Seasonal dynamics and effects of nitrogen supply rate on nitrogen and carbohydrate reserves in cutting-derived *Salix viminalis* plants

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Abstract: Nutrient storage is an important aspect of resprouting potential and production of *Salix viminalis* L., a pioneer species used for biomass production in Sweden. Seasonal dynamics of nitrogen (N), protein, soluble carbohydrates, starch, and lipids were studied in roots, cuttings, stems, and leaves during a full growth cycle induced by varying photoperiod and temperature in a growth chamber. Nitrogen was supplied at two rates. Both season and N availability significantly affected storage of N and carbohydrates. Reserves peaked in dormancy, and plants grown at a higher N availability were able to build up larger N reserves, whereas carbohydrate reserves were similar in the two N treatments. All perennial plant organs functioned as storage sites for N. Roots were prominent in carbohydrate storage, in good agreement with the notion of a pioneer species adapted to recurring disturbance by a pronounced resprouting capacity. Roots differed from aboveground plant parts in their exceptionally high starch levels, and in that N storage in roots to a greater extent involved nonprotein compounds. Triglycerides contributed to carbon storage in aerial plant parts but not in roots. Our results suggest that an increased N supply enhance both the accumulation of nutrient reserves and early season growth.

Résumé : Le stockage de nutriments est un facteur important dans la production de rejets chez le *Salix viminalis* L., une espèce pionnière utilisée pour la production de biomasse en Suède. La dynamique saisonnière de l'azote (N), des protéines, des hydrates de carbone solubles, de l'amidon et des lipides a été étudiée dans les racines, les boutures, la tige et les feuilles durant un cycle complet de croissance induit en faisant varier la photopériode et la température en chambre de croissance. Les plants ont reçu deux niveaux d'azote. La saison et la disponibilité de N ont affecté de façon significative le stockage de N et des hydrates de carbone. Les réserves ont atteint le maximum pendant la période de dormance et les plants qui avaient plus d'azote disponible ont accumulé plus de N tandis que les réserves d'hydrates de carbone étaient semblables quel que soit le niveau de N. Tous les organes pérennes des plants ont agi comme sites d'emmagasinage de N. Les racines se démarquaient par le stockage d'hydrates de carbone conformément à la notion d'espèce pionnière, adaptée à des perturbations récurrentes grâce à une forte capacité de produire des rejets. Les racines se distinguaient des parties aériennes des plants par leur niveau exceptionnellement élevé d'amidon et par le fait que N était emmagasiné dans les racines sous forme de composés non protéiques. Les triglycérides contribuaient au stockage du carbone dans les parties aériennes des plants, mais non dans les racines. Les résultats suggèrent qu'un apport élevé de N favorise à la fois l'accumulation de réserves de nutriments et la croissance tôt en saison.

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Introduction

The risk for global climatic changes resulting from anthropogenic CO_2 emissions warrants the development of carbon (C) neutral energy sources, e.g., within the bioenergy sector. In Swedish short-rotation forestry, shrub-form *Salix* species are used in a coppice plantation system for biomass production (Sennerby-Forsse and Christersson 1994). The selected, high-yielding clones are propagated from dormant stem cuttings and regenerate new shoots after coppicing in 3- to 5-year rotations. Potential production, exceeding 10 t/ha (Christersson 1986), is high for Swedish conditions and is, in part, due to rapid foliage development early in the season and the high growth rate of regenerating coppice shoots after harvest (McDonald 1984; Sennerby-Forsse and Zsuffa 1995).

The *Salix* species posses several traits typical for pioneer plants. They are adapted to disturbed environments with a high nutrient availability, where rapid resource acquisition and height growth are means of competition against neighbors and to escape browsing. Repeated disturbance, e.g., severe browsing by mammals, has selected for belowground storage (mineral nutrients and carbohydrates) to support rapid regrowth of shoots (Bryant et al. 1983).

Nutrient reserves are important for early season leaf development and shoot growth (Tromp 1983). Regrowth after loss of shoot biomass has been shown to rely on carbohydrate reserves in the roots (Kozlowski 1992; Zasada et al. 1994; Landhäusser and Lieffers 1997) before photosynthetic

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capacity is restored in new foliage. Little is known about the nitrogen (N) sources for regrowth in trees, but N reserves are important for regrowth in grasses and herbs (Cyr and Bewley 1990; Volenec et al. 1996). Nutrient reserves in trees include nitrogenous compounds, starch, sugars, fats, and hemicellulose. Annual cycles of total nonstructural carbohydrates (TNC) and N are particularly distinct in deciduous trees (Kramer and Kozlowski 1979; Chapin et al. 1980), because the lack of photosynthetic tissue in early spring increases the asynchrony between supply and demand (Fischer and Höll 1991). In addition to seasonality, mineral nutrition is a potentially important factor in reserve regulation (Chapin and Kedrowski 1983; Birk and Matson 1986).

Root reserves have been studied in horticultural crops, but data for forest trees grown in short-rotation forestry is scarce, especially in connection to coppicing. Few studies include the whole plant, despite that translocation of nutrients between plant organs may accompany reserve accumulation and mobilization (Dickmann and Pregitzer 1992; Isebrands and Burk 1992). Furthermore, experiments on the effect of nutrient supply on plant nutrient status have mostly covered the growing season, and there is considerably less information on the persisting effects during and after plant dormancy. Thus, there is a need for plant nutrition studies, encompassing the entire growth cycle and a whole-plant approach, to elucidate reserve dynamics of resprouting species.

Nutrient storage has been studied using various methodological approaches. The physiological basis for storage is a nutrient availability in the tissue that exceeds the current utilization for growth (Kramer and Kozlowski 1979). This excess may arise from a high external nutrient-supply rate during the growing season or from retranslocation from internal sources, as during development of plant dormancy in climates with a marked seasonality. The resulting accumulation can be detected as the presence of specific storage compounds or as increasing concentrations without a corresponding growth rate increase (Ingestad 1982). The contribution of external and internal nutrient sources for reserve formation has often been studied using nutrient budgets, which however, only disclose the net effect of contributing fluxes. This limitation can be overcome by the use of isotopic labeling (Millard 1996). However, under controlled experimental conditions, nutrient budgets may be used to estimate individual fluxes. For example, in the absence of external N supply, resorption from senescing leaves is the sole contributor to formation of mineral nutrient reserves in perennial tissues.

The aim of this study was to determine the changes in nutrient status (N, protein, soluble carbohydrates, starch, and triglycerides) throughout an entire growth cycle in roots, cuttings (propagule), stems, and leaves of a basket willow (*Salix viminalis* L.) clone, selected for superior coppice characteristics. In addition, the effect of N supply on nutritional dynamics was tested. The hypotheses were that nutrient reserves are enhanced by an increasing N supply and that storage function primarily resides in the root system.

