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## Photosynthetic ecophysiology of evergreen leaves in the woody angiosperms – a review

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**Abstract:** Evergreen plants are an important component of many ecosystems of the world and occur in numerous evolutionary lineages. In this article we review phenotypic traits of evergreen woody angiosperms occurring in habitats that regularly experience frost. Leaf anatomical traits such as sclerenchymatic tissues or prominent cuticles ensure mechanical strength while often enhancing tolerance of water deficit. The low ratio of photosynthetic to nonphotosynthetic tissues as well as modified cell wall structure and nitrogen allocation patterns in evergreen leaves result in lower mass-based photosynthetic rate and photosynthetic nitrogen use efficiency in comparison with deciduous leaves. Their photosynthetic apparatus is adapted for the survival of frost in a down-regulated state with potential for photosynthetic activity in winter during periods of permissive temperatures. Leaf structure interacts with the mechanisms of frost survival. Stem xylem in evergreen plants tends to contain smaller diameter conduits incurring greater resistance to freeze/thaw induced cavitation than in deciduous plants, although at the cost of reduced hydraulic efficiency. In contrast, no such differences in hydraulic conductivity have been documented at the leaf level. There is evidence for reduced structural plasticity of evergreen leaves in response to variability in irradiance, however photosynthetic downregulation occurs in mature leaves in response to self shading. Some evergreen species exhibit slow leaf development and “delayed greening”, while in many species aging is also a very protracted process. Finally, evergreen leaves may participate in carbohydrate and, less obviously, in nitrogen storage for the support of spring shoot and foliage growth, although the importance of this function is under debate. In conclusion, the evergreen leaf habit is correlated with numerous structural and functional traits at the leaf and also at the stem level. These correlations may generate trade-offs that shape the ecological strategies of evergreen plants.

**Additional key words:** internal conductance to CO<sub>2</sub>, leaf anatomy, sclerophylly, winter photosynthesis, winter photoinhibition.

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List of Abbreviations:  $A_{\max(\text{area})}$  – photosynthetic rate per unit leaf area ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ );  $A_{\max(\text{mass})}$  – photosynthetic rate per unit leaf mass ( $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ );  $g_m$  – mesophyll CO<sub>2</sub> diffusion conductance

( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ ); LMA – leaf mass per unit leaf area ( $\text{g m}^{-2}$ );  $N_{\text{area}}$  – nitrogen concentration per leaf area ( $\text{g N m}^{-2}$ ); PNUE – photosynthetic nitrogen-use efficiency ( $\mu\text{mol CO}_2 \text{ mol N}^{-1} \text{ s}^{-1}$ )

## Introduction

Leaf life span is a key ecophysiological trait, greatly influencing the photosynthetic capacity, biomass productivity potential, tolerance to biotic and abiotic stresses and role in ecosystem-level interactions of each plant species (Chabot and Hicks 1982; Reich et al. 1992; Kikuzawa and Lechowicz 2011). Even though across the plant kingdom the life span of leaves varies in a continuous manner from weeks to 45 years (Ewers and Schmid 1981; Wright et al. 2004), plant ecologists usually recognize the dichotomy between deciduous vs. evergreen plant habit and treat these as distinct phenological traits. The former group is defined as shedding foliage at the onset of a dormant period, whether caused by drought, cold temperatures or intrinsic growth rhythm and the latter as having no leafless period (Kikuzawa and Lechowicz 2011). The evergreen plant habit is usually achieved through a long leaf life span. However, in Mediterranean-type climates where summers are dry and hot, and winters cool and wet, many species produce successions of short-lived leaves, including ones that function during the winter (Ackerly 2004). In such species, although the life span of individual leaves may be shorter than a single season, because of the production of overlapping leaf cohorts the whole plant is still classified as evergreen (Gratani and Crescente 1997). A number of other variants can be distinguished, blurring the distinction between deciduous and evergreen categories (Kikuzawa and Lechowicz 2011). A recent analysis has indicated, however, that, since leaf life span differentiated the two habits in over 90% of cases, they should be considered as representing two viable, alternative strategies (van Ommen Kloeke et al. 2012).

Leaf habit is a species-specific trait. Instances when a single species contains genetically determined deciduous and evergreen variants are rare, although climatic factors, especially low temperatures, may prevent or reduce green leaf retention over the winter in genetically evergreen plants. Within the evergreen plant group, however, there is a considerable intraspecific variability in leaf life span. In several species of European dwarf evergreen shrubs leaf life span was longer at higher latitudes (Karlsson 1992). Few studies have addressed the proximal causes of this variability, however, based on common garden results for *Pinus sylvestris* and *Picea abies*, it may be mainly environmentally controlled (Reich et al. 1996). Leaf life span may be extended or shortened by shade, depending on circumstances of the entire plant. When the whole plant is shaded, individual leaves live longer than in full sun (Nilsen 1986; Katahata et al. 2007; Yasumura and Ishida 2010), whereas when part of the shoot or crown is well illuminated, the lowermost, shaded leaves frequently undergo ab-

scission earlier than the leaves receiving full sun (Kikuzawa and Lechowicz 2011). Leaf life span may be shortened in plants growing on nitrogen-poor soils (e.g. by 3.5 months in *Rhododendron ferrugineum*; Marty et al. 2009) as the onset of leaf abscission may be induced by the demand for nitrogen from growing parts (Ono et al. 2001; Oleksyn et al. 2003; Marty et al. 2009). In intraspecific and biogeographic analyses, evergreen habit and extended life span are associated with poor nutrient availability (Monk 1966; Givnish 2002; Wright et al. 2002) and low temperature (Reich et al. 2003, 2014; van Ommen Kloeke et al. 2012). Evergreen habit is thus an adaptive trait in many environments. Its consequences may occur at leaf, shoot and whole plant levels and may be environment-specific (Givnish 2002).

Understanding the selective advantages and disadvantages of different leaf habits and variation in leaf life span has been the objective of numerous ecophysiological and biogeographical studies and reviews (e.g. Monk 1966; Chabot and Hicks 1982; Reich et al. 1992; Aerts 1995; Givnish 2002; Kikuzawa and Lechowicz 2011; van Ommen Kloeke et al. 2012). The consensus view is that an extended life span ensures a greater total photosynthetic carbon gain for the amount of nutrients invested in construction of the leaf in comparison to short-lived leaves (Chabot and Hicks 1982). The purpose of this article is to provide an overview of the current issues in ecophysiology of photosynthetic carbon gain by evergreen leaves with emphasis on links between morpho-anatomical and functional characteristics of leaves (and, where relevant, also stems) of evergreen plants, and trade-offs that result from the evergreen leaf habit. With the vast number of evergreen species worldwide, we focus on species from the temperate and Mediterranean climatic zones and preferably use examples of plants that regularly endure temperatures below freezing. The taxonomic coverage has been limited to angiosperms except where only gymnosperm data were available. We first review the functional interpretations of structural features of evergreen leaves. Next, we examine the relationships between leaf structure and photosynthesis in evergreen and deciduous species and discuss photosynthetic adaptations to winter conditions. Finally, we focus on some less well known functional aspects of the evergreen leaf habit such as rate of leaf development, leaf plasticity and specialization for resource storage.

## Anatomy and morphology

Structural differences between evergreen and deciduous leaves can be quantified using leaf mass per area (LMA,  $\text{g m}^{-2}$ ), a parameter that in interspecific comparisons covaries with leaf life span (Nardini



