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Ontogenetic patterns of leaf CO_2 exchange, morphology and chemistry in *Betula pendula* trees

Abstract In order to explore ontogenetic variation in leaf-level physiological traits of *Betula pendula* trees, we measured changes in mass- (A_{mass}) and area-based (A_{area}) net photosynthesis under light-saturated conditions, mass- (RS_{mass}) and area-based (RS_{area}) leaf respiration, relative growth rate, leaf mass per area (LMA), total nonstructural carbohydrates (TNC), and macro- and micronutrient concentrations. Expanding leaves maintained high rates of A_{area}, but due to high growth respiration rates, net CO_2 fixation occurred only at irradiances >200 µmol photons m^{-2} s^{-1}. We found that full structural leaf development is not a necessary prerequisite for maintaining positive CO_2 balance in young birch leaves. Maximum rates of A_{area} were realized in late June and early July, whereas the highest values of A_{mass} occurred in May and steadily declined thereafter. The maintenance respiration rate averaged ≈8 nmol CO_2 g^{-1} s^{-1}, whereas growth respiration varied between 0 and 65 nmol CO_2 g^{-1} s^{-1}. After reaching its lowest point in mid-June, leaf respiration increased gradually until the end of the growing season. Mass and area-based dark respiration were significantly positively correlated with LMA at stages of leaf maturity, and senescence. Concentrations of P and K decreased during leaf development and stabilized or increased during maturity, and concentrations of immobile elements such as Ca, Mn and B increased throughout the growing season. Identification of interrelations between leaf development, CO_2 exchange, TNC and leaf nutrients allowed us to define factors related to ontogenetic variation in leaf-level physiological traits and can be helpful in establishing periods appropriate for sampling birch leaves for diagnostic purposes such as assessment of plant and site productivity or effects of biotic or abiotic factors.

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Introduction

Measurements of CO_2 exchange and several other leaf characteristics show promise for use in assessment of plant productivity, response to predicted global climatic changes and to the effects of biotic and abiotic stress factors (Oleksyn et al. 1998a,b; Reich et al. 1991, 1994; Rey and Jarvis 1998). In recent years substantial progress has been made in assessment of factors affecting interspecific variation of CO_2 exchange. It has been shown that photosynthetic capacity, defined as the light-saturated rate of net photosynthesis, is highly correlated with leaf mineral nutrients (Field and Mooney 1986; Gower et al. 1993; Oleksyn et al. 1998b) and influenced by such traits as leaf mass per unit area (LMA), or leaf life-span (Reich et al. 1991, 1997).

Substantially less attention has been devoted towards understanding and characterizing ontogenetic changes in photosynthesis, respiration, leaf N, and morphology during leaf development. There are also potential complications in comparison of data on CO_2 exchange expressed on area and mass bases which can be affected by seasonal variation in leaf total nonstructural carbohydrates (TNC) concentration, because changes in carbohydrate concentration could affect mass-based rates without affecting photosynthesis or respiration rates per unit leaf area (Niinemets 1997; Reich et al. 1998). Attempts to address interrelations between physiological and morphological traits during woody plant leaf ontogeny have been limited in number and often originate from the insertion profiles on leaves developed from different primordia under varying microenvironmental conditions. Therefore, changes in insertion gradients frequently do not resemble those observed during the leaf ontogeny

(for review see: Šesták and Čatský 1985; Tichá et al. 1985; Čatský and Šesták 1997).

The objective of our study was to examine ontogenetic patterns of leaf-level gas exchange, morphology, macro- and micronutrients and TNC for sun leaves of open-grown trees of silver birch (*Betula pendula*) over the course of the entire growing season. By conducting an assessment of seasonal and developmental patterns in the above parameters, we attempt to identify underlying traits related to variation in CO_2 exchange during leaf ontogeny.

Materials and methods

Study site and plant material

Measurements were taken 17 times, between 26 April and 11 October 1996 (Fig. 1), on two neighboring 40-year-old silver birch (*Betula pendula* Roth., syn. *B. verrucosa* Ehrh.) trees of local origin grown at the Arboretum of the Institute of Dendrology in Kórnik, Poland (52°15'N and 17°04'E, elevation 85 m). The trees were planted on well-drained, sandy loam soil. The average annual precipitation is 626 mm and average temperature 7.7°C, with a mean growing season length (measured as the number of days with temperature reaching >5°C) of 220 days. In 1996, growing season precipitation was evenly distributed and slightly above the long-term average.

The trees were 18 and 22 m tall and were 23.6 and 24.3 cm in diameter respectively at a height of 1.3 m. During the sampling period measurements of CO_2 exchange were taken on 54–62 leaves per sampling date on unshaded branches from the southeastern sides of the crowns. Since we sampled open-grown trees, leaves from these positions adequately represented most of the leaves formed at the beginning of the growing season. Less than 0.5% of the foliage had been sampled by the end of the year.

B. pendula is a species with sylleptic growth where a phase of fixed growth is followed by a phase of free growth each season (Harper 1989). As a result, the distribution of leaf age within a birch shoot varies widely at the end of the growing season ranging from 80 to 140 days (Koike 1990). To sample leaves of comparable age all measurements were made for leaves that developed at the beginning of the growing season.

Foliar growth and gas exchange and changes of leaf mean dry mass and area over time were analyzed using a functional approach to plant growth analysis (Hunt 1982) using the Morgan-Mercer-Florin Growth Model (JMP, SAS Institute, Cary, N.C., USA). By definition, the slope of a function describing changes in natural log-transformed leaf mass or area over time is the relative growth rate (RGR). Taking the first derivative of the functions provided estimates of RGR at discrete points of time (Hunt 1982).

Gas exchange rates of *B. pendula* leaves were measured with portable photosynthetic systems (LCA-3, Analytical Development Corporation, Hoddesdon, England) used in the differential mode. The system was calibrated against known CO_2 standards. Net photosynthesis was measured according to previously described protocols (Oleksyn et al. 1998a) on intact leaves under field conditions using the Parkinson leaf chamber PLC-B. Measurements of net photosynthesis were always taken on sunny days between 0900 and 1300 hours. Light-response curves were determined under field conditions using multiple layers of neutral density shade cloth to alter the incident photosynthetic photon flux density (PPFD). In order to avoid possible adverse effects of prolonged leaf enclosure in the leaf chamber, during measurements of light response only one to four measurements at different PPFD were taken on each leaf. During the measurements, air temperature ranged from 17.5°C in early spring and late autumn to 28°C in mid-summer (average 24°C), ambient relative humidity varied from 37% to 84% (average 64%), and ambient CO_2 concentration averaged 379 μmol mol⁻¹. Light-response curves fitted a nonlinear
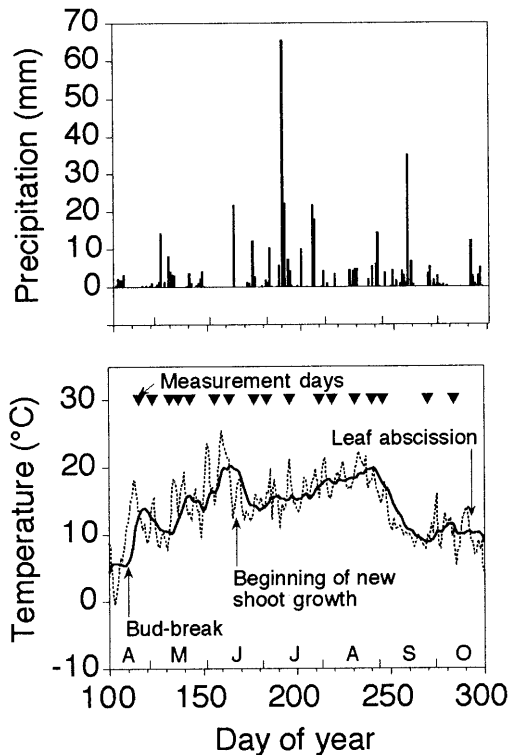

