Interplay of Water and Nutrient Transport: A Whole-Plant Perspective

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Contents

Abstract This review aims to summarise the many facets of how water flow in higher plants affects nutrient transport and vice versa. Initially, some theoretical background is given on physico-chemical concepts to describe fluxes and their (in) direct coupling, followed by a brief overview on some of the relevant methods (pressure probes, ZIM probe, MIFE technique, radioactive and stable isotopes, MRT flow imaging, heat balance technique, modelling of nutrient fluxes). This essay focusses on roots, on vascular tissues and on the whole-plant level, with only occasional in-depth reference to the molecular scale. Radial water and nutrient transport in roots are discussed in analogy to processes in mammalian epithelia,

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including a possible role of salt/water cotransporters for generating the "non-osmotic" component of root pressure. Moreover, the significance of low reflection coefficients for the coupling of water and solute transport in roots is critically addressed. Separate sections deal with interactions of water and nutrient transport in vascular tissue (xylem, phloem). Finally, a whole-plant perspective is taken; the significance of transpiration for plant nutrition in general, and for the nutrients N, Ca and K in particular, is discussed.

1   Introduction

In the preceding volume of Progress in Botany, Lüttge (2013) pleaded for a revival of an organismic approach to (whole) plant physiology. Examples for a holistic view on plant science treating vascular plants as a highly integrated functional unit were presented. The author recurred to earlier work from the 1970s and 1980s when whole-plant physiology flourished, and also referred to more recent literature standing in this tradition. Systemic responses of the plant, e.g. to stress or to nutrient availability are highlighted that are brought about by electric, hydraulic and chemical signaling. The opposing concept that currently seems to dominate plant sciences considers the plant as an assembly of more or less autonomously acting "modules". According to this view, it is sufficient to study physiological process at the molecular or cellular scale, since the key processes shaping plant life are supposed to take place at this level: Gene expression can be seen entirely as a cellular process with each cell being fully equipped with the required biochemical machinery. The same is true for chemical interaction of a cell with its environment, mediated by membrane proteins like receptors and ion channels; the information is subsequently transduced, e.g. by transcription factors, leading, via an impact on gene expression, to a modification of the chemical properties of cells (and, in turn, of plant organs and the whole organism). Frequently, it is tacitly implied that features of the organism can largely be extrapolated by simply scaling up cellular processes. A separate discipline, systems biology, has evolved that is concerned with the integration of molecular process at a higher organisational level. However, rather than analysing data obtained by hypothesis-driven research, this discipline is largely concerned with treating datasets obtained by "omics" approaches, using statistical tools to find correlations among them. As pointed out by Lüttge, these concepts of physiology tend to undervalue and neglect synergistic emergence as a key feature of living organisms.

Here I want to elaborate on Lüttge's view, referring to an aspect of emergence that was given less attention in his preceding essay: Integration is not only an issue with respect to different parts of the plant (e.g. root–shoot) or different tissues or cell types. Another aspect is the integration of fluxes, namely those of water, ions, energy and metabolites. Fluxes of energy and matter are key features of living organisms, essentially operating away from thermodynamic equilibrium. These fluxes are highly interactive. The focus of this paper, following my own expertise,

will be on the interaction of water and ion (nutrient) fluxes, with occasional references to the other two.

The choice to focus on water and nutrient *fluxes* in this review article, rather than on processes at the molecular level, was also guided by the following more specific considerations:

- Several excellent reviews have been published in the last 5 years that provide a comprehensive overview on our knowledge of different classes of transport proteins, including ion channels (Hedrich 2012) and aquaporins (Maurel et al. 2008). In order to avoid just being repetitive, it was preferable to take a different viewpoint in this essay. By contrast, integrative features of the plant, such as nutrient and water fluxes, received comparatively little attention recently.
- Physiological processes are frequently organised in a highly redundant way with respect to the contribution of various types of proteins. This contributes to the plasticity of the organism and serves as a protection against a complete loss of function by mutations. Hence, it may be difficult to quantify the contribution of a particular protein to a physiological process (even though there are also striking counter-examples; see, e.g. Gajdanowicz et al. (2011)).
- Fluxes are quantitative features of a biological system based on a firm physico-chemical concept (see below) that are relatively easy to integrate into computational modelling of physiological processes. Theoretically, trans-membrane fluxes can be traced back to the contribution of individual membrane proteins, but in practice this link is still out of reach in many cases, among other things because of the plasticity of transport processes (see previous bullet point). Generally speaking, extreme environmental conditions provide the best opportunity to link physiological processes to the activity of a limited number of proteins, since the plant's response to a stress situation clearly dominates all other processes. But such a situation may be a rare event in the life of a plant.
- Not all transport phenomena are mediated by proteins! Although membranes (and membrane proteins) are major sites of control, nutrients and water on their way from root to shoot pass through the bulk of various cellular and extracellular compartments. (Electro)diffusion and mass-flow-driven transport of ions, e.g. are important transport mechanisms.
- Fluxes can readily be interpreted in an ecological context, e.g. when the soil–plant continuum is considered.

Of course, focussing on fluxes and their interaction does by no means preclude highlighting the contribution of individual proteins in a transport process where this is appropriate. Rather, the integrative approach that is favoured here implies that processes at the higher organisation level (plant tissues, organs and the whole organism) and emergence phenomena are given at least equal attention.

In this essay, I will focus on the macronutrients K, N (provided either as NO_3^- or as NH_4^+) and Ca and ion-specific aspects of their interaction with water transport [for their physiological roles, see Marschner (1995)]. Moreover, I will discuss aspects of flux coupling relevant for all nutrients in a more general way.

2   Physico-Chemical Concepts to Describe Interactions of Water and Nutrient Transport

Generally speaking, a flux, J_i, denotes the number of molecules n of a species i moving across a boundary area, A, (e.g. a membrane surface) per time increment:

$$J_i = -\frac{1}{A}\frac{dn_i}{dt} \tag{1}$$

In order to quantify the transport of water, it is more convenient from an experimental point of view to consider volume flow (J_v) rather than transport of water molecules:

$$J_v = -\frac{1}{A}\frac{dn_{H_2O}}{dt}\,\bar{V}_w \tag{2}$$

$\bar{V}_w =$ molar volume of water (Note that for a dilute aqueous solution, the contribution of the solutes to volume flow can be neglected).

A flux cannot be treated as a singular event, but is part of a complex pattern of interconnected processes. In most textbooks dealing with this matter, coupling of water and nutrient transport is solely considered with respect to the osmotic force that solutes dissolved in water exert on water transport. Net transport of nutrients across a membrane will alter concentration gradients and, via the concomitant effect on the osmotic balance, will induce passive water transport. In plant cells, water flow between a cell and its environment also affects the cell turgor P, i.e. the hydrostatic overpressure inside the cell. In order to take the interplay of hydrostatic and osmotic pressure into account, the water potential, Ψ, was defined that is usually considered as the key parameter for water transport:

$$\Psi = P - \pi \tag{3}$$

with the osmotic pressure, π, calculated according to van't Hoff's law: $\pi = RT\,c$ (R being the gas constant and T the absolute temperature in Kelvin). Where appropriate, this equation has to be extended by a gravitational term $\rho{*}g{*}h$ (ρ being the density of the media, g the gravitational constant and h the height) and a matrix potential τ (with a negative sign) taking into account the capillary forces exerted by the cell wall material that have to be overcome when this material is (partly) dehydrated.

Water is thought to follow the Ψ gradient, flowing from higher to lower potential. However, this approach is not unambiguous, and some notes of caution are required:

- Water flow is not induced by an osmotic gradient per se, but only between compartments differing in osmotic pressure *and* separated by a semi-permeable membrane. This second, mandatory prerequisite is fulfilled when water

exchange between cells and their environment is considered, but, e.g. usually not for water transport within the apoplast. Osmotic gradients within a compartment (in the absence of a semi-permeable transport barrier) dissipate by diffusion, not by volume flow!

