Chapter 8
The Role of Xylem Parenchyma in the Storage and Utilization of Nonstructural Carbohydrates

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1 The Structure, Abundance, and Function of Ray and Axial Parenchyma in Wood

1.1 The Structure of Ray and Axial Parenchyma in Wood

The fact that most of the cells present in mature wood are dead is often highlighted. Indeed, all xylem conduits (vessels and tracheids) found in the functional sapwood undergo cell autolysis, forming hollow tubes made of lignified secondary cell wall. However, cells with a protoplasm are also present in secondary xylem. These cells are referred to as wood parenchyma because their cell wall is often much thinner than that of fibers. Depending on their arrangement and orientation with respect to the main stem axis, parenchyma cells can be divided into two distinct types—radial (ray) and axial parenchyma.

Ray parenchyma consists of ribbon-like aggregates of cells that are produced by ray initials, extending radially from the cambial zone in the xylem and phloem. Xylem rays can be classified depending on their width as uniseriate (Fig. 8.1a–d), biseriate (Fig. 8.1e), and multiseriate, referring to rays that are one-, two-, or more cells wide. Rays can also be subdivided according to the dimensions of individual cells viewed in a radial section. While most of the ray parenchyma cells have their longest axis oriented radially (procumbent cells), vertically elongated (upright) or isodiametric (square) cells also occur. Rays comprised exclusively of procumbent cells are called homocellular, while rays made of more than one parenchyma cell type are termed heterocellular (Carlquist 2001; Evert 2006). The entire ray system can consist of a single ray type, but a combination of different ray types commonly

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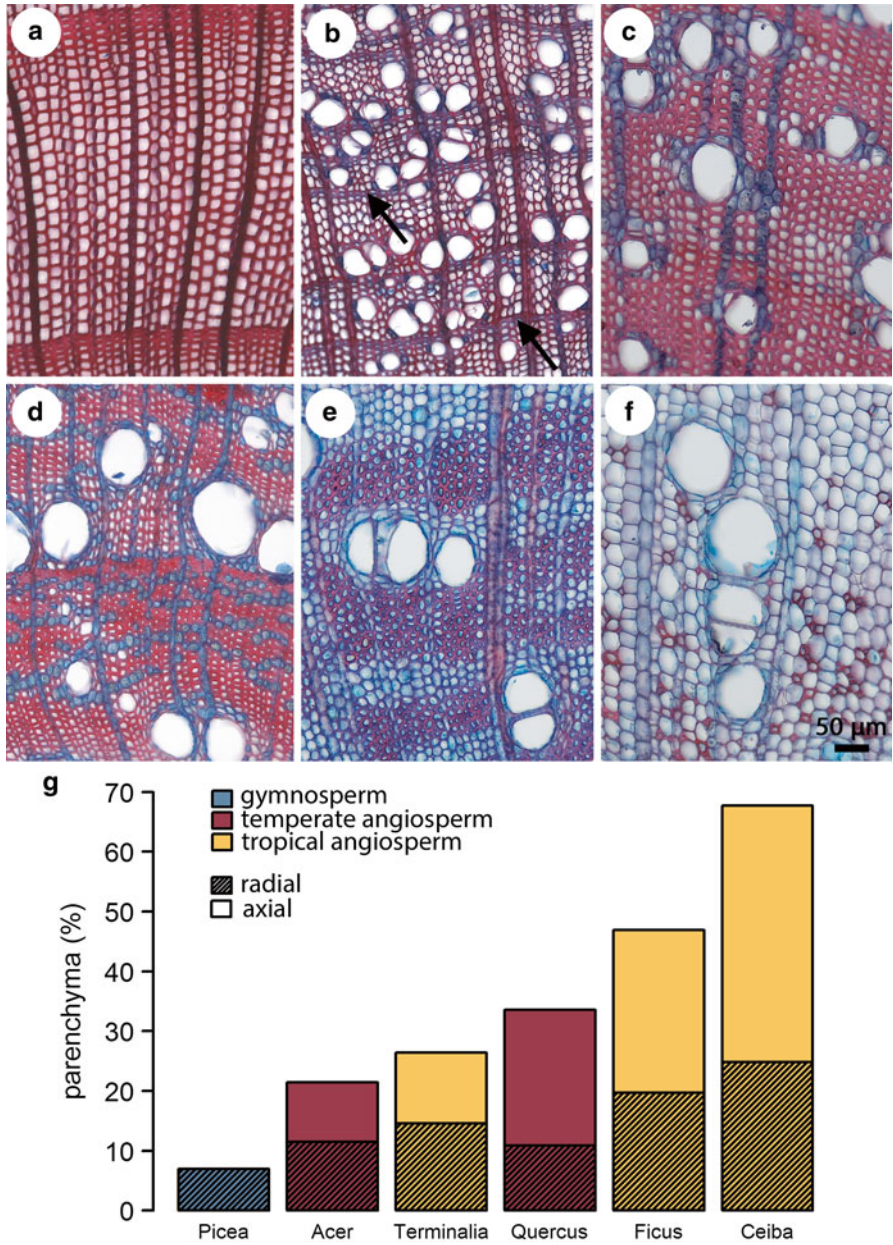e-mail: lenka.plavcova@gmail.com

Fig. 8.1 Diversity in ray and axial parenchyma patterns as viewed in transverse wood sections stained with *safranin* and *alcian blue*. Thick lignified secondary cell walls of fibers and vessels stain *pink*, while protoplasts and less extensively lignified cell walls of parenchyma cells appear *blue*. All images were taken at the same magnification; a scale bar is shown in (**f**). (**a**) A conifer species (*Picea abies*) showing the lowest proportion of parenchyma; rays are uniseri-ate, axial parenchyma is absent, (**b**) a temperate, diffuse-porous tree (*Acer pseudoplatanus*)

occurs. In addition to the ray types described above, other specialized ray systems such as aggregated and interconnected rays have been identified. Interestingly, a temporal or permanent absence of rays occurs in the wood of several species (Barghoorn 1941; Carlquist 1970). Nevertheless, the complete absence of rays is a rarity restricted to small plants, in which woodiness is not pronounced and has evolved secondarily (Carlquist 1970).

Axial parenchyma is produced by fusiform cambial initials that undergo transverse divisions, resulting in a parenchyma strand or axial series of two or more parenchyma cells. In some species, axial parenchyma is absent or sparse. This condition is characteristic of conifers (Fig. 8.1a); however, it can be found in many angiosperms as well. For instance, sparse axial parenchyma occurs in *Populus*, *Aesculus*, *Berberis*, *Magnolia*, and *Eucalyptus*, to name a few examples. If present, axial parenchyma can be arranged in different patterns as distinguished in transverse sections (Carlquist 2001; Kribs 1937). Traditionally, axial parenchyma is classified as apotracheal if it appears distributed without a direct connection to vessels (Fig. 8.1d), and paratracheal if it is strongly associated with xylem vessels (Fig. 8.1b, c). These two basic categories can be further subdivided. For instance, parenchyma cells can be randomly scattered within the vessels and fibers (diffuse apotracheal parenchyma), be in contact with vessels but not ensheathing them completely (scanty paratracheal parenchyma, Fig. 8.1b), form a complete sheath surrounding vessels (vasicentric paratracheal parenchyma, Fig. 8.1c), or be arranged in distinct tangential bands (banded parenchyma, Fig. 8.1d, e). In addition, an increased occurrence of parenchyma cells at the tree ring boundary is often found in temperate species and referred to as marginal parenchyma (Fig. 8.1b). Last but not least, wood of some trees such as *Adansonia* (Chapotin et al. 2006) or *Ceiba* (Fig. 8.1f) is extremely parenchymatous, with axial parenchyma comprising most of the matrix between vessels and rays. The categorization outline above is useful, but to a certain extent arbitrary. Thus, intermediary patterns and co-occurrence of more than one type of axial parenchyma are frequently observed. Besides thin-walled parenchyma cells, some thick-walled axially oriented cells, which could be hence termed fibers, also retain living protoplasts (Fahn and Leshem 1963). Axial cells that can be classified as living fibers rather than parenchyma occur, for instance, in *Acer* (Fig. 8.1b),

