Research paper

Whole-plant allocation to storage and defense in juveniles of related evergreen and deciduous shrub species

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In evergreen plants, old leaves may contribute photosynthate to initiation of shoot growth in the spring. They might also function as storage sites for carbohydrates and nitrogen (N). We hence hypothesized that whole-plant allocation of carbohydrates and N to storage in stems and roots may be lower in evergreen than in deciduous species. We selected three species pairs consisting of an evergreen and a related deciduous species: *Mahonia aquifolium* (Pursh) Nutt. and *Berberis vulgaris* L. (Berberidaceae), *Prunus laurocerasus* L. and *Prunus serotina* Ehrh. (Rosaceae), and *Viburnum rhytidophyllum* Hemsl. and *Viburnum lantana* L. (Adoxaceae). Seedlings were grown outdoors in pots and harvested on two dates during the growing season for the determination of biomass, carbohydrate and N allocation ratios. Plant size-adjusted pools of nonstructural carbohydrates in stems and roots were lower in the evergreen species of Berberidaceae and Adoxaceae, and the slope of the carbohydrate pool vs plant biomass relationship was lower in the evergreen species of Rosaceae compared with the respective deciduous species, consistent with the leading hypothesis. Pools of N in stems and roots, however, did not vary with leaf habit. In all species, foliage contained more than half of the plant's nonstructural carbohydrate pool and, in late summer, also more than half of the plant's N pool, suggesting that in juvenile individuals of evergreen species, leaves may be a major storage site. Additionally, we hypothesized that concentration of defensive phenolic compounds in leaves should be higher in evergreen than in deciduous species, because the lower carbohydrate pool in stems and roots of the former restricts their capacity for regrowth following herbivory and also because of the need to protect their longer-living foliage. Our results did not support this hypothesis, suggesting that evergreen plants may rely predominantly on structural defenses. In summary, our study indicates that leaf habit has consequences for storage economics at the whole-plant level, with evergreen shrub species storing less carbohydrates (but not N) per unit plant biomass than deciduous species.

Keywords: biomass allocation, carbohydrate storage, evergreen leaves, phenolic compounds, resource allocation.

Introduction

Leaf lifespan constitutes a major axis of differentiation of plant functional types, with deciduous and evergreen species forming strikingly distinct categories in many biomes (Wright et al. 2004, Van Ommen Kloeke et al. 2012). Functional consequences of the evergreen leaf habit have been studied extensively, revealing a pattern of structural, nutritional and photosynthetic correlations (Chabot and Hicks 1982, Reich et al. 1992, Givnish 2002, Wyka and Oleksyn 2014). Although our understanding of evergreen plants has largely concentrated on leaf traits, it is clear

that this phenological syndrome potentially influences plant functioning at other levels of organization, including whole-plant resource partitioning (Givnish 2002).

Plants usually allocate part of the carbohydrate acquired through photosynthesis to storage (Chapin et al. 1990, Kozlowski 1992). Seasonal variability of carbohydrate levels in plant tissues indicative of accumulation and utilization has been found in numerous woody and herbaceous perennials from a variety of habitats (e.g., Mooney et al. 1992, Newell et al. 2002, Hoch et al. 2003, Palacio et al. 2007a, Sanz-Pérez et al. 2009,

Richardson et al. 2013). Stored carbohydrates may be used to support initiation of shoot growth after dormancy (Sanz-Pérez et al. 2009, Hoch et al. 2013), reproduction (Mooney and Hays 1973) or early-season production of new wood (Michelot et al. 2012). These roles should be especially important in deciduous plants that enter the new growing season in a leafless state. Conversely, in evergreen plants, the reserves are potentially less important at the start of the growing season because of the availability of carbohydrates from current photosynthesis (Larcher 2003, Epron et al. 2012). The lower reliance of growth processes in evergreen plants on stored reserves suggests that their allocation of carbohydrates to storage pool should be smaller in comparison with deciduous species.

The lower allocation to storage in evergreen species has been hypothesized (Kozlowski 1992, Hoch et al. 2003, Fajardo et al. 2013) based on comparison of carbohydrate concentrations between plants with contrasting habits. Studies of storage patterns in evergreen and deciduous species have usually revealed lower total nonstructural carbohydrate (TNC) concentrations in evergreen plants; however, they have often been based on comparisons between evergreen conifers and deciduous broadleaves with the associated phylogenetic differences confounding the effects of differences in leaf habit (Kobe 1997, Hoch et al. 2003, Machado and Reich 2006, Michelot et al. 2012, Richardson et al. 2013, Zhang et al. 2014). Several reports, however, have compared evergreen and deciduous species sharing closer phylogenetic backgrounds. Greater TNC concentration in deciduous than in evergreen species has been reported in studies of arctic shrubs (Chapin and Shaver 1988), *Nothofagus* species (Fajardo et al. 2013, Piper and Fajardo 2014) and drought-deciduous vs evergreen Mediterranean (Mooney and Hays 1973) and neotropical (Newell et al. 2002) woody species. Also among gymnosperms, stemwood (but not necessarily branch) TNC concentrations were higher in the deciduous *Larix* compared with evergreen conifers (Hoch et al. 2003, Fajardo et al. 2013). Only a few studies show lower TNC concentrations in deciduous than evergreen (Zhang et al. 2014) or semi-evergreen (Palacio et al. 2007a) species or report similar concentrations between contrasting plant types (Sanz-Pérez et al. 2007). Thus, according to the majority of reports, evergreen species build lower TNC concentrations than deciduous species.

The allocation to storage, however, is best evaluated not on the basis of carbohydrate concentration in the storage organs but rather based on size of the whole-plant carbohydrate pool in relation to the plant size (Imaji and Seiwa 2010, Kobe et al. 2010, Poorter and Sack 2012, Zhang et al. 2013). This is because biomass partitioning to reserve-containing organs, such as roots and stems, may vary independently of the reserve concentration. Moreover, for a given species, organ biomass ratios are highly plastic and are influenced by developmental stage and growth conditions (Poorter et al. 2012). Data sets accounting for whole-plant TNC pools in woody species are not common. They

are available for juvenile (e.g., Canham et al. 1999, Schaberg et al. 2000, Cerasoli et al. 2004, Myers and Kitajima 2007, Poorter and Kitajima 2007, Piper et al. 2009) and, less commonly, for adult (Barbaroux et al. 2003) individuals of woody plants, but they usually do not compare species differing in leaf habit. As an exception, a multispecies study of fire-prone savanna tree seedlings reported that root TNC in evergreen species accounted for a larger fraction of plant biomass than in deciduous species at a common plant mass, while no data were given for stem carbohydrates (Tomlinson et al. 2013). In another study, stem TNC pools of plant size-normalized evergreen conifers and deciduous angiosperms showed little difference between plant types while root pools were not studied (Zhang et al. 2014). Both of these studies have considered storage pools of only single organ type. To our knowledge, the hypothesis of reduced allocation to storage in evergreen vs deciduous species has not been explicitly tested at the whole-plant level.