- Most importantly, direct interaction of water and solute transport during membrane passage is neglected by this approach. A number of studies published in the 1980s of the last century seemed to justify this approach (e.g. Palta and Stadelmann 1980). Evidence was provided that plant membranes are ideally semi-permeable for physiologically relevant salts and sugars, implying that direct interaction of water and solute transport is negligible, and solutions exert their full osmotic pressure as a driving force for water transport. However, recently Wegner (2014) has argued that water-solute cotransport across plant cellular membranes needs to be re-considered in the light of previous advances in our understanding of water secretion in mammalian epithelia [for a review, see Zeuthen and MacAulay (2012)]. In specialised cell types like xylem parenchyma, transport proteins, e.g. those of the CCC class that carry a stoichiometrically fixed number of water molecules together with a salt (e.g. KCl) could account for the generation of root pressure and refilling of embolised xylem vessels (for a more detailed discussion of those physiological processes, see below). Gradients in chemical potential of solutes could drive water secretion across a membrane in the absence of a water potential gradient, or even against it. Salt or sugar-driven water transport is not easy to detect, since it is necessarily associated with a subsequent retrieval of the "lost" solutes by the cell to "recharge the battery" and thus keep the process going. This will, at some step, require metabolic energy. Only constant cycling of solutes can drive sustained water secretion, and net solute gradients may even not be affected by this process. Seemingly futile cycling of solutes has indeed been demonstrated to occur between symplast and apoplast in plant tissues, using sophisticated radioactive labelling techniques (Britto and Kronzucker 2006). Although osmotica are involved in this process, water flow is not driven by (local) osmotic forces as hypothesised by Diamond and Bossert (1967). In their "standing osmotic gradient" hypothesis that is fundamentally different from the one proposed here, local cavities play a crucial role that are in free exchange with the bulk via one end, whereas the other end is closed. Salt is actively transported into the cavity at the closed end by adjacent cells. Due to passive water flow into the cavity, the osmotic pressure is decreasing along its length; progressive dilution towards the open end results in the constant export of a medium of low osmotic pressure into the bulk. A similar mechanism was also proposed to operate in plants, e.g. in salt glands (Pate and Gunning 1972; Lüttge 1975) and to drive root pressure exudation (Katou et al. 1987), but neither in mammalian epithelia nor in plant tissues clear experimental evidence in favour of this hypothesis could be obtained.

Co-transport of water and solutes may, in fact, fundamentally change our view of many processes in plants that involve a shift of water. Inside the water-filled bladder traps of carnivorous *Utricularia* species, e.g. a hydrostatic underpressure of

up to 17 kPa is generated with respect to the ambient pond water (Bentrup 1979). Water efflux occurs against an osmotic gradient across the bladder walls consisting of two cell layers. Hence, water is secreted against the water potential gradient that favours water influx into the lumen of the trap. This could be brought about by water-solute cotransport across the cellular membrane of the wall cells facing the ambient medium, thus overcoming passive water influx. Consistently, generation of underpressure is correlated with the export of K^+, Na^+ and Cl^- from the trap lumen (Bentrup 1979). Fast movements, e.g. stomatal closure, as well as nastic responses of leaves and leaflets to touch that are associated with water shift in pulvini, e.g. in *Mimosa pudica*, may also operate by a water secretion mechanism involving cotransport (Zeuthen 1996 p. 109; Morillon et al. 2001).

It should be noted that direct coupling of water and solute flow also occurs in other transport proteins. Ion channels allow passage of ion *and* water, and it has been demonstrated, e.g. for K^+ channels that water flow can drive K^+ transport through the pores (Homblé and Véry 1992). Moreover, some aquaporins are not only permeable to water but also to other small solutes such as urea or CO_2 (Uehlein et al. 2003).