Materials and methods

Plants were propagated from standardized cuttings prepared from dormant 1-year-old stems of *S. viminalis* clone 78183. Stem sections were soaked in water for 48 h and cut into pieces 5 cm long and 1 cm in diameter. Average dry mass (DM) of cuttings was 1.8 ± 0.2 g (mean ± SD, n = 20) and average N content 7.5 ± 0.5 mg (n = 4). One hundred and fifteen cuttings were planted in pots with 0.5 dm^3 quartz sand with a mean particle diameter of 0.6 mm. Plants were established in a growth chamber (18 h light : 6 h dark photoperiod, 25°C during the light period and 15°C during the dark period) without fertilization. Light intensity was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, relative humidity was 70%, and plants were kept well watered throughout the experiment. Each cutting developed one to three shoots. After 3 weeks the shoots were 5–10 cm, and the leaves started to turn yellow-green, indicating that N reserves in the cuttings were becoming depleted. This was the predetermined starting point for the climate and fertilization programmes, because a low internal N status would enhance the nutrient treatment effect. In prefertilization plants the DMs (n = 15) of roots, cuttings, and shoots (stems + leaves) were 0.12 ± 0.04, 1.74 ± 0.23, and 0.20 ± 0.03 g, respectively, and the corresponding N conentrations (n = 5) were 7.6 ± 1.8, 2.1 ± 0.3, and 13.5 ± 0.9 $mg \cdot g^{-1}$ DM.

Photoperiod and temperature were varied according to a predetermined climate programme to induce an entire seasonal growth cycle (Table 1; modified for *Salix* from Jonsson et al. 1997). The criteria for defining the end of each climate phase (CP) were as follows: CP I, visibly different growth response to N treatments; CP II, cessation of shoot elongation; CP III, all leaves senesced; CP IV, bud swell; and CP V, spring flush.

Nutrients were supplied once daily during CP I and CP II and the first 7 days of CP III using a complete nutrient solution in which N was slightly underrepresented in relation to other essential nutrients (Ericsson 1981). It was assumed that the plants took up the entire nutrient supply. This assumption gains support by the fact that no N was detected in leacheate or sand substrate at harvests. Daily nutrient doses were low in the beginning and in the end of the fertilization period, according to

$$[1] \qquad N(t) = N_0 t^p (t_m - t)$$

where t is the number of days after the start of fertilization, $N(t)$ is the amount of N added on day t, and t_m is the fertilization period (77 days). The parameter N_0 was adjusted to the desired cumulative N dose (60 and 30 mg N per plant in the +N and –N treatment, respectively) and calculated by integrating eq. 1. The parameter p was calculated by equating the derivative of $N(t)$ (eq. 1) to zero, and choosing day 21 for maximum nutrient dose. With this N supply regime, actively growing plants (CP I) received an average relative addition rate of N (R_N) of 0.079 $g \cdot g^{-1}$ per day in the +N treatment and 0.059 $g \cdot g^{-1}$ per day in the -N treatment, as given by

$$[2] \qquad R_N = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

where N_1 and N_2 were total plant N amounts at the start of fertilization (t_1) and at the end of CP I (t_2), respectively (Ingestad and Lund 1986). N_1 was estimated as the N amounts in leaves, stems, and roots (3.5 mg), excluding the remaining cutting N, which was assumed not to be available for growth. Within CP I, eq. 1 gives a closer to linear curve than eq. 2.

At the end of each climate phase, 10 randomly selected plants from each N treatment were harvested and separated into roots, cuttings (the hardwood cutting used for propagation remains a substantial part of the young willow plant), stems and, if present, leaves and spring shoots. Root systems were submerged in tap water to remove adhering sand. Any abscising leaves in CP III were collected by enclosing the yellowed shoot in a net. Leaf area was measured using a surface area meter (LI-COR 3100, Lincoln, Neb.). Fresh masses were determined. For protein and lipid analyses, fresh tissue from three plants was combined into one sample

Table 1. Climate programme (modified for *Salix* from Jonsson et al. 1997).

Climate phase	Photoperiod (h)	Light period temperature (°C)	Dark period temperature (°C)	Duration (days)
(I) Active growth	18	20	15	28
(II) Growth retardation	14	20	10	7
	12	20	10	7
	10	20	10	7
	8	20	10	21
(III) Growth cessation and hardening	8	15	4	35
	8	4	4	16
(IV) Dormancy to bud swell	8	20	15	5
	18	20	15	48
(V) Bud break to spring flush	18	20	15	52

Note: Light intensity was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and relative humidity was 70%.

per organ, treatment, and harvest and immediately frozen in liquid nitrogen. Remaining plant material was dried for 3 days at 70°C before dry mass determination (dry masses of plants sampled for protein and lipid analyses were corrected using fresh masses before and after sampling). For N and carbohydrate analyses, nine plants were randomly combined into groups of three, yielding three replicates per organ, treatment, and harvest. In spring flush shoots, only two replicates were obtained. The reason for combining organs from several plants was to obtain a minimum amount of material for the analyses.

Total N was analyzed by gas chromatography in a Carlo Erba Elemental Analyzer NA 1500 (Kirsten and Hesselius 1983). Total nonstructural carbohydrates (TNC) were extracted in two steps (Steen and Larsson 1986): Samples were extracted in acetate buffer (pH 5) at 60°C; after withdrawal of an aliquot for determination of soluble carbohydrates, a thermostable α-mylase (Termamyl 300L, type DX, Novo Nordisk, Denmark) was added, and the extraction proceeded at 90°C for determination of residual starch. The aliquot of the first extract was divided into three portions for the determination of (*i*) glucose and fructose, (*ii*) sucrose and fructans, and (*iii*) maltodextrins. Sucrose and fructans were determined after acid hydrolysis as the increase in glucose and fructose, respectively. Maltodextrins and starch were determined after hydrolysis by amyloglucosidase as the increase in glucose in the first and second extract, respectively. Glucose and fructose were determined by the glucose phosphate dehydrogenase method (Bergmeyer et al. 1974; Bernt and Bergmeyer 1974), using a Hitachi U1100 spectrophotometer (Hitachi, Tokyo, Japan) and a Hitachi AS 3000 autosampler. Protein was extracted from the tissue by homogenizing the tissue at 4°C using a 0.2 M NaCl, 2.0% sodium dodecyl sulphate (SDS), 25 mM potassium phosphate buffer at pH 7.0. Protein was quantified using the DC protein assay (Bio-Rad, Rockford, Ill.). Triglycerides were extracted with 95% ethanol containing activated aluminum purifier and subsequently saponified with KOH. The liberated glycerol was measured using a coupled enzyme system (Sigma diagnostics kit No. 320a; Sigma, St. Louis, Mo.).

The effects of N supply and climate phase on DM and on concentrations and amounts of N and TNC were tested for each plant organ separately using a two-factor analysis of variance (SAS Institute Inc. 1982). Dry mass was analyzed after $\log_{10}$ transformation because heteroscedastic variances were detected using Bartlett's test ($p < 0.02$). Treatment combination means were compared by using mean square error (MSE) as estimate of variance and, in case of climate phase means, using Tukey multiple pairwise comparisons. The effects of N supply, climate phase, and plant organ on the proportional within-plant distributions of DM, N, and TNC during CP I – CP III were analyzed using a three-factor analysis of variance, and the means were compared following estimation of variance by MSE. Differences between N treatments in terms of leaf area, leaf-N retranslocation, and spring-flush data were assessed using Student's *t* test. The difference in protein concentration between organs was tested using analysis of covariance with N concentration as covariate, according to the model

$$[3] \qquad y_{ij} = \mu + \alpha_i + (\beta_1 + \beta_{1i}^{*})x_{ij} + e_{ij}$$

where y is protein concentration, x is N concentration, and β_1^* is the treatment (levels of organ, climate phase, or N treatment) divergence from the average slope (β_1). In analogy, triglyceride concentrations were tested using TNC or starch as covariate.