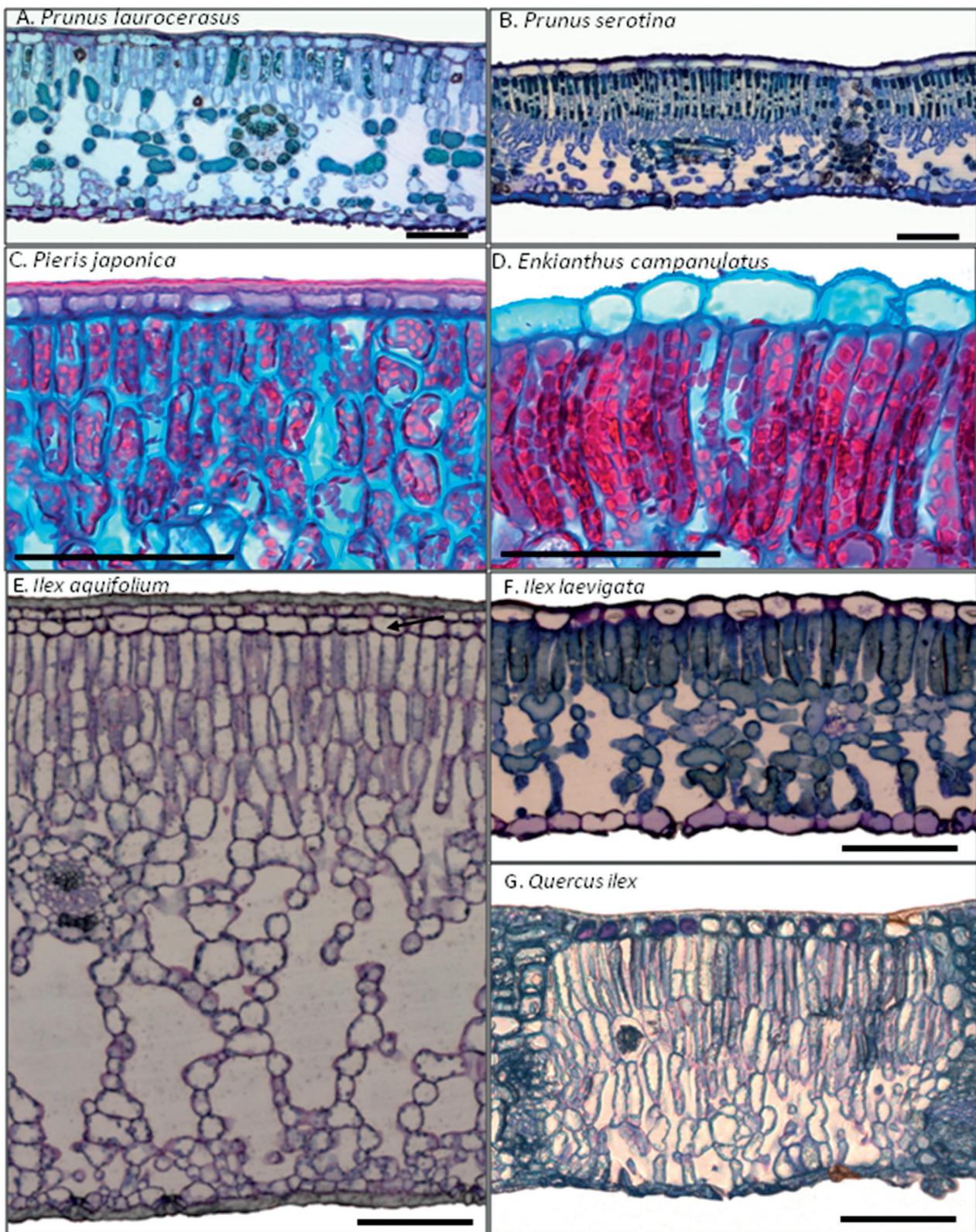


Fig. 1 Cross-sections through evergreen (A, C, E) and deciduous (B, D, F) leaves, belonging to pairs of related species (A, B – Rosaceae; C, D – Ericaceae; E, F – Aquifoliaceae). An evergreen leaf of *Quercus ilex* (G – Fagaceae) is also shown. Note features of evergreen leaves: greater lamina thickness (A, E vs. B, F), greater cuticle thickness (C, E vs. D, F), the presence of a collenchymatous hypodermal layer in E (arrow) and the densely packed mesophyll in G. Also note the abundance of air spaces in spongy mesophyll in A, E. Sections A, B, E, F were prepared in Technovit resin and stained with toluidine blue and sections C, D were prepared in paraffin and stained with fast green and safranin. Adaxial indumentum was present in G but was destroyed during section processing. Bars indicate 100 μm



2001; Reich et al. 1992). LMA is an important leaf attribute depicting a compromise between the capture of light and CO<sub>2</sub> fixation, and the limitations imposed by leaf structure, resistance to herbivores and pathogens, and the mitigation of water loss (Sefton et al. 2002). Higher LMA of evergreen leaves may be caused by their greater thickness, but also by greater tissue density (Witkowski and Lamont 1991). The density of leaf lamina is influenced by the packing of cells and by the thickness and composition of the cell walls and, to a lesser extent, by the composition of the protoplasts. Evergreen leaves tend to be both thicker and denser than deciduous leaves (Mediavilla et al. 2001; Mediavilla et al. 2008; Poorter et al. 2009; see Fig. 1 A vs. B, E vs. F) with much interspecific variability (Rotondi et al. 2003; Villar et al. 2013). Studying pairs of closely related evergreen and deciduous species, Villar et al. (2013) concluded that the higher LMA of evergreen leaves is attributable primarily to their greater volume of mesophyll tissue per leaf area, with leaf thickness 60% higher but leaf density only 10% higher than in the deciduous species group. Within each leaf type, however, higher LMA is explained by a higher tissue density, due to higher volumetric fractions of mesophyll and lower fractions of air spaces and epidermis.

## Mechanical properties

Anatomical design of evergreen leaves is responsible for their mechanical endurance, rendering them less attractive to herbivores and resistant to physical hazards (Wright and Cannon 2001). Leaf mechanical properties have been shown to depend on LMA (e.g. see data from Kitajima et al. (2012) plotted in Fig. 2 A, B, C). In a global multispecies leaf sample, LMA was a strong predictor of forces needed to shear, punch and tear leaves (Onoda et al. 2011). Much variability of mechanical properties was, however, not explained by bulk LMA indicating that structural reinforcement may result from finer anatomical features.

One extreme of structural leaf variability is represented by sclerophyllous (“hard leaved”) species. Measurements of several mechanical characteristics in a range of leaves differing in the degree of sclerophylly have clearly showed that this trait is correlated with mechanical strength and flexural stiffness (Read and Sanson 2003). In typical sclerophyllous leaves mesophyll is densely packed (Fig. 1G), palisade parenchyma is well developed and multilayered, epidermis is thick-walled and covered with a thick cuticle (Fig. 1 C vs. D, E vs. F), and various types of sclerenchymatic tissues are prominent (Rotondi et al. 2003; Jordan et al. 2005). Sclerenchyma fibers may be associated with the midrib and major veins, in some species forming bundle extensions that di-

vide the leaf into self-contained sectors (heterobaric leaves). Fibers may also occur as a hypodermal layer or form a bundle along the edge of the lamina as in *Buxus sempervirens* (Gostin and Ivanescu 2008) and *Vaccinium vitis-idaea* (Piwczyński et al. 2013). Remarkably diverse arrangements of sclerenchyma fibers are found in various species of Proteaceae (Jordan et al. 2005, 2013). Another category of sclerenchymatic tissue are various sclereids, developing as idioblasts throughout the mesophyll (Fahn and Cutler 1992; Jordan et al. 2005, 2013).

Many evergreen species, especially those from mesic habitats, lack the abundant sclerenchyma component and instead depend on a tough epidermis, subepidermal collenchyma (Fig. 1 E) and/or cuticular layer for structural reinforcement (Fig. 1 C,E; Balsamo et al. 2003). An investigation of mechanical properties of isolated foliar cuticles of 13 Australian evergreen species has shown that cuticle thickness was correlated with resistance to tearing and that tensile strength and modulus of elasticity were much higher than those of leaf laminae (Onoda et al. 2012). Intracuticular wax has been shown to increase the stiffness of *Hedera helix* and *Ilex aquifolium* leaves (Khanal et al. 2013).

Leaf toughness is also increased by greater tissue density per se through an increased concentration of wall cellulose in a unit of leaf volume (Onoda et al. 2011; Kitajima et al. 2012). Other wall fractions such as hemicellulose and lignin did not significantly influence toughness as measured in the latter study, however lignin is known to enhance bending resistance – another aspect of leaf mechanical resistance (Alvarez-Clare and Kitajima 2007). Bending resistance and other leaf mechanical traits are also increased by the greater thickness of the lamina, independently of its density (Onoda et al. 2011; see also Fig. 2 D, E, F for data from Kitajima et al. 2012).