A discussion of fundamental aspects of the coupling of water and nutrient transport would be incomplete without mentioning unstirred layer effects. When a transport barrier is more permeable to water than to solutes (as is the case with semipermeable membranes), volume flow across that barrier will generate a local concentration gradient across the membrane (and, in turn, an osmotic gradient that counteracts volume flow). Solutes accumulate at the membrane in the "upstream compartment" (concentration-polarisation) whereas in the "downstream compartment" solutes are diluted close to the membrane (sweep-away effect). Unstirred layer effects are most pronounced in compartments that equilibrate by comparatively slow diffusion processes rather than by convection. For membrane transport processes, actual concentrations at the membrane surface are more relevant than bulk concentrations, but are also more difficult to measure! Unstirred layer effects are frequently undervalued. They can have a strong impact on transport processes and may be the actual cause of many interactions of water and solute transport reported in the literature that are ascribed to membrane proteins.

A formalism taking co-transport of water and solutes across membranes into account is provided by the following equation. (For the derivation of this equation and the theoretical framework provided by the thermodynamics of irreversible processes, the reader is referred to Zimmermann and Steudle (1978)):

$$J_v = L_P(\Delta P - \sigma \Delta \pi) \tag{4}$$

with L_P being the hydraulic conductance of the transport barrier. Direct interaction of water and solute transport is accounted for by the reflection coefficient σ. If the membrane is ideally semipermeable, σ is close to unity and water and solutes move independently across the membrane; σ values <1 provide evidence for an interaction of solute and water fluxes, e.g. by solute–water cotransport (Wegner 2014). Solute-driven water flow counteracts water flow driven by osmotic forces (that is

largely mediated by aquaporins) and, hence, reduces the apparent impact of the osmotic pressure gradient (whereas turgor-driven water flow remains unaffected).

Correspondingly, solute transport, J_s, can be described as a sum of two components, one of them comprising the interaction with water flow ("solvent drag"), whereas the other is equivalent to diffusion, i.e. flux driven by a concentration gradient across the membrane (other forces are not taken into account):

$$J_s = \underbrace{J_v \bar{c}_s (1 - \sigma)}_{\text{solvent drag}} + \underbrace{\varpi \Delta c_s}_{\text{diffusion}} \tag{5}$$

$\bar{c}_s$ and ω denote the averaged concentration of the solute s in both compartments separated by a transport barrier (membrane) and the permeability for this solute, respectively. These equations are unsuitable to describe the complex transport phenomena at membrane level in all details (e.g. they neglect electrical forces). However, they provide a simple mathematical framework for taking the interaction of water and nutrient transport into account and can potentially be extended on the basis of the simple formalism provided by the thermodynamics of irreversible processes (Zimmermann and Steudle 1978). Interestingly, Eq. (5) is also valid in the absence of a semipermeable membrane, e.g. when mass flow in the capillaries of the cell wall matrix and in xylem vessels is considered. Under these conditions, σ attains a value of zero, and J_s is equal to $J_v \times c_s$ (ignoring the relatively slow diffusion process that will make a small contribution to J_s under these conditions).

Further refinement of our physico-chemical concepts will be required in the future, e.g. to take non-linearities in the relationships between fluxes and driving forces into account (Ciancio and Verhás 1994).

3 A Brief Survey of Techniques Used to Measure Nutrient and Water Fluxes in Plants

In experimental sciences, progress is bound to the available methods, their advantages and limitations. Hence, it appears appropriate to introduce some of the methods that are routinely used for recording nutrient and water fluxes and their interactions. This summary and the information given on each of these methods cannot be comprehensive, and suggestions for further reading will also be provided.

Pressure Probe Techniques In plants, hydrostatic pressures can vary over a wide range, with overpressures of up to about 1 MPa prevailing in the cells (the "turgor pressure") to values below vacuum in functional xylem vessels [for a review, see Zimmermann et al. 2004)]. Pressure gradients provide a major driving force for volume flow; frequently, hydrostatic pressure data can be used to obtain information on changes in osmotic pressure and to determine the hydraulic conductivity of a biological structure. The basic concept of the turgor pressure probe was initially