Apart from carbohydrates, plants form nutrient, especially nitrogen (N), reserves (Chapin et al. 1990, Millard and Grelet 2010). Their role in supporting growth, regrowth and reproduction is parallel to that of carbohydrates, even though a number of important physiological differences between these two reserve types are apparent (Pasche et al. 2002, Millard and Grelet 2010). Based on a limited number of reports, Millard and Grelet (2010) have not found a consistent difference between evergreen and deciduous species in their dependence of new growth on remobilized N. It is also not known whether the levels of N reserves differ between the two functional types.

Stored resources may be needed to support plant survival and regeneration of damaged organs following a severe herbivory event (Myers and Kitajima 2007). As such, storage carbohydrates or nutrients may be considered parts of the integrated defense system, enhancing plant tolerance to herbivory (Piper and Fajardo 2014). Consequently, a trade-off may be expected between the level of reserves and other components of the defensive system, such as tough leaf structure or concentration of defensive compounds (Imaji and Seiwa 2010). Leaf lifespan is usually considered to be positively related to the level of carbon-intensive defenses including fiber, tannin and lignin content (Coley 1988). However, few studies have examined the relationship between storage and defense, in spite of the importance of each for species' survival strategy in a variety of habitats (Coley et al. 1985, De Jong and van der Meijden 2000).

In this study, we searched for whole-plant differences in nonstructural carbohydrate and N levels between evergreen and deciduous woody plants. To avoid a possible phylogenetic bias, we used three species pairs, each consisting of an evergreen and a related deciduous species. Specifically, we tested the hypotheses that (i) for a given plant biomass, evergreen species contain a smaller quantity of storage carbohydrates in roots and stems than do deciduous species and (ii) the same trend is reflected in the allocation of N. Additionally, we tested the

hypothesis (iii) that allocation to storage is inversely related to the concentration of defensive phenolic compounds.

Materials and methods

Plant cultivation

Seeds of *Berberis vulgaris* L. and *Mahonia aquifolium* (Pursh) Nutt. (Berberidaceae), *Prunus laurocerasus* L. and *Prunus serotina* Ehrh. (Rosaceae), and *Viburnum lantana* L. and *Viburnum rhytidophyllum* Hemsl. (Adoxaceae) were collected in 2009 from at least five parental individuals per species using cultivated specimens as seed sources (wild specimens in *B. vulgaris*). For presowing treatment, seeds were mixed with a stratification medium (a 1 : 1 v/v mixture of fine peat and moist sand) and stratified according to species requirements: *B. vulgaris* for 4 months at 3 °C, *M. aquifolium* for 4 months at 20 °C, followed by 3 months at 3 °C, *P. serotina* for 2 weeks at 20 °C, followed by 14 weeks at 3 °C, *P. laurocerasus* for 3 months at 3 °C, *V. lantana* for 6 months at 3 °C and *V. rhytidophyllum* for 10 months at 3 °C. Stratification was followed by exposure of seeds to temperature cycles of 16 h (8 h at 20 °C alternating with 16 h at 3 °C) for various durations (4–17 weeks, depending on species) to stimulate germination.

To obtain seedlings, germinated seeds were transferred to a growing room, maintained at 20 °C under 60 μmol m^{-2} s^{-1} irradiance for 16 h a day until shoot elongation was evident. This procedure resulted in raising seedlings at various times during the 2010 growing season. Seedlings were planted in 200-ml pots containing horticultural peat substrate (pH 5.5) with the addition of 20% perlite (v/v) and 2 g l^{-1} of slow release fertilizer (Osmocote 15-10-12) and maintained in an outdoor enclosure covered by a neutral density shade cloth transmitting 50% of solar irradiance. Plants overwintered in the enclosure with pots and stem bases protected by a layer of sawdust. In the spring, seedlings bearing signs of winter damage as well as the smallest individuals were rejected. Only healthy seedlings showing vigorous growth initiation were selected for further cultivation. Certain size variability within and between species was not considered a problem since the aim of our experiment was to study a spectrum of individual plant biomass values using allometric analysis. On 10 May 2011, seedlings were planted into 3-l pots containing substrate composed of forest soil (60% v/v), peat (pH 5.5; 20%) and perlite (20%) enriched with 3 g l^{-1} of Osmocote 15-10-12. Plants of each species were randomly divided among three blocks, with equal number of individuals assigned to each block. A randomized complete block design with subsampling was thus used. All plants were randomly rearranged within each block at ~2-week intervals. Plants were regularly watered to field capacity. None of the plants produced flowers in the course of the experiment.

Morphology and biomass

Whole plants were harvested on 10 July (day-of-year 191) and then on 7 September (day-of-year 250) to produce a range of plant sizes within each species. At harvests, plants had healthy leaves without symptoms of senescence and did not form winter buds. Any plants with signs of slug damage or disease spots were rejected. Numbers of harvested plants are given in Table S1 available as Supplementary Data at *Tree Physiology* Online. At each harvest, soil was thoroughly washed off and the entire root system was recovered and cleaned using forceps. Above-ground parts were separated into stems (including buds), leaf laminas and petioles or rachises. The separate collection of petioles and rachises was necessitated by their prime involvement in support rather than photosynthesis, yet they were not included in the stem fraction because in deciduous species, they abscise together with laminas. Two mature laminas per plant were scanned and their areas were measured using WinFolia software (Regent Instruments, Quebec City, QC, Canada). Total area of the remaining leaf laminas on the plant was measured separately. Immediately after harvest and scanning, all plant material was placed in a forced-circulation drier, dried at 65 °C for 72 h and then weighed. Leaf mass per area (LMA, g m^{-2}) was calculated as LMA = leaf mass × leaf area^{-1}.

Chemical analyses

Biomass samples (except for petioles, for which too little biomass was available) were ground to <1 mm particles using a Culatti Mikro-Feinmühle (IKE Labortechnik, Staufen, Germany). Total nonstructural carbohydrates (sum of soluble carbohydrates and starch, TNC) were determined spectrophotometrically according to Hansen and Møller (1975) and Haissig and Dickson (1979). Soluble carbohydrates were extracted three times for 10 min in methanol–chloroform–water (6 : 2.5 : 1.5 v/v) at room temperature and quantified at $\lambda = 625$ nm following a color reaction with anthrone. For determination of starch, the precipitate was incubated in NaF buffer (pH 4.5) at 100 °C for starch gelatinization, followed by digestion with amyloglucosidase, oxidation with the peroxidase–glucose oxidase and color reaction with *o*-dianisidine ($\lambda = 450$ nm). The total amount of soluble carbohydrates and starch (using glucose as a standard), and their sum (TNC), was expressed as percentage of dry mass (% d.m.). Nitrogen concentration (% TNC-free d.m.) was determined using an Elemental Combustion System CHNS-O 4010 (Costech Instruments, Milan, Italy).

For analysis of total phenolic compounds, large sample quantities were required; therefore, in several cases, composite samples were assembled by pooling samples from two to three similar-sized plants in each species and only plants from the final harvest were used. Number of samples per species was thus between 4 and 10 (Table S1 available as Supplementary Data at *Tree Physiology* Online). Concentrations of phenolic compounds were determined using Folin Ciocalteu's Phenol Reagent (Sigma F-9252) at $\lambda = 660$ nm (Sukovata et al. 2015), and results were expressed as μmol chlorogenic acid g^{-1} d.m.