Fig. 1 Pattern of precipitation and average daily (*dashed line*) and running 10-day mean temperature (*solid line*) for the study site in 1996

regression model (Hanson et al. 1987). Each measurement lasted until a new steady-state rate of photosynthesis was reached.

Dark respiration rates were measured on intact leaves between 0900 and 1300 hours after darkening the cuvette with a double-layer dark cloth for at least 15 min. Evaluation of RS rates taken from 15 to 45 min after cuvette darkening indicated no obvious short-term changes in RS sometimes observed as a result of the light-enhanced dark respiration (Collier and Grodzinski 1997). After gas exchange measurements, leaves were oven-dried at 65°C and weighed. The dark respiration rates values were adjusted to 20°C applying a temperature-correlated Q_{10} (Tjoelker 1997). Throughout the growing season the Q_{10} for the range of RS measurement temperatures ranged from 2.0 to 2.4 (average 2.17±0.03 SE).

Leaf-level maintenance respiration rates were calculated by regressing RS_{mass} (mass-based rate of dark respiration at 20°C, nmol CO_2 g⁻¹ s⁻¹) against RGR, where the intercept m is the rate of maintenance respiration (RS_{maint} nmol CO_2 g⁻¹s⁻¹) (Tjoelker et al. 1999). The rates of growth respiration (RS_{growth}) were calculated by subtracting maintenance respiration from the total respiration rate,

$$RS_{growth} = RS_{mass} - RS_{maint} \tag{1}$$

Data on gas exchange are expressed on area, mass, and mass without TNC basis, since each provides different information (Niinemets 1997; Reich and Walters 1994; Reich et al. 1998). The projected leaf area was determined using an image analysis system and the WinFOLIA Pro Software (Regent Instruments, Quebec, Canada). Leaf mass per area (LMA, defined as the leaf dry mass divided by projected leaf area) was calculated for the same leaves used for CO_2 exchange.

Measurements of foliage nonstructural carbohydrates and nutrients

Total soluble and nonstructural carbohydrate (TNC) concentrations were determined using a modification of the method de-

scribed by Haissig and Dickson (1979) and Hansen and Møller (1975). Data are means of two replications (one per tree) from composite samples for each date. Leaf tissue for TNC was analyzed for 16 sampling dates, since there was not enough leaf tissue to conduct carbohydrate analyses on April 26.

Nitrogen (N) was measured on dried (65°C for 48 h) tissue powdered in a Microdysmembrator (Melesungen, Germany), using the same leaves that were used in CO_2 exchange and TNC measurements. The samples were digested by the micro-Kjeldahl method and analyzed.

Analyses of foliar concentrations of P, K, Ca, Mg, Mn, Fe, Cu, Zn, Al, B, Pb, Ni, Cr and Cd were done simultaneously with an inductively coupled plasma emission spectrometer (ICP-AES, model ARL 3560) at the University of Minnesota Research Analytical Laboratory, St. Paul, Minn., USA. The sampling procedure and number of replications for nutrient analyses were the same as for the TNC measurements except for the 3 May sampling date for which we did not have enough tissue to conduct analyses. In order to eliminate the variation of dry mass due to seasonal changes in nonstructural carbohydrates, all nutrient concentrations are expressed on TNC-free mass and on area basis.

N retranslocation was calculated as:

$$N_{ret} = \frac{N_{max} - N_{sen}}{N_{max}} \cdot 100\%, \tag{2}$$

where N_{max} is the maximal N concentration for mature leaves (after leaf mass reached plateau) and N_{sen} is the concentration obtained from the last measurement conducted in autumn in senesced leaves.

For all variables, differences among leaf development stages were calculated using analysis of variance (GLM procedures). Relationships between the day of year, leaf N, and other studied traits were made using correlation and regression analyses. All statistical analyses were conducted using JMP software (version 3.1.5, SAS Institute, Cary, N.C., USA).

Results

Leaf age effects on the patterns of leaf CO_2 exchange and morphology

There were differences in growth patterns of leaf area versus leaf mass (Fig. 2). The initial increase of leaf area was faster than for leaf mass and there was a 35 day difference between the culmination of leaf area and mass growth, defined as the day of year when 99% of final leaf mass or area was completed. These differences are reflected in changes of leaf mass per area which increased almost linearly until the end of June and beginning of July, when it reached a plateau (Fig. 2). Leaf area RGR reached ≈0.14 cm^2 cm^{-2} day^{-1} at the beginning of the growing season and steadily declined until the beginning of June (data not shown). Leaf area growth rate changed from ≈0.8 cm^2 day^{-1} in April to ≈0 in mid-July (Fig. 2). A similar pattern was observed for leaf mass growth rate.