introduced by Zimmermann et al. (1969); a fine-tipped glass capillary suitable for impaling a cell is attached to a microbaric chamber carrying a miniature pressure sensor. The interior of the chamber and the capillary are filled with silicone oil forming—once being inserted—a meniscus with the cells sap that is kept at a constant position close to the cell surface to eliminate artefacts related to the elasticity of the probe during pressure recording (Tomos and Leigh 1999). Later, this tool was modified in order to measure root pressure in excised roots ("root pressure probe"; Steudle and Jeschke 1983), and to record xylem pressure in individual vessels ("xylem pressure probe"; Balling and Zimmermann 1990). The latter is filled with degassed water (or electrolyte solution) instead of oil (for experimental details on this type of probe, see Zimmermann et al. (2004); Wegner (2012)). Still later, an advanced version of the xylem pressure probe, the multifunctional xylem probe, was developed (Fig. 1a). In addition to measuring xylem pressure, this tool allows simultaneous recording of the electrical potential in a xylem vessel with respect to a reference electrode outside the plant (the so-called trans-root potential) and of ion concentrations (or rather, activities) in the xylem. For multifunctional xylem probes, double-barreled electrodes are used; one barrel serves for measuring xylem pressure and electrical potential. This barrel is attached to the body of the microbaric probe (now additionally containing an Ag/AgCl electrode), whereas the other one is prepared as an ion-selective electrode. For more technical details, the reader is referred to a recent review by Wegner (2012). Note that this instrument is particularly well suited to measure interactions of water and nutrient transport, since the main driving forces for water and nutrient transport are accessible at a particular site in the vascular system; probes measuring K^+ and pH have been published; moreover, a nitrate selective probe has been designed (S. Scherzer and L.H. Wegner, unpublished).

The types of pressure probes introduced so far were predominantly devised for use in the laboratory or greenhouse. They are hardly suitable for field studies, though, and their use requires some effort and skill. Recently, however, a novel type of pressure probe, the ZIM probe, has been introduced by Zimmermann and coworkers, which offers a simple and inexpensive way for measuring relative values of turgor pressure (changes) both in the field and the laboratory in real time and with high precision. The ZIM probe makes use of a miniature pressure sensor embedded in a polymeric matrix that is pressed to a leaf by magnetic force (Fig. 1b); a magnetic counterpad is required at the opposite side of the leaf. Turgor pressure of cells in the leaf patch covered by the probe tends to oppose the magnetic force exerted upon the clamped leaf area. As a consequence, the pressure sensed by the pressure sensor (the "output pressure") is relieved, i.e. turgor pressure and output pressure are inversely related [for details on the measuring principle, see Zimmermann et al. (2013); Westhoff et al. (2009); Zimmermann et al. (2008); Rüger et al. (2010)]. The ZIM probe has great potential for application, e.g. in plant phenotyping and irrigation scheduling (Bramley et al. 2013), but it is also a very useful tool for basic research. Rüger et al. (2010) could, e.g. monitor water shifting in the canopy of Avocado and Eucalyptus trees when branches were differently
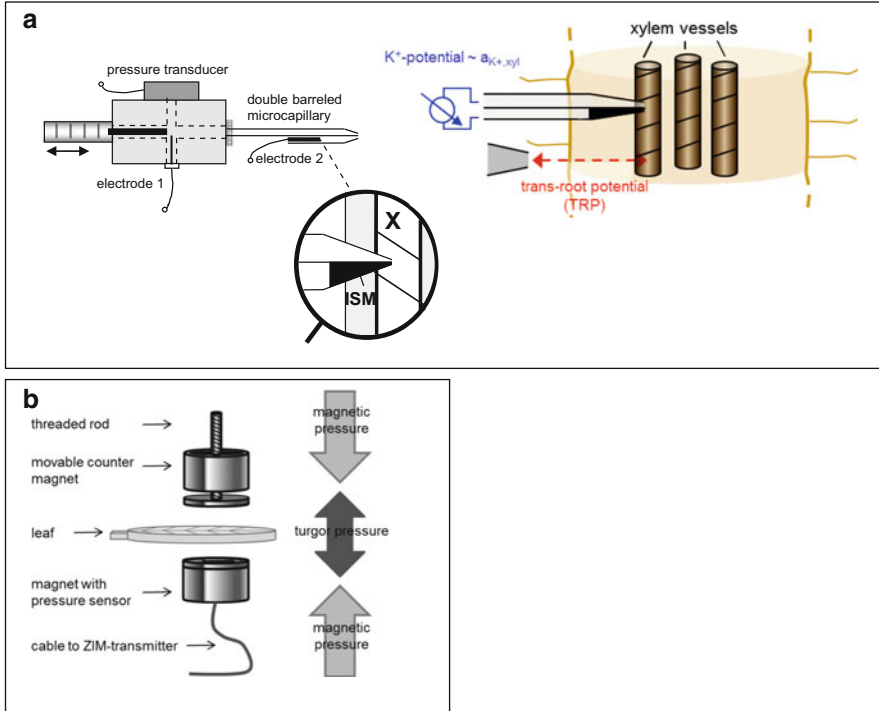
Fig. 1 Diagrams showing advanced pressure probe techniques. (**a**) Multifunctional xylem pressure probe (*left*), consisting of a microbaric Perspex chamber with miniaturised pressure sensor attached to it. An Ag/AgCl electrode (electrode 1) is integrated into the probe for simultaneous recording of hydrostatic pressure and electrical potential in an individual xylem vessel (x). A double-barreled microcapillary is used. One barrel connects (in the impaled state) the lumen of the xylem conduit with the probe for recording pressure and electrical potential, the second one is designed as an ion-selective electrode (electrode 2) to measure, e.g. K^+ or H^+. The very tip of this barrel is filled with an ion-selective matrix (ISM; under the "magnification glass"). An Ag/AgCl electrode is inserted into this barrel to record the K^+ potential. The experimental design for roots is shown in the scheme on the right side. The potential difference of the two barrels corresponds to the K^+ potential; this potential is used to calculate the xylem K^+ activity ($a_{K+,xyl}$) based on pre-/post-calibration of the ion-selective electrode. Moreover, the electrical potential difference between a xylem vessel and an external electrode, the trans-root potential (TRP), is recorded. For further details, see Wegner (2012). (**b**) Schematic diagram of the measuring principle of the magnetic ZIM-probe. For more details, see the text