## Xeromorphism

While mechanical design of the leaf is an exciting and promising subject of botanical research, anatomical features providing mechanical reinforcement may be multifunctional, with sclerophylly often considered to be primarily a xeromorphic feature as suggested by its prevalence in Mediterranean-type environments (Turner 1994). Mechanical and hydraulic properties of evergreen leaves are closely related. First, the vein network used for water transport contributes to structural strengthening of the leaf, because of the presence of sclerenchymatic sheaths and extensions around the bundles and because of rigid cell walls of the xylem conduits. Second, properties of the extra-xylary pathway of water transport to transpiring surfaces may be influenced by the lignification of mesophyll cell walls

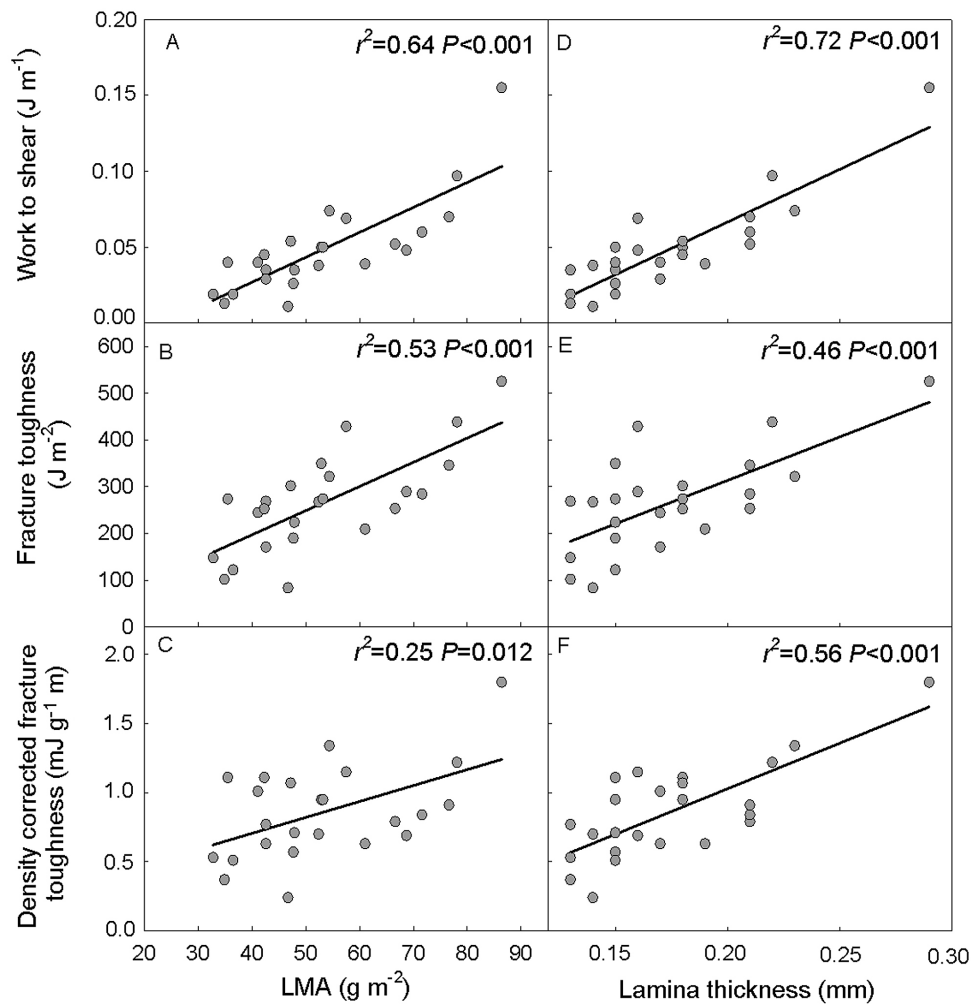


Fig. 2. Dependence of mechanical properties of leaf laminae (work to shear – A, B; fracture toughness – C, D; density corrected fracture toughness – E, F) on leaf mass per area (LMA; A, C, E) and lamina thickness (B, D, F) in seedlings of 24 neotropical tree species. Data are from Table 3 of Kitajima et al. 2013. Determination coefficients  $r^2$  and associated  $t$  values are shown

(increasing their resistance to water movement) and by the high ratio of mesophyll surface area to leaf area in the thickened or dense leaves (Zwieniecki et al. 2007; Simonin et al. 2012). Integrated properties of individual components of the water transport pathway determine the hydraulic conductance of the whole leaf.

Thickening and secondary modifications of the cell walls increase their elastic modulus, i.e. make the cells more rigid. Bulk elastic modulus in three tropical evergreen species averaged 15 MPa, comparing to 10 MPa in three deciduous species (Sobrado 1986) and was 22 MPa in the evergreen *Heteromeles arbutifolia* compared with 17 MPa in the deciduous *Prunus serrulata* (Balsamo et al. 2003). On dehydration, the rigid cells of evergreen leaves undergo smaller volume change for a similar amount of turgor drop compared to cells of deciduous leaves (Sobrado 1986; Salleo and Lo Gullo 1990; Salleo et al. 1997). Leaves of two sclerophyllous evergreens

*Viburnum tinus* and *Ilex aquifolium* lost turgor after losing only 13% of water compared with deciduous *Sambucus nigra* and a soft-leaved evergreen *Hedera helix*, that both lost turgor after losing over 20% of water (Salleo et al. 1997). Thus, the less elastic mesophyll of evergreen leaves also stores relatively less water available for withdrawal without losing turgor. However, such rigid cells may tolerate lower water potentials, facilitating water uptake from the drying soil without suffering damage to the photosynthetic apparatus that might be caused by major cell volume changes (Lo Gullo and Salleo 1988; Turner 1994). Their mechanical tissues provide an additional protection from distortion of the leaf structure allowing for an easier recovery from dehydration. Scleromorphic leaves may also be adaptive in high light environments, with hypodermal fibers and thick cuticle increasing the tolerance to high irradiance (e.g. in the Proteaceae; Jordan et al. 2005).

## Construction costs

The abundance of strengthening features in evergreen leaves suggests a high cost of leaf construction. Chemical analyses of evergreen leaves usually yield higher cellulose and hemicellulose concentrations, and, often but not always, a higher lignin concentration compared to deciduous species (Villar and Merino 2001; Mediavilla et al. 2008). The metabolic cost per unit of leaf biomass has been estimated by measuring the heat of combustion corrected for ash content and additionally calculating the cost of protein synthesis (Villar and Merino 2001). The average cost of construction of evergreen leaves from diverse ecosystems was  $1.55 \text{ g glucose g}^{-1}$ , i.e. 6% higher than the construction cost of deciduous leaves ( $1.46 \text{ g glucose g}^{-1}$ ). Leaves of evergreen species had a higher heat of combustion, and lower nitrogen and ash concentration, however these costs were reduced by the lower contribution of protein synthesis to construction costs (16% vs. 26% in deciduous species). Lignin, phenolic and lipid concentrations contributed especially strongly to construction costs (Villar and Merino 2001). The costs are high especially where the cuticle is very thick (in some cases accounting for up to 24% of lamina dry mass), because its main component (cutin) is over two times more costly to synthesize than carbohydrates (Onoda et al. 2012). Variability in leaf chemical composition and construction costs, however, has a significant phylogenetic component, with leaf habit explaining only a minor fraction (Villar et al. 2006). When expressed on the basis of projected leaf area, the difference in construction costs is much larger than on the basis of leaf mass, [e.g.  $237 \text{ vs. } 130 \text{ g glucose m}^{-2}$  in, respectively, evergreen and deciduous species studied by Villar and Merino (2001)], because of the multiplicative effect of greater LMA and a higher cost of leaf biomass in evergreen species.

Calculation of construction costs allows estimation of the energy investment in leaves, but does not fully reflect integrated lifetime costs that, in addition to costs of synthesis of leaf dry matter, should also include respiratory costs. At present there is not enough information to compute a complete lifetime energy budget of evergreen vs. deciduous leaves, however respiration per dry mass in evergreen leaves is lower than that in deciduous leaves under both dark and light conditions (Villar et al. 1995; Reich et al. 1998a; Tjoelker et al. 1999; Wright et al. 2005a). This slower respiration is related to lower N concentration as an index of leaf metabolic activity (Reich et al. 1998a), however for a given N concentration evergreen leaves still respire more slowly than deciduous leaves (Wright et al. 2005a).

## Photosynthetic rates

A large number of studies have addressed the consequences of the evergreen leaf habit for photosynthetic capacity at various time scales and under different environmental conditions. According to the global meta-analyses published by Reich et al. (1997; 1998a), Wright et al. (2004; 2005a, b) and many individual studies (e.g. Hassiotou et al. 2009, 2010; Hikosaka and Shigeno 2009), photosynthetic rate expressed per leaf mass ( $A_{\text{max(mass)}}$ ) is negatively correlated with LMA, i.e. the thicker or denser the leaf, the lower its mass-based photosynthetic rate. Because of the strong coupling of leaf longevity with LMA (Reich et al. 1992; Wright et al. 2005b), evergreen leaves as a group have lower  $A_{\text{max(mass)}}$  than deciduous leaves (Reich et al. 1998a,b; Hikosaka and Hirose 2000; Wright et al. 2005a). On a leaf area basis this relationship is less straightforward because, on the one hand, the greater amount of photosynthetic tissue per unit leaf area results in a higher  $A_{\text{max(area)}}$  and on the other hand, anatomical modifications associated with increased LMA may lead to the lowering of  $A_{\text{max(mass)}}$  (see below), with the net effect on  $A_{\text{max(area)}}$  dependent on the relative strength of these opposing effects. Globally, however, evergreen leaves have lower  $A_{\text{max(area)}}$  than deciduous species (Reich et al. 1998a; Wright et al. 2005a).