Bud-break in *B. pendula* took place on 20 April 1996, when daily temperature had averaged 5°C for ≈10 days (Fig. 1). Six days later the area-based rate of net photosynthesis under light-saturated irradiance (A_{area}) was 30% of the highest seasonal value (Fig. 3). There were differences in the seasonal pattern of area versus mass-based rates of light-saturated net photosynthesis (A_{mass}) in *B. pendula* leaves. Maximum rates of A_{area} ≈10 µmol m^{-2} s^{-1} occurred
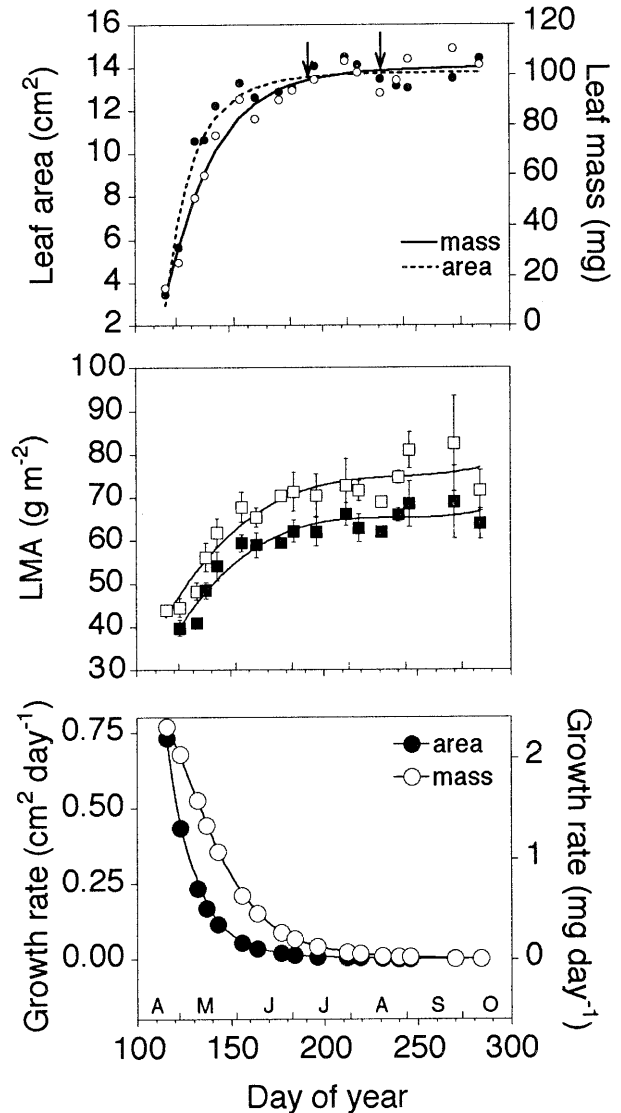

Fig. 2 *Top two graphs* Leaf area, mass and leaf mass per area (LMA, g m^{-2}) in relation to the day of the year for *Betula pendula* leaves. LMA is shown both on TNC-free leaf mass (*closed squares*) and leaf mass with TNC (*open squares*). *Bottom graph* Leaf area and mass growth rate in relation to the day of the year. Here and in all other figures standard errors are shown

in late June and early July (≈170–180th day of year), whereas the highest values of A_{mass} (expressed on TNC-free mass) ≈180 nmol g^{-1} s^{-1} occurred very early in the growing season on May 12 and steadily declined with time to ≈120 nmol g^{-1} s^{-1} in late autumn (Fig. 3). There was also a significant positive relationship between the leaf K:Ca ratio and A_{mass} and a negative correlation between A_{mass} and Ca concentration (r^2, ≥0.53, P≤0.002, data not shown). The Ca concentration can be used as a measure of the physiological age of leaves (Meier-Dinkel and Kleinschmit 1990). Both A_{mass} and A_{area} remained relatively high until the end of the growing season. Light-saturated photosynthesis on a leaf N basis did not vary significantly throughout the growing season (P=0.25) and averaged 57±2 µmol CO_2 mol N^{-1} s^{-1} (data not shown).

Fig. 3 Measured *in situ* (**a**) area-based and (**b**) mass-based light saturated net photosynthesis and dark respiration rates in relation to day of year for *B. pendula* trees. Values of A_{mass} are expressed on TNC-free mass. All RS rates were adjusted to 20°C. Each point is a mean of 3–13 measurements taken on individual leaves from both of two trees
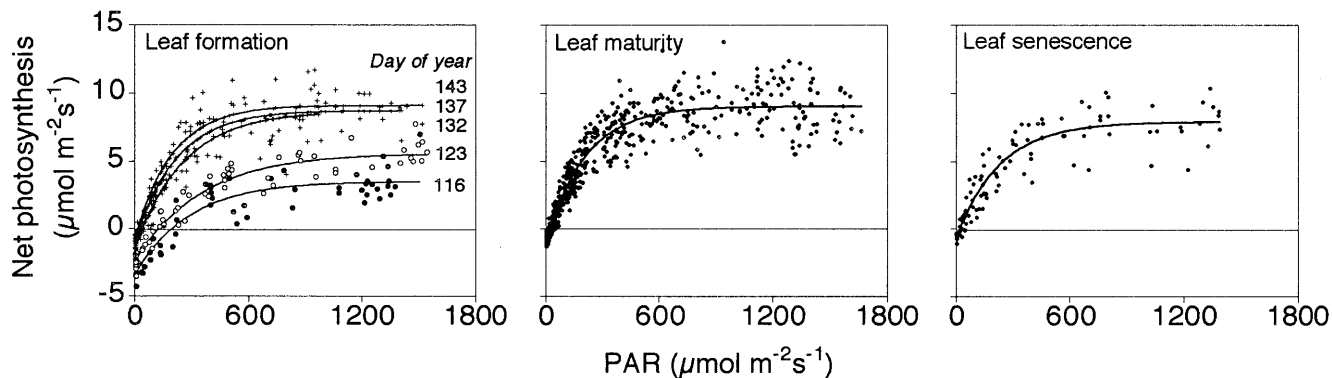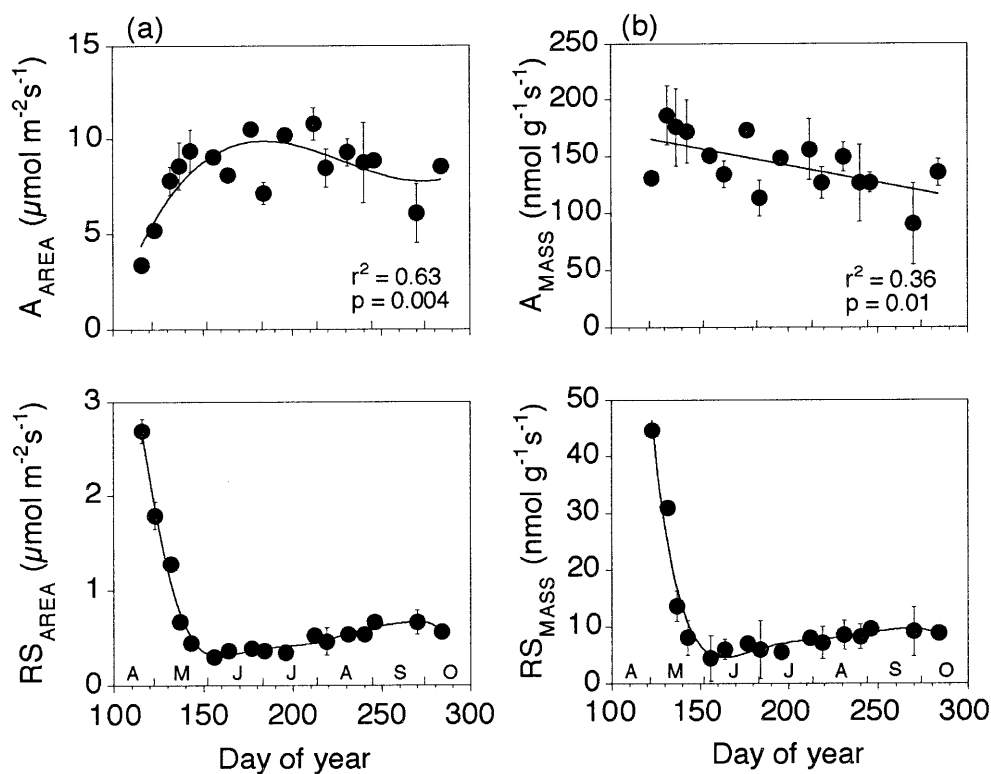