exposed to light; water was re-directed to those branches that suffered from temporary drought stress. Uneven allocation of water to different branches was also observed in oak trees during the course of the day (Fig. 2).

The MIFE Technique An elegant way to quantify ion fluxes at the surface of plant organs or isolated cells is provided by the Microelectrode Ion Flux Estimate (MIFE) technique originally designed by Ian Newman (for reviews, see Newman 2001; Shabala et al. 2012). The basic concept relies on the fact that ion fluxes across
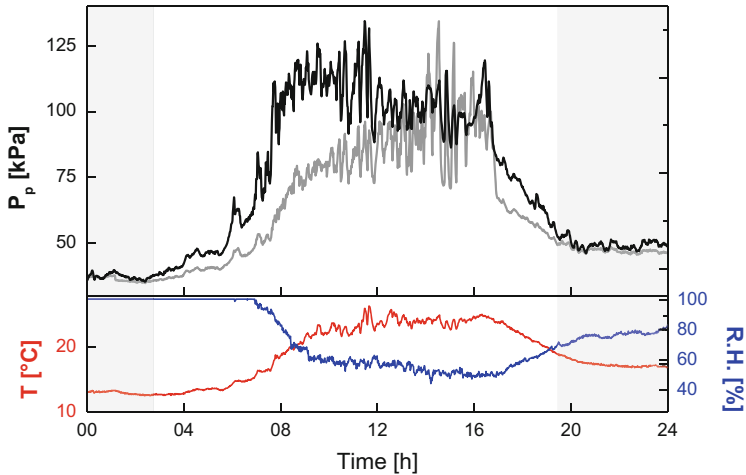
Fig. 2 Multiple probe readings with the ZIM probe (Fig. 1b) on leaves of a 30-m tall oak tree for one day (02 July 2013, Germany) together with the corresponding profiles of local air temperature (T; *lower panel*; *red line*) and relative humidity (R.H.; *lower panel*, *blue line*; unpublished data, U. Zimmermann and S. Rüger). P_p values (that are inversely proportional to leaf turgor pressures) were normalised to the P_p range of the respective probes located on the west side. Nocturnal hours are marked by *grey bars*. Probes were clamped in the east (*black line*) and in the west (*grey line*). Please note the P_p oscillations due to stomatal oscillations during the day. For details see also Zimmermann et al. (2008) and Rüger et al. (2010)