Data analysis

Allocation ratios (leaf mass ratio (LMR) = leaf mass × whole-plant mass^{-1}, stem mass ratio (SMR) = stem mass × whole-plant mass^{-1}, root mass ratio (RMR) = root mass × whole-plant mass^{-1}) were calculated for individual plants. Differences of means between species with contrasting leaf habits were evaluated separately for each family, using analysis of variance with harvest day and leaf habit as fixed factors. Since the effects of blocks and interactions of blocks with leaf habit on plant and organ biomass were all insignificant, the block effect was dropped from analyses. A posteriori comparisons of means were based on contrasts within confamiliar species pairs. Analysis of covariance on data pooled across both sampling days with leaf habit as a discrete factor, individual plant or organ biomass as a continuous predictor variable and an interaction term was used to evaluate differences in allocation ratios and storage pools at a common size. When biomass × leaf habit interaction was nonsignificant (homogenous slopes), another analysis of covariance was run with plant biomass and leaf habit as the only effects. JMP (v. 8.0.2) program (SAS Institute Inc., Cary, NC, USA) was used for all data analysis.

Results

Mean biomass values of organ fractions at each harvest for the six studied species are reported in Table S2 available as Supplementary Data at *Tree Physiology* Online. We were, however, principally interested in biomass allocation ratios (shown in Figure 1). Leaf laminas constituted the largest biomass fraction in all species at both harvests, ranging from 48% in *P. serotina* at first harvest to 68% in *V. rhytidophyllum* at second harvest. Evergreen species had larger lamina fractions than deciduous species only in genera *Prunus* and *Viburnum* and only at the second harvest. However, when laminas were considered jointly with petioles (or rachises in case of *M. aquifolium*), leaf mass fraction was larger in evergreen than in deciduous species in all three species pairs but only at the second harvest (*P* < 0.01 in all pairs). Leaf structural characteristics indicated by LMA showed a rather low degree of differentiation between evergreen and deciduous species; however, where differences were significant, LMA was higher in the evergreen species (Table 1). Average RMR did not show clear trends between the habits, whereas SMR was smaller in evergreen species, except in *Viburnum* at the first harvest (Figure 1). Analysis using individual plant biomass as a covariate confirmed that SMR was smaller in evergreen than in deciduous species (significant leaf habit effect in Berberidaceae and Adoxaceae and a difference in slopes in Rosaceae; Figure 2a–c) and that root mass and lamina mass ratios were not linked to the leaf habit (Figure 2d–i).

In evergreen species, concentrations of starch and TNC in dry mass of stems and roots tended to be lower (six significant contrasts out of eight in Berberidaceae, seven out of eight in Adoxaceae and two out of eight in Rosaceae) or not statistically different (remaining contrasts) in comparison with deciduous species, and the pattern held at both harvests (Table 1). When compared on a common mass basis, pools of TNC in roots and stems were significantly larger in deciduous than in evergreen species of Berberidaceae and Adoxaceae (Figure 3a, c, d and f). In the Rosaceae, where all plants of the deciduous *P. serotina* were larger than the evergreen *P. laurocerasus* plants, the slope of the root TNC vs root mass relationship was significantly higher in the deciduous species (Figure 3e), but slopes were not different in the case of stem TNC (Figure 3b). Combined pools of root and stem carbohydrates analyzed against whole-plant structural biomass were larger in deciduous than in evergreen species of Berberidaceae and Adoxaceae, and the slope of this relationship was steeper in *P. serotina* than in *P. laurocerasus* (Figure 3g–i).
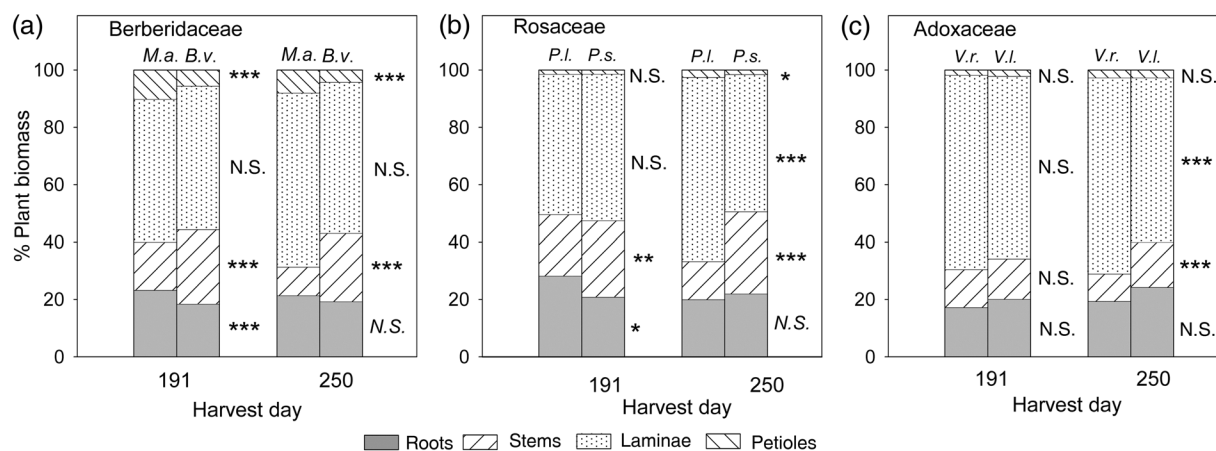

Figure 1. Biomass partitioning in seedlings of three pairs of deciduous and evergreen shrub species representing different families on two harvest days. Mean allocation ratios are shown. In each pair of bars, the left-hand bar is for the evergreen species and the right-hand bar is for the deciduous species. Species abbreviations are M.a. (*M. aquifolium*), B.v. (*B. vulgaris*), P.l. (*P. laurocerasus*), P.s. (*P. serotina*), V.r. (*V. rhytidophyllum*) and V.l. (*V. lantana*). Significance of contrasts between allocation ratios for the same biomass fraction within each species pair and harvest day is indicated by asterisks (*P < 0.05; **P < 0.01; ***P < 0.001; N.S., not significant).

Table 1. Traits of leaves, stems and roots in seedlings (means ± SD) of three pairs of deciduous (D) and evergreen (E) shrubs representing different families. LMA and N concentration are expressed on a TNC-free biomass basis. Statistical significance levels are given for contrasts between species within the same family (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, N.S., not significant).