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Fig. 8.1 (continued) and biseriate rays, axial cells that can be, due to their thick secondary cell wall, classified as living fibers are in a scanty paratracheal and marginal arrangement (*arrows*), (**c**) a tropical dry-deciduous tree (*Terminalia catappa*) with uniseriate rays and paratracheal vasicentric parenchyma, (**d**) a ring-porous temperate species (*Quercus robur*) with apotracheal axial parenchyma arranged in narrow bands and scanty paratracheal parenchyma contacting the vessels, rays are uniseriate (but multiseriate, up to 30-cells wide rays are common in older stems of this species), (**e**) a tropical evergreen tree (*Ficus rubiginosa*) with uniseriate and biseriate rays and axial parenchyma arranged in wide bands, (**f**) a tropical dry-deciduous tree (*Ceiba aesculifolia*) showing highly parenchymatous wood composed of multiseriate rays and thin-walled axial parenchyma cells. (**g**) The relative proportion of ray and axial parenchyma cells measured using transverse sections for all six species shown above

Robinia pseudoacacia (Yamada et al. 2011), or *Cactaceae* (Mauseth and Plemons-Rodriguez 1997), although the distinction between these two cell types has often been neglected. When these cell types are distinguished, it has been hypothesized that living fibers substitute for, or complement, the function of axial parenchyma cells (Carlquist 2001; Yamada et al. 2011; Wheeler et al. 2007).

1.2 How Much Ray and Axial Parenchyma Occurs in Wood?

Ray and axial parenchyma cells make up a substantial proportion of all wood cells (Fig. 8.1g). The volumetric content is hard to measure directly; however, the proportion of parenchyma cells can be estimated from the area measurements taken on a transversal or tangential section. In gymnosperms, the total parenchyma proportions are commonly between 5 and 10 % and compose mainly of radial parenchyma (Fig. 8.1a). In angiosperms, the total amount or parenchyma ranges typically between 20 and 40 % (Fig. 8.1b–d); however, values between 40 and 60 % are not uncommon among tropical angiosperms (Fig. 8.1e). The proportion of ray parenchyma is typically around 10–20 %, while the axial parenchyma proportions between 1 and 30 % are common in angiosperms. The aforementioned numbers represent values typically encountered in wood (Fig. 8.1g) (Von Frey-Wyssling and Aeberli 1942; Wagenführ 2007; Ruelle et al. 2006; Zieminska et al. 2013); however, more extreme values also occur. For example, very low ray proportions of around 7 % were reported for two *Acacia* species (Zieminska et al. 2013), while very high axial parenchyma proportions of 67 % were measured in *Ceiba aesculifolia* (Fig. 8.1f) and several species of the genus *Adenia* (Hearn 2009).

1.3 The Function of Ray and Axial Parenchyma in the Storage of Nonstructural Carbohydrates

The function of wood parenchyma in storage is often highlighted and put in contrast with the main role of vessels in facilitating water conduction and the role of fibers in providing the mechanical support. Nonstructural carbohydrates (NSC) represent the most abundant reserves stored in wood parenchyma. The importance of NSC storage for tree growth and functioning has been known for many decades (Kozlowski 1992; Kramer and Kozlowski 1979). Recently, this topic has received renewed attention because the size and the dynamics of the NSC pool might represent factors potentially limiting tree growth (Palacio et al. 2014) and affecting tree survival under drought stress (McDowell et al. 2008).

The total volume of ray and axial parenchyma can be viewed as a finite compartment potentially available for storage. Given the large volume of woody trunks, the size of this storage pool is substantial from the whole plant perspective. For instance,

Würth et al. (2005) calculated that the carbon stored in the above ground woody biomass accounts for 80 % of the total carbon pool present in a seasonally dry tropical forest and that this amount would be sufficient to completely regrow the entire canopy. However, the storage capacity provided by wood parenchyma is not always completely filled up. Instead, the NSC levels fluctuate, reflecting the dynamic balance between carbohydrate production and utilization.

Besides the total amount of NSCs, their partitioning into starch and soluble sugar fraction is of importance. Out of these two components, starch can be considered as the primary long-term storage form of NSCs. Its molecules are large and cannot move freely between cells; however, they can be readily hydrolyzed to produce soluble sugars. Soluble sugars constitute a plethora of mono- and oligosaccharides that are mobile and fulfill more active physiological roles.

As ray and axial parenchyma are the main sites of NSC accumulation in wood, the NSC status of these cells should be directly mirrored in the NSC content and composition measured in the bulk sapwood. The sapwood NSCs have been analyzed in a number of ecological studies (Hoch et al. 2003; Sauter and Wellenkamp 1998; Ashworth et al. 1993; Palacio et al. 2007; Carbone et al. 2013), providing insights into the size and dynamics of the wood parenchyma carbohydrate pool. In the following sections, we will review the NSC accumulation patterns observed in different tree species and different woody organs and discuss changes in NSC concentration and composition that occur throughout the growing season. These aspects of sapwood NSC dynamics will be linked to the anatomy and physiology of ray and axial parenchyma cells.

2 Patterns in NSC Accumulation Across Different Woody Species, Organs, and Time

2.1 Variation in NSC Across Different Tree Species

As already mentioned, a positive relationship between the NSC content and the proportion of ray and axial parenchyma in wood can be expected. To the best of our knowledge, this assumption has not been confirmed empirically, except for the notion that conifer wood tends to have lower NSC concentrations than the wood of angiosperms, which is in agreement with the lower proportion of parenchyma found in conifer wood (Johnson et al. 2012). Considering the large differences in wood parenchyma content across angiosperms, as illustrated by the more than threefold variation shown by the five angiosperms in Fig.8.1a–g, it would be interesting to see if the tendency for a higher NSC content with increasing volume of parenchyma holds true also within this group. While the meta-analysis of published data can provide useful insights (Johnson et al. 2012), the finer-scale patterns are likely to be confounded by the different sampling schemes employed by different authors and the high variation in NSC concentration found across different woody organs and
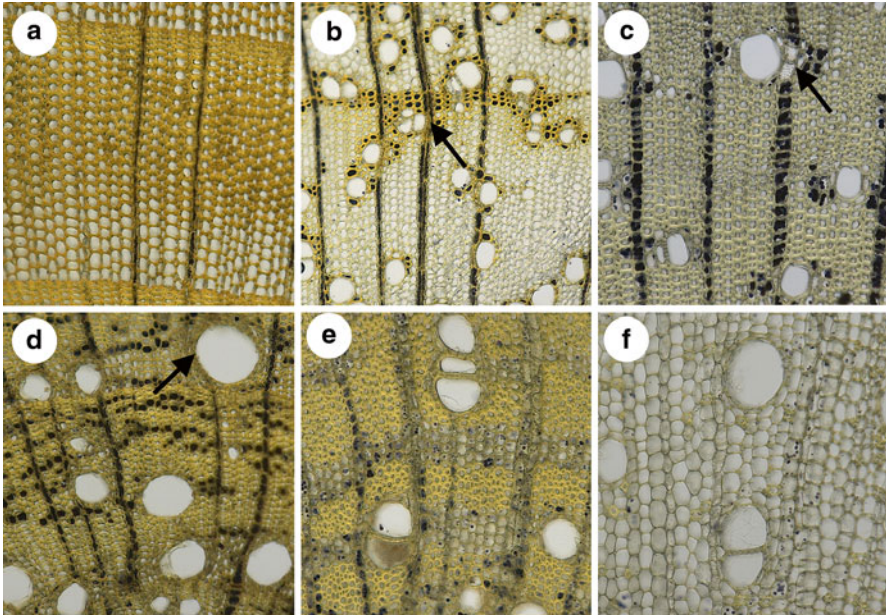
Fig. 8.2 Distribution of starch in wood parenchyma cells in the same species as shown in Fig. 8.1. The wood samples of the temperate species *Picea abies* (**a**), *Acer pseudoplatanus* (**b**), and *Quercus robur* (**d**) were collected at the Ulm University campus in October. In these species, most of the wood parenchyma cells appear densely packed with starch grains. The samples of the tropical plants *Terminalia catappa* (**c**), *Ficus rubiginosa* (**e**), and *Ceiba aesculifolia* (**f**) were obtained from the living collections of the botanical garden of Ulm University in early June. Despite growing under the same conditions of a tropical greenhouse, these species show marked differences in starch deposition. While *Terminalia* accumulates large amounts of starch in xylem parenchyma cells (**c**), the wood parenchyma in *Ficus* (**e**) and in particular in *Ceiba* (**f**) show much lower starch content. Note the absence of starch in contact cells in *Acer*, *Terminalia,* and *Quercus* (*arrows*)