Fig. 4 Seasonal changes in light-response of photosynthesis in *B. pendula* leaves. In the left graph (Leaf formation) light saturation curves are shown separately for different days of year; in the middle graph (Leaf maturity) for days from 156 to 240, and in the right graph (Leaf senescence) for days from 246 to 280. See Table 1 for parameter values

Leaf respiration rate declined rapidly within the first 6 weeks of leaf development (Fig. 3). During that period of time, area- and mass-based respiration adjusted to 20°C (RS_{area} and RS_{mass}, adjusted for TNC) declined ≈10-fold from 2.7 to ≈0.3 μmol m^{-2} s^{-1} and 45 to 4.4 nmol g^{-1} s^{-1}, respectively (Fig. 3). After reaching its lowest point in mid-June leaf respiration increased until the end of the growing season ($r^2 \geq 0.42$, $P \leq 0.02$). The ratios of A_{mass}:RS_{mass} and A_{area}:RS_{area} increased linearly with leaf age, reaching highest values in mid-June and declined thereafter (data not shown).

Fitted light-response model parameters indicated that most changes in A_{mass} and A_{area}, light compensation point (LCP) and dark respiration occurred during leaf formation, defined as the period from bud-break to the time when full photosynthetic capacity was reached and leaves were fully expanded, i.e. between 10 April and 23 May (Fig. 4). During that period A_{area} increased from 3.5 to 9.1 μmol m^{-2} s^{-1}, LCP decreased more than 10-fold from 196 to 18 μmol m^{-2} s^{-1} and RS_{area} decreased 4-fold from 3.5 to 0.9 μmol m^{-2} s^{-1} (Figs. 3–5, Table 1). Differences in these parameters during the photosynthetic maturity period (between 3 June and 3 September), defined as a period which begins when leaves reached maximum leaf area and full photosynthetic capacity to the time when the first visual symptoms of senescence were observed, were relatively small (Table 1, Fig. 4). The two sets of measurements taken during the senescence period (27 September and 11 October 1996)

Table 1 Light-saturation curve parameter estimates (±asymptotic standard error) for *Betula pendula* leaves

[a] See Fig. 4 and text for description
[b] Number of all CO_2 exchange measurements taken on a given day of year or leaf development stage

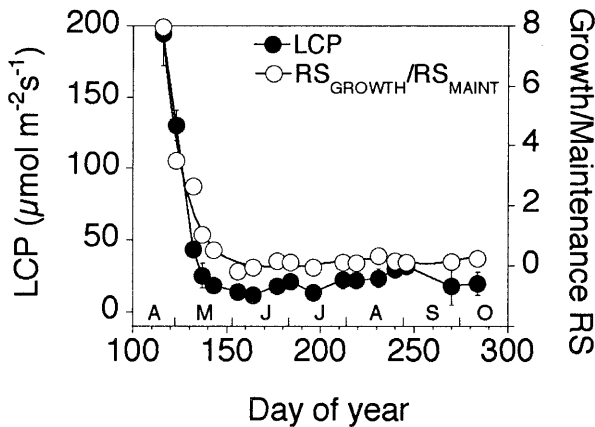| Leaf development Stage[a] and day of year		N^b	A_{area} ($\mu mol\ m^{-2}s^{-1}$)	RS_{area} (20°C) ($\mu mol\ m^{-2}s^{-1}$)	LCP ($\mu mol\ m^{-2}s^{-1}$)
Leaf formation	116	54	3.5±0.3	3.5±0.4	196±22
	123	64	5.6±0.3	2.5±0.2	128±11
	132	58	8.6±0.3	1.6±0.2	43±4
	137	64	8.7±0.4	1.1±0.4	25±7
	143	60	9.1±0.3	0.9±0.3	18±5
Maturity	156–246	584	9.1±0.1	0.9±0.1	20±2
Senescence	270–284	113	7.9±0.3	0.7±0.2	20±5

Fig. 5 Seasonal changes in light compensation point (LCP) and growth respiration (RS_{growth}) to maintenance respiration (RS_{maint}) ratios in *B. pendula* leaves
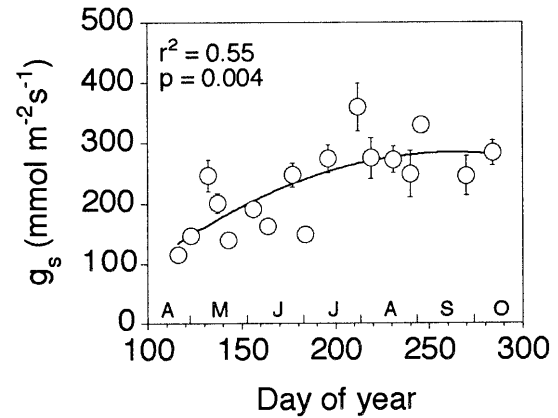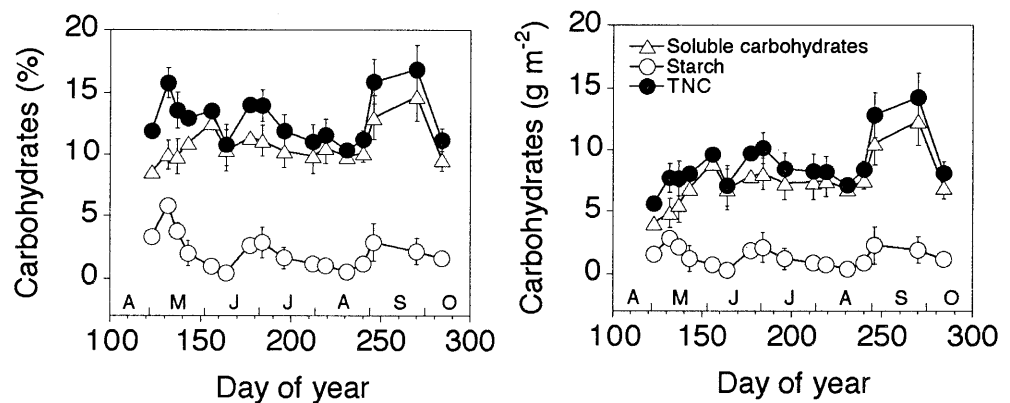

Fig. 6 Stomatal conductance to water vapor diffusion (g_s) in relation to day of year for *B. pendula* trees

Fig. 7 Seasonal pattern of mass- and area-based concentrations of soluble carbohydrates, starch and TNC

showed signs of declining photosynthesis and N mobilization (Table 2, Fig. 4).