a membrane or the surface of a plant organ (such as the root) will establish an ion gradient in a dilute medium bordering on that surface. This gradient is experimentally accessible by using ion-selective microelectrodes that constantly migrate between two positions (at a distance of a few μm). From the known diffusion coefficient of the dilute medium, ion fluxes can directly be calculated at a high temporal and spatial resolution. An important prerequisite of this approach is, however, that volume flow across the membrane is negligible, since volume flow tends to build up local unstirred layers [see above, sect. 2. In fact, Pohl and co-workers even made use of this fact to quantify volume flow across an ideally semi-permeable membrane; Pohl et al. (1997)]. These volume-flow induced ion gradients would tend to be misinterpreted in the theoretical framework of the MIFE theory.

Use of Isotopes to Label H₂O or Certain Nutrients An early boost in plant transport physiology in the 1960s and 1970s profited from the availability of radioactive tracers for chemical elements relevant for plant nutrition, particularly H (^{3}H, to label water), K (^{86}Rb), N (^{13}N), S (^{35}S) and ^{32}P. When fed, e.g. to the root, distribution of these tracers in the plant could easily be followed by local recording of radiation, e.g. by scintillation. Moreover, techniques for measuring and interpreting washout kinetics of radioactive tracers (Metzner et al. 2008) were developed (Britto and Kronzucker 2013). A particular challenge is provided by

short-lived radioisotopes such as ^{42}K (half life about 12 h). In the meantime, rare stable isotopes also gained popularity as tracers (e.g. ^{41}K, ^{26}Mg, ^{15}N, ^{44}Ca, ^{18}O and ^{2}H (=deuterium) for labelling water); these isotopes can be identified by mass spectrometry, e.g. with high spatial resolution in combination with cryo-microscopy (*S*econdary *I*on *M*ass *S*pectrometry = SIMS-technique; Metzner et al. 2008). A definite advantage of using isotopes as tracers is that unidirectional fluxes can (initially) be measured. This can provide information, e.g. about rapid cycling of ions across membranes (Britto and Kronzucker 2006) that is unavailable by any other technique.

Isotope labelling proved to be ineffective for measuring water flow in plants because of the extremely fast exchange kinetics of water, but other techniques are available to measure volume flow.

Flow Imaging by MRT Nuclear magnetic resonance tomography (MRT) provides a convenient way to monitor, among other things, volume flow with a high temporal and spatial resolution. It is a big advantage of this technique that it is truly non-invasive. The technique has been used, among other things, to monitor xylem and phloem flow simultaneously (Rokitta et al. 1999; Peuke et al. 2001). Flow imaging takes advantage of the fact that non-aligned H-spins move into the measuring plane during scanning. From the data, a flow velocity can be calculated; by multiplication with the conducting area (that can be obtained from flow images) volume flow becomes accessible (e.g. Schulze-Till et al. 2009).