Results

Growth

Dry masses (Fig. 1) of all organs were significantly affected by climate phase and N supply ($p < 0.0001$ for both factors in all organs except cutting, where $p = 0.02$ for N supply), and the significant climate phase × N supply interaction ($p < 0.005$ in stems and leaves) mirrored the faster mass accumulation in response to a higher N supply. In addition, the two N-addition rates generated significant differences in growth rate, leaf area, and in the distribution of dry mass among plant organs. Relative growth rates (R_G) in CP I were 0.080 and 0.069 $g \cdot g^{-1}$ per day in the +N and –N treatments, respectively, which corresponded to R_N. R_G was calculated in analogy with eq. 2 based on plant DM on the first and last day of CP I, excluding the prefertilization cutting DM. Leaf area in CP I and II was larger ($p < 0.001$) in the +N treatment, 90 and 156 cm^2, respectively, than in the –N treatment, 61 and 91 cm^2, respectively. Distribution of dry mass among perennial organs was significantly affected by climate phase and N supply ($p < 0.0001$ for both factors). The proportional distributions of the dry mass gained during CP I – CP III were 40 and 37% ($p = 0.009$) for stems, 13 and 17% ($p < 0.0001$) for cuttings, and 47 and 45% ($p = 0.14$) for roots in the +N and –N treatments, respectively.

No phenological differences, e.g., development and break of dormancy, between the N treatments were observed. Growth continued in CP III, but after 14 days the leaves started senescing, and after 35 days the entire leaf mass was yellow. Dry masses generally declined slowly during CP IV and V. Breaking of dormancy required 7 weeks before the buds swelled, whereafter an additional 7 weeks elapsed before the spring flush. Bud burst and flushing occurred in 6 of 10 plants in the +N treatment and 7 of 10 plants in the –N

treatment. Average accumulation of DM in spring shoots was enhanced in the +N treatment, 0.34 ± 0.30 g, compared with 0.19 ± 0.09 g in the –N treatment (Fig. 1).

Nitrogen

Nitrogen concentration was significantly affected by N supply ($p < 0.005$ for all plant organs). Average N concentrations in all plant organs and climate phases were higher in +N plants (Fig. 2). Nitrogen concentrations also showed significant seasonal fluctuations ($p < 0.0001$ for all plant organs). During CP I, N concentrations remained at prefertilization levels. After decreasing in stems and roots during CP II, N concentrations in all perennial organs increased during CP III and CP IV. Average N concentrations in spring shoots of flushing plants were 16 ± 0.66 mg·g^{-1} DM in the +N treatment and 13 ± 6.2 mg·g^{-1} DM in the –N treatment.

Nitrogen amounts in perennial organs increased rapidly during CP I – CP III in response to fertilization and leaf senescence (Table 2). Average leaf N amounts decreased by 75 and 71% during CP III in the +N and –N treatments, respectively. This N resorption was not significantly affected by N supply ($p = 0.15$, $n = 3$; resorption was calculated as percent loss in CP III plants in relation to CP II treatment averages). The amount of N lost from senescing leaves corresponded to that gained by perennial organs. The sinks for resorbed leaf N may be estimated as the increase in N amounts in perennial organs during CP III, because no other significant N source was present (the contribution from current root uptake may be neglected, because the N dose in CP III only corresponded to 12% of the amount redistributed from senescing leaves, and no residual N was detected in the substrate): 50% was imported by the stems; 35%, by the roots; and 15%, by the cuttings. During CP IV and CP V there were only minor changes in N amounts in perennial organs.

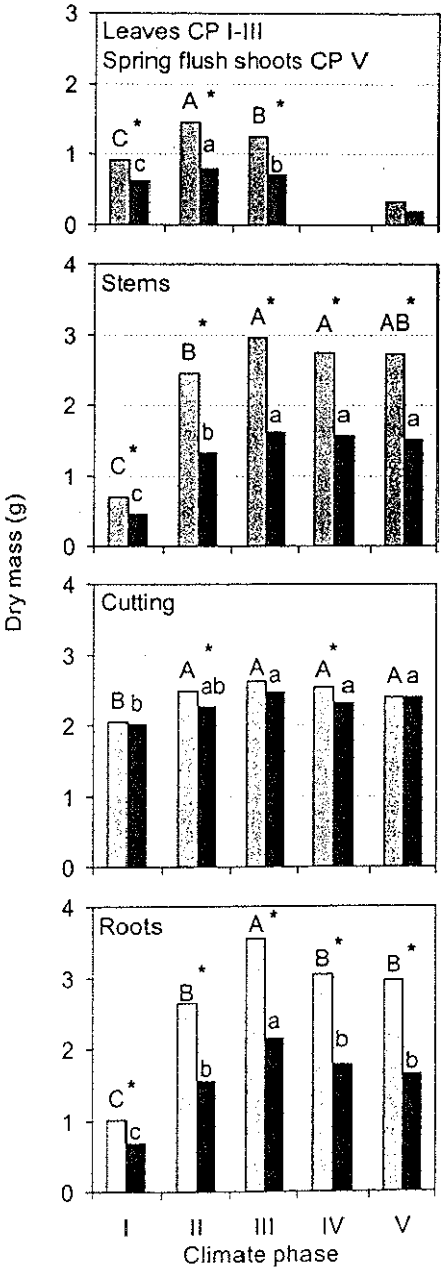
The proportional allocation among perennial organs of fertilizer N was calculated after subtracting the N amounts at the start of fertilization (data on prefertilizer shoot N was recalculated assuming the same stem/leaf N ratio as in CP I). Allocation varied between climate phases but did not differ significantly between N treatments. Roots were the largest sink for fertilizer N allocation (average over N treatments), but their proportion decreased from 62 to 47% ($p \leq 0.0001$) between CP I and CP III, whereas it increased from 28 to 42% ($p \leq 0.0001$) in stems. Cuttings contained 9% of the acquired N throughout the period.

The relation between protein and N concentrations differed between organs ($p = 0.05$) but not between climate phases or N treatments (Fig. 3). Protein concentrations in stems and cuttings increased with N concentration, whereas in roots the relation was negative. The protein/N ratios in roots were higher in CP I and CP II, intermediate in CP III, and lower in CP IV and CP V.

Carbohydrates and lipids

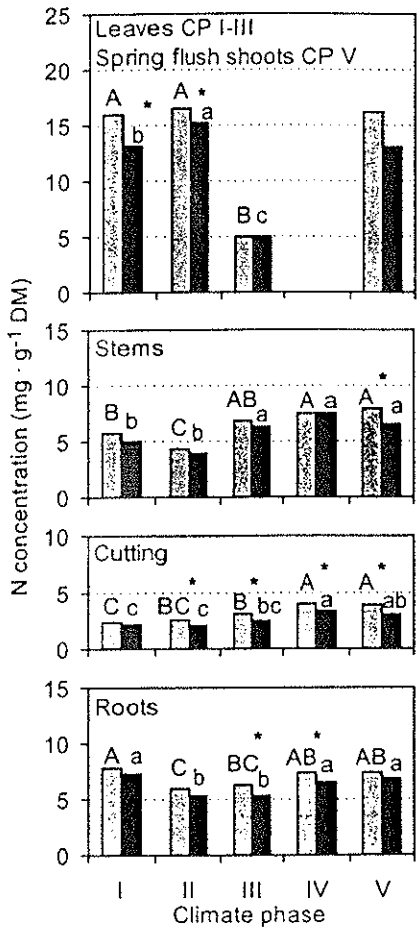
All analyzed carbohydrates (glucose, fructose, sucrose, fructans, maltodextrins, and starch; Fig. 4) showed significant variation between climate phases in all organs ($p < 0.0001$). Also N supply affected concentrations of individual carbohydrates: sucrose and starch in roots ($p < 0.003$); all analyzed carbohydrates except glucose in cuttings ($0.01 < p < 0.05$); sucrose and fructans in stems ($p = 0.04$ and $p = 0.0007$, respectively); and glucose, fructose, sucrose, and fructans in leaves ($0.0001 < p < 0.01$).