The lower  $A_{\text{max(mass)}}$  in evergreen leaves is thought to be largely caused by the “dilution” of photosynthetic tissues due to the abundance of structural elements (Harrison et al. 2009). This dilution effect leads to a lower leaf concentration of nitrogen, a nutrient principally involved in photosynthetic functions and a strong predictor of photosynthetic capacity (Field and Mooney 1986). However, photosynthetic rate expressed per unit of foliar nitrogen (known as instantaneous photosynthetic nitrogen use efficiency, PNUE) is usually also lower in evergreen than in deciduous species (Reich et al. 1998a; Takashima et al. 2004; Warren and Adams 2004; Juárez-López et al. 2008) but see Hikosaka and Hirose (2000). Several hypotheses have been proposed to explain this difference in PNUE, emphasizing the role of leaf structure (hypotheses 1 and 2 below) or nitrogen allocation pattern (hypotheses 3 and 4).

### Hypothesis (1)

Structural characteristics of the high-LMA evergreen leaf may cause lower conductance to  $\text{CO}_2$  transfer between the sub-stomatal cavity and chloroplasts ( $g_m$ ), leading to lower availability of  $\text{CO}_2$  at rubisco carboxylation sites (Field and Mooney 1986). Measurements of  $g_m$  using online discrimination against  $^{13}\text{CO}_2$  or combined gas exchange and fluorescence techniques in connection with photosyn-

thetic modeling have so far allowed estimation of  $g_m$  in over 100 species including numerous evergreens (Niinemets et al. 2009a,b; Hassiotou et al. 2010). In agreement with this hypothesis,  $g_m$  in four species of evergreen fruit trees was lower than in the deciduous peach (Syvertsen et al. 1995; Marchi et al. 2008) and in a set of 35 species of Australian sclerophylls  $g_m$  was negatively correlated with both LMA and leaf longevity (Niinemets et al. 2009a). A similar finding was reported for seven species of sclerophyllous *Banksia* (Hassiotou et al. 2009) and in a literature-derived data set of 81 diverse species including many evergreens (Niinemets et al. 2009b). As an exception, leaves of an evergreen and a deciduous *Quercus* species had similar  $g_m$  (Mediavilla et al. 2002).

The generally low  $g_m$  in evergreen or sclerophyllous leaves might be potentially attributed to resistances in both the gas (intercellular spaces) and the liquid (cell wall to chloroplast stroma) phase (Syvertsen et al. 1995). The denser packing of cells and greater mesophyll thickness cause a greater tortuosity of the diffusion pathway. However, because of the large diffusion coefficient of  $\text{CO}_2$  in the gas phase relative to the liquid phase, the influence of gas phase conductance on total mesophyll conductance is not major except in very dense mesophyll (Terashima et al. 2006). Consequently, the relationship between mesophyll porosity and  $g_m$  is ambiguous and varies from positive (Loreto et al. 1992; Syvertsen et al. 1995) to negative (Hanba et al. 1999). In contrast, mesophyll cell walls constitute the major diffusional resistance estimated at 50% of total mesophyll resistance for herbaceous leaves and potentially more in leaves with thicker cell walls (Terashima et al. 2011). For a group of 32 sclerophyllous taxa the contribution of cell wall resistance to total mesophyll resistance was estimated to be between 50 and 70% and was correlated with cell wall thickness (Tosens et al. 2012). In an evergreen *Quercus*, mesophyll cell walls were indeed thicker than in a deciduous congeneric species (Terashima et al. 1995), similarly to five evergreen vs. five deciduous woody species from diverse families (Tomás et al. 2013). Although simultaneous measurements of cell wall thickness and  $g_m$  are sparse, in three multi-species studies cell wall thickness appeared to be positively correlated with LMA and negatively with  $g_m$  (Hassiotou et al. 2010; Tosens et al. 2012; Tomás et al. 2013). Moreover, the porosity of mesophyll cell walls is expected to be lower in evergreen leaves because of their potentially greater lignification, thus further restricting  $g_m$  (Tosens et al. 2012). Furthermore, perennial evergreen leaves undergo extensive secondary age- and stress- induced changes in their mesophyll wall structure, including thickening and hardening, that lead to a decrease in  $g_m$  and restriction of photosynthetic capacity (Karlsson 1985; Niinemets et al. 2005).

Having crossed the cell wall,  $\text{CO}_2$  diffuses to rubisco carboxylation sites also in the liquid phase, including plasma membrane, cytoplasm, chloroplast envelope and stroma. Shortening of this pathway greatly increases  $g_m$  and, on a leaf area basis, may be achieved by increasing the surface area of chloroplasts adjacent to intercellular air spaces (Terashima et al. 2006; Terashima et al. 2011). The thick laminae of evergreen leaves provide additional mesophyll cell wall area available for chloroplast packaging, thus potentially compensating for thicker cell walls (Hanba et al. 1999). However, where walls are very thick the additional impact of chloroplast distribution on  $g_m$  may be negligible (Tomás et al. 2013). Remarkably, species with thick cell walls had thinner chloroplasts, suggesting that they have been subjected to selection pressure to optimize anatomical requirements of long leaf life span and efficient photosynthesis (Tosens et al. 2012). Regardless of which specific structural features contribute to restricting  $g_m$ , chloroplasts in evergreen leaves function under lower  $\text{CO}_2$  concentrations and experience a greater degree of limitation of photosynthesis by mesophyll conductance relative to deciduous species (Niinemets et al. 2009a,b). With the progressing understanding of the rapid modulation of  $g_m$  due to changes in activity of carbonic anhydrase and water channels, further work is required to partition the controls of  $g_m$  between structural and physiological factors in the context of leaf form and longevity (Evans et al. 2009).

## Hypothesis (2)

Evergreen leaves may be less efficient than deciduous leaves at ensuring penetration of light down the mesophyll profile, leading to reduced photosynthetic rates in the abaxial mesophyll layers. Light attenuation in the leaf profile is influenced by anatomical structure (Vogelmann 1993; Terashima and Hikosaka 1995). Species of *Eucalyptus* with multilayered palisade mesophyll contained more chlorophyll per leaf area than species with thin palisade, but light absorbance was similar, yielding reduced efficiency of light capture per unit chlorophyll (Sefton et al. 2002) and probably increasing shading of the abaxially located cells. Leaves illuminated at a saturating level from the adaxial direction will often increase photosynthesis if additional light is also provided from abaxial side, suggesting that chloroplasts in the abaxial mesophyll are normally undersaturated (Evans et al. 1993). When deciduous angiosperm and evergreen gymnosperm leaves were subjected to bilateral illumination, photosynthesis increased relative to measurements with adaxial illumination only, and magnitude of the increase was positively correlated with LMA, confirming greater mesophyll self-shading in high-LMA evergreen leaves (Green and Kruger



2001). Certainly the prominent development of cuticle and the abundance of mechanical tissues would further decrease the efficiency of light utilization in evergreen leaves, although certain types of sclereids may help to guide light into the deep mesophyll as shown in *Phillyrea latifolia* (Karabourniotis 1998).

### Hypothesis (3)

It has been proposed that nitrogen partitioning to non-photosynthetic functions, such as structural wall proteins, N-intensive defensive compounds and other compounds increasing longevity, may be greater in evergreen than in deciduous leaves (Field and Mooney 1986; Warren and Adams 2004). In agreement with this hypothesis, two evergreen species of *Quercus* allocated greater fractions of nitrogen to detergent insoluble (i.e. wall) proteins in comparison to two deciduous species, suggesting a trade-off between leaf habit and PNUE (Takashima et al. 2004). This result, however, has not been validated for other species. When a diverse sample of only evergreen species was analyzed, LMA was not correlated with the wall nitrogen fraction (Harrison et al. 2009). The fraction of nitrogen allocated to rubisco, on the other hand, has been shown to decline with LMA in some studies (Ellsworth et al. 2004; Harrison et al. 2009; Hikosaka and Shigeno 2009), while in Australian sclerophylls it was highly variable and was not lower than in other, more mesophyll C3 species (Warren et al. 2000). In contrast to metabolism-associated proteins, the concentration of wall proteins does not decrease with leaf age because the insoluble wall proteins are resistant to degradation and undergo hardly any resorption. Their fraction therefore increases, contributing to the age-related decline in PNUE (Yasumura and Ishida 2010). Thus, at least in aging evergreen leaves, the low PNUE may be partially attributed to low relative allocation of N to rubisco, whereas the trade-off between photosynthetic and wall nitrogen is questionable in fully active leaves. More measurements are certainly needed to strengthen this finding.