seasons. In addition, the sapwood NSC content is usually expressed as a mass-based concentration, and therefore depends on wood density, which varies substantially between different species (Chave et al. 2009) and to some extent also within a single tree (Domec and Gartner 2002; McCulloh et al. 2012). This problem could be solved by expressing the NSC concentration on a wood volume rather than a wood mass basis. Unfortunately, the studies looking at the sapwood NSC content do not usually report wood density values to allow this conversion. Thus, additional research is required to demonstrate conclusively whether the proportion of ray and axial parenchyma is an important driver of the amount of NSC stored in wood. Here, we will provide some initial hints on answering this question.

Using the same species as shown in Fig. 8.1a–g, we visualized the starch deposition by staining with Lugol's solution (Fig. 8.2a–f). The iodine test revealed obvious differences in starch accumulation in these species that differ greatly in the amount of wood parenchyma. The parenchyma was packed with starch in all three temperate

species sampled at the end of the growing season (Fig. 8.2a, b, d). In contrast, the tropical plants showed varying patterns in starch accumulation, despite growing under the same conditions in a tropical greenhouse (Fig. 8.2c, e, f). The starch abundance was highest in *Terminalia* in which almost all parenchyma cells were filled with starch grains (Fig. 8.2c). The amount of starch was much lower in *Ficus* (Fig. 8.2e). In this species, the starch grains were sparse and almost absent in the regions closer to the cambium. The lowest starch accumulation was observed in the highly parenchymatous wood of *Ceiba aesculifolia* (Fig. 8.2f). Based on this simple iodine test, we cannot say whether most of the NSC were present in the form of soluble sugars in the wood of *Ficus* and *Ceiba*, or whether the total levels of NSC were low in spite of the high parenchyma content found in these two species. More than threefold differences in wood NSC concentration have recently been reported in a study encompassing 17 tropical trees from 10 different families (Würth et al. 2005). It would be interesting to see if this variability is at least partially explained by the amount of wood parenchyma. Marked differences in NSC concentration were observed not only between species but also between different tree parts. Thus, we will continue this review by comparing the patterns in NSC content found between woody branches, trunks, and roots.

2.2 Variation in NSC Across Different Woody Organs

Most studies monitoring the NSC levels in more than one woody organ report values for the main trunk and small terminal branches. Three times higher NSC concentrations in branch wood than in trunk wood were observed in eleven temperate trees (Hoch et al. 2003; Sala and Hoch 2009); however, such trend was much less pronounced in a tropical environment (Würth et al. 2005; Newell et al. 2002). The proportion of parenchyma does not differ greatly between branches and trunks (Bhat et al. 1985; Koch 1985); therefore, any differences in NSC concentrations between these two tissues are likely caused by physiological rather than anatomical drivers. Alternatively, it is possible that the branch wood NSC values are overestimated because of the inclusion of pith in the samples used for branch wood NSC measurements. Besides affecting the total branch biomass, medullary (i.e., pith) tissue is known to accumulate starch (Essiamah and Eschrich 1985). Both of these phenomena could bias the NSC concentrations toward higher values.

Nevertheless, the branch wood NSC pool consistently appears to be more dynamic than the trunk wood pool. This was manifested by a more dramatic seasonal change (Würth et al. 2005; Hoch et al. 2003; Newell et al. 2002), a higher proportion of the soluble sugar fraction (Sala and Hoch 2009), and a steeper increase in NSC levels with increasing tree height (Sala and Hoch 2009; Woodruff and Meinzer 2011) in branches as compared to trunks. The aforementioned differences between branches and trunks are consistent with the more proximal position of branches to the source of photoassimilates and developing buds and fruits that act as strong carbon sinks. Therefore, it can be suggested that branch wood parenchyma helps to buffer against a short-term imbalance between carbon supply and demand.

In contrast, the trunk wood parenchyma might be more specialized for long-term storage. This idea is supported by a recent study showing surprisingly long residence times of NSC extracted from the outermost 2 cm of sapwood in red maple and eastern hemlock. Using radiocarbon dating, the mean age of sapwood NSC has been estimated to be about a decade, although a substantial fraction of carbon seemed to have much faster turnover (Richardson et al. 2013). This faster fraction likely represents carbohydrates used to support parenchyma metabolism and cambial growth (Carbone et al. 2013; Hill et al. 1995).

In older trunks, the NSC concentration is known to decline radially with increasing distance from the cambium (Würth et al. 2005; Hoch et al. 2003). This decline is likely linked with parenchyma cell death and transition to heartwood. While low values of NSC concentrations are typically reached at the depth of 15–20 cm in trunks that showed a stem diameter of 30–100 cm, the radial patterns show interesting interspecific differences and variation with tree age (Würth et al. 2005; Hoch et al. 2003; Barbaroux and Bréda 2002). More specifically, the decrease in NSC concentration with increasing sapwood depth was particularly sharp in ring-porous oak, while diffuse porous trees exhibited a more gradual decline (Hoch et al. 2003; Barbaroux and Bréda 2002). The tropical tree, *Luehea seemanii*, exhibited remarkably constant NSC content throughout the entire 12-cm-thick sapwood (Würth et al. 2005).

Besides above-ground xylem biomass, woody roots also accumulate large amounts of NSC (Würth et al. 2005; Palacio et al. 2007; Loescher et al. 1990; Pate et al. 1990). High NSC storage capacity of belowground woody tissue is in accordance with a higher proportion of ray and axial parenchyma typically found in small woody roots compared to branches (Lens et al. 2000; Pratt et al. 2007). However, in coarser roots, the relative proportion of wood parenchyma might not be significantly different from stems due to an increase in inter-ray distance with increasing root diameter (Koch 1985; Wargo 1976).

The below-ground storage pool is particularly important when coping with disturbances that destroy a substantial portion of the above-ground plant biomass. A greater dependence on below-ground storage is characteristic for plants resprouting after disturbance, as opposed to plants that regenerate from seeds. In agreement, a higher proportion of wood parenchyma, paralleled by a greater amount of starch reserves, have been observed in resprouters than seeders growing in fire-prone habitats of Western Australia and South Africa (Pate et al. 1990; Verdaguer and Ojeda 2002). Interestingly, however, the overall starch tissue content was more strongly driven by the starch packing density than the amount of parenchyma tissue (Pate et al. 1990). Furthermore, not all the plants under study, including some of the resprouters, accumulated starch in ray and axial parenchyma cells. While some species accumulated starch only in their root cortex, other plants deposited starch in both or only one of the wood parenchyma subsystems. Yet other species had starch grains distributed in all three tissues. Unfortunately, it is not known if the same patterns occurred consistently throughout the season and what the levels of soluble sugars in the roots of these plants were.