Fig. 1. Dry mass (g/plant) of *Salix viminalis* L. plant organs measured in connection with active growth (I), cessation of shoot elongation (II), leaf senescence (III), bud swell (IV), and spring flush (V). Spring flush shoots in climate phase V are included in the figure but not in the statistical model. Plants were grown at a higher (shaded bars) or lower (solid bars) N availability, both at a suboptimal nutrition level. Climate phase means with different letters are significantly different: uppercase letters show a higher N supply; and lowercase, a lower N supply. Asterisks give significant differences between N treatments within climate phases ($\alpha = 0.05$, $n = 10$).



Concentrations of TNC (Fig. 4), the combined nonstructural carbohydrates, fluctuated markedly between climate phases in perennial organs ($p < 0.0005$) and leaves

Fig. 2. Nitrogen concentrations (mg·g^{-1} DM) in *S. viminalis* plant organs. See Fig 1 for details (α = 0.05, n = 3).



(p = 0.035). Starch concentrations in roots, cuttings, and stems peaked in CP II, whereas soluble carbohydrates accumulated during CP III, causing a culmination of TNC concentrations. A fourfold increase in TNC concentrations was registered in roots during hardening. Carbohydrate concentrations in perennial organs decreased during CP IV and CP V. Nitrogen supply had a significant effect on TNC concentration in perennial organs ($0.001 < p < 0.01$) but not in leaves. The –N treatment enhanced average concentrations of TNC (Fig. 4) in roots, stems, and green leaves, except in CP III when the two N treatments attained the same levels. The N treatment difference tended to recur in CP IV and CP V in roots and stems and in spring shoots of flushing plants. Average TNC concentrations in spring shoots were 148 ± 6.4 mg·g^{-1} DM in the +N treatment and 168 ± 30.4 mg·g^{-1} DM in the –N treatment. In contrast, average TNC concentrations were enhanced by a higher N supply in cuttings and senesced leaves.

Amounts of starch and TNC differed between climate phases and N treatments (Table 2). The proportion of perennial-organ TNC allocated to roots increased between CP I and CP III from 33 to 59% ($p \leq 0.0001$). Concomitantly, the proportion allocated to cuttings decreased from 45 to 21% ($p \leq 0.0001$) and to stems, from 24 to 20% (p = 0.03). The effect of N supply on TNC allocation was similar to its effect on DM distribution; stems accounted for 24 and 20% (p = 0.002) of the TNC and cuttings 29 and 33% (p = 0.0004) in +N and –N treatments, respectively, and there was no significant difference in roots.

The relation between triglyceride and TNC concentrations differed between organs (p = 0.04) but not between climate phases or N treatments (Fig. 5). The corresponding relation between triglyceride and starch concentrations was not significant. Triglyceride concentration increased with TNC concentration in aboveground organs, whereas in roots there was no apparent relation between the variables.

Discussion

Nitrogen storage

Nitrogen concentrations in the plants depended on the stage in the seasonal cycle, on N supply, and on the organ examined, thereby varying the prerequisites for N reserve formation. Actively growing plants could not accumulate N reserves because N status was low and growth clearly N limited in both N treatments (cf. Ericsson et al. 1992). The continuous N supply stimulated corresponding increases in biomass, which diluted N uptake. As apical shoot growth ceased in response to short photoperiod, starch accumulation further diluted N (Nelson and Dickson 1981). Conditions favorable for N reserve formation did not occur until leaf senescence, which supported accumulation in two ways. First, the growth-related sink strength declined, and second, catabolism in senescing leaves gave rise to an N source for translocation to perennial organs. In response, N concentrations increased in stems (Fig. 2) and also in roots and cutting if expressed on a structural basis (i.e., by subtracting TNC from the DM base). A similar influence of the seasonal growth pattern on the response of N storage to N supply was demonstrated in pear (*Pyrus communis* L.; Taylor et al. 1975) and apple (*Malus pumila* L.; Tromp and Ovaa 1985), where N concentrations in woody organs increased more after late-season fertilization than after early season applications. These findings support the conclusion that accumulation of nutrients in plants is regulated by the relation between supply rate and the utilization capacity (Ingestad 1982; Millard 1988).

Despite the dilution by an increasing biomass, the treatment with higher N supply enhanced N status in growing plants. In addition, the effects of N supply prevailed, allowing +N plants to show higher N concentrations in perennial organs also during dormancy. Such enduring fertilizer effects have previously been reported for *Salix dasyclados* Wimm. (Sennerby-Forsse and von Fircks 1987), *Populus deltoides* Bartr. × *Populus nigra* L. (Wetzel et al. 1995), peach (*Prunus persica* (L.) Batsch.; Taylor 1967, Stassen et al. 1981), and pear (Taylor et al. 1975). Since a higher N supply during the growing period also tended to increase N concentrations in spring shoots, there were indications of N supply effects both on accumulation and mobilization of N reserves.

The interpretation of the pattern of N-reserve allocation in the whole-plant perspective depended on the criteria chosen for storage, and all perennial organs were indicated as having a function in N storage. Seasonal fluctuations in N concentration were larger in stems and cuttings, but in terms of

Table 2. Amounts of nitrogen, starch, and total nonstructural carbohydrates (TNC) in *S. viminalis* L. plant organs sampled in connection with active growth (I), cessation of shoot elongation (II), leaf senescence (III), bud swell (IV), and spring flush (V).

Plant organ	Climate phase	Nitrogen (mg/plant)			Starch (mg/plant)			TNC (mg/plant)		
		+N	–N	LSD	+N	–N	LSD	+N	–N	LSD
Roots	I	8.0	5.0	1.3	17	20	45	100	90	79
	II	16	8.3		390	290		620	440	
	III	23	11		340	210		1300	780	
	IV	22	11		210	140		740	460	
	V	22	11		180	120		460	280	
Cuttings	I	4.9	4.2	1.6	80	63	31	140	120	54
	II	6.3	4.6		210	190		310	270	
	III	8.2	6.0		140	110		400	320	
	IV	10	7.6		79	43		310	210	
	V	9.4	7.2		43	44		190	170	
Stems	I	4.0	2.3	0.7	20	19	22	77	58	32
	II	11	5.1		170	88		340	180	
	III	20	10		100	62		450	250	
	IV	21	12		49	36		350	270	
	V	22	10		41	21		320	200	
Leaves	I	15	8.1	0.6	86	75	29	160	120	38
	II	24	12		72	48		230	120	
	III	6.2	3.5		29	13		220	98	
Spring shoots	V	3.3	1.8		11	10		30	23	
Whole plant	I	32	20	2.2	200	180	72	480	390	110
	II	57	30		840	610		1500	1000	
	III	57	31		620	400		2300	1500	
	IV	53	31		340	220		1400	940	
	V	56	30		280	190		1000	660	

Note: Plants were grown at a higher (+N) or lower (–N) N availability, both at a suboptimal nutrition level. Least significant difference (LSD) was calculated for climate phases I–III ($\alpha = 0.05$, $n = 3$). Means for climate phases IV and V were calculated based on means of dry mass and nutrient concentrations. Data on variation in climate phases IV and V was not available.