### Hypothesis (4)

Some of the rubisco present in evergreen leaves may not be active in carboxylation, instead playing the role of a storage molecule as suggested by the presence of excessive amounts of this enzyme in relation to actual CO<sub>2</sub> uptake rate (Warren et al. 2000; Warren and Adams 2004). A study of 8 species of co-occurring evergreen forest trees in Japan, however, found no connection between leaf life span (0.5 to 2 years) and photosynthetic rate per unit rubisco (Hikosaka and Hirose 2000). While this hypothesis is still under consideration a case has been reported

of rubisco accumulation in expanding chloroplasts of *Aucuba japonica* prior to winter (Muller et al. 2009). Although the latter authors favored the interpretation of rubisco accumulation as a photosynthetic acclimatory preparation for higher irradiance during winter, the reduced winter activity of rubisco nevertheless suggests a storage function.

Taken together, studies investigating the question of low  $A_{\max(\text{mass})}$  and PNUE in evergreen leaves have produced ample evidence for trade-offs between photosynthetic efficiency and structural and functional requirements of long leaf life span. Such trade-offs are the basis of coexistence of evergreen and deciduous strategies (Chabot and Hicks 1982). To understand the advantages and disadvantages of the evergreen leaf form, integrated lifetime carbon income needs to be weighed against leaf construction and maintenance costs at the leaf and the plant levels (Aerts 1995; Givnish 2002). This complex task has been recently approached by Falster et al. (2012) who, by modeling lifetime carbon gain in leaves of temperate Australian woody plants experiencing progressive self-shading, and correcting it for dark respiration, showed a positive relationship between leaf longevity and life-time carbon return. Under this scenario, evergreen leaves with their lower photosynthetic rate are superior over deciduous leaves in terms of carbon return per unit of resource invested in the leaf, but deciduous species match that output by quickly re-investing resources into making new, more efficient leaves ("time discounting effect", Falster et al. 2012).

## Adaptation of evergreen leaves to winter conditions

### Winter photosynthesis and photoinhibition

Maintenance of functional leaves through the unfavorable period of low temperatures allows evergreen plants to capitalize on additional windows of photosynthetic opportunities when the potentially competing deciduous species are leafless. However, photosynthetic carbon uptake, as an enzymatic process, is temperature dependent, therefore during cool winters the net CO<sub>2</sub> uptake is temperature limited. Additionally, seasonal downregulation of photosynthetic capacity causes a decrease of CO<sub>2</sub> uptake rate also during brief periods of potentially permissive temperatures. Winter declines of the capacity for CO<sub>2</sub> uptake have been documented e.g. in *Hedera helix* (Oberhuber and Bauer 1991), *Mahonia repens* (Logan et al. 1998; but see Adams et al. 2002), *Phillyrea angustifolia* (Arena et al. 2006), *Nerium oleander* and *Laurus nobilis* (Meletiou-Christou and Rhizopoulou 2012).



This regulated acclimation process occurs in response to the onset of low temperatures and is followed by springtime de-acclimation and photosynthetic recovery (Oberhuber and Bauer 1991; Verhoeven et al. 1998; Neuner et al. 1999; Öquist and Huner 2003). Winter decline of photosynthesis thus results from both environmental and endogenous controls. It may, however, be aggravated by frost damage (Bauer and Kofler 1987).

Although snow cover may protect evergreen leaves from frost damage, low light and near or below zero temperatures under snow prevent photosynthesis. However, when snow cover partly melts allowing access of sufficient light, positive net photosynthesis resumes, as in low-statured arctic evergreen shrubs *Vaccinium vitis-idaea*, *Ledum palustre* and *Cassiope tetragona* (Starr and Oberbauer 2003). In snow covered *Vaccinium vitis-idaea* photosynthetic capacity dropped to 4% of annual maximum during the coldest period but recovered to 25% of maximum before snow melt (Lundell et al. 2008). Also *Rhododendron ferrugineum* retained substantial photosynthetic capacity under a heavy snowpack (Larcher and Siegwolf 1985). In leaves not covered by snow, winter photosynthetic decline is stronger in leaves exposed to higher irradiance, indicating the prevailing influence of photo-oxidative stress (Oberhuber and Bauer 1991; Logan et al. 1998; Adams et al. 2002).

The mechanism of photosynthetic downregulation involves a synchronous decrease in carboxylation capacity of rubisco and photosynthetic electron transport (Miyazawa and Kikuzawa 2005a; Miyazawa et al. 2007). The downregulation of electron transport capacity is typically measured as a decline in photochemical efficiency of PSII correlated with an enhanced thermal dissipation of absorbed light energy (Adams et al. 2004; Hormaetxe et al. 2007). Such a trend has been reported e.g. for *Cistus albidus* (Oliveira and Peñuelas 2002), *Ilex aquifolium* (Groom et al. 1991), *Hedera helix* (Oberhuber and Bauer 1991), *Euonymus kiatchovicus* (Verhoeven et al. 1998), *Eucalyptus pauciflora* (Blennow et al. 1998), *Mahonia repens* (Logan et al. 1998), *Rhododendron catawbiense* (Harris et al. 2006), *Quercus ilex* ssp. *ballota* (Corcuera et al. 2005) and many other evergreen species. It is accompanied by the accumulation of photoprotective xanthophylls, zeaxanthin and antheraxanthin, and their permanent retention in the de-epoxidized state (Oberhuber and Bauer 1991; Verhoeven et al. 1998; García-Plazaola et al. 2003; Corcuera et al. 2005). The mechanism of sustained winter quenching via de-epoxidized xanthophylls in evergreen leaves is thus distinct from the dynamic engagement of non-photochemical quenching operating at the scale of minutes to hours in response to excessive irradiance at mild temperatures, and is much less understood (Verhoeven et al. 1998; reviewed in Öquist and Hun-

er 2003). The second photoprotective mechanism involves the degradation of D1 protein in the PSII core and proteins of the oxygen-evolving complex, with the resulting inhibition of photosynthetic electron transport (Adams et al. 2004; Zarter et al. 2006).

While nonphotochemical quenching and down-regulation of electron transport reduce the formation of reactive oxygen species, further protection of the cellular environment is provided by antioxidative enzymes (Logan et al. 1998) or several categories of antioxidative compounds, some of which, e.g.  $\alpha$ -tocopherol (García-Plazaola et al. 1999, 2003; Kypris et al. 2000), glutathione (Logan et al. 1998) or phenylpropanoids (Grace et al. 1998), have been shown to accumulate during winter. Whole leaves may transiently change color from green to red due to accumulation of carotenoids (e.g. retro-carotenoids in *Buxus sempervirens*, Hormaetxe et al. 2004) or anthocyanins (Hughes et al. 2012). Red leaf coloration is enhanced by solar radiation and is accompanied by the decrease of the chlorophyll *a/b* ratio, suggesting a shading effect of pigments on the chloroplasts. Although light screening is the favored explanation for the presence of anthocyanins, their role as antioxidants is also possible (Hughes 2011). A variety of other acclimatory metabolic changes have been described, e.g. estrification of xanthophylls with fatty acids in *Buxus sempervirens* (Hormaetxe et al. 2004), conversion of chlorophyll into an inactive storage form in *Eucalyptus pauciflora* (Gilmore and Ball 2000), accumulation of dehydrins (Harris et al. 2006) and early light-induced proteins (ELiPs; Peng et al. 2008b), and downregulation of aquaporins (Peng et al. 2008a) in *Rhododendron catawbiense*.

In response to low temperature, leaves of some evergreen species perform thermonastic movements, involving reversible lamina drooping and rolling. Examples of thermonastic species include *Rhododendron catawbiense* (Wang et al. 2008), *Rhododendron maximum* (Bao and Nilsen 1988), some other evergreen Ericaceae (Russell et al. 2009) and *Viburnum rhytidophyllum* (Fig. 3). Such change in leaf orientation reduces photooxidative damage by decreasing lamina exposure to light but also protects the leaf from desiccation and allows rapid recovery of photosynthetic activity in the spring (Nilsen 1992; Russell et al. 2009). The benefits of the thermonastic movements occur only in the exposed parts of the crown (Russell et al. 2009). This and other photoprotective mechanisms contribute to the preservation of structural and functional integrity of chloroplasts through the winter when energy loads received by the leaf exceed its capacity for carboxylation.

