B. pendula* was non-significantly related to N_M, these data fit the same regression surface with *B. pubescens* (Fig. 4A), indicating a functional convergence in foliar characteristics. These non-significant relationships may be a manifestation of lower plasticity, but may have also resulted from clustering of M_A data of *B. nana* and *B. pendula* at relatively low N contents (Fig. 4A).

The relationship between N_M and M_A was qualitatively identical when a whole-plant average M_A or M_A of the uppermost leaves was used in the regressions. This consistency demonstrates that the negative effect of site nutrient conditions on M_A was not solely caused by increased self-shading within the canopy. Given that self-shading was ruled out as the only determinant of low M_A at higher N_M, it is pertinent to ask by which additional mechanisms the changes in M_A in *B. pubescens* may have occurred along the nutrient gradients. Fundamental insight into the nutrient availability effects on M_A may be obtained by distinguishing between the two components of M_A, leaf thickness (T) and density (D, $M_A = T \cdot D$), that may vary independently (Witkowski and Lamont, 1991; Niinemets, 1999). The strong positive relationship between lateral expansion growth and N_M suggests that the growth in thickness may also respond to enhanced N availability in a similar
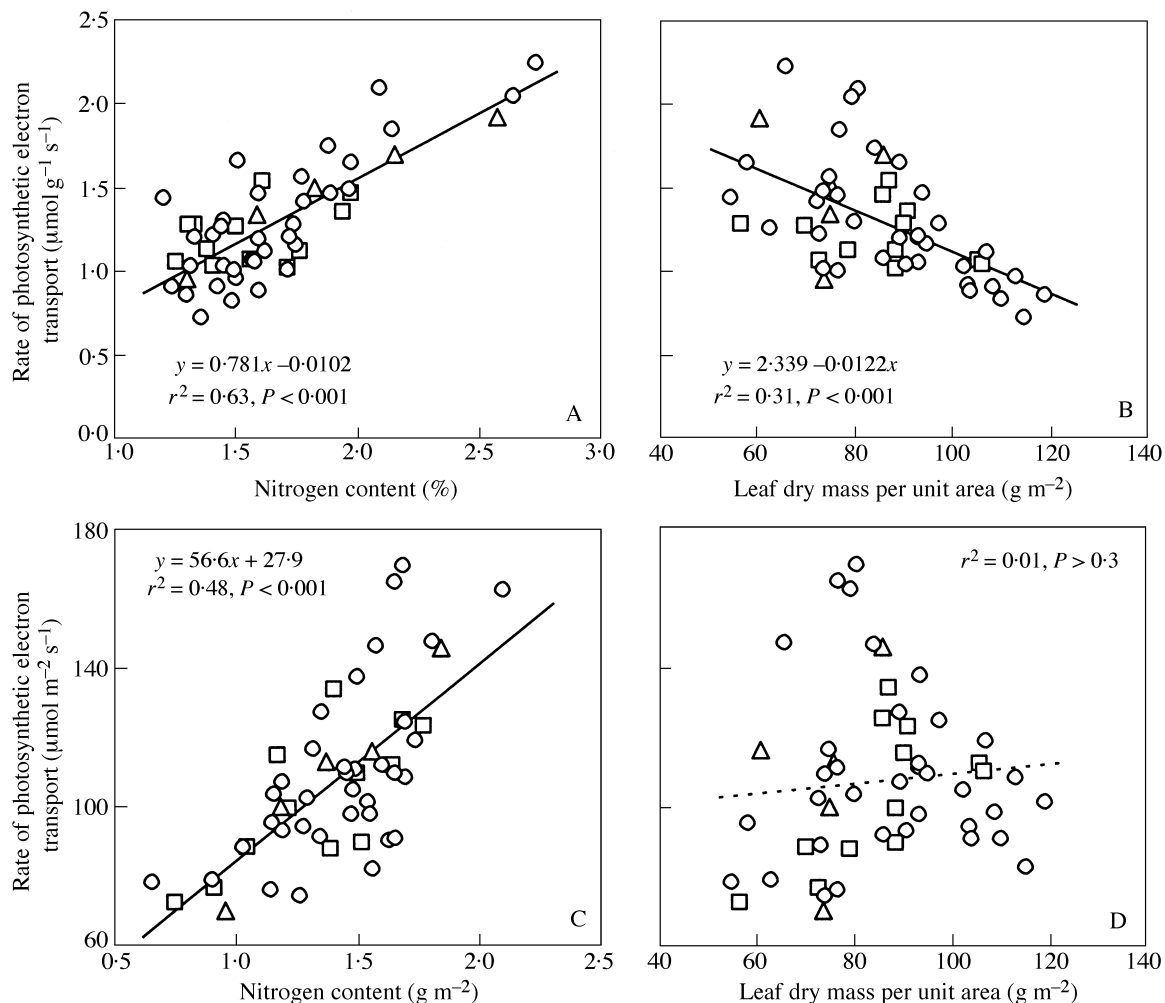
FIG. 6. Relationships between the rate of light-saturated photosynthetic electron transport (*J*) per unit leaf dry mass in dependence on leaf nitrogen content per unit dry mass (A) and M_A (B); and *J* per unit leaf area in relation to nitrogen content per unit area (C) and M_A (D). The rate of photosynthetic electron transport was determined from chlorophyll fluorescence measurements (eqn. 2). The data were fitted by linear regressions. Symbols as in Fig. 1.