Taken together, the comparison between species and woody organs suggests that the amount of parenchyma is important for the overall capacity to store carbohydrates;

however, the differences in starch accumulation patterns, possibly tied to the concentration of soluble sugars, provide another layer of complexity.

2.3   Seasonal Variation in NSC

The NSC content and its partitioning between starch and soluble sugars is also known to vary seasonally. The seasonal dynamics of wood NSC is most widely studied in temperate winter-deciduous trees (Kozlowski 1992; Ashworth et al. 1993; Sauter and van Cleve 1994), providing the following general picture. The total NSC concentration usually peaks at the end of the growing season and declines throughout winter, reaching its minimum during or shortly after bud break. Importantly, starch is often almost completely hydrolyzed during winter months in response to low temperatures, resulting in an increased concentration of soluble sugars (Fig. 8.3a) (Sauter and Wellenkamp 1998; Schoonmaker 2013). At the end of winter, the starch is transiently resynthesized, only to be hydrolyzed again shortly before bud break (Essiamah and Eschrich 1985; Sauter and van Cleve 1994). Starch and the total NSC levels then recover over the growing season. In contrast to deciduous trees, the peak in NSC concentration commonly occurs before bud break and remains low throughout the growing season in both temperate and boreal conifers (Hoch et al. 2003; Schoonmaker 2013). The aforementioned patterns make intuitive sense in terms of the typical progression of photosynthetic activity and growth and likely hold true on a large scale. However, recent studies indicate that this view might be an oversimplification and that various modifications of this general pattern can be found across different woody tissues, species, sites, and seasons (Hoch et al. 2003; Richardson et al. 2013). For instance, Hoch et al. (2003) did not observe a considerable reduction of NSC concentration during bud break in most of the angiosperm species studied. In some years, Richardson et al. (2013) even found higher NSC concentrations in March than in November in maple and oak, suggesting that a redistribution of NSC took place during the dormant season.

The patterns in carbohydrate concentration appear even more variable in a seasonally dry tropical forest. In a study on four trees differing in their leaf phenology (Newell et al. 2002), the branch wood of a truly drought-deciduous species (*Cecropia longipes*) exhibited a fourfold higher NSC concentration during the dry season, which was driven by a large increase in starch concentration (Fig. 8.3b). In contrast, the brevi-deciduous trees such as *Anacardium excelsum* showed much smaller seasonal variation in their NSC levels and a slight increase in soluble sugar fraction during the dry season (Fig. 8.3c). The tendency for higher levels of NSC in branch wood during the dry season was confirmed by a follow-up study encompassing 17 species (Würth et al. 2005). However, the seasonal effect was relatively weak in comparison with a striking interspecific variability.

The seasonal changes in sapwood NSC reflect the balance between carbon supply by photosynthesis and carbon utilization for various physiological needs such as growth, reproduction, or stress mitigation. The structure of ray and axial parenchyma
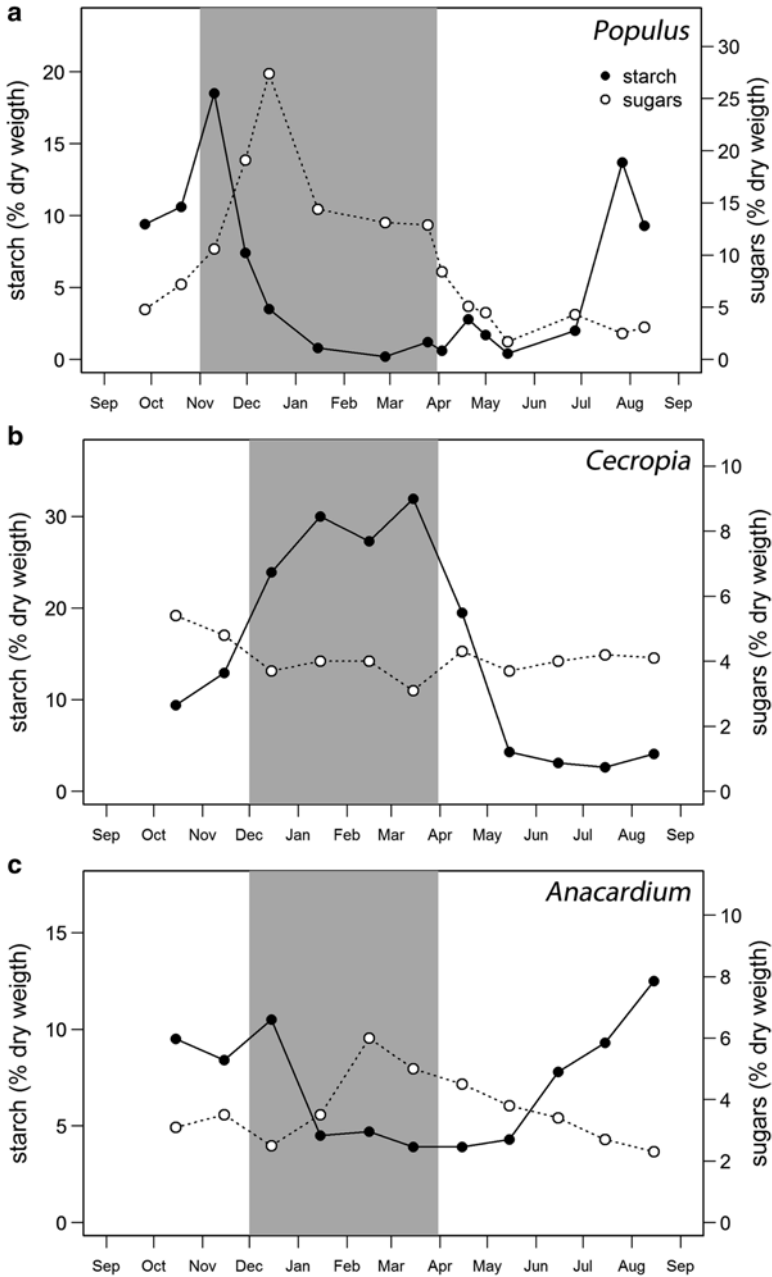
Fig. 8.3 Seasonal course of starch and soluble sugar concentrations in branch wood of winter-deciduous *Populus×canadensis* (**a**), drought-deciduous *Cecropia longipes* (**b**), and brevi-deciduous *Anacardium excelsum* (**c**). The shaded area in each graph indicates the period with unfavorable growing conditions (i.e., the winter in case of *Populus* and the dry season in the case of *Anacardium* and *Cecropia*). Data redrawn from Sauter and van Cleve (1994) (**a**) and Newell et al. (2002) (**b, c**)

and their biochemical machinery likely have a great influence on the NSC dynamics described above. The total parenchyma volume should be the parameter most closely related to the seasonal maximum in NSC content, assuming that the storage capacity of parenchyma cells is fully used during this period. In contrast, the dynamics of NSC is driven by physiological activity of ray and axial parenchyma cells and surrounding source and sink tissues (e.g., leaves, flushing buds, developing fruits, the cambium). Furthermore, the spatial proximity and connectivity between wood parenchyma and these sinks and sources is important for facilitating the NSC translocation within the plant body. While mechanisms underlying the buildup of NSC stores remain poorly understood, several studies have focused on processes involved in the mobilization and utilization of starch stored in wood parenchyma cells. The underlying cellular processes will be discussed in the following section.