The maintenance respiration rate averaged 8.2 nmol $CO_2\ g^{-1}\ s^{-1}$, whereas growth respiration changed between 0 and 65 nmol $CO_2\ g^{-1}\ s^{-1}$. The pattern of RS_{maint}/RS_{growth} was similar to that of LCP (Fig. 5). As predicted by the model of growth and maintenance respiration, rates of respiration were an approximately linear function of leaf RGR_{mass} ($r^2=0.91$, $P<0.0001$; data not shown).

Stomatal conductance to water vapor diffusion (g_s) increased from the beginning of leaf extension to early September. g_s varied from ≈100 mmol $m^{-2}\ s^{-1}$ in young leaves in early spring to ≈300 mmol $m^{-2}\ s^{-1}$ at the end of the growing season (Fig. 6).

Pattern of nonstructural carbohydrate changes

Soluble carbohydrate concentration increased from ≈9% of leaf mass at the beginning of the growing season in early May to ≈13% 1 month later (Fig. 7). After mid-May, the soluble carbohydrate concentration steadily declined until the end of August ($r^2=0.54$, $P<0.02$). However, this decline was not statistically significant

Table 2 Element concentrations in *B. pendula* foliage for the leaf formation, maturity and senescence periods (see Table 1 for description). Values (±SE) calculated on a leaf mass basis are expressed on TNC-free mass

Nutrient	Units	Leaf development stage			ANOVA effects $P>F$	Optimal or common concentration range[a]
		Formation	Maturity	Senescence		
N	mg g^{-1} g m^{-2}	39.0±0.9 1.8±0.1	34.9±0.6 2.2±0.0	28.4±1.2 1.9±0.1	<0.0001 <0.0001	25–40
P	mg g^{-1} g m^{-2}	3.1±0.2 0.14+0.01	2.2±0.1 0.14±0.01	2.5±0.3 0.16±0.01	0.003 0.29	1.5–3.0
K	mg g^{-1} g m^{-2}	14.1±0.9 0.66±0.05	10.6±0.5 0.67±0.03	13.3±1.2 0.68±0.06	0.006 0.93	10–15
Ca	mg g^{-1} g m^{-2}	8.5±1.0 0.41±0.08	12.8±0.6 0.82±0.05	19.9±1.4 1.30±0.11	<0.0001 <0.0001	3–15
Mg	mg g^{-1} g m^{-2}	2.0±0.2 0.09±0.01	2.5±0.1 0.16±0.01	3.3±0.2 0.21±0.02	0.002 0.0001	1.5–3.0
Mn	µg g^{-1} mg m^{-2}	237±34 11.5±2.5	357±19 22.8±1.4	421±46 27.8±3.3	0.008 0.0007	30–100
Al	µg g^{-1} mg m^{-2}	78.8±7.9 3.8±0.5	76.9±4.3 4.9±0.3	91.9±10.2 6.0±0.6	0.67 0.03	103–120
Fe	µg g^{-1} mg m^{-2}	95.5±5.2 4.5±0.3	98.4±2.9 6.2±0.2	103.0±9.6 6.7±0.4	0.68 0.0003	25–33
Na	µg g^{-1} mg m^{-2}	33.1±4.0 1.50±0.19	27±2.0 1.76±0.11	32.6±S. 3 2.14±0.26	0.45 0.17	85–98
Zn	µg g^{-1} mg m^{-2}	33.l±4.0 1.5±0.2	27.7±2.2 1.8±0.1	32.6±5.3 2.1±0.3	0.45 0.17	15–50
Cu	µg g^{-1} mg m^{-2}	6.9±0.3 0.32±0.01	5.9±0.2 0.40±0.03	6.2±0.4 0.40±0.03	0.03 0.07	6–12
B	µg g^{-1} mg m^{-2}	34.9±4.1 1.6±0.3	56.l±2.1 3.6+0.2	65.6±5.5 4.2±0.4	0.0004 0.0001	15–40
Pb	mg m^{-2}	3.4±0.6	2.7±0.3	4.1±0.8	0.24	
Ni	mg m^{-2}	1.4±0.2	0.8±0.1	1.0±0.2	0.01	0.99–1.4
Cr	mg m^{-2}	0.74±0.11	0.67±0.06	0.79±0.15	0.78	0.36–0.55
Cd	mg m^{-2}	0.37±0.04	0.25±0.02	0.35±0.05	0.009	0.03–0.4
K/Ca		1.7±0.1	0.8±0.1	0.5±0.1	<0.0001	

[a] Based on Bergmann 1992; Hrdlicka and Kula 1998; and unpublished data of Karolewski et al. Reported values are calculated on total dry mass (with TNC)

when calculated on a leaf area basis (*P*=0.2). The highest concentration of soluble carbohydrates, ≈15% of leaf dry mass or 12 g m^{-2}, occurred at the end of growing season in September (Fig. 7). Overall variation in soluble carbohydrate concentrations on leaf mass and area bases differed significantly within the growing season (*P*≤0.02).

Seasonal patterns of starch (*P*=0.02 and 0.28 on mass and area basis, respectively) were much more variable. The highest concentration of starch (6% of leaf dry mass) occurred very early in the growing season on 12 May. After that it declined continuously reaching the lowest point of less than 0.5% of leaf dry mass on 13 June, concurrent with new shoot growth (Fig. 1). Seasonal patterns in starch concentrations were roughly similar when calculated on a mass or area basis (*r²*=0.57, *P*=0.0005). Throughout the entire growing season, total nonstructural carbohydrate concentrations varied from 10% to 17% of leaf dry mass, or from 6 to 14 g m^{-2} (*P*≤0.1).

During leaf formation and maturity stages starch concentrations declined significantly with increasing mean

temperature for the 10 days prior to measurement (Fig. 8). Concentrations measured during leaf senescence were lower than expected based on this pattern.

Relationships between leaf structure, CO_2 exchange, N and nonstructural carbohydrate concentration

During leaf ontogeny LMA was correlated with CO_2 exchange. However, this relationship varied with leaf development stage. For the period of leaf formation RS_{area} and RS_{mass} decreased with increasing LMA ($r^2 \geq 0.78$, $P \leq 0.0005$), and this trend was reversed at stages of leaf maturity and senescence ($r^2 \geq 0.19$, $P \leq 0.04$). A_{mass} declined with increasing LMA for all data pooled across leaf development stages (Table 3).