Much environmentally induced and interspecific variation exists in the employment of the various strategies (Adams et al. 2004). For example, winter downregulation of photochemistry and photo-



Fig. 3 Evergreen leaves of *Viburnum rhytidophyllum* in a state of thermonastic drooping during freezing temperature conditions

synthetic  $O_2$  evolution in *Arctostaphylos uva-ursi* was complete at a high mountain site but nearly absent at a low elevation site, reflecting the difference in the harshness of weather conditions (Zarter et al. 2006). It has been hypothesized that variation in the extent of downregulation of photosynthesis may be related to differences in carbohydrate sink activity. While overwintering herbaceous plants often retain the capacity for growth during periods of permissive temperatures and retain or even enhance their photosynthetic capacity, typical woody evergreens cease growth during winter, thus cutting down the utilization of photosynthates and simultaneously downregulating photosynthesis (Adams et al. 2002, 2004). Intermediate behaviors also occur, depending on the activity of sinks other than shoot growth, such as root growth or carbohydrate storage. It has been also suggested that sensitivity of the phloem transport to cold might constrain photosynthetic activity, especially in species with symplastic phloem loading (Adams et al. 2002).

During periods of subfreezing temperatures photosynthesis is inhibited completely, however during warmer periods winter leaves often have significant photosynthetic activity. Where temperature allows, winter  $CO_2$  uptake may be an important contribution to the annual  $CO_2$  budget, e.g. because of favorable

moisture conditions in Mediterranean vegetation (Varone and Gratani 2007) or abundant light in the understory of temperate deciduous forests (Miyazawa and Kikuzawa 2005b). Saplings of six understory evergreen species in central Japan showed an early winter decline and a late winter recovery of photosynthetic capacity, with *Camellia japonica* and *Ilex pedunculosa* maintaining as much as ca. 50% and 25% of their respective summer capacities even during the coldest period in January (Miyazawa and Kikuzawa 2005b). When integrated over the whole year, assimilation during the 4.5 months of winter in three of these six species exceeded that during the rest of the year, illustrating the advantages of an evergreen life strategy under a deciduous forest canopy.

### Structural adaptations for frost tolerance

Plants with wintergreen leaves occur and sometimes even dominate in environments where frost occurs regularly, including boreal, alpine, and some temperate and Mediterranean areas. Tolerance of winter conditions may be enhanced by some of the structural leaf traits associated with mechanical endurance and xeromorphy that could prevent damage by snow or ice. The thick, wax-rich cuticles protect evergreen leaves from winter desiccation by ensuring a very low cuticular water permeability, while stiffness of the cell walls and whole leaf may slow down dehydration of the protoplasts (Rajashekar and Burke 1996; Burghardt and Riederer 2003). Whereas the physiological mechanisms of cellular low temperature tolerance are beyond the scope of this review (except for photoprotective mechanisms discussed above), their functioning in the leaf may be influenced by the anatomical structure.

Avoidance of freezing damage requires that ice is not formed in the protoplasts. This end may be achieved by two complementary mechanisms: the supercooling effect allowing the protoplasmic (and sometimes, also the apoplastic) water to remain liquid in spite of subzero temperatures and extracellular ice nucleation, whereby water is partly withdrawn from protoplasts into the apoplast where it forms an ice layer on the outer surface of the cell walls or between the wall and the protoplast (Sakai and Larcher 1987; Körner 2003). Leaves of several evergreen species have been found to supercool down to  $-10^\circ C$  or  $-12^\circ C$  (Sakai and Larcher 1987) and in the evergreen bamboo *Sasa senanensis* supercooling was effective down to  $-22^\circ C$  (Ishikawa 1984). The effectiveness of supercooling appears to be influenced by the internal leaf structure. The preferred anatomical environment for supercooling is provided by leaves with small, densely packed mesophyll cells and small intercellular spaces (Neuner and Bannister



1995). Sclerenchymatous bundle sheath extensions that divide the air space of the leaf into small compartments further favor the supercooling mechanism by restricting the spreading of ice from its nucleation sites as shown for *Nothofagus menziesii* (Neuner and Bannister 1995), *Sasa senanensis* (Ishikawa 1984) and a tropical tree *Cinnamomum camphora* (Hacker and Neuner 2007). Abundant intercellular spaces, on the other hand, provide space to accommodate extracellular ice (Ball et al. 2004).

The exact sites of initial ice nucleation or pathways of ice propagation have been subject of much discussion and research, typically revealing consistent, species specific patterns. For example, anatomical zones for massive ice accumulation have been identified in the midribs of cold-acclimated *Eucalyptus pauciflora* where ice deposits form in intercellular spaces of parenchyma strands located on the upper and lower side of the vascular bundle (Ball et al. 2004). In recent years, the employment of high-resolution infrared differential thermal analysis has allowed to further link leaf anatomy with ice nucleation and propagation pathways in evergreen leaves (Hacker and Neuner 2007; Hacker and Neuner 2008). Whereas in deciduous species ice propagated inside the leaf from the petiole following the venation network, in 1-year old leaves of the evergreen *Buxus sempervirens*, ice nucleation took place in a lacunar space separating palisade and spongy mesophyll, followed by the formation of a central ice lens. This lacuna was absent from current year leaves in which, accordingly, ice propagated along the vein network. The basis for such ordered patterns of ice formation has not been elucidated but an involvement of anatomical ice propagation barriers, localized presence of heterogenous nucleation agents and antifreeze proteins (AFPs) that adhere to ice crystal surfaces and direct their growth have all been implicated (Wisniewski et al. 2014).

## Winter xylem dysfunction

Maintenance of leaves through periods of subfreezing temperatures in a sufficiently hydrated state is a prerequisite for their further photosynthetic functionality and depends on the safety and efficiency of water transport to the leaf and within the leaf. Exposure to freezing/thawing cycles may disrupt water flow in the xylem by causing conduit cavitation (Zimmermann 1983). A cavitated stem xylem cannot supply water to evergreen leaves, inducing stomatal closure and a reduction of photosynthesis. If cavitation persists, photosynthetic recovery after the cold period may be delayed (Nardini and Salleo 2000). In severe cases leaves may desiccate. Since vulnerability to freeze/thaw-induced cavitation is highest in conduits with large diameter and volume (Davis et al. 1999; Zanne et al. 2014), evergreen species are expected to have smaller

conduits relative to deciduous species (Cochard and Tyree 1990; Cordero and Nilsen 2002). Moreover, the size of pit pores determines the maximal size of particles that may pass into conduits and later cause ice nucleation (Lintunen et al. 2013). Small conduits with narrow pit pores, however, are hydraulically inefficient, potentially restricting gas exchange rates in evergreen species during warmer parts of the year (Tyree and Sperry 1989; Johnson et al. 2009).

According to the majority of reports, resistance of stem xylem to freeze-thaw induced cavitation is indeed correlated with leaf habit. Evergreen oaks had narrower vessels than deciduous oaks and were also less vulnerable to freeze induced cavitation both in the Mediterranean and in Florida (Cavender-Bares and Holbrook 2001; Cavender-Bares et al. 2005), similarly to a representative sample of arctic evergreen vs. deciduous shrubs and cushion plants (Gorsuch et al. 2001). Most evergreen chaparral species have relatively small-diameter vessels making them resistant to winter cavitation, however evergreen plants with larger vessels such as *Rhus ovata* and *Malosma laurina* suffered near complete loss of stem conductivity in subfreezing temperatures (Pratt et al. 2005). Two wide-vesselled Japanese evergreens underwent a severe reduction of stem conductivity and leaf dieback during winter, in contrast to two species with narrow-vessels (Taneda and Tateno 2005). Likewise, in an Australian high-altitude heath, evergreen species had variable conduit diameters and there was a close correlation between that trait and vulnerability to winter cavitation (Choat et al. 2011). Finally, based on a global study of plant radiation into freezing environments, among evergreen species only those with narrow ( $<44\ \mu\text{m}$ ) conduits managed to colonize areas experiencing temperatures  $<0^\circ\text{C}$  (Zanne et al. 2014). Although not all studies cited here included deciduous species for comparison, the trend for narrower vessels in evergreens and a negative correlation between stem hydraulic efficiency and resistance to freeze-induced cavitation seems well established (Fu et al. 2012).