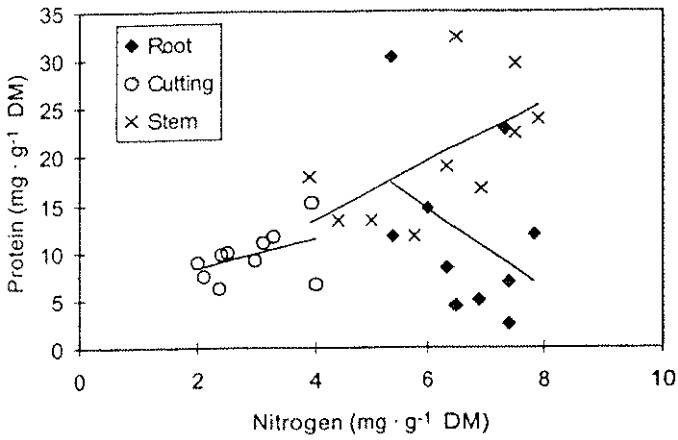
amounts the seasonal changes were equally large in roots. The highest N concentrations, expressed on a structural basis, were observed in roots. During active growth, the greatest proportion of perennial-organ N was in roots, but this proportion declined during hardening. Instead the proportion in stems increased, because they sequestered half of the N exported from senescing leaves. In field-grown willow, increases in stem N concentration generally coincide with leaf fall, reflecting a preferential aboveground storage (Ericsson and von Fircks 1985; Nilsson and Ericsson 1986). Tromp (1970) suggested that all of the translocated leaf N remained aboveground in young apple trees, but Shim et al. (1973) found that it was distributed among all perennial plant parts. Aerial plant parts have generally been considered more than roots with regard to N storage (Titus and Kang 1982). The results from our study support reports demonstrating belowground N storage (Taylor and May 1967; Loescher et al. 1990; Pregitzer et al. 1990).

Although all *Salix* organs functioned in N storage, they differed in respect to the chemical compounds involved. Roots were indicated to employ nonprotein compounds to a higher degree than aerial organs. In stems, the relation between protein and N concentrations was positive over the five climate phases, and the protein/N ratio was higher than in roots, indicating stem N storage in protein form. Autumnal protein accumulation in branch bark and wood has been reported for several temperate hardwoods, including *Salix*, and was linked to specific storage proteins (Sauter and Wellenkamp 1988; Greenwood et al. 1990; Sauter and van Cleve 1990; Wetzel and Greenwood 1991). The lower protein/N ratio in roots during the period from dormancy to bud burst may be explained by preferential N storage in nonprotein form, e.g., amino acids and (or) an early season mobilization of root protein. In the latter case, although twigs are important sites of N mobilization for growth in spring (Sauter et al. 1989), the whole-plant perspective would indicate a more complicated pattern of internal N translocation. Little is known about the N storage forms in roots. Sauter et al. (1989) measured 10–25 mg protein·g^{-1} DM in autumnal roots and slightly higher levels in branches of *Populus* ×*canadensis* Moench. Langheinrich (1993) referred to unpublished data showing that storage proteins accumulated in the bark of older poplar roots. In fruit trees, roots differ from aerial parts by storing N primarily as amino acids (Tromp 1983), which is in agreement with our results for *Salix*.

Carbon storage

Concentrations of nonstructural carbohydrates varied markedly between seasons and plant organs, while N supply had a smaller but still significant effect. Throughout the

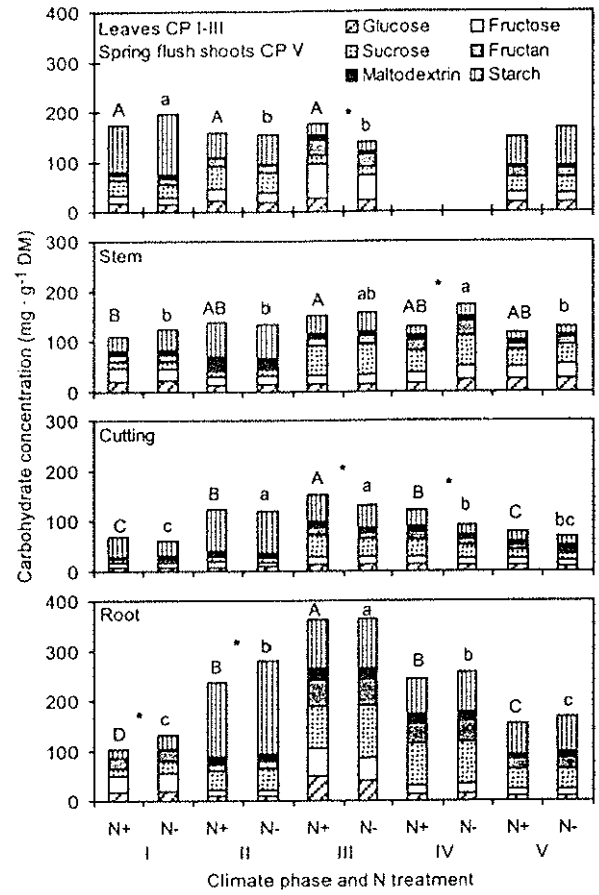
Fig. 3. Covariance between total protein and nitrogen concentrations (mg·g^{-1} DM) in stems, cuttings, and roots of *S. viminalis* L. plants sampled throughout the growth cycle (slope of relations differed significantly between plant organs, $p = 0.05$).



growth cycle there was considerable variation in concentrations of all measured nonstructural carbohydrate compounds but especially in starch and sucrose. Before dormancy, soluble carbohydrates remained constant, whereas starch levels showed some variation. The starch accumulation in CP II was localized to phloem, cortex, and ray tissues in stems and roots (von Fircks and Sennerby-Forsse 1998) and associated with the gradual cessation of shoot elongation and leaf formation (cf. Sennerby-Forsse and von Fircks 1987; Dickson 1991). Probably, this shift in sink strength from growth to storage was a response to short photoperiod, as has been shown for stems of *Populus deltoides* (Nelson and Dickson 1981). After shoot-tip abscission, *Salix* leaves remained green and actively photosynthesizing for several weeks, as can be seen from the increase in DM and TNC in CP III. The importance of such late-season photosynthesis for carbohydrate storage has been demonstrated for *Populus nigra* × *Populus laurifolia* Lebed. (Nelson and Isebrands 1983). The decline in starch concentrations measured in CP III coincided with increases in soluble carbohydrates, and probably resulted from a starch–sugar conversion in response to cool temperatures (Nelson and Dickson 1981; Sennerby-Forsse and von Fircks 1987; Sauter and van Cleve 1991; Witt and Sauter 1994). This conversion was evident also in roots, contrary to observations in sugar maple (*Acer saccharum* Marsh.; Wargo 1979). TNC concentrations decreased after CP III. Since the declines in TNC amounts and DM during CP IV were approximately equal, this TNC probably was used for maintenance respiration, an important function for survival during the dormant period. Alternatively, TNC may have been lost through production and concurrent decay of fine roots because willow roots may continue to grow when aerial plant parts are dormant (Rytter and Rytter 1997). During CP V, TNC amounts decreased two to three times faster than DM, indicating that part of the utilized TNC was allocated to growth of spring flush shoots.