manner. Such a positive relationship has been found in some investigations (Jurik *et al.*, 1982; Thompson *et al.*, 1992; Sims *et al.*, 1998*a*), and may be the consequence of larger cells in leaves produced at higher N availability (Roggatz *et al.*, 1999). Yet, there are consistent negative effects of N on *D* (Witkowski and Lamont, 1991; Niinemets *et al.*, 2001, recalculated from Jurik *et al.*, 1982; Thompson *et al.*, 1992) that are generally larger than the positive effects of N on *T*, such that the product variable, M_A, decreases with increasing nutrient availability (Jurik *et al.*, 1982; Thompson *et al.*, 1992). Data demonstrate that the number of cells per unit area is larger (Roggatz *et al.*, 1999), and the fraction of intercellular air space lower (Jurik *et al.*, 1982) in nitrogen-limited leaves, providing an explanation of greater density of these leaves. Moreover, starch (Waring *et al.*, 1985; Bowler and Press, 1996; Sims *et al.*, 1998*b*) and lignin (Waring *et al.*, 1985) contents may accumulate, further increasing leaf density at a common thickness.

Modification of leaf size and M_A by tree size

Average leaf area was positively related to total tree height in three *Betula* species (Fig. 2), as in previous studies on young trees of *Betula pubescens* ssp. *tortuosa* (Sveinbjörnsson, 1987; Senn *et al.*, 1992). Apart from the ecological benefits of larger leaves in terms of the efficiency of height growth, shorter plants have branches and stems with lower diameter. Accordingly, both mechanical and hydraulic constraints may be responsible for the limited maximum leaf size in smaller plants. Leaf size generally scales positively with the diameter of supporting branchlet (White, 1983). Although we observed a continuous increase in leaf size with increasing plant height (Fig. 2), other investigations conducted over a larger range of plant height and age indicate that the leaf size *vs.* plant age or height relationship may be a curve with a maximum, with the leaf size either levelling off or decreasing after the leaves have reached the maximum size (Montfort and Müller, 1951; Steele *et al.*, 1989). Increasing hydraulic limitations and

greater water stress in taller trees (Ryan and Yoder, 1997; Mencuccini and Magnani, 2000) may provide an explanation for this curvilinearity.

In contrast to the non-linear relationships between leaf size and plant height, M_A increases linearly with increasing tree size (Steele *et al.*, 1989; Niinemets 1997*a*; Niinemets *et al.*, 1999), as was also observed in our study in *B. nana* and *B. pubescens* (Table 2, Fig. 4A). Similarly to M_A increases in nutrient-limited leaves, the positive scaling of M_A with tree size primarily results from increases in leaf density (Niinemets, 1997*a*, 1999) rather than from increases in thickness (but cf. Malkina, 1983; Niinemets *et al.*, 1999). Because increases in density are compatible with larger tissue elastic moduli that allow a greater change in leaf water potential with a lower tissue water loss (Niinemets, 2001), it has been suggested that the tree-size related increase in M_A is an acclimation response to a more severe water stress in the canopies of larger trees (Niinemets 1997*a*).

Biomass partitioning in relation to N and tree size

Increases in M_A with increasing tree size and with decreasing nitrogen content (Fig. 4A) in *B. pubescens* evinced that construction of extensive foliar area in terms of foliar biomass investment in leaves may be more expensive in taller trees and at low N (Figs 4B and 5). Our study indicates that, in all species, changes in LAR (LAR = F_L/M_A) resulted primarily from modifications in fractional biomass allocation to leaves, F_L, rather than in M_A. Because the plants grow faster at a greater nutrient supply, the plants of the same age are also larger at higher nutrient availability. Given the scaling of plant size with nutrient availability and M_A with tree size (Fig. 4A), we hypothesize that the plastic modifications in M_A in response to nutrient availability may be constrained by tree size effects on M_A.