Trait	Day of harvest	Berberidaceae			Rosaceae			Adoxaceae		
		B. vulgaris (D)	M. aquifolium (E)	P	P. serotina (D)	P. laurocerasus (E)	P	V. lantana (D)	V. rhytidophyllum (E)	P
LMA mature leaves (g m^{-2})	191	65.89 ± 19.08	73.44 ± 13.12	N.S.	59.71 ± 3.61	86.57 ± 32.43	**	95.60 ± 22.71	81.02 ± 15.35	N.S.
	250	80.25 ± 11.22	79.66 ± 15.77	N.S.	77.12 ± 11.72	86.94 ± 21.57	N.S.	78.53 ± 9.88	97.64 ± 5.70	*
LMA all leaves (g m^{-2})	191	68.51 ± 10.51	76.32 ± 8.69	N.S.	53.84 ± 3.07	90.64 ± 20.50	***	95.42 ± 16.05	82.85 ± 13.76	N.S.
	250	70.08 ± 7.77	77.90 ± 17.27	N.S.	76.83 ± 13.18	83.74 ± 14.00	N.S.	72.69 ± 15.93	95.96 ± 4.75	***
Starch leaves (%)	191	3.22 ± 2.33	1.27 ± 0.66	**	0.85 ± 0.03	2.92 ± 4.24	N.S.	6.17 ± 4.17	4.43 ± 1.55	N.S.
	250	0.96 ± 0.15	3.42 ± 3.41	**	2.88 ± 2.97	1.46 ± 0.43	N.S.	2.00 ± 0.83	2.40 ± 0.73	N.S.
Starch stems (%)	191	6.38 ± 2.87	1.90 ± 1.28	***	1.01 ± 0.39	0.81 ± 0.04	N.S.	4.21 ± 1.07	1.72 ± 0.68	***
	250	3.60 ± 1.88	1.93 ± 1.56	*	1.37 ± 0.74	0.93 ± 0.19	N.S.	3.29 ± 2.68	1.82 ± 0.51	*
Starch roots (%)	191	4.27 ± 2.16	0.98 ± 0.24	***	0.92 ± 0.05	1.16 ± 0.56	N.S.	3.06 ± 1.85	0.81 ± 0.08	***
	250	1.06 ± 0.33	0.94 ± 0.21	N.S.	6.11 ± 2.56	0.87 ± 0.09	***	1.06 ± 0.36	0.84 ± 0.03	N.S.
TNC leaves (%)	191	11.29 ± 2.19	9.41 ± 1.40	*	8.33 ± 1.45	13.18 ± 4.94	**	17.78 ± 3.18	12.62 ± 2.22	***
	250	10.10 ± 0.53	10.72 ± 4.33	N.S.	11.50 ± 3.10	8.67 ± 1.46	N.S.	13.15 ± 1.59	9.23 ± 1.22	***
TNC stems (%)	191	11.30 ± 2.86	5.23 ± 1.41	***	4.17 ± 0.69	4.39 ± 0.76	N.S.	12.46 ± 1.61	9.20 ± 1.56	***
	250	8.40 ± 2.51	4.72 ± 2.25	***	4.42 ± 0.96	3.87 ± 0.65	N.S.	9.53 ± 2.75	6.94 ± 1.12	**
TNC roots (%)	191	7.95 ± 2.92	2.26 ± 0.28	***	3.07 ± 0.45	2.98 ± 0.11	N.S.	7.53 ± 2.89	3.89 ± 0.40	***
	250	3.27 ± 1.19	2.43 ± 0.45	N.S.	12.77 ± 3.52	2.61 ± 0.21	***	5.93 ± 1.43	4.26 ± 0.93	**
N leaves (%)	191	2.56 ± 0.31	2.36 ± 0.51	N.S.	4.99 ± 0.74	3.75 ± 1.41	*	2.15 ± 0.45	2.51 ± 0.16	**
	250	3.21 ± 0.59	3.17 ± 0.77	N.S.	3.99 ± 0.68	5.22 ± 0.71	*	2.93 ± 0.52	2.70 ± 0.17	N.S.
N stems (%)	191	1.46 ± 0.17	1.71 ± 0.28	*	2.00 ± 0.41	2.85 ± 0.75	**	1.56 ± 0.08	1.61 ± 0.24	N.S.
	250	2.10 ± 0.46	1.77 ± 0.30	*	1.29 ± 0.25	3.53 ± 0.56	***	1.57 ± 0.31	1.50 ± 0.19	N.S.
N roots (%)	191	3.42 ± 0.64	3.53 ± 0.66	N.S.	3.07 ± 0.40	3.02 ± 0.77	N.S.	2.50 ± 0.10	3.63 ± 0.16	**
	250	4.32 ± 0.77	4.65 ± 0.72	N.S.	3.17 ± 0.60	3.62 ± 0.54	N.S.	3.96 ± 0.69	3.63 ± 1.17	N.S.

Together, these results show a tendency for greater accumulation of carbohydrates in axial organs of the three deciduous species compared with their evergreen relatives.

In contrast to carbohydrates, allometric relationships between N pools in roots and stems and plant structural biomass did not differ between species with contrasting leaf habits (Figure 4a–c). When whole-plant N was considered, results of the analysis were slightly modified in Berberidaceae and Rosaceae, in which slopes of plant N vs plant biomass were slightly steeper in the evergreen species (Figure 4d–f).

The contribution of TNC and N pools in particular organs to whole-plant pools is presented in Figure 5. In all species, the majority of the plant TNC pool was distributed in leaves (up to 83% in V. rhytidophyllum). The fraction of plant TNC in leaves was greater in evergreen than in deciduous species except for the first harvest in Rosaceae (Figure 5a–c), even though starch and TNC concentrations in leaves were not associated with leaf habit (Table 1). On the other hand, fractions of TNC contained in stems and roots were either similar in both plant types or significantly smaller in the evergreen species (Figure 5a–c). Leaves also contained more than half of total plant N, except for P. laurocerasus at first harvest (Figure 5d–f). The second large pool of N was contained in roots, followed by stems; however, no pattern was apparent with regard to differences in root and stem N fractions between deciduous and evergreen species.

Concentrations of total phenolics differed significantly among families both in leaves and in roots with highest concentrations occurring in the Berberidaceae in both organs (Figure 6). Within the Berberidaceae, leaf phenolic concentration was similar in both species, whereas root phenolics were at a lower level in the deciduous B. vulgaris. In both leaves and roots of the evergreen Prunus and Viburnum species, phenolics occurred at significantly lower concentrations than in their deciduous congenerics. Concentrations of phenolic compounds in roots and leaves of individual plants (pooled across species) were correlated ($r^2 = 0.37$, $P < 0.001$, $N = 36$).