3 Metabolic Activity of Wood Parenchyma Underlying the Dynamics of Sapwood NSC

3.1 Starch Mobilization and Metabolism of Soluble Sugars

The mobilization of starch reserves is initiated by the depolymerization of its molecules (Fig. 8.4). In plants, starch breakdown can be catalyzed by various enzymes such as amylases, glucosidases, and glucanohydrolases (Zeeman et al. 2010). To the best of our knowledge, only one study has looked at the starch hydrolyzing machinery acting in wood parenchyma (Witt et al. 1995). In this study, numerous enzymes with a potential amylolytic activity have been investigated in the ray parenchyma of *Populus × canadensis* and the main effect on starch degradation has been attributed to α-amylase and it has been hypothesized that the high temperature sensitivity of this enzyme underlies the mid-winter starch degradation and its resynthesis in early spring.

Simple sugars originating from starch hydrolysis are metabolized in a myriad of ways. Typically, the sugar molecules need to be phosphorylated before they can participate in further biochemical reactions. The phosphorylation of sugars is catalyzed by phosphotransferases. One of these enzymes is a plant hexokinase catalyzing the phosphorylation of hexoses, most importantly glucose. In addition, hexokinase also plays a prominent role in sugar-mediated signaling (Jang et al. 1997). While we are not aware of any studies on hexokinase activity in wood parenchyma, a gene encoding for this enzyme has recently been shown to exhibit a xylem parenchyma-specific expression in leaf petioles of tobacco (Giese et al. 2005).

Phosphorylation of glucose is the first step of glycolysis that can be followed by aerobic respiration. Respiration represents the key process through which the chemical energy contained in nutrients is released and made available for fueling the cellular metabolism. Respiration of wood parenchyma has been studied in a series of interesting experiments (Spicer and Holbrook 2005, 2007a, b). The respiration rates expressed per unit sapwood volume were between 0.4 and 1 μmol O_2 cm^{-3} h^{-1}
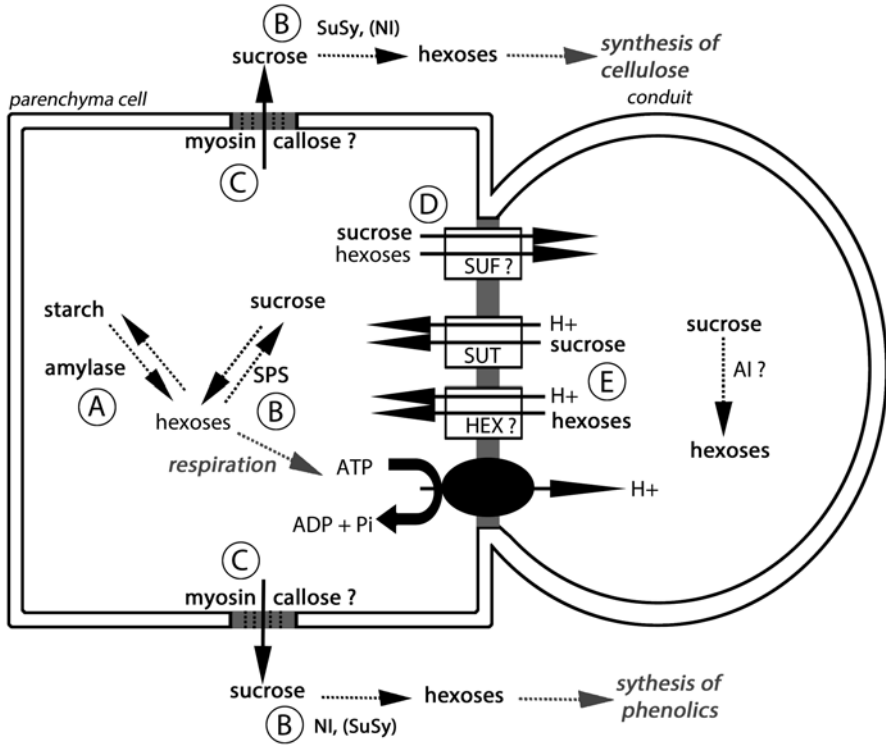
Fig. 8.4 NSC dynamics at the cellular level. Enzymes and transporters involved in the biochemical transformation and intercellular trafficking of NSC are shown. Starch hydrolysis is catalyzed by α-amylase (*A*). The synthesis of sucrose at the sites of starch mobilization is catalyzed by sucrose-6-phosphate-synthase (SPS). Sucrose breakdown at the sink sites is catalyzed by sucrose synthase (SuSy) and neutral invertase (NI) (*B*). Sucrose might also be hydrolyzed in the apoplast by acid invertase (AI). Soluble sugars are transported within the parenchyma network symplastically through pit plasmodesmata. The permeability of plasmodesmata might be affected by myosin contraction and callose deposition (*C*). Soluble sugars can move across a parenchyma-vessel pit membrane to the apoplast. The efflux of sugars to a conduit lumen is passive and driven by the concentration gradient. Putative membrane channels (SUF) likely facilitates the efflux (*D*). Soluble sugars can also be retrieved from the conduit apoplast to the parenchyma symplast by active transport via proton-sugar symporters (SUT, HEX). The electrochemical proton gradient required for the sugar uptake is generated by ATP-dependent proton pumps (*E*)

and did not differ greatly between three angiosperm and two conifer species studied. However, when expressed per living parenchyma volume, the two conifers showed one order of magnitude higher respiration than the angiosperms, with the respiration rates being around 12 and 3 μmol O_2 cm^{-3} h^{-1}, respectively. Such respiration rates are much lower than those typically found in meristems, but considerably higher than those measured in tissues purely devoted to storage, such as tubers. This suggests that wood parenchyma cells have a more active role than just being a simple storage compartment. Based on cytochemical staining, respiratory activity appears

particularly high in parenchyma cells that are in a direct contact with xylem conduits. These so-called contact or vessel-associated cells are characterized by high mitochondrial counts, high activity of respiratory enzymes (Sauter et al. 1973; Alves et al. 2001), and reduced starch accumulation (Fig. 8.2b, c, d, Braun 1984). Interestingly, wood parenchyma cells of some species contain chloroplasts and are photosynthetically active (Langenfeld-Heyser 1989; Cocoletzi et al. 2013; Larcher et al. 1988). The carbon assimilation rates exhibited by woody stems are low and usually not sufficient to result in a net carbon uptake; nevertheless, they may be involved in the refixation of CO_2 released during parenchyma respiration, thereby reducing respiratory carbon loss (Pfanz et al. 2002).

Sucrose is usually the most abundant component of soluble sugar fraction found in wood. There are three key enzymes governing the metabolism of sucrose in plants—sucrose-6-phosphate-synthase (SPS), sucrose-synthase (SuSy), and inverase (Fig. 8.4). While SPS catalyzes the synthesis of sucrose molecules from phosphorylated monomers, the other two enzymes, SuSy and invertase, are responsible for sucrose catabolism. The difference between these two enzymes is that SuSy catalyzes the conversion of sucrose into fructose and UDP-glucose, while invertase catalyzes the hydrolysis of sucrose into nonphosphorylated monomers. In plants, several types of invertases can be distinguished based on their subcellular localization and pH optimum. While netural invertase (NI) is localized in the cytoplasm, acid invertases (AI) are found in vacuoles and cell walls (Sturm 1999).

Seasonal changes in the activity of all three enzymes, SPS, SuSy, and invertase, which play an important role in sucrose metabolism, were studied along a radial profile in the sapwood of *Robinia pseudoacacia*, providing interesting insights into the coordination of NSC mobilization and utilization (Hauch and Magel 1998). An increased activity of sucrose synthesizing SPS was indicative of starch mobilization. Thus, a high SPS activity was observed throughout the entire width of sapwood during cold winter months when starch is being hydrolyzed into soluble sugars. The high SPS activity persisted in the middle and outermost sapwood during bud break. In contrast, the activities of SuSy and neutral invertase peaked in sink tissues to which sucrose was transported and subsequently catabolized. In spring, SuSy was highly active in sapwood regions close to the cambium, producing UDP-glucose for the synthesis of cell walls of newly developing xylem cells. By contrast, NI was mostly active in the sapwood-to-heartwood transition zone during autumn, likely providing precursors for the synthesis of heartwood phenolic. Similar patterns in SPS and SuSy activity as observed in *Robinia pseudoaccacia* have also been detected in the wood of *Populus × canadensis* (Schrader and Sauter 2002).