The stem hydraulic pathway is extended into the leaf vein system. Although similar biophysical principles govern the processes of water conduction and cavitation in leaves and in stems, leaves are a more complex system because they constitute a network of parallel and serial hydraulic resistors with much redundancy and large safety margins (Roth-Nebelsick et al. 2001). Whole leaf conductance integrates xylary and extra-xylary pathways of water movement and, depending on species, may or may not be constrained by hydraulic properties of the xylem conduits (Lo Gullo et al. 2003; Salleo et al. 2003). Declines of leaf hydraulic conductance may be caused by cavitation of xylem conduits but also by their collapse and by changes in the extra-xylary part of the pathway, such as drops in cell turgor or downregulation of mem-



brane permeability (Sack and Holbrook 2006). Although loss of conductivity has been demonstrated under laboratory conditions in dehydrating leaves of *Laurus nobilis* (Salleo et al. 2001), *Prunus laurocerasus* (Nardini et al. 2001), *Pieris japonica* and *Castanopsis chrysophylla* (Johnson et al. 2009), much less information is available on leaf hydraulic disruption due to freeze/thaw events. Leaves of *Eucalyptus pauciflora* subjected to a  $-6^{\circ}\text{C}$  treatment followed by thawing experienced embolization of 1/3 of vessels (Ball et al. 2006). There is also no clear evidence for superior resistance to hydraulic disruption in evergreen leaves. In contrast to stems, there is also generally no connection between leaf habit or LMA and leaf hydraulic conductance (Sack and Holbrook 2006). The few published studies specifically comparing leaf hydraulic properties of evergreen and deciduous species indicate that leaf habit is not a major determinant of leaf hydraulic conductance expressed on the leaf area basis (Lo Gullo et al. 2005; Sack and Frolé 2006; Simonin et al. 2012).

This apparent absence of hydraulic specialization in evergreen leaves could be explained by the ultimate dependence of leaves on water supplied by stems. If stems, with their wider conduits, become embolized before the leaves, excessive hydraulic safety of leaf xylem would be of little value to the plant because leaves would become dehydrated anyway (Davis et al. 1999). In general, however, the leaf vein network is hydraulically safer than stems and, since cavitation first occurs in the midrib, its effect on whole leaf conductance may be mitigated by hydraulic bypasses formed by lateral veins (Nardini et al. 2001).

## Photosynthetic decline with leaf age

Aging of evergreen leaves is typically accompanied by progressive self-shading by consecutive leaf cohorts (Niinemets et al. 2006; Wright et al. 2006; Falster et al. 2012). A gradual decrease of photosynthetic potential with age has been measured e.g. in *Vaccinium vitis-idaea* (by 75% in 6-year-old leaves, Karlsson 1985), *Laurus nobilis*, *Olea europaea* and *Quercus ilex* (Niinemets et al. 2005). Such a decrease is in part caused by the thickening of cell walls that cause a greater internal resistance to  $\text{CO}_2$  diffusion (Niinemets et al. 2005; Niinemets 2007). In some species, such as *Laurus nobilis*, the decline in photosynthesis is concurrent with nitrogen withdrawal, but nitrogen concentration per area changed little during this process in *Olea europaea* or *Quercus ilex* (Niinemets et al. 2005).

Since the downregulation of photosynthetic capacity with age is, at least in some cases, independent of withdrawal of nitrogen to younger leaves, it is debat-

able whether it represents an acclimative response to shade or merely a symptom of leaf aging (Hikosaka 2005; Niinemets 2007). In *Quercus ilex* both declining irradiance and leaf aging resulted in decreased photosynthetic capacity caused by lower nitrogen partitioning to rubisco and electron transport (Niinemets et al. 2006; Rodríguez-Calcerrada et al. 2012). Since both carboxylation and electron transport capacities were more closely related to current than to past irradiance, and an increase in leaf absorptance was reported, these changes may be considered to at least partly represent active shade acclimation (Niinemets et al. 2006; Niinemets 2007).

## Leaf acclimation to altered irradiance

Leaves of evergreen understory species often develop and function in deep shade after the deciduous canopy closes. They become exposed to high irradiance after overstory leaves have fallen in the autumn. Acclimative adjustments of leaf structure and photosynthetic potential allow the plants to deal with spatial and seasonal variability of ambient irradiance.

In response to altered light intensity, evergreen species show plastic modifications of leaf structure that are similar in direction to those of deciduous species [e.g. see Letts et al. (2012) for data on *Buxus sempervirens*, Valladares et al. (2005) for *Ilex aquifolium*, Gratani et al. (2006) and Tattini et al. (2006) for *Quercus ilex*, *Phillyrea latifolia*, *Pistacia lentiscus* and *Myrtus communis*]. Leaves formed in low light are thin, have low LMA and tissue density, and a low photosynthetic capacity per area, consistent with the need to adjust carbon investment costs to anticipated photosynthetic benefits in a low light environment (Poorter et al. 2009; Lusk et al. 2010). Extended life span, however, requires high LMA and tough leaf structure also under shaded conditions, therefore it may be hypothesized that the capacity for plastic modification of leaf structure should be lower in evergreen than in deciduous leaves (Lusk et al. 2008). Surprisingly few comparisons of quantitative reaction norms of these contrasting leaf types have been conducted. Data from Valladares et al. (2000) for 16 species of neotropical *Psychotria* shrubs indicate no connection between leaf longevity and the plasticity index measuring the response of LMA to irradiance. Likewise, plasticity of LMA in the evergreen *Quercus suber* was similar to that in the deciduous *Q. canariensis* (Daas-Ghrib et al. 2011). On the other hand, response of LMA to a canopy light gradient in *Ilex aquifolium* was smaller than for most deciduous species studied by Aranda et al. (2004). In a greenhouse experiment, average plasticity index for LMA was

35% in 36 deciduous species and only 18% in 35 evergreen species from subtropical China (Böhnke and Bruelheide 2013). Given the large number of species included in the latter study, the hypothesis that evergreen leaf habit is associated with reduced plasticity of leaf structure in comparison with deciduous species, seems valid.

The conflict between longevity and plasticity may be attenuated by several factors. A detailed analysis of leaf plasticity in tropical evergreen species conducted by Lusk et al. (2010) showed that the reduction of LMA in shade did not cause a proportional decrease in leaf mechanical properties. In fact, at similar LMA, shade leaves were stronger than sun leaves, reflecting their slightly higher leaf density and cell wall fraction while specific cell wall strength was unchanged. Further, the decrease of LMA (a trait contributing to herbivore resistance) in shade, could be compensated for by the lower attractiveness of shade leaves to herbivores due to their lower nutritional contents per unit force needed to ingest the leaf tissue (Lusk et al. 2010).

Interestingly, even constitutive shade tolerance in woody evergreens does not require the expression of the typical low LMA syndrome. Numerous evergreen species, especially in tropical forests, are primarily adapted to shade while retaining structural characteristics normally associated with longevity (Lusk et al. 2008). This strategy is viable because evergreen plants in low light achieve a favorable lifetime carbon balance not by maximally reducing leaf construction costs, but rather by extending their life spans, decreasing respiratory costs and plant growth rate (Walters and Reich 1999; Lusk 2008).

Regardless of the leaf habit, there are few exceptions to the rule that structural adjustment to ambient irradiance takes place during the expansion phase of the leaf lamina. Surprisingly, one of the most remarkable of those exceptions has been described in the evergreen *Hedera helix*, in which mature, previous-year, shade-acclimated leaves responded to increased light by the division and expansion of cells in the upper mesophyll layer (Bauer and Thöni 1988). In some evergreen species, lamina development is slow and the maturation of photosynthetic apparatus even slower (“delayed greening”) which allows a longer time window when structural acclimation to increased light is possible (Ishii and Ohsugi 2011). Reports of late structural adjustments are, however, rare, in contrast to physiological acclimation. Even mature, previous-season leaves may respond to increased irradiance by increasing their N content and photosynthetic capacity without undergoing structural modifications (Ishii and Ohsugi 2011).

## Leaf development rate

Leaf development consists of lamina expansion, development of internal and external anatomical structures and acquisition of photosynthetic competence. Leaves tend to reach peak  $A_{\max(\text{area})}$  at, or soon before, the completion of the lamina expansion (Pantin et al. 2012). The rates of lamina expansion and acquisition of functional maturity tend to be slow in species with high LMA (Miyazawa et al. 1998; Gratani and Bonito 2009). An extreme case of this phenomenon has been termed “delayed greening” and involves a slow build-up of leaf chlorophyll content and photosynthetic capacity after full lamina expansion. It has been widely observed in many, especially shade tolerant, tropical trees (Kursar and Coley 1992a). This phenomenon has rarely been reported from northern temperate areas but it is common e.g. in warm temperate forests in Japan where it is a feature of many evergreen trees. Between 15 and 44 days were required to achieve full photosynthesis after completion leaf area expansion in *Neolitsea sericea*, *Quercus glabra*, *Q. myrsinifolia*, *Machilus thunbergii*, *Castanopsis sieboldii* and *Cinnamomum japonicum* (Miyazawa et al. 1998). The delay was correlated with the final LMA, suggesting that longer time is needed to form a fully functional internal structure in the tough, evergreen leaves (Miyazawa et al. 1998). Delayed greening has also been interpreted as an adaptive mechanism minimizing losses to herbivory by producing sufficiently resistant leaf tissues before investing resources in photosynthesis (Kursar and Coley 1992b). The development of leaf area and photosynthetic capacity seem, however, to be well synchronized in other evergreens, e.g. the Mediterranean *Arbutus unedo* (Gratani and Ghia 2002) or *Quercus ilex* (Gratani and Bonito 2009).