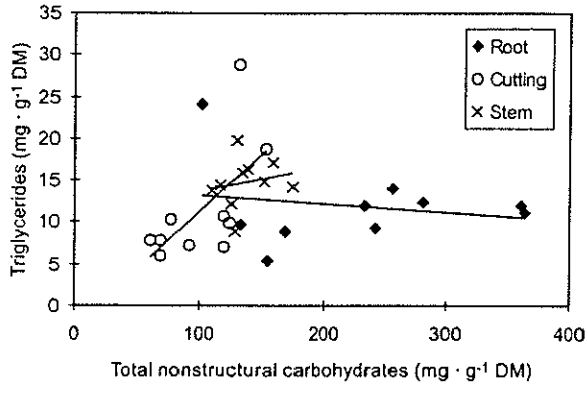
Nitrogen supply caused significant variation in sucrose concentrations in all organs and in starch concentrations in roots and cuttings. Since these were the major pools of

Fig. 4. Concentrations of total nonstructural carbohydrates (mg·g^{-1} DM) in *S. viminalis* plant organs. See Fig. 1 for details ($\alpha = 0.05$, $n = 3$).



nonstructural carbohydrates, it may be concluded that N availability affected TNC status in the whole plant. Nutrient limitation generally promotes carbohydrate accumulation in leaves of actively growing plants (Waring et al. 1985; McDonald et al. 1986; Rytter and Ericsson 1993). A greater access to N allowed our plants to assimilate more C per unit time, as evidenced by the faster DM increase, but TNC concentrations in roots were lower than in more N-limited plants. Stem TNC showed the same tendency. In response to a higher internal N status, the proportion of carbohydrates partitioned into the structural components of growth increases, as long as growth is not restricted by other factors (Birk and Matson 1986). The relation between N and TNC status did not, however, remain negative in our *Salix* plants. During CP III, TNC amounts in perennial organs increased by a factor of 1.7 and 1.5 in the +N and –N treatments, respectively, whereas DM increased by 1.2 in both treatments. When growth declined in the autumnal climate, the larger leaf area and possibly higher photosynthetic capacity allowed +N plants to accumulate TNC faster than –N plants. Likewise, the negative effect of N supply on TNC status observed during the growing season disappeared or turned positive after growth cessation in loblolly pine (*Pinus taeda* L) needles (Birk and Matson 1986), in leaves of field-grown *S. viminalis* (Rytter and Ericsson 1993), and in stems and

Fig. 5. Covariance between concentrations (mg·g^{-1} DM) of triglyceride and total nonstructural carbohydrate in stems, cuttings, and roots of *S. viminalis* L. plants sampled throughout the growth cycle (slope of relations differed significantly between plant organs, $p = 0.04$).



large roots of *Populus nigra* × *Populus deltoides*, clone *Eugenei* (recalculated from Nguyen et al. 1990). In addition to effects on C assimilation rate, the positive effect of N availability on autumnal TNC accumulation may be attributed to prolongation of the growing season (Stassen et al. 1981). Increased plant N status has been shown to delay leaf senescence in *Salix dasyclados* (Sennerby-Forsse and von Fircks 1987), but no such effect was observed in the present study. The N-treatment differences recurred after CP III, possibly as a result of higher respiration rates in plants of the +N treatment (Waring et al. 1985; Margolis and Waring 1986).

Cuttings, in contrast to other organs, responded to higher N availability by building up higher TNC concentrations also during the growing period. In plants grown at a higher N availability, larger amounts of TNC were translocated from leaves to roots and were thus available during transit in the cutting. Since the cuttings did not respond by growth to this TNC supply, as evidenced by the lack of significant change in structural DM during the experiment, more TNC was available for storage in cutting tissues in the +N treatment. Results concerning the role of the cutting in growth and development of coppice plants are not previously available in the literature.

Roots were prominent as carbohydrate storage organs, exhibiting both the highest levels and the largest seasonal fluctuations of starch and TNC concentrations. Late-season accumulation of root carbohydrates has also been reported from field-grown, young poplar trees, *Populus trichocarpa* Torr. & Gray × *Populus deltoides*, cv. Raspalje, and hybrid poplar, clone *Eugenei*, (Bonicel et al. 1987; Nguyen et al. 1990). Even though TNC concentrations are generally higher in roots than in other parts of the tree, the special role of roots in storage has been questioned. Nutrient amounts may still be greater in aboveground tree parts, owing to low biomass root/shoot ratios, especially for older trees. Further, this allocation pattern often remains unchanged over the year (Loescher et al. 1990). In the young plants used in our experiment and by Nguyen et al. (1990), however, the dominating site for carbohydrate storage was in roots, and this role was strengthened during hardening.

Plants partition C for storage into a wide array of compounds, including starch, sugars, and lipids. In *Salix*, starch and sucrose were the predominant nonstructural carbohydrate compounds on a whole-plant basis, while glucose and fructose intermittently reached high levels in some organs. The selected analytical method probably detected all major nonstructural C compounds. Additional substances have been detected using gas chromatography, e.g., 10–20 mg·g^{-1} DM *myo*-inositol in *Salix* leaves (A. Flower-Ellis, Sveriges Lantbruksuniversitet, Uppsala, Sweden, personal communication). In hybrid poplar roots, *myo*-inositol and salicin each constituted 1.5 mg·g^{-1} DM (Tschaplinski and Blake 1989). Lipids may have a storage function in aerial parts of *Salix* plants, because of the positive relation to carbohydrate storage compounds over the growth cycle in these organs. In roots, however, there was no apparent relation. Lipid concentrations have been shown to fluctuate seasonally in stems, but not in roots, of *Tilia cordata* Mill. (Höll and Priebe 1985) and *Populus deltoides* × *Populus nigra* (Wetzel et al. 1995). In poplar, lipid concentration was higher in stems than in roots, but in *Tilia* the levels were comparable. The contribution of lipids to *Salix* C storage, if any, would have been small because the amounts of lipids were 10 times lower than those of TNC. A ratio of 1:10 to 1:5 has been measured for poplar stems and branches (Nelson and Dickson 1981; Sauter and van Cleve 1994) and even less for branch wood of *Betula pendula* Roth (Harms and Sauter 1992).

Spring flush

Nitrogen-supply effects tended to carry over between growth periods, i.e., on average, plants that had received a higher N supply before dormancy showed higher N concentrations and lower TNC concentrations in spring flush shoots. The reason for the lack of statistical significance of N treatment effects in spring-flush shoots probably relates to the low number of replicates in the chemical analyses (n = 2) and to plants being harvested at different developmental stages. Furthermore, the changes in nutrient amounts in perennial organs did not give a clear picture of which organs exported resources to the new shoots because sampling was performed before significant reserve depletion had occurred. The utilization of C and N reserves for spring flush has been demonstrated using isotopic labeling. In mature apple trees, TNC reserves were utilized for development of the first five or six leaves of current-year extension shoots (Hansen 1971), and in young apple trees, 25% of the estimated TNC reserve consumption was incorporated into the early season shoots (Hansen and Grauslund 1973). The N utilized for early season leaf growth in apple and sycamore (*Acer pseudoplatanus* L.) was supplied by reserves in perennial, woody tissues, and the mobilization was unaffected by the current N supply (Millard and Neilsen 1989; Millard and Thomson 1989; Millard and Proe 1991). Since the +N plants in our study tended to grow faster, it can be tentatively concluded that N reserves rather than TNC reserves were limiting for growth during spring flush. The ample access to carbohydrates may be related both to high TNC accumulation under N-limited conditions in the previous growing season, and to the contribution of new leaves once they reach photosynthetic maturity during spring flush.