3.2 Translocation of Soluble Sugars

In order to supply carbon and energy to the cambium and the sapwood-to-heartwood transition zone, sucrose and other soluble sugars arising from starch mobilization have to move radially within the stem (Fig. 8.4). Rays provide an ideal path for such a translocation (Van Bel 1990). Ray parenchyma cells are interconnected via pits
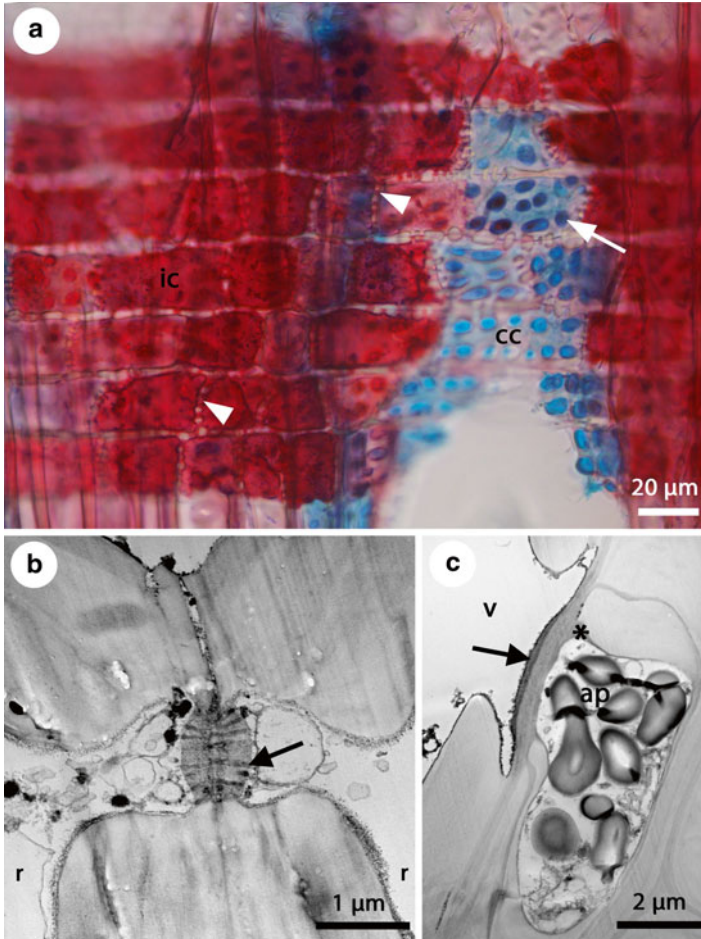
Fig. 8.5 Parenchyma–conduit and parenchyma–parenchyma cell connections in *Quercus robur*. (**a**) A radial section showing a vessel-ray interface observed with a light microscope. Numerous pit connections are apparent at the interface between the ray and vessel (*arrow*) and between the different files of ray cells (arrowheads). A clear distinction between contact (cc) and isolation cells (ic) within the ray is obvious in the picture. (**b**) A slightly bordered pit between two ray cells (r) observed with a transmission electron microscope. The pit membrane is penetrated by plasmodesmata (*arrow*). Various cytoplasmic bodies and vesicles are abundant in the pit channel, suggesting intense transport activity across the pit membrane. (**c**) Cross-section through an axial parenchyma cell (ap) connected to an adjacent vessel (v) via a half-bordered pit. The pit membrane is without plasmodesmata. The amorphous (or protective) layer between the pit membrane and the plasmalemma is thin but still apparent (*asterisk*). From the vessel lumen side, the pit membrane is covered by an electron dense plug, also known as the "black cap" (*arrow*)

perforated by numerous plasmodesmata, such that a symplastic continuum exists within the ray (Fig. 8.5a, b). This continuum can be visualized with symplastic fluorescence tracers (Sokolowska and Zagórska-Marek 2012); however, very little is known about the rate, selectivity, and regulation of this transport pathway. The rate

and direction of the bulk symplastic transport of carbohydrates are likely driven by the concentration gradient, similarly to the movement of sucrose during symplastic phloem loading. Based on the dynamics of starch deposition, Sauter and Kloth (1986) calculated a carbohydrate flow rate of 800 pmol cm^{-1} s^{-1} across the tangential ray walls of *Populus × canadensis* and concluded that much of this flux must have occurred via plasmodesmata.

Moreover, the presence of highly ordered microfilaments and microtubules running parallel to the longer (i.e., radial) axis of the ray cells provides possibility for active directional transport (Chaffey and Barlow 2001). Myosin, belonging to the family of ATP-dependent molecular motors, and the polysaccharide callose are localized at the plasmodesmal faces within the pit membranes. Both of these compounds are known to influence the permeability of plasmodesmata (Reichelt et al. 1999; Zavaliev et al. 2011; White and Barton 2011) and could hence facilitate an active regulation of the ray-to-ray cell conductance. Nevertheless, even if plasmodesmata are present and unblocked, the passage of molecules through pits will be associated with a certain resistance. Thus, more efficient radial conduction is expected in rays composed of procumbent rather than square or upright cells, because the number of cell-to-cell connections that need to be crossed is smaller in case of procumbent cells (Carlquist 1975).

Sugars can also move out of the parenchyma cells and enter the conduit lumen. The exchange of carbohydrates between parenchyma cells and conduits is facilitated by conduit–parenchyma pits, which exhibit a different structure than the simple or slightly bordered parenchyma–parenchyma pit pairs (Fig. 8.5a, c). When observed with a transmission electron microscope, the conduit–parenchyma pit membranes appear compact, rather electron dense and free of plasmodesmata (Fig. 8.5c). In addition, a specialized cell wall layer is deposited underneath the pit membrane, lining the entire conduit–parenchyma interface between the plasmalemma and the lignified wall. This so-called amorphous or protective layer may enlarge the actual area available for the exchange of substances (Barnett et al. 1993); however, other functions such as providing a buffer against xylem pressure oscillations were also proposed (Van Bel and Van der Schoot 1988). Both the pit membrane and the amorphous layer are rich in pectins (Wisniewski and Davis 1995; Plavcová and Hacke 2011). The amorphous layer contains also arabinogalactan-rich glycoproteins (AGPs) (Wisniewski and Davis 1995). These extracellular proteins prevent a tight alignment of pectin molecules (Lamport et al. 2006) and hence may increase the porosity and permeability of the amorphous layer. Moreover, AGPs are known to interact with the plasma membrane and act as receptors (Seifert and Roberts 2007), which points to interesting possibilities for a more active role of the amorphous layer in sensing and signaling. Another feature of conduit–parenchyma pits is the formation of an additional pectinaceous plug during winter months observed in several temperate trees (Wisniewski and Davis 1995; Wisniewski et al. 1991a). Because of its high electron density this plug is sometimes referred to as the "black cap." The exact function of the black cap is not known but it might hinder the growth of ice crystals or prevent uncontrolled loss of water and other substances from parenchyma cells during winter dormancy.