## Evergreen leaves as storage organs

Seasonal growth rhythms in perennial plants are accompanied by rhythms of resource uptake, accumulation and mobilization (Chapin et al. 1990). Early season initiation of new shoot growth is supported to various extents by stored carbohydrates. Whereas in deciduous plants the only source of carbohydrates at that time is the storage pool, evergreen species rely both on storage and on current assimilation by preexisting leaves. Moreover, in addition to carbohydrates accumulated in axial organs, as is typical for all woody plants, evergreen species may store part of their carbohydrate pool in overwintering leaves. According to Miyaké (1902), this idea was historically suggested by Sachs in his 1862 paper published in *Flora*. A semi-quantitative study by Miyaké (1902), however, showed, that during the coldest months

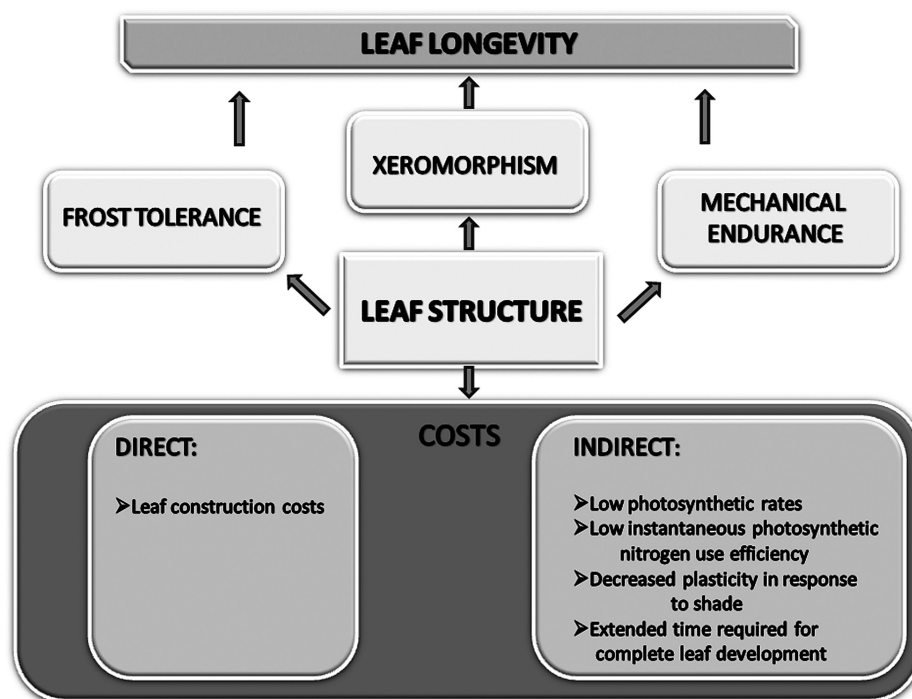


Fig. 4. A synthetic view of structural modifications found in evergreen leaves (enhanced mechanical endurance, frost tolerance and water deficit tolerance) required for leaf longevity and incurring direct and indirect costs to the plant

starch concentration in overwintering leaves is usually lower than during the rest of the year, and in many species starch is nearly absent. It does, however accumulate during early spring, before shoot growth begins. Soluble carbohydrates may also be present acting as osmotica in winter and later used as energy sources (Reyes-Díaz et al. 2005). The role of leaves as storage site has since been suggested by numerous phenological and manipulative studies. For example, the period of springtime shoot extension in *Quercus ilex* coincided with a decrease in soluble sugar, hemicellulose, and lipid pools in at least some age classes of preexisting leaves (Cherbuy et al. 2001). Total nonstructural carbohydrate level in a Mediterranean plant *Salvia lavandulifolia* leaves increased between November and February and declined thereafter when new shoots were expanding (Palacio et al. 2007). Winter carbohydrate levels were high in recent-leaf cohorts of *Aucuba japonica*, *Camellia rusticana*, *Ilex crenata* and *Daphniphyllum macropodium* and declined later in the year presumably because of export and consumption by growing plant parts (Ino et al. 2003). Removal of mature leaves from *Kalmia polifolia*, *Ledum groenlandicum* (Reader 1978) and *Quercus ilex* (Cherbuy et al. 2001) at the end of winter decreased the storage pools and resulted in a decreased growth of new shoots and foliage. Such results are suggestive of significant resource storage in leaves. However, in the case of defoliation treatments, the plant also loses part of its assimilatory surface that would otherwise contribute to regrowth.

In addition to carbohydrates, defoliation treatments remove certain amounts of nutrients that might possibly also function as a storage pool. The storage of nitrogen in protein form in leaves is suggested by the elevated fraction of inactive rubisco (Takashima et al. 2004). Also, the patterns of seasonal variability in leaf nitrogen concentration often indicate an accumulation of this nutrient during the growth season followed by springtime translocation into new growing shoots, as documented e.g. for *Satureia montana* (Palacio et al. 2007), *Daphniphyllum humile* (Katahata et al. 2007) and *Quercus ilex* (Cherbuy et al. 2001). The withdrawal of nutrients from mature leaves may also be interpreted as nutrient recycling accompanying senescence, similar as in deciduous species but less rapid. This is especially likely in species where timing of leaf senescence and nutrient remobilization do not match the timing of new growth (Milla et al. 2005). In *Rhododendron ferrugineum*, tracing the  $N^{15}$  label revealed that nearly 70% of nitrogen resorption from leaves took place within the first 12 months of their life (leaf life span was up to 25 months in this species) and 26% of the label was recovered from new shoots (Pornon and Lamaze 2007). Essentially, in this species the second and final season of life was an extended senescence accompanied by a decline in photosynthetic rate and chlorophyll concentration. The blurry distinction between mobilization of nitrogen from the storage pool and its resorption from senescing leaves, is further complicated by the fact that in young, nonse-



nescent leaf cohorts, resorption and replenishment of nutrient pools may occur concurrently (Pasche et al. 2002).

A convincing case of nitrogen storage has been described in *Quercus myrsinifolia*, in which nitrogen accumulated during the first season of leaves' life was partially resorbed in the spring, followed by another period of moderate accumulation and resorption before leaf abscission at the end of the second season (Yasumura and Ishida 2010). Leaves of this tree thus performed a full accumulation-mobilization-accumulation cycle. A potential physiological mechanism for seasonal storage of nitrogen has been documented in high-light grown *Aucuba japonica* in which  $N_{ar-ear}$  increased before the winter followed by a spring decrease (Muller et al. 2009). Much mesophyll cell wall area was initially free of chloroplasts. That vacant space was filled by expanding chloroplasts as winter approached, accompanied by the accumulation of rubisco with reduced activity (Muller et al. 2009). In this case authors favored the interpretation of rubisco accumulation as a photosynthetic acclimatory mechanism, compensating for limitation of photosynthesis by low winter temperatures. Nitrogen storage in leaves would clearly be a side effect of such acclimation since nitrogen was withdrawn from leaves in the following spring (Muller et al. 2011). In the understory shrub *Kalmia latifolia*, gradual accumulation of nitrogen during summer when plants are shaded may be viewed as building up of the storage pool, perhaps facilitated by lower demand from other sinks or by nutrient recycling from other senescing organs (Xu et al. 2007).

The role of resources accumulated in preexisting leaves for initiation of growth will vary from crucial to null depending on the diversity of growth phenologies, leaf longevities and species specific strategies and allocation patterns (Eckstein et al. 1998; Milla et al. 2005; Palacio et al. 2006). It is certainly different for different resource types (with little information available e.g. on phosphorus storage) and on habitat characteristics. Especially where leaf damage during the winter is likely, other storage sites within the plant should become more important (Palacio et al. 2007).

## Summary

In seasonal climates, adaptation of leaves to survive more than one growing season requires the presence of special structural, developmental and physiological adaptations, most of which involve costs and trade-offs with other plant functions (Fig. 4). Direct costs take the form of additional resource investment into making leaf mechanical tissues. Indirect costs may include reduced photosynthetic effi-

ciency and PNUE, slower leaf development, smaller leaf plasticity and greater self shading as well as lower hydraulic efficiency of the xylem. With the rapidly increasing understanding of adaptive physiology of particular traits and the associated costs, progress is being made towards answering key questions in evolutionary ecology of plants differing in leaf habit.

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