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References

Bergmeyer, H.U., Bernt, E., Schmidt, F., and Stork, H. 1974. *In* Methods of enzymatic analysis. *Edited by* H.U. Bergmeyer. Verlag Chemie, Weinheim, Germany, and Academic Press Inc., New York and London. Vol. 3. pp. 1196–1201.

Bernt, E., and Bergmeyer, H.U. 1974. *In* Methods of enzymatic analysis. *Edited by* H.U. Bergmeyer. Verlag Chemie, Weinheim, Germany, and Academic Press Inc., New York and London. Vol. 3. pp. 1304–1307.

Birk, E.M., and Matson, P.A. 1986. Site fertility affects seasonal carbon reserves in loblolly pine. Tree Physiol. **2**: 17–27.

Bonicel, A., Haddad, G., and Gagnaire, J. 1987. Seasonal variations of starch and major soluble sugars in the different organs of young poplars. Plant Physiol. Biochem. **25**: 451–459.

Bryant, J.P., Chapin, F.S., and Klein, D.R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos, **40**: 357–368.

Chapin, F.S., and Kedrowski, R.A. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. Ecology, **64**: 376–391.

Chapin, F.S., Johnson, D.A., and McKendrick, J.D. 1980. Seasonal movement of nutrients in plants of differing growth form in an Alaskan tundra ecosystem: implications for herbivory. J. Ecol. **68**: 189–209.

Christersson, L. 1986. High technology biomass production by *Salix* clones on a sandy soil in southern Sweden. Tree Physiol. **2**: 261–272.

Cyr, D.R., and Bewley, J.D. 1990. Proteins in the roots of the perennial weeds chicory (*Cichorium intybus* L.) and dandelion (*Taraxacum officinale* Weber) are associated with overwintering. Planta, **182**: 370–374.

Dickmann, D.I., and Pregitzer, K.S. 1992. The structure and dynamics of woody plant root systems. *In* Ecophysiology of short rotation forest crops. *Edited by* C.P. Mitchell, J.B. Ford-Robertson, T. Hinckley, and L. Sennerby-Forsse. Elsevier, London and New York. pp. 95–123.

Dickson, R.E. 1991. Assimilate distribution and storage. *In* Physiology of trees. *Edited by* A.S. Raghavendra. John Wiley & Sons, Inc., New York. pp. 51–85.

Ericsson, T. 1981. Growth and nutrition of three *Salix* clones in low conductivity solutions. Physiol. Plant. **52**: 239–244.

Ericsson, T., and von Fircks, H.A. 1985. Nitrogen cycling and development of frost hardiness in *Salix dasyclados* Wimm. in relation to nutrient supply. Poster abstract. Physiol. Plant. **64** (Fasc. 2).

Ericsson, T., Rytter, L., and Linder, S. 1992. Nutritional dynamics and requirements of short rotation forests. *In* Ecophysiology of short rotation forest crops. *Edited by* C.P. Mitchell, J.B. Ford-Robertson, T. Hinckley, and L. Sennerby-Forsse. Elsevier, London and New York. pp. 35–65.

Fischer, C., and Höll, W. 1991. Food reserves of Scots pine (*Pinus sylvestris* L.). I. Seasonal changes in carbohydrate and fat reserves of pine needles. Trees, **5**: 187–195.

Greenwood, J.S., Demmers, C., and Wetzel, S. 1990. Seasonally dependent formation of protein-storage vacuoles in the inner bark tissues of *Salix microstachya*. Can. J. Bot. **68**: 1747–1755.

Hansen, P. 1971. ^{14}C-studies on apple trees. VII. The early seasonal growth in leaves, flowers and shoots as dependent upon current photosynthates and existing reserves. Physiol. Plant. **25**: 469–473.

Hansen, P., and Grauslund, J. 1973. ^{14}C-studies on apple trees. VIII. The seasonal variation and nature of reserves. Physiol. Plant. **28**: 24–32.

Harms, U., and Sauter, J.J. 1992. Changes in content of starch, protein, fat and sugars in the branchwood of *Betula pendula* Roth during fall. Holzforschung, **46**: 455–461.

Höll, W., and Priebe, S. 1985. Storage lipids in the trunk- and rootwood of *Tilia cordata* Mill. from the dormant to the growing period. Holzforschung, **39**: 7–10.

Ingestad, T. 1982. Relative addition rate and external concentration: driving variables used in plant nutrition research. Plant Cell Environ. **5**: 443–453.

Ingestad, T., and Lund, A.-B. 1986. Theory and techniques for steady state mineral nutrition and growth of plants. Scand. J. For. Res. **1**: 439–453.

Isebrands, J.G., and Burk, T.E. 1992. Ecophysiological growth process models of short rotation forest crops. *In* Ecophysiology of short rotation forest crops. *Edited by* C.P. Mitchell, J.B. Ford-Robertson, T. Hinckley, and L. Sennerby-Forsse. Elsevier, London and New York. pp. 231–266.

Jonsson, A., Ericsson, T., Eriksson, G., Kähr, M., Lundkvist, K., and Norell, L. 1997. Interfamily variation in nitrogen productivity of *Pinus sylvestris* seedlings. Scand. J. For. Res. **12**: 1–10.

Kirsten, W.J., and Hesselius, G.U. 1983. Rapid, automatic, high capacity Dumas determination of nitrogen. Microchem. J. **28**: 529–547.

Kozlowski, T.T. 1992. Carbohydrate sources and sinks in woody plants. Bot. Rev. **58**: 107–222.

Kramer, P.J., and Kozlowski, T.T. 1979. Physiology of woody plants. Academic Press, Orlando, Fla.

Landhäusser, S.M., and Lieffers, V.J. 1997. Seasonal changes in carbohydrate storage and regrowth in rhizomes and stems of four boreal forest shrubs: applications in *Picea glauca* understory vegetation. Scand. J. For. Res. **12**: 27–32.

Langheinrich, U. 1993. Clonal variation in apical growth and content in vegetative storage proteins in *Populus*. Trees, **7**: 242–249.

Loescher, W.H., McCamant, T., and Keller, J.D. 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience, **25**: 274–281.

Margolis, H.A., and Waring, R.H. 1986. Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism. Can. J. For. Res. **16**: 897–902.

McDonald, A.J.S., Ericsson, A., and Lohammar, T. 1986. Dependence of starch storage on nutrient availability and photon flux density in small birch (*Betula pendula* Roth). Plant Cell Environ. **9**: 433–438.

McDonald, J. 1984. A summary criticism of photosynthetic studies and stemwood production. *In* Ecology and management of forest biomass production systems. *Edited by* K. Perttu. Department of Ecology and Environmental Research, Sveriges Lantbruksuniversitet, Uppsala, Sweden, Rep. No. 15. pp. 167–178.

Millard, P. 1988. The accumulation and storage of nitrogen by herbaceous plants. Plant Cell Environ. **11**: 1–8.

Millard, P. 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. Z. Pflanzenernähr. Bodenkd. **159**: 1–10.

Millard, P., and Neilsen, G.H. 1989. The influence of nitrogen supply on the uptake and remobilization of stored N for the seasonal growth of apple trees. Ann. Bot. (London), **63**: 301–309.

Millard, P., and Proe, M.F. 1991. Leaf demography and the seasonal internal cycling of nitrogen in sycamore (*Acer pseudoplatanus* L.) seedlings in relation to nitrogen supply. New Phytol. **117**: 587–596.