Our knowledge of molecular mechanisms involved in the sugar movement between parenchyma cells and conduits is rather limited, with most information coming from a few temperate deciduous trees. In these trees, two opposing sugar fluxes have been identified, namely the sugar efflux from and the sugar influx to the parenchyma cells (Fig. 8.4). The balance between these two fluxes drives the sugar composition of the xylem sap. High sugar concentrations, indicative of high efflux and/or low influx rates, are often found in xylem sap during winter and early spring. For instance, the spring sap concentration of sugar maple (*Acer saccharum*) reaches typically values of 2–3 % (Taylor 1956), while a concentration of about 0.6 % was measured in *Acer platanoides* (Schill et al. 1996) and *Populus × canadensis* (Sauter 1988). In contrast, the summer concentrations are close to 0.1 %.

The efflux of soluble sugars out of parenchyma cells (Fig. 8.4) occurs passively along a concentration gradient (Sauter 1982; Améglio et al. 2004; Münch 1930). Therefore, high efflux rates are expected when the concentration of soluble sugars in parenchyma cells is high. In agreement, high sugar efflux is observed during winter when most of the starch stored in parenchyma cells is hydrolyzed. In walnut, the sap sugar concentration was indeed highest during winter, with sucrose representing the most abundant xylem sap saccharide (Améglio et al. 2002, 2004). The dynamics of sap sugars are different in poplar. In this species, a rapid increase in sap sugar levels was observed during bud break, reaching levels more than three times higher than those measured in winter. Interestingly, hexoses comprised the major fraction of xylem sap sugars during this time, suggesting that sucrose might be hydrolyzed in the apoplast by acid invertase (Sauter 1988). The rapid increase in sap sugar levels indicates the sugar efflux is not just a mere leakage but rather an actively regulated process. The sugar efflux rates are sensitive to inhibitors, suggesting that the efflux is facilitated by membrane channels (Sauter 1982; Améglio et al. 2004). Thus, the modulation of efflux rates can be achieved by changing the abundance and activity of these hitherto uncharacterized proteins.

If sap flow occurs, sugars released into the conduit lumen can be carried via the low-resistance apoplastic pathway toward the canopy. This additional amount of carbon can be valuable to support flushing buds in spring (Bonhomme et al. 2010). However, as vascular connections are often not fully developed during the initial phase of bud reactivation (Ashworth 1982), sugars need to be reabsorbed by parenchyma cells and move to the bud tissue via extraxylary pathways.

In contrast to sugar efflux, the uptake of sugars from the xylem sap by parenchyma cells is an active process facilitated by proton-sugar symporters (Fig. 8.4). Transcript and protein levels of several of these putative transporters have been studied in walnut (Decourteix et al. 2006, 2008). While the sucrose transporter JrSUT1 was strongly up-regulated in xylem parenchyma cells during bud break, two hexose transporters, JrHT1 and JrHT2, were abundant during the period of intense radial growth. This suggests that the sugar uptake is selective and likely tailored to suit specific physiological needs. The symport of sugars is powered by the electrochemical gradient generated by ATP-dependent proton pumps also known as H^+-ATPases (Alves et al. 2007). High expression of H^+-ATPase coincides with a high activity of respiratory enzymes, indicating that the sugar retrieval is

energetically demanding but also remarkably efficient. For instance, in willow (*Salix*), the rates of sugar influx have been estimated to be more than five times higher than the rates of sugar efflux (Sauter 1983).

In this section, we described the cellular processes that underlie the dynamics of NSC in sapwood (Fig. 8.4), starting with the conversion of starch into soluble sugars, continuing with the in situ use of sugars, and finishing with their radial and axial transport into more distant sink tissues. We showed that NSC dynamics are driven by the activity of key sugar-modifying enzymes and transport systems, acting within the anatomical and physiological boundaries provided by wood parenchyma cells. In the next section, we will briefly discuss potential implications of these processes for whole plant physiology.

4   The Role of Sapwood NSC at the Whole Plant Level

Carbohydrate storage is important for a tree's ability to withstand periods of unfavorable environmental conditions and to reactivate its growth when favorable conditions are reestablished. Interestingly, the NSC reserves are rarely depleted in trees, leading to the suggestion that tree growth and survival is not limited by carbon supply (Körner 2003). Alternatively, it has recently been proposed that trees actively maintain high NSC concentration at the expense of growth in order to sustain plant functioning under environmental stress (Sala et al. 2012a; Wiley and Helliker 2012). We believe that the wide array of tightly regulated physiological processes taking place in ray and axial parenchyma cells fits well into this picture. While starch accumulation in wood parenchyma at the end of the growing season can be viewed as manifestation of a long-term storage function, the complex dynamics of soluble sugars can be perceived as a suite of active physiological processes—some are related to maintenance respiration and growth while others are mostly involved in stress mitigation (Fig. 8.6). For the sake of simplicity, we will outline these functions as consecutive events progressing over seasons typical for a temperate climate. However, we recognize that not all of these functions are relevant all the time. Instead, different functions can be more important in different tree species or under particular circumstances, resulting in different requirements on structural and physiological properties of wood parenchyma cells.

In winter, two important physiological roles of soluble sugars can be identified, namely the protection of parenchyma cells from freeze injury and reversal of freeze-induced embolism. Subzero temperatures can damage or even kill wood parenchyma cells. Therefore, two strategies for coping with freezing temperatures evolved in these cells—they either tolerate extracellular ice formation or avoid freezing by deep supercooling (Sakai et al. 1987; Kuroda et al. 2003; Burke et al. 1976). In the case of freeze tolerance, an increased concentration of soluble sugars resulting in higher osmotic potential of the cytoplasm helps to prevent cellular dehydration driven by extracellular freezing (Yuanyuan et al. 2009; Cavender-Bares 2005). In case of freeze avoidance, soluble sugars may help to inhibit the formation
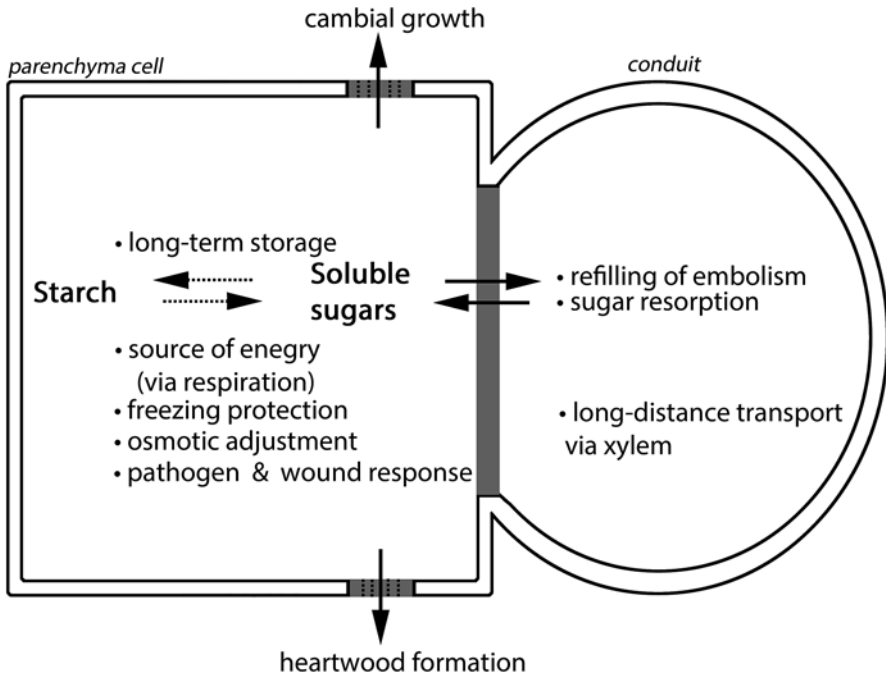
Fig. 8.6 Schematic representation of various functions that starch and soluble sugars fulfill in sapwood. Starch represents the primary form of nonstructural carbohydrates used for the long-term storage. Starch can be converted into soluble sugars that fulfill more active physiological roles. Soluble sugars are used for respiration. They are important for defense against pathogens by providing energy and material for the synthesis of defense chemicals. Increased concentration of soluble sugars in wood parenchyma cells may prevent freeze and desiccation damage. Soluble sugars can move radially via the symplastic continuum in ray parenchyma cells toward the cambium or toward the center of the stem to supply carbon and energy for the formation of new xylem or for the synthesis of heartwood extractives. Soluble sugars are also secreted into the apoplast where they can drive refilling of xylem embolism. Sugars released to the xylem sap can be carried upstream and retrieved closer to the plant apex, thereby supplying carbon to flushing buds

of ice crystals and to stabilize the plasmatic membrane. Furthermore, the integrity of the amorphous layer and its pectin composition are important for the ability of parenchyma cells to undergo supercooling (Wisniewski and Davis 1989; Wisniewski et al. 1991b).