Discussion

In temperate climates with a cold dormancy period, photosynthetic activity of evergreen leaves extends into the period of springtime bud burst and shoot leafing (Miyazawa and Kikuzawa 2005). Whereas in deciduous plants the carbohydrates needed to initiate new growth come exclusively from the storage pool accommodated in overwintering stems and roots, evergreen plants can supplement this source by simultaneously conducting photosynthesis in leaves from previous-years leaf cohorts (Epron et al. 2012, Wyka and Oleksyn 2014). The requirements for carbohydrate storage in evergreen plants may thus be lower than in deciduous plants (Mooney and Hays 1973). In agreement
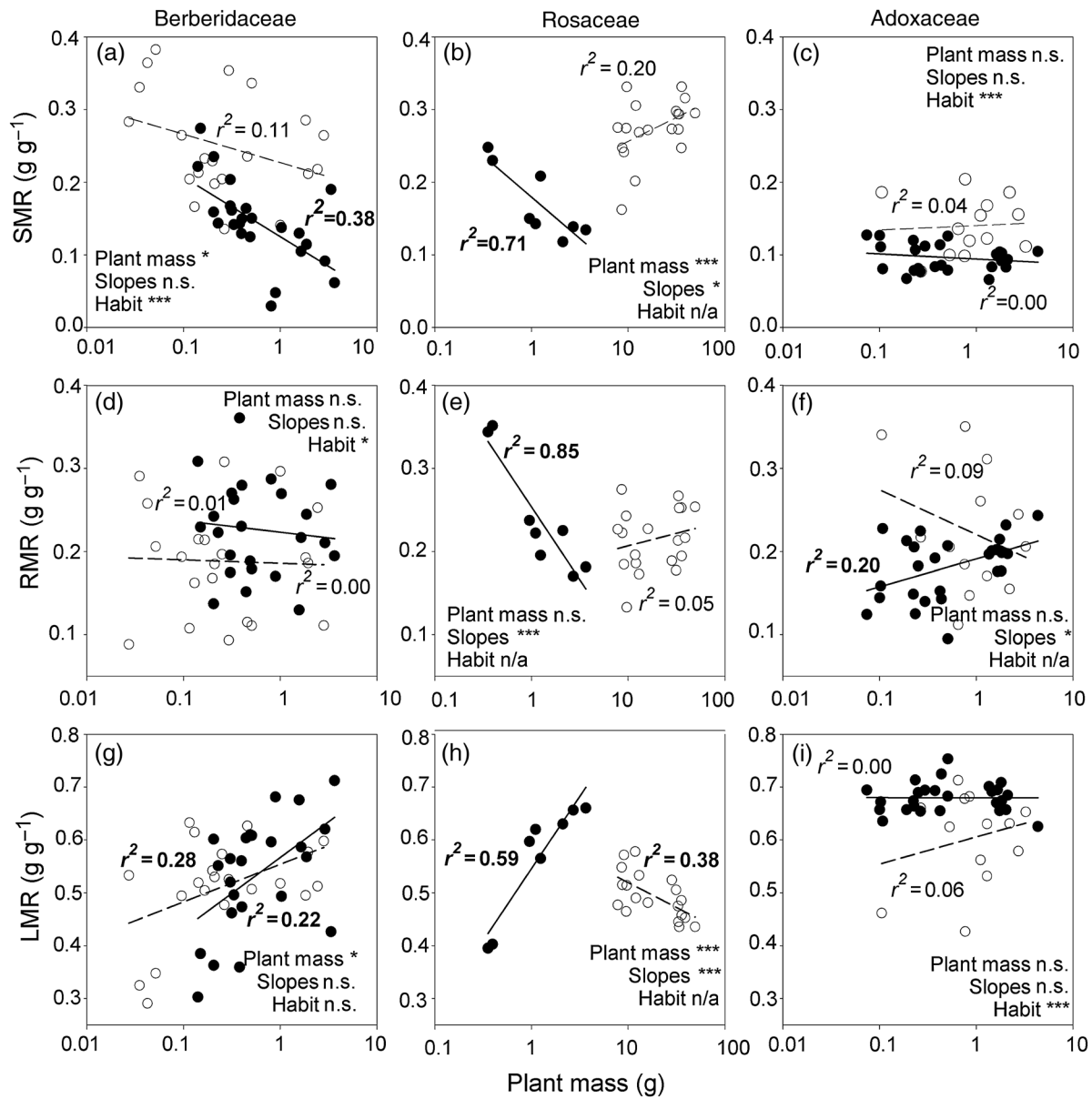
Figure 2. Relationships between biomass allocation ratios and whole-plant biomass in three pairs of deciduous and evergreen shrub species representing different families (a–c, SMR; d–f, RMR; g–i, LMR). Data from both harvest days are shown together. Filled symbols and solid regression lines are for evergreen species, and open symbols and dashed lines are for deciduous species. Pearson's correlation coefficients (r^2) are shown separately for evergreen and deciduous species in each family and are given in bold font where significant ($P < 0.05$). Analysis of covariance (ANCOVA) effects are shown. If significant slope difference (i.e., interaction between biomass and leaf habit) was detected, effect of leaf habit was not included in the ANCOVA model (n/a). Significance symbols as in legend to Figure 1. Note log scales on horizontal axes.

with this hypothesis, our study revealed a lower allocation of photosynthates to storage in evergreen (when compared with deciduous) species, at least at the seedling stage. This finding is in line with the previously reported tendency for lower TNC concentrations in evergreen species (Mooney and Hays 1973, Chapin and Shaver 1988, Kobe 1997, Newell et al. 2002, Fajardo et al. 2013, Piper and Fajardo 2014) and, significantly, demonstrates this trend at the scale of the whole-plant TNC pool.

Although in our study both TNC concentrations and TNC pools tended to be smaller in evergreen plants, it is clear that concentrations alone do not always indicate whole-plant allocation

patterns. Storage tissues in woody plants are distributed in stems (trunks, branches and twigs) and roots, i.e., winter hardy axial organs. The whole-plant level of reserves is thus influenced by the biomass allocation ratios SMR and RMR. Deciduous species in the Berberidaceae and Adoxaceae had both higher SMR and stem TNC concentrations, resulting in larger stem TNC pools in comparison with evergreen species. In the Rosaceae, where TNC concentrations were similar and species size ranges did not overlap (therefore, SMR could not be meaningfully compared), the greater TNC pool size in deciduous *P. serotina* could be attributed entirely to larger plant sizes. Even in this species,
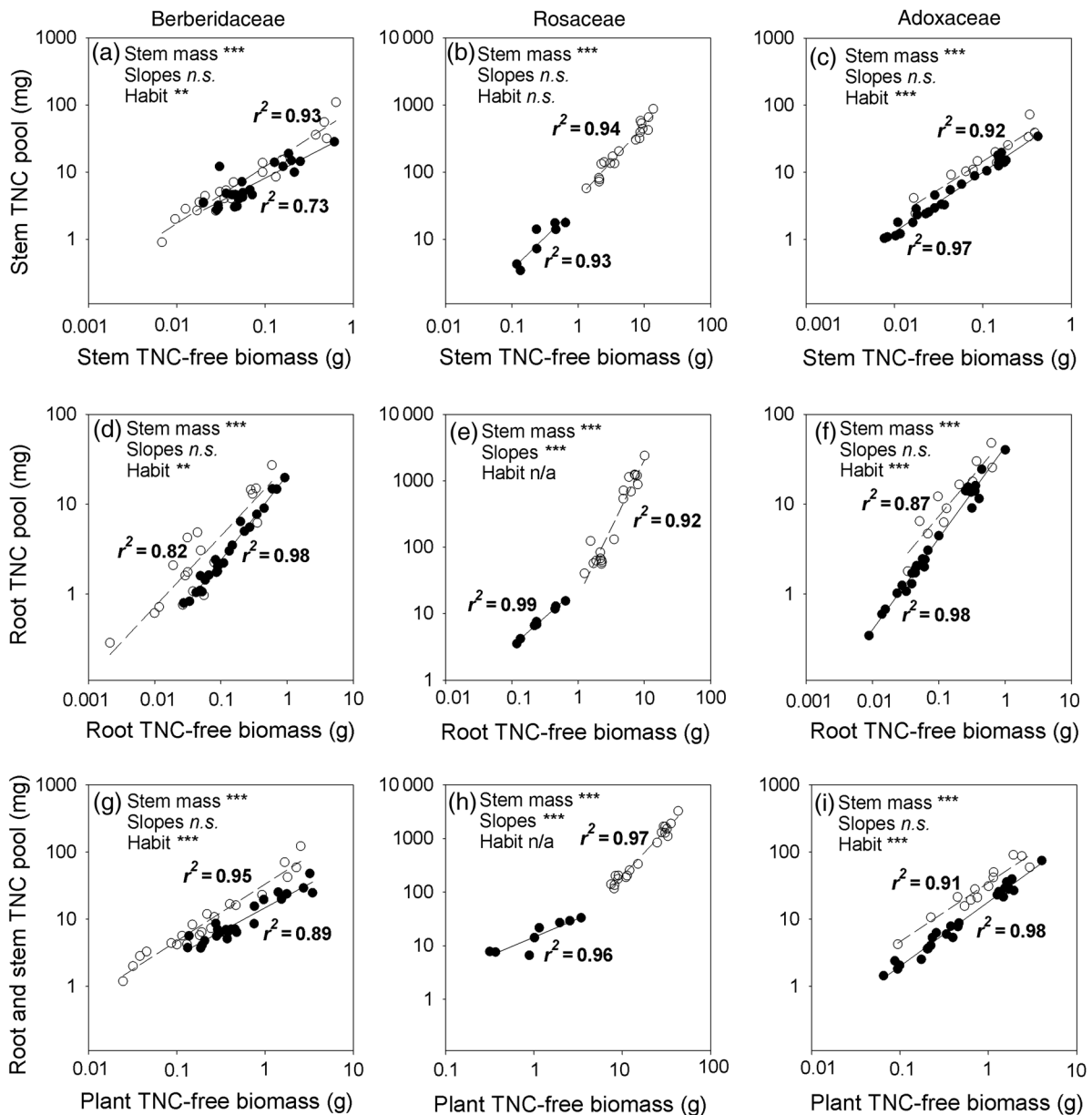
Figure 3. Relationships between pools of TNC contained in stems (a–c), roots (d–f) and combined stems and roots (g–i) and the dry, TNC-free biomass of stems, roots and whole plants, respectively. Data from both harvest days are shown together. Filled symbols and solid regression lines are for evergreen species, and open symbols and dashed lines are for deciduous species. Pearson's correlation coefficients (r^2) are shown separately for deciduous and evergreen species in each family and bold font indicates significance ($P < 0.05$). ANCOVA effects are shown. If significant slope difference (i.e., interaction between biomass and leaf habit) was detected, effect of leaf habit was not included in the ANCOVA model (n/a). Significance symbols as in legend to Figure 1. Note log scales.