RS_{mass} and RS_{area} were unrelated to A_{mass} or A_{area} at leaf maturity and senescence ($P \geq 0.4$, data not shown). During leaf formation, rates of respiration were negatively correlated with A_{mass} and A_{area} ($r^2 \geq -0.96$, $P \leq 0.04$). For the entire growing season, mass-based N
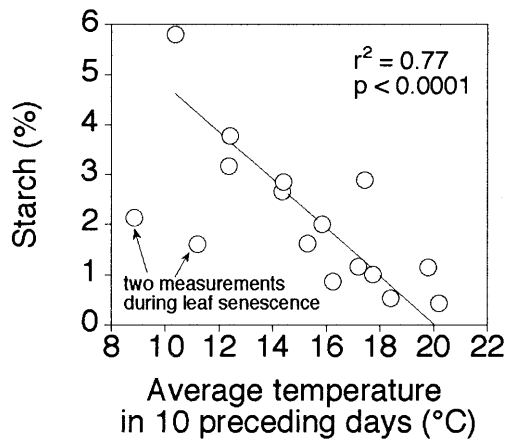
Fig. 8 Starch concentrations in *B. pendula* leaves in relation to the average temperature of the 10 days preceding measurements. When the last two measurements before leaf abscission are included $r^2=0.44$, $P=0.005$
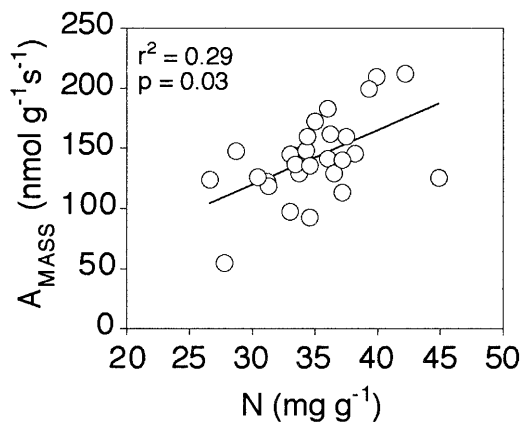

Fig. 9 A_{mass} in relation to leaf nitrogen concentration (N) for *B. pendula* leaves. Average values of both trees sampled over the entire growing season are shown

concentration (N_{mass}) was significantly related to A_{mass} (Fig. 9).

There was a close relationship between leaf area growth and A_{area} during leaf formation and a portion of the leaf maturity period. However, there was no relation-

Table 3 Summary of regression relationships ($P<0.05$) between area and mass-based gas exchange rates, nitrogen concentration and various morphological and chemical traits in *Betula pendula* leaves. The coefficients of determination (r^2), are given for linear regressions between the dependent and independent variables. Abbreviation and units: A_{mass} light-saturated net photosynthesis, nmol $g^{-1}s^{-1}$; A_{area} light-saturated net photosynthesis, μmol $m^{-2}s^{-1}$; g_s stomatal conductance, mmol $m^{-2}s^{-1}$; N_{mass} nitrogen concentration, mg g^{-1}; N_{area} nitrogen concentration, g m^{-2}; SC_{area} soluble carbohydrates, g m^{-2}; SC_{mass} soluble carbohydrates, % of dry mass; TNC_{area} total nonstructural carbohydrates, g m^{-2}; leaf area, cm^2; leaf mass, g; LMA leaf mass per area, g m^{-2}. All traits which are expressed on a dry mass basis are calculated on TNC-free mass (except those for carbohydrates). [−] and [+] after r^2 indicate direction of changes

Dependent variable	Independent variable	r^2 for leaf development stage		
		Formation	Maturity	All
A_{mass}	N_{mass}	NS	NS	0.29[+]
A_{mass}	TNC_{area}	NS	NS	0.31 [−]
A_{mass}	SC_{mass}	NS	NS	0.22[−]
A_{mass}	SC_{area}	NS	NS	0.36[−]
A_{mass}	g_s	0.61 [+]	0.35[+]	NS
A_{mass}	LMA	NS	NS	0.29[−]
A_{area}	g_s	NS	0.61[+]	0.44[+]
A_{area}	Leaf area	0.86[+]	NS	0.49[+]
A_{area}	Leaf mass	0.58[+]	NS	0.15[+]
RS_{mass}	Leaf area	0.80[−]	NS	0.76[−]
RS_{mass}	Leaf mass	0.90[−]	NS	0.67[−]
RS_{mass}	LMA	0.81[−]	0.26[+]	0.56[−]
RS_{area}	Leaf area	0.90[−]	NS	0.84[−]
RS_{area}	Leaf mass	0.91[−]	0.19[+]	0.56[−]
RS_{area}	LMA	0.78[−]	0.43 [+]	0.41[−]
N_{mass}	LMA	0.63[−]	0.35[−]	0.57[−]
N_{area}	LMA	NS	0.29[+]	0.47[+]

ship between A_{mass} and leaf area or mass growth. Peak A_{area} was reached at ≈90% of final leaf mass, 30 days before leaf mass growth was complete (Fig. 10).

Leaf N_{mass} decreased linearly with LMA for all data pooled across leaf development stages as well as for periods of leaf formation and maturity separately (Table 3). Leaf N_{area} increased linearly with LMA for all data pooled across leaf development stages and for the period of leaf maturity separately. However, overall this relationship was weaker than for N_{mass} (Table 3). There were no relationships between area- and mass-based starch concentrations and net photosynthetic rates.