The evidence that N_M effects on M_A, LAR and F_L were non-significant in *B. nana* but significant in *B. pubescens* (Table 2) confirms quantitatively the suggestion that more competitive species with larger growth rates are more responsive to nutrients (Coomes and Grubb, 2000). However, the non-significant effect of N_M on M_A in *B. pendula* apparently contradicts this explanation. Nevertheless, although we did not study LAR and F_L in *B. pendula*, previous investigations have demonstrated that LAR and F_L are plastically modified along nutrient availability treatments in this species (McDonald *et al.*, 1986, 1992). Thus, the allocation patterns suggest that *B. pendula* fits the general hypothesis of a larger plasticity in more competitive species.

Nitrogen status and tree size vs. the foliage photosynthetic characteristics and the construction cost

As observed previously (for a review, see Field and Mooney, 1986), increased foliar N contents strongly enhanced foliar photosynthetic capacities (Fig. 6A, C). Studies dealing with the leaf structure *vs.* photosynthetic capacity relationships along light gradients have further found a strong positive correlation between M_A and foliage

photosynthetic capacity per unit area (e.g. Ellsworth and Reich, 1993; Niinemets *et al.*, 1998*a*). This correlation has been interpreted as indicative of greater thickness of leaves with larger M_A (Niinemets *et al.*, 1999), and consequently, characterizing an accumulation of photosynthetic biomass per unit leaf area with increasing M_A (Niinemets *et al.*, 1998*a*). However, we found no relationships between the capacity of photosynthetic electron transport (J) per unit area (J_A) and M_A (Fig. 6D), and a negative correlation between J per unit dry mass (J_M) and M_A (Fig. 6B). We hypothesize that the negative scaling of J_M with M_A at a common foliar nitrogen content resulted from increases in leaf density with decreasing nutrient availability. Leaves with a greater density have larger intercellular gas- and liquid-phase transfer resistances to CO_2 (Syvertsen *et al.*, 1995), and consistently lower photosynthesis rates at common stomatal limitations (Niinemets, 1999).

Studies have brought evidence of a negative scaling of foliar nitrogen contents per unit dry mass (Niinemets, 1997*a*) and leaf assimilation potentials with increasing tree size (Grulke and Miller, 1994). Although the positive effects of tree size on M_A led to a greater nitrogen content per unit leaf area in our study, J_A was independent of tree size, and J_M decreased with increasing tree size. Greater density and diffusive limitations in leaves with greater M_A may also be responsible for the negative effects of tree height on J_M in the three *Betula* species studied.

Despite the extensive modifications in M_A (Fig. 4A), foliage construction cost (G) changed little across different foliar morphologies. Although carbon-rich lignin (63·3 %, calculated according to Nimz, 1974) and tannins may accumulate at lower nutrient availabilities (Waring *et al.*, 1985), there is also evidence of higher contents of non-structural carbohydrates in nutrient-limited leaves (Waring *et al.*, 1985). Given that the non-structural carbohydrates have low carbon content (44·4 % for starch), simultaneous accumulation of both the expensive and cheap compounds at low nutrient availability may be a reason for lower G at low nutrient availability. Yet, we found that G was strongly related to N_M, which provides an estimate of foliar protein contents. Because proteins are rich in carbon (53·5 %), the construction of leaf photosynthetic apparatus with high N content is also costly in terms of carbon investments in unit foliar biomass. Although G should not always be related to site fertility (Chapin, 1989), our finding agrees with the majority of studies (Lafitte and Loomis, 1988; Griffin *et al.*, 1993; Griffin *et al.*, 1996; Poorter and Villar, 1997) demonstrating a larger construction cost in leaves with higher N content.

CONCLUSIONS

The information summarized indicates that the effects of internal plant N on M_A are complex in temperate deciduous woody species, and may partly be mediated through enhanced self-shading and increased plant size at higher nutrient availability. Yet, there are also important independent effects of nitrogen on foliar structure, which seem mainly to result in denser leaves at lower N. Increases in density may provide an explanation for a stronger reduction

in foliar photosynthetic capacities with decreasing nitrogen content than may be predicted from decreases in N_M alone. High-density mesophyll is generally an adaptation to water-limited environments such as Mediterranean communities and semi-deserts (Niinemets, 2001), and the functional significance of dense leaves in nutrient-limited habitats remains to be discovered.

The biomass allocation responds more readily to differences in N than M_A, indicating a larger plasticity in allocation than in foliar structure. However, as the trees increase in size, the allocation in foliage consistently decreases. Thus, the positive effect of high internal nutrient availability on foliar biomass investment in leaves becomes buffered by concomitant increases in plant size that reduce F_L. Given the strong controls of plant size, the studies on N influences on foliar structure and plant biomass allocation should also include plant size as a covariate in statistical comparisons.

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