however, SMR was higher in plants only slightly larger than the largest *P. laurocerasus*. Overall, the lower allocation to carbohydrate storage in stems of evergreen vs deciduous species could be partly explained by the smaller contribution of stems to plant biomass in the former, in connection with lower or similar TNC concentration. The tendency for smaller SMR in seedlings of evergreen species was also shown in a study of eight pairs of species with contrasting leaf habit (Antúnez et al. 2001). In contrast, the smaller pool of TNC in roots of evergreen vs deciduous species studied by us could be explained by the tendency for deciduous species to contain higher levels of carbohydrates without evidence for a systematic difference in RMR. On the other hand, Antúnez et al. (2001) and Tomlinson et al. (2013) reported a larger contribution of root biomass to plant biomass in deciduous than in evergreen species.

By considering a range of sizes, we were able to show that, at a common plant biomass, TNC pool is greater in evergreen than in deciduous species of at least two of the three studied species pairs. Other reports on evergreen and deciduous woody plants allow only comparison of carbohydrate pools between plants of
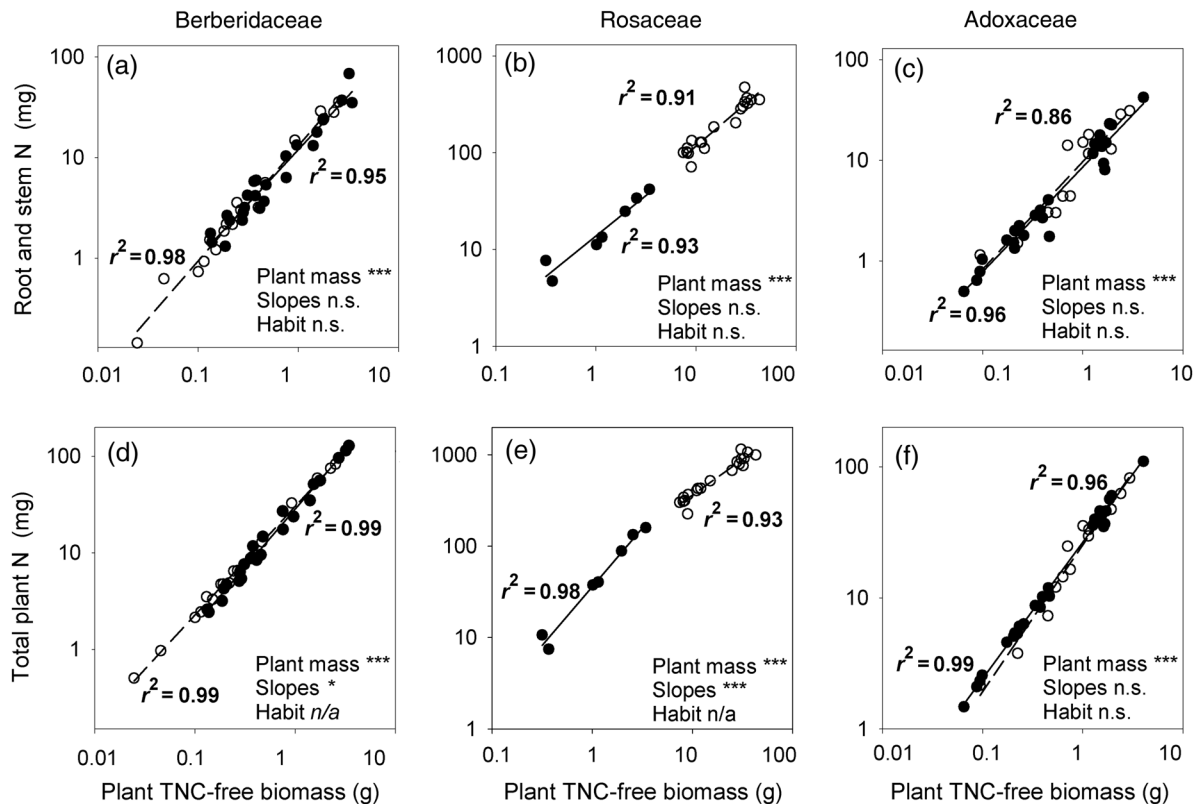
Figure 4. Relationships between pools of N contained in roots and stems (a–c), and whole plants (d–f) and the dry TNC-free whole-plant biomass. Data from both harvest days are shown together. Filled symbols and solid regression lines are for evergreen species, and open symbols and dashed lines are for deciduous species. Pearson's correlation coefficients (r^2) are shown separately for evergreen and deciduous species in each family, and bold font indicates significance ($P < 0.05$). ANCOVA effects are shown. If significant slope difference (i.e., interaction between biomass and leaf habit) was detected, effect of leaf habit was not included in the ANCOVA model (n/a). Significance symbols as in legend to Figure 1. Note log scales.

roughly similar but not identical biomass. For example, in an experiment described by Vanderklein and Reich (1999), control seedlings of deciduous *Larix* had 19% greater biomass than the evergreen *Pinus*, but contained 42% more TNC. Well-watered seedlings of semi-deciduous *Quercus faginea* were 45% larger than seedlings of evergreen *Q. ilex* but contained 128% more starch (Sanz-Pérez et al. 2007). Similarly, woody biomass of a single evergreen adult *Pinus strobus* tree was 30% smaller than that of a deciduous *Quercus rubra* tree but contained about four times less carbohydrates (Richardson et al. 2015). Qualitatively, these three studies yielded conclusions similar to ours. Quantification of the relationship between allocation ratios and plant size provides, nevertheless, a more reliable measure of resource allocation patterns by controlling the ontogenetic drift in biomass allocation (Poorter and Sack 2012).