Fig. 10 Seasonal pattern of relative values of area- and mass-based net photosynthesis at light saturation, leaf mass, area and nitrogen
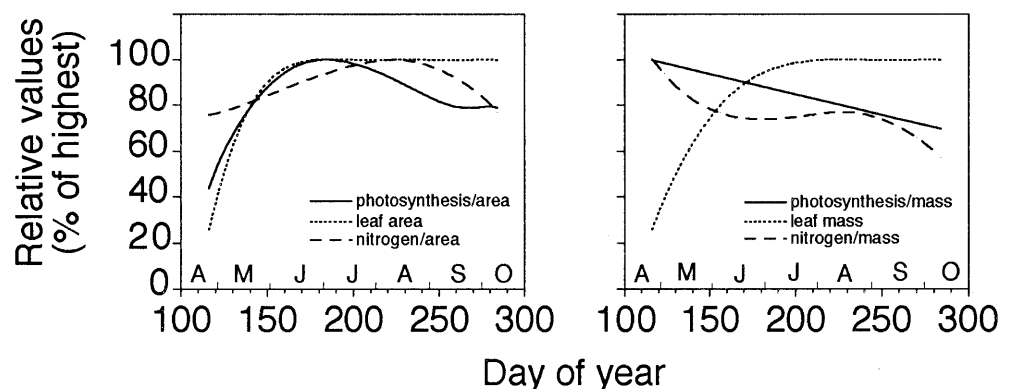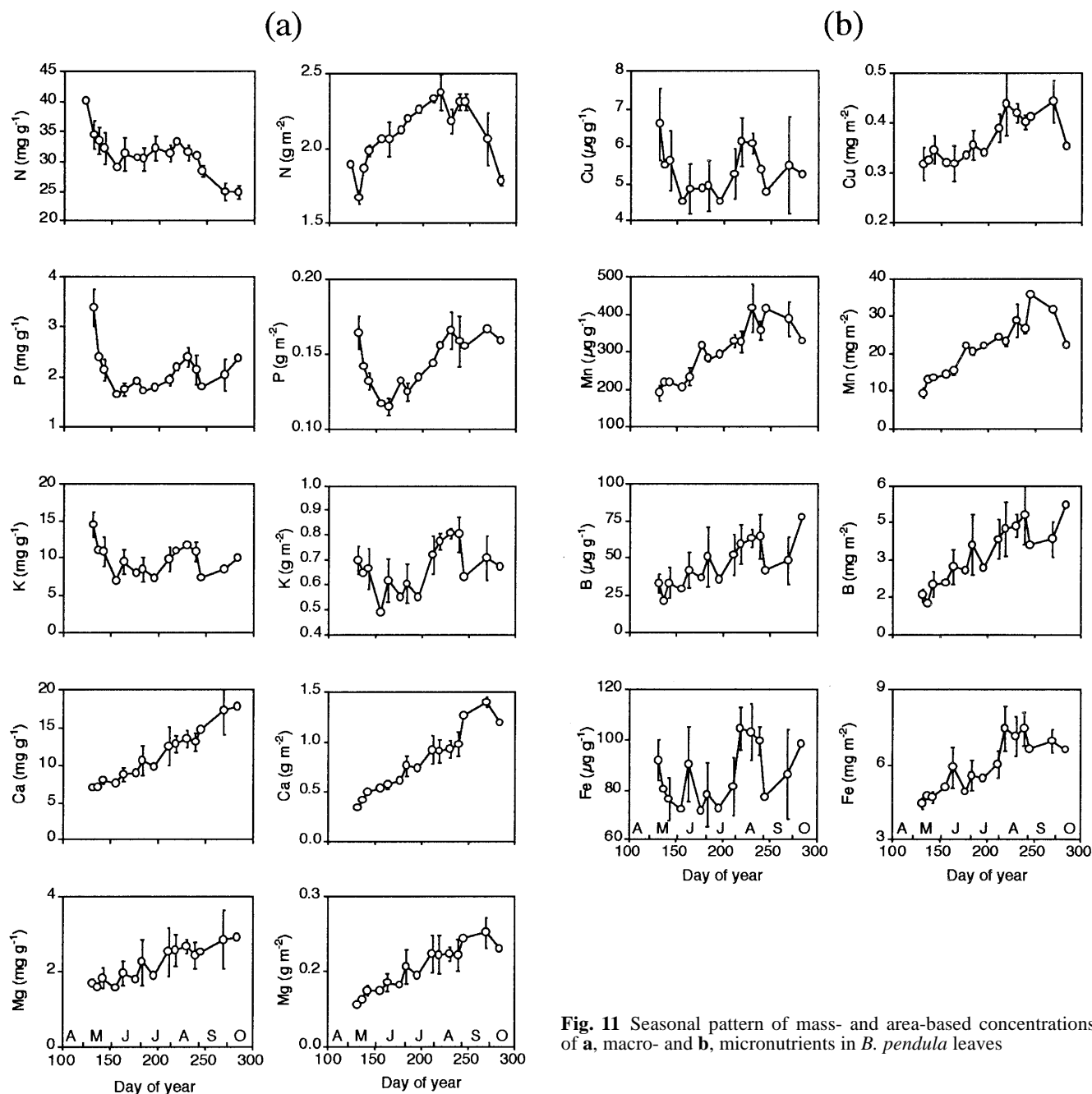
Fig. 11 Seasonal pattern of mass- and area-based concentrations of **a**, macro- and **b**, micronutrients in *B. pendula* leaves

Ontogenetic changes in leaf mineral composition

All concentrations of nutrients in mature leaves were in a range sufficient for normal growth noted for *B. pendula* (Table 2). Except for Al, Fe, Zn, Pb and Cr concentrations of all other mineral elements varied significantly throughout leaf development stages in *B. pendula* (Table 2, Fig. 11). Mass-based concentrations of N, P and K were higher in newly formed leaves than in mature or senescing leaves. During the period of leaf formation, mass-based N, P and K concentrations in leaves decreased by 24, 63 and 27%, respectively. Both area- and mass-based concentrations of Ca, Mn and B increased almost linearly with leaf age ($r^2 \geq 0.67$, $P \leq 0.0002$), while concentrations of Al, Fe, Cu and Zn increased significantly with age only when expressed on an area basis ($r^2 \geq 0.36$, $P \leq 0.02$).

Among all measured mineral elements only N showed clear signs of retranslocation at the end of the growing season. Both mass- and area-based retranslocation values were similar, at 35 and 33%, respectively (Fig. 11).

Discussion

Gas exchange

The data revealed that very early in the growing season, 6 days after bud-break, unfolding *B. pendula* leaves had relatively high rates of photosynthesis under light-saturated conditions. However, due to high growth respiration rates, positive net CO_2 fixation was observed only at irradiances >200 µmol m^{-2} s^{-1}. Our data corroborate laboratory observations of Valanne et al. (1981) who measured the CO_2 exchange of *B. pendula* buds and expanding leaves under low irradiance (130 µmol m^{-2} s^{-1}) and found that the positive CO_2 balance occurred 5 days after leaf opening.

In *B. pendula* differentiation of palisade tissue and the intercellular spaces takes ≈20 days from leaf unfolding (Valanne et al. 1981). Therefore, the high rates of A_{area} ranging from 3 to 9 µmol m^{-2} s^{-1} observed in early spring indicate that full structural development is not a necessary prerequisite for maintaining a positive CO_2 balance in young birch leaves. However, the data also indicate that the maximal rate of A_{area} was reached at the time when leaf area extension was completed (Fig. 10). Mid-season A_{mass} and A_{area} rates (Tables 1, 2, Figs. 3, 10) were within the range usually noted for *B. pendula* (from 8 to 14 µmol m^{-2} s^{-1}, Maurer et al. 1997; Pääkkönen et al. 1996; Öquist et al. 1981; Taylor and Davies 1988).

Leaf area development was much faster than that of leaf mass and was completed more than 1 month earlier (Fig. 2). A decline in A_{area} started shortly after the leaves had reached their final area, during a period of continued mass gain due to thickening of leaves. An increase of leaf mass at that time is mainly due to secondary thickening of epidermis, cell wall, cuticle and other surface structures which can lead to an increase in diffusion pathways for CO_2 (Fitter 1986). Greater investment in leaf structural tissue was found to be correlated with a decrease in rates of photosynthesis (Oleksyn et al. 1997). At leaf maturity, g_s and A_{area} were well correlated (Table 3).