Millard, P., and Thomson, C.M. 1989. The effect of the autumn senescence of leaves on the internal cycling of nitrogen for the spring growth of apple trees. J. Exp. Bot. **40**: 1285–1289.

Nelson, E.A., and Dickson, R.E. 1981. Accumulation of food reserves in cottonwood stems during dormancy induction. Can. J. For. Res. **11**: 145–154.

Nelson, N.D., and Isebrands, J.G. 1983. Late-season photosynthesis and photosynthate distribution in an intensively-cultured *Populus nigra* × *laurifolia* clone. Photosynthetica, **17**: 537–549.

Nguyen, P.V., Dickmann, D.I., Pregitzer, K.S., and Hendrick, R. 1990. Late-season changes in allocation of starch and sugar to shoots, coarse roots, and fine roots in two hybrid poplar clones. Tree Physiol. **7**: 95–105.

Nilsson, L.O., and Ericsson, T. 1986. Influence of shoot age on growth and nutrient uptake patterns in a willow plantation. Can. J. For. Res. **16**: 185–190.

Pregitzer, K.S., Dickmann, D.I., Hendrick, R., and Nguyen, P.V. 1990. Whole-tree carbon and nitrogen partitioning in young hybrid poplars. Tree Physiol. **7**: 79–93.

Rytter, L., and Ericsson, T. 1993. Leaf nutrient analysis in *Salix viminalis* (L.) energy forest stands growing on agricultural land. Z. Pflanzenernähr. Bodenkd. **156**: 349–356.

Rytter, R.-M., and Rytter, L. 1997. Seasonal growth patterns of fine roots and shoots in lysimeter-grown basket willows and grey alders. Department of Short Rotation Forestry, Sveriges Lantbruksuniversitet. Uppsala, Sweden. Rep. No. 59.

SAS Institute Inc. 1982. SAS User's guide. statistics. SAS Institute Inc., Cary, N.C.

Sauter, J.J., and van Cleve, B. 1990. Biochemical, immunochemical, and ultrastructural studies of protein storage in poplar (*Populus* ×*canadensis* 'robusta') wood. Planta, **183**: 92–100.

Sauter, J.J., and van Cleve, B. 1991. Biochemical and ultrastructural results during starch–sugar conversion in ray parenchyma cells of *Populus* during cold adaption. J. Plant Physiol. **139**: 19–26.

Sauter, J.J., and van Cleve, B. 1994. Storage, mobilization and interrelations of starch. sugars, protein and fat in the ray storage tissue of poplar trees. Trees, **8**: 297–304.

Sauter, J.J., and Wellenkamp, S. 1988. Protein storing vacuoles in ray cells of willow wood (*Salix caprea* L.). Int. Assoc. Wood Anat. Bull. New. Ser. **9**: 59–65.

Sauter, J.J., van Cleve, B., and Wellenkamp, S. 1989. Ultrastructural and biochemical results on the localization and distribution of storage proteins in a poplar tree and in twigs of other tree species. Holzforschung, **43**: 1–6.

Sennerby-Forsse, L., and Christersson, L. 1994. The role of energy forestry in alternative energy planning, waste recycling and agriculture in Sweden. World Resour. Rev. **6**: 395–405.

Sennerby-Forsse, L., and von Fircks, H.A. 1987. Ultrastructure of cells in the cambial region during winter hardening and spring dehardening in *Salix dasyclados* Wim. grown at two nutrient levels. Trees, **1**: 151–163.

Sennerby-Forsse, L., and Zsuffa, L. 1995. Bud structure and resprouting in coppiced stools of *Salix viminalis* L., *S. eriocephala* Michx., and *S. amygdaloides* Anders. Trees, **9**: 224–234.

Shim, K.-K., Titus, J.S., and Splittstoesser, W.E. 1973. The fate of carbon and nitrogen from urea applied to foliage of senescing apple trees. J. Am. Soc. Hortic. Sci. **98**: 360–366.

Stassen, P.J.C., Terblanche, J.H., and Strydom, D.K. 1981. The effect of time and rate of nitrogen application on development and composition of peach trees. Agroplantae, **13**: 55–61.

Steen, E., and Larsson, K. 1986. Carbohydrates in roots and rhizomes of perennial grasses. New Phytol. **104**: 339–346.

Taylor, B.K. 1967. The nitrogen nutrition of the peach tree: I. Seasonal changes in nitrogenous constituents in mature trees. Aust. J. Biol. Sci. **20**: 379–387.

Taylor, B.K., and May, L.H. 1967. The nitrogen nutrition of the peach tree: II. Storage and mobilization of nitrogen in young trees. Aust. J. Biol. Sci. **20**: 389–411.

Taylor, B.K., van den Ende, B., and Canterford, R.L. 1975. Effects of rate and timing of nitrogen applications on the performance and chemical composition of young pear trees, cv 'Williams Bon Chretien.' J. Hortic. Sci. **50**: 29–40.

Titus, J.S., and Kang, S.-M. 1982. Nitrogen metabolism, translocation, and recycling in apple trees. Hortic. Rev. **4**: 204–246.

Tromp, J. 1970. Storage and mobilization of nitrogenous compounds in apple trees with special reference to arginine. *In* Physiology of tree crops. *Edited by* L.C. Luckwill and C.V. Cutting. Academic Press, New York. pp. 143–159.

Tromp, J. 1983. Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen. Plant Soil, **71**: 401–413.

Tromp, J., and Ovaa, J.C. 1985. Response of young apple trees to time of nitrogen fertilization with respect ot nitrogen, potassium, and calcium levels in xylem sap, new growth and the tree as a whole. J. Plant Physiol. **119**: 301–309.

Tschaplinski, T.J., and Blake, T.J. 1989. Water-stress tolerance and late-season organic solute accumulation in hybrid poplar. Can. J. Bot. **67**: 1681–1688.

Volenec, J.J., Ourry, A., and Joern, B.C. 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. Physiol. Plant. **97**: 185–193.

von Fircks, Y., and Sennerby-Forsse, L. 1998. Seasonal fluctuations of starch in root and stem tissues of coppiced *Salix viminalis* plants grown under two nitrogen regimes. Tree Physiol. **18**: 243–249.

Wargo, P.M. 1979. Starch storage and radial growth in woody roots of sugar maple. Can. J. For. Res. **9**: 49–56.

Waring, R.H., McDonald, A.J.S., Larsson, S., Ericsson, T., Wiren, A., Arwidsson, E., Ericsson, A., and Lohammar, T. 1985. Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition. Oecologia, **66**: 157–160.

Wetzel, S., and Greenwood, J.S. 1991. A survey of seasonal bark proteins in eight temperate hardwoods. Trees, **5**: 153–157.

Wetzel, S., Sennerby-Forsse, L., and Burgess, D. 1995. Storage compounds in *Populus* cuttings in response to two different nitrogen regimes. *In* Nutrient uptake and cycling in forest ecosystems. *Edited by* L.O. Nilsson, R.F. Hüttl, and U.T. Johansson. Kluwer Academic Publishers. Dordrecht, the Netherlands. pp. 677–685.

Witt, W., and Sauter, J.J. 1994. Starch metabolism in poplar wood ray cells during spring mobilization and summer deposition. Physiol. Plant. **92**: 9–16.

Zasada, J.C., Tappeiner, J.C., III, Maxwell, B.D., and Radwan, M.A. 1994. Seasonal changes in shoot and root production and in carbohydrate content of salmonberry (*Rubus spectabilis*) rhizome segments from the central Oregon Coast Ranges. Can. J. For. Res. **24**: 272–277.