Data presented here were collected during the active growing season. It is, however, possible that TNC pool and allocation pattern may be modified later. On one hand, deciduous species may exhibit a period of increased allocation to storage soon before leaf fall, and on the other, substantial photosynthetic gains by evergreen plants have been documented during winter (Miyazawa and Kikuzawa 2005, Morin et al. 2007, Woodruff

and Meinzer 2011). Moreover, reserves may be depleted during winter due to respiration and root growth, and starch may be depleted to supply soluble carbohydrates to enhance freezing tolerance. Such potential differences in storage phenology between evergreen and deciduous species that were not captured by our sampling might add temporal complexity to the observed pattern. The tendency for lower TNC pools in evergreen plants during the summer might then not occur at other times of the year.

In both deciduous and evergreen seedlings, the majority of a plant's TNC pool was contained in the leaves. We did not observe a higher LMR in evergreen species in contrast to the report by Antúnez et al. (2001), owing possibly to the low differentiation of LMA between evergreen and deciduous seedlings observed in our study. Although in the evergreen species leaves persist on the plant during winter, their importance in long-term carbohydrate storage has been disputed because leaf carbohydrate concentration during cold periods in winter, especially the concentration of starch, is variable and frequently very low (Miyaké 1902, Schaberg et al. 2000, Wyka and Oleksyn 2014). Moreover, leaves maintain a pool of soluble carbohydrates, often chemically diverse, that participate in frost tolerance and may
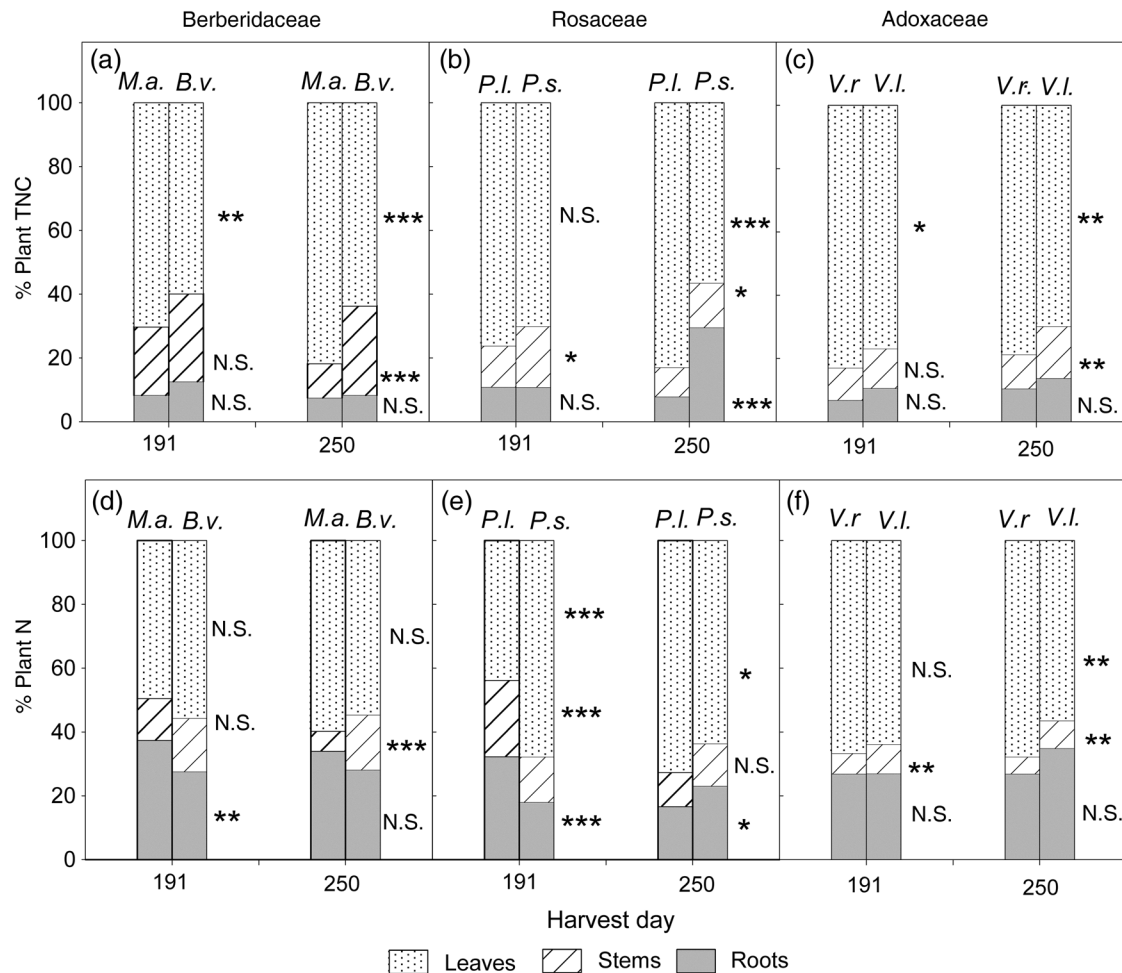
Figure 5. Distribution of nonstructural carbohydrates (a–c) and N (d–f) among biomass fractions. Mean ratios are shown. In each pair of bars, the left-hand bar is for the evergreen species and the right-hand bar is for the deciduous species. Species abbreviations are M.a. (*M. aquifolium*), B.v. (*B. vulgaris*), P.l. (*P. laurocerasus*), P.s. (*P. serotina*), V.r. (*V. rhytidophyllum*) and V.l. (*V. lantana*). Significance of contrasts between allocation ratios for the same biomass fraction within each species pair and harvest day is indicated by asterisks (see legend to Figure 1). Note that biomass does not include the petiole fraction.

only secondarily serve as energy reserves (Leborgne et al. 1995, Reyes-Díaz et al. 2005). On the other hand, overwintering leaves may accumulate substantial amounts of TNC before shoot growth starts in springtime (Ino et al. 2003). Such short-term storage along with current photosynthesis may later support new growth. Accordingly, experimental wintertime defoliation of evergreen plants lowered the available carbohydrate pool and caused a decrease or a delay in spring shoot expansion (Cherbuy et al. 2001, Palacio et al. 2007b).