We also tested whether the K:Ca ratio or leaf Ca concentrations alone show the same pattern as net CO_2 exchange with leaf age. Several nutrients like N, P, S and especially K are readily mobile in plants and their concentrations decline with the age of foliage, whereas the ions of alkaline earths, especially Ca, accumulate steadily in the leaves at the end of transpiration routes (Fig. 11, Larcher 1995). Therefore, the K:Ca ratio or the concentration of Ca alone can be a measure of the physiological age of leaves (Meier-Dinkel and Kleinschmit 1990). We found that all relationships which were significant between CO_2 exchange and leaf age were also significant for the K:Ca ratio or Ca concentrations. However, since much of the K can be removed by leaching (Chapin 1980), leaf Ca concentration can be a better reflection of physiological age of leaves when compared within one tree or similar soil conditions.

Dark respiration and net photosynthesis rates have been found to be positively correlated with leaf N among species within plant functional groups, within species and populations (Reich et al. 1996, 1998; Oleksyn et al. 1998b). Reich et al. (1991) also found that net photosynthesis and leaf N of open grown sun-lit leaves of *Acer rubrum*, *A. saccharum* and *Quercus ellipsoidalis* were highly linearly correlated within each species from late spring until leaf senescence in fall. Data presented in this study confirm the existence of a relationship between leaf N and net photosynthesis throughout leaf ontogeny in *B. pendula* (Table 3). However, we found that only the relationship between N_{mass} and A_{mass} calculated for the entire growing season was statistically significant, and even then could explain less than a third (r^2=0.29) of the A_{mass} variation. The strength of A_{mass}-N_{mass} relationship is likely to be controlled by such factors as differences in N availability and concentration in foliage, with plants growing in N-limited soils showing the strongest response of A_{mass} to N (Field and Mooney 1986; Reich and Walters 1994; Reich et al. 1995b). Results of our study indicate that on sites with an adequate nutrient supply, N limitation is a less important component of the A_{mass}-N_{mass} relationship during leaf ontogeny. There was also no evidence that at the study site nutrients other than N might limit photosynthesis during *B. pendula* leaf ontogeny.

Nutrients

Throughout the leaf life-span, the concentration of individual nutrients changes, depending on their chemical nature, supply, physiological function, and environmental factors. The highest concentrations of N_{mass} were observed during the period of rapid leaf growth in the spring (Fig. 11, see also Chapin and Kedrowski 1983; Drossopoulos et al. 1996). At that time there is a strong demand for N for production of new biomass and building photosynthetic structures. The major portion of the leaf N is in Rubisco and other photo- and biochemical machinery involved in photosynthesis (Dietz and Harris 1997). The decline in N_{mass} is not the result of its loss or resorption from leaves, but primarily of dilution due to the large increase in dry mass at that period of time (Guha and Mitchell 1966). The plateau of N_{mass} observed from the beginning of June until the end of August (Fig. 11) seems to be typical for leaves of birch (Ferm and Markkola 1985). Since leaf N scales well with potential carbon gain or loss within and among species and biomes (Reich et al. 1991, 1997, 1998; Oleksyn et al. 1998b), the period from June to the end of August seems to be the most appropriate for sampling birch leaves for modeling species photosynthetic capacity.

A different ontogenetic pattern was observed for N_{area}, in which a gradual increase in N content occurred until the mid-summer, followed by a rapid decrease (Fig. 11). This type of seasonal pattern in N_{area} seems to be typical for broadleaved tree species as evidenced

from studies of *Alnus crispa*, *Betula papyrifera* (Chapin and Kedrowski 1983), *Acer rubrum*, *A. saccharum*, *Quercus ellipsoidalis* (Reich et al. 1991) or *Juglans regia* (Drossopoulos et al. 1996).

The concentrations of P and K decreased during the leaf formation stage and stabilized or increased during the maturity stage (Fig. 11). Similarly to N_{mass} the pattern of P and K during these stages is governed by changes in leaf mass, and the initial decline is due to the diluting effects of cell wall growth (Chapin 1980). At the same time the concentration of another mobile element, Mg, increased with time.

Changes of partially mobile elements such as Mn and Zn reveal an asymptotic increase with time. Such increases are typical for sites with high supplies of these elements (Nilsson et al. 1995). The concentration of immobile elements such as Ca and B increased during the growing season with leaf age (Fig. 11). This type of pattern for Ca, Mn and B is characteristic of temperate zone plants (Marschner 1995).

N retranslocation

We found that senescing leaves of *B. pendula* retranslocated more than 30% of N (Fig. 11). This level of N resorption is in the medium to low range of values reported in the literature for different species of woody plants (Chapin and Kedrowski 1983; Reich et al. 1995a; Ryan and Bormann 1982). The mechanisms that allow that reallocation of resources are considered essential for efficient functioning of woody plants, unless external sources of mineral nutrient supplies are readily available and can be absorbed at lower cost (Eckstein and Karlsson 1997; Harper 1989; Marshner 1995). Since processes of breakdown of organic products, their transport to new growth or to storage organs and synthesizing of storage substances involve considerable costs in terms of energy and resources (Thomas and Stoddart 1980), relatively small retranslocation of N and other macro- and micronutrients from *B. pendula* trees in the present study is perhaps related to a favorable supply of nutrients from the soil as evident in adequate leaf nutrient concentrations (Table 2).

Nonstructural carbohydrates

Increase in soluble carbohydrate concentration in spring was accompanied by a decrease in starch content. High starch content in *B. pendula* at the beginning of the growing season is associated with its resynthesis at the end of dormancy (Valanne et al. 1981). Our data indicated that starch concentration during leaf formation and maturity was inversely related to temperature (Fig. 8). Low temperature along with other factors can cause an accumulation of assimilates in leaves. Accumulation of starch and other photoassimilates frequently leads to inhibition of CO_2 assimilation, a long-term adjustment of

photosynthetic capacity, a decrease of enzyme protein levels, or an initiation and acceleration of senescence (Stitt et al. 1990; Dietz and Keller 1997).

The rate of photosynthesis is often decreased due to inadequate sink demand (Geiger 1979; Stitt and Quick 1989). Although this phenomenon is well established at the whole plant level, very little is known to what extent leaf-level ontogenetic changes in TNC concentration are associated with rates of photosynthesis. We found a statistically significant negative correlation between total nonstructural carbohydrate concentration in *B. pendula* leaves and A_{mass} when calculated for the entire growing season (Table 3).

In summary, the results of this study show distinctive ontogenetic differences in leaf-level CO_2 and H_2O exchange, development and chemistry in *B. pendula*. The ontogenetic pattern of leaf structure, composition, and metabolic functions is genetically controlled, but their expression depends on environmental conditions such as irradiance, temperature, and nutrient availability. We found that among the factors related to variation in net CO_2 exchange rates during the leaf development were changes in leaf-N, TNC, g_s, and growth (RGR, LMA). The seasonal patterns of such traits as photosynthesis, respiration or nutrient concentrations were different when expressed on a leaf dry mass or area basis. Knowledge of ontogenetic changes in physiological and chemical leaf traits could be helpful in identifying proper periods for leaf sampling for diagnostic purposes such as evaluation of site and plant productivity, the effects of biotic and abiotic factors on plants, or revealing genetic differences among plant populations.

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