Repeated freeze–thaw cycles are known to induce embolism of xylem conduits even under modest tensions. Ring-porous trees cope with this phenomena by producing new conduits in spring, whereas many diffuse porous species are capable of refilling embolized conduits (Hacke and Sauter 1996). Refilling can be driven by positive root or stem pressure or a combination of the two. While the accumulation of inorganic nutrients in the root apoplast seemed to underlie the development of root pressure in *Juglans*, soluble sugars released by the parenchyma cells were critical for the generation of positive stem pressure in both *Juglans* (Améglio et al. 2002, 2004;

Ewers et al. 2001) and *Acer* (Sauter et al. 1973; Hacke and Sauter 1996). Thus, in climates where freezing occurs, wood parenchyma can be important for the restoration of vascular integrity at the start of a new growing season. On the other hand, a high amount of parenchyma represents a challenge because freeze damage to the living tissue needs to be prevented.

In spring, the main function of soluble sugars is to support new growth in order to quickly reestablish photosynthetic production. Expanding buds and the active cambial zone represent the strongest sinks during this period. Both of these tissues receive carbohydrates stored in sapwood (Hill et al. 1995; Bonhomme et al. 2010; Decourteix et al. 2008). While little is known about the partitioning of reserves between these two tissues, it can be expected that it is closely related to the offset between the cambium and bud phenology. The cambium likely represents a more important sink for stored carbohydrates in ring-porous than diffuse-porous species because a large proportion of the radial stem growth occurs before the onset of photosynthetic activity in ring-porous trees (Barbaroux and Bréda 2002; Panchen et al. 2014). In species that bloom before the leaf-out, the opening flower buds draw strongly on stored reserves as indicated by a pronounced decline in branch wood NSC levels (Hoch et al. 2003). Higher allocation of NSC reserves into the cambium should put more requirements on the radial transport mechanisms via the ray symplast, while the axial transport pathway, which involves sugar exchange between wood parenchyma and xylem apoplast, should be accentuated in case of higher needs for sugar translocation into the buds.

In summer, when the canopy is fully developed and photosynthetically active, soluble sugars found in the sapwood could help to prevent and repair damage caused by environmental stress. In the more traditional sense, the importance of sapwood NSC reserves should be seen in the possibility to regrow leaves in case of severe defoliation caused by environmental stress. From the less traditional point of view, the high NSC pool may be needed for a continuous maintenance of hydraulic integrity that is constantly being perturbed. Drought and the attack of pathogens arguably represent the two most important environmental challenges frequently encountered by trees.

The importance of carbohydrates in the repair of drought-induced embolism has been widely recognized. Despite some recent concerns calling the routine occurrence of refilling under tension into question (Wheeler et al. 2013; Sperry 2013), the active release of both sugars and water into the conduit lumen by xylem parenchyma cells is believed to be at the heart of the putative mechanism that may facilitate rapid reversal of drought-induced embolism (Salleo et al. 1996; Tyree et al. 1999; Hacke and Sperry 2003; Secchi et al. 2011; Secchi and Zwieniecki 2011; Brodersen et al. 2010). Moreover, abundant wood parenchyma, as found in many tropical trees, can help to delay the onset of cavitation by providing high water storage capacity (Borchert and Pockman 2005). It is not known if excessive water loss from parenchyma cells during drought can compromise their physiological functions, although desiccation-induced damage to the protoplasm has been documented in wood parenchyma cells during cold stress (Ristic and Ashworth 1994). If the maintenance of turgor pressure is important, for instance for biomechanical reasons (Chapotin

et al. 2006), increased concentration of soluble sugars could provide means for reducing the capacitive discharge from wood parenchyma cells.

The importance of ray and axial parenchyma for wound and pathogen responses in wood is also well documented. Most importantly, parenchyma cells produce tyloses and gums that plug old or damaged xylem conduits, thereby preventing uncontrolled spread of pathogens within the xylem pipeline (Bonsen and Kucera 1990; Nicole et al. 1992). The production of these vascular occlusions involves active secretory processes (Rioux et al. 1998) and hormonal signaling (McElrone et al. 2010), and thus is likely associated with high demands for energy that can be drawn from NSC reserves. On the other hand, a higher proportion of thin-walled parenchyma cells that are rich in carbohydrates can make wood more attractive for nutrient-seeking pathogens and herbivores (Schwarze 2007; Martín et al. 2009). This could result in a faster progression of infection once the pathogen succeeds in overcoming the initial defense mechanisms.

In fall, the wood parenchyma NSC stores should be replenished and available to support the tree's physiological functions in winter and during the next growing season. However, some of the NSC can still be consumed for heartwood formation, which is known to occur predominantly during the period of early dormancy (Taylor et al. 2002). As suggested in a recent review (Spicer 2005), heartwood formation should be viewed as an active developmental program during which a conductive but vulnerable sapwood is transformed in a nonconductive but durable heartwood. This process, initiated within wood parenchyma cells, involves a suite of biochemical reactions that use, at least in part, energy and carbon from carbohydrates stored in sapwood (Hauch and Magel 1998; Magel et al. 1994).

In this section, we summarized the most important ways of how sapwood NSC are used in growth, development, and stress mitigation (Fig. 8.6) and showed the tight links to the well-known functions of ray and axial parenchyma cells. However, it is important to note that reserves other than NSC are also stored in wood parenchyma cells, with nitrogen and phosphorous representing the most important ones (Hoch et al. 2003; Langheinrich and Tischner 1991; Sauter and van Cleve 1991). Thus, it is likely that the tree performance is, at least in some occasions, more strongly limited by the availability of these nutrients than by the availability of carbon (Millard and Grelet 2010; Sala et al. 2012b). Nevertheless, we believe that our analysis of NSC dynamics provides a useful conceptual basis that can be applied to better understand the dynamics of other nutrients as well.

5 Future Perspectives

Research on xylem has a great tradition in integrating structure and function and great advances in understanding the plant water transport have been made by linking the anatomy of xylem conduits to functional hydraulic traits (Hacke et al. 2001, 2006; Jansen et al. 2009). We can envision similar progress in elucidating the functional role of parenchyma cells in carbohydrate storage and dynamics, paved by

uncovering the great diversity in ray and axial parenchyma structure and their spatial distribution. Such research would greatly benefit from integrating approaches traditionally used in studies on xylem hydraulics (e.g., perfusion experiments, analysis of pit structure) with methods used to examine phloem physiology (e.g., the application of symplastic and apoplastic tracers, radioactive labelling, molecular methods). Moreover, ecological data on sapwood NSC concentration and composition will help to upscale the processes and imply their importance for whole plant functioning.

Most research to date has been made on temperate species. However, wood structure exhibits great diversity and ray and axial parenchyma seems to be more abundant and exhibit more elaborated patterns in tropical trees. Similarly, carbohydrate metabolism in sapwood seems to be more complex, dynamic, and shifted further from the role in long-term storage in the tropics. Therefore, studies conducted on tropical trees might provide further valuable insights.

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