Whereas leaves in young seedlings may contain a substantial fraction of the plant TNC pool, in larger evergreen plants, the contribution of leaves to the whole-plant storage pool may be minor since LMR tends to be strongly negatively related to plant biomass (Poorter et al. 2012). On the other hand, mobilization of TNC during bud burst may be predominantly local, as some evidence suggests significant twig autonomy in woody plants (Lippu 1998, Landhäusser 2011). Bursting buds would then be predominantly served by the nearest stem segments and the

adjacent evergreen leaves. Our estimates of foliar TNC fraction indicate that leaves have the potential to constitute an important storage compartment in evergreen plants, at least at the seedling stage, as well as in those species in which bud activation does not rely on long distance mobilization of carbohydrates.

In contrast to deciduous species that recover part of their N before leaf abscission in the fall, leaves of evergreen plants carry over their N content into the next growing season. Leaf proteins may undergo partial degradation during the new flush of growth in the spring as suggested by dynamics of leaf N content (Cherbuy et al. 2001, Katahata et al. 2007, Palacio et al. 2007b, Muller et al. 2009, Yasumura and Ishida 2011). The presence of this N reserve would reduce the need for accumulation of N in stems and roots. However, we did not find evidence for differential specialization in N allocation to stems and roots of deciduous and evergreen species, at least during the growing season. Also, variability of leaf N concentration in our species was not explained by leaf habit, even though on a broad
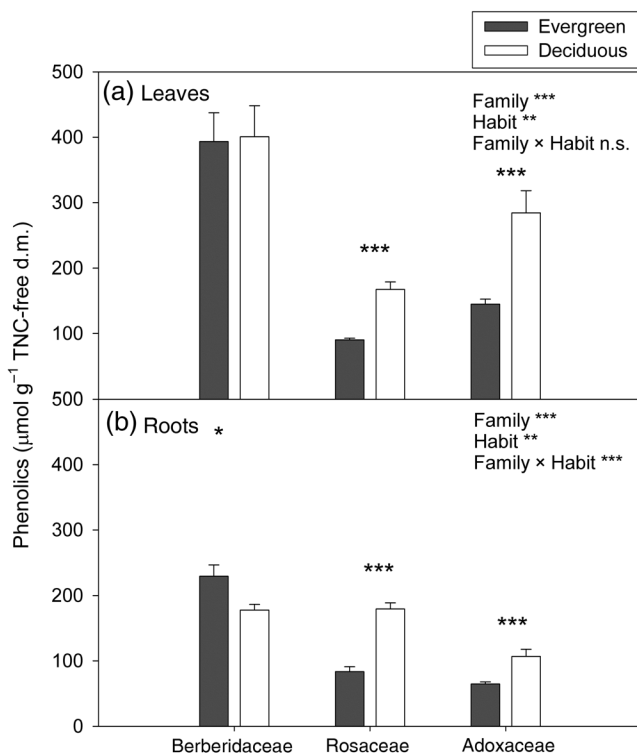
Figure 6. Concentration of phenolic compounds (means ± standard error; expressed as μmol chlorogenic acid g^{-1} TNC-free d.m.) in (a) leaves and (b) roots of three pairs of evergreen and deciduous species representing different families. Only plants collected on Day 250 were analyzed. Analysis of variance effects are shown. Asterisks above bars indicate significant contrasts between species within each family (see legend to Figure 1).

interspecific scale, leaf lifespan is negatively correlated with mass-based N concentration (Reich et al. 1992, Wright et al. 2005). This result can probably be explained by the overall small differences in LMA between plant types observed in our seedlings. Nevertheless, it appears that nutrient conservation afforded by the evergreen leaf habit may provide an important storage mechanism, especially in young seedlings in which the majority of plant N is contained in the leaves.

The usefulness of stored carbohydrates is often associated with the plant's ability to survive incidents of herbivory and to regenerate lost foliage or, less obviously, roots (Piper and Fajardo 2014). It was hypothesized that such an herbivory tolerance mechanism represents an alternative strategy to carbon-intensive defense mechanisms, such as construction of particularly tough tissues or accumulation of phenolic compounds. Leaf mechanical properties are correlated with the LMA ratio, and evergreen leaves typically have a higher LMA than deciduous leaves (Onoda et al. 2011, Kitajima et al. 2012, Wyka and Oleksyn 2014). We expected longer-living leaves of evergreen species to show both greater LMA and higher phenolic concentrations compared with deciduous species, consistent with the resource availability hypothesis (Coley et al. 1985, Endara and Coley 2011). In the seedlings studied, the differences in LMA between

evergreen and deciduous species were, however, largely insignificant. Surprisingly, phenolic compounds occurred at lower concentrations in the evergreen species, except for roots in species of Berberidaceae. This trend was later confirmed by analysis of adult foliage of the same and several additional confamiliar evergreen/deciduous species pairs (T.P. Wyka, P. Karolewski, J. Oleksyn, unpublished). Since evergreen species tended to also contain smaller TNC storage pools, our data set provides no evidence for a trade-off between carbon investments into storage vs defense. It is, however, possible that the tough leaf structure of evergreen species provides an efficient defense against generalist herbivores that allows reduction in investment in phenolic compounds.

Consideration of defensive compounds may be important from the point of view of resource economy, since contents of phenolic compounds were of similar magnitude as those of TNC. For example, since molecular weight of chlorogenic acid is 354.31 g, and given that, e.g., at second harvest leaves of *M. aquifolium* constituted, on average, 61% of seedling biomass and leaf phenolic concentration was 393 μmol g^{-1} d.m., the foliar phenolic pool in this species accounted for as much as 8.5% of the plant's TNC-free biomass. We did not investigate other classes of compounds, such as terpenoids or alkaloids that might provide alternative or supplementary defense mechanisms (Goodger et al. 2013). Especially the carbon-intensive terpenoid compounds might weigh significantly on the plant's carbon budget; however, examination of leaf anatomy in our species did not reveal internal secretory structures needed to house significant quantities of these toxic chemicals (Goodger et al. 2013).

A majority of studies of storage patterns in plants with contrasting leaf habits have compared evergreen gymnosperms and deciduous angiosperms (Hoch et al. 2003, Machado and Reich 2006, Michelot et al. 2012, Richardson et al. 2013, Zhang et al. 2014 but see Tomlinson et al. 2013). Admittedly, our data were collected for only three species pairs, all members of temperate floras where evergreen species are a minority among woody angiosperms. Nevertheless, since the contrast between evergreen and deciduous habit was replicated in three widely separated lineages, our results are suggestive of a wider pattern and justify examination of more examples from other phylogenetic backgrounds and climatic zones.

In spite of a growing number of studies searching for a connection between the size of the storage pool and species' ecological strategy, few definitive patterns have emerged. Several reports have identified a correlation between species' shade tolerance and size of storage pool (Kobe 1997, Imaji and Seiwa 2010, Poorter et al. 2010). Among fire-prone communities, resprouters store more carbohydrates than reseeders (Verdaguer and Ojeda 2002). Species showing continuous or extended growth store less than those growing by a brief flush (Canham et al. 1999). Our study supports yet another such pattern: one correlating evergreen leaf habit with reduced allocation to

carbohydrate storage with respect to both concentrations and whole-plant pools. It remains to be determined whether this trend is supported in older individuals and under other sets of conditions.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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