Sapflow Measurements Based on Local Heating While the MRT technique provides very detailed information on volume flow, but is challenging from a technical point of view and requires sophisticated equipment, the heat dissipation or heat balance techniques provide a simpler alternative suitable for field studies (Smith and Allen 1996; Renninger and Schäfer 2012). Local, constant heating of a shoot (e.g. a tree trunk) leads to subsequent convection in the tissue until a constant temperature (measured with respect to a second thermocouple some 10–15 cm below the first one, serving as a reference) is attained. At this point of time energy input equals dissipation. Sap flow passing the electrode will lower this temperature, and the temperature drop is a direct measure of volume flow (also called thermal dissipation or Granier-style probe). Alternatively, sapwood is heated locally and the asymmetrical temperature increase below and above that site (upstream and downstream, respectively) is monitored that is related to volume flow (heat balance technique in the strict sense, or heat field deformation). Because of their technical simplicity these as well as related methods have frequently been used especially to measure transpirational flow in trees, but there is some debate on the reliability of volume flow data obtained this way (Shackel et al. 1992; Renninger and Schäfer 2012).

Flow Modelling According to the Method of Pate and Jeschke An amazingly simple and efficient way of *ex post* modelling of fluxes of nutrients between root and shoot is provided by tapping xylem and phloem sap from individual plants and subsequently harvesting root and shoot biomass to analyse them with respect to the

particular nutrient of interest (Jeschke and Pate 1991). From these data, xylem and phloem fluxes as well as total net uptake by the root can be estimated for the whole lifetime of the plant [summarised, e.g. by Peuke (2010)]. A critical point is that the composition of vascular saps may be highly variable with time and data just represented a—somewhat arbitrary—snapshot, whereas the nutrient content of root and shoot tissue has evolved over the whole lifetime of that plant.

4 Radial Transport of Water and Nutrients in Roots: Transpiring Plants

Uptake of water and nutrients by roots and subsequent radial transport into xylem vessels provides a good case study to illustrate the more general considerations outlined in Sects. 1 and 2. The cellular membrane of cortex cells is frequently considered to be the primary interface separating the plant from its local environment, the adjacent soil (Schroeder et al. 2013; Wang et al. 2012). A plethora of transport proteins co-located in this membrane has been studied in much detail. However just focusing on the membrane level would be too reductionist a view on transport processes in this highly complex organ. It is more adequate to adopt a concept well known from animal physiology and to consider root tissue as an epithelium consisting of several cell layers that separate two extracellular compartments: The lumens of the (dead) xylem vessels in the root centre, and the soil solution at the periphery of the root. In analogy to mammalian epithelia, transfer of water and nutrients across this barrier can occur symplastically—via the cells and plasmodesmata connecting them—or apoplastically, i.e. via the cell walls. Apoplastic transport is limited by the Casparyian band, with suberin depositions in the cell wall of the endodermis in its mature state, separating the cortex from the stele in the root centre. In some species the exodermis forms an additional, more peripheral transport barrier in the root apoplast. Some research effort has been invested into quantifying the relative contributions of the two transport pathways—symplastic and apoplastic—to radial water and nutrient transport. Many researchers favoured the "composite transport model" that was originally proposed and promoted by E. Steudle and coworkers (Steudle and Peterson 1998; Steudle 2000a), based on results obtained with the root pressure probe. When the root is challenged with a hyper-osmotic shock by adding various osmotica to the ambient medium, in many cases the root pressure drop did not match the change in external osmotic pressure, indicating that the radial reflection coefficient (calculated from the ratio $\sigma_r = \Delta P/\Delta \pi$) is significantly lower than unity. Following the "composite membrane" model, the root reflection coefficients were considered a measure to quantify the contribution of the cellular pathway (for which $\sigma_r = 1$ was assumed for many osmotica) to overall volume flow; consistently, a σ_r value of 0 was ascribed to the apoplastic pathway (Steudle 2000b). Steudle and co-workers concluded from their root pressure probe measurements that the apoplastic pathway contributed

significantly to radial water transport in many species. However, more recently the composite transport model and the experimental approach on which it was based were heavily critisised for various reasons. Bramley et al. (2007) identified technical flaws in the use of the root pressure probe and the interpretation of the probe data when performing experiments with two probes each attached to one cut surface of a root segment. Moreover, Bramley et al. insisted that the experimental procedures to determine hydraulic conductivity and reflection coefficient of the root were prone to produce artefacts, since they tend to affect local gradients in water potential (and gradients in solute concentrations) and give rise to unstirred layers (see also the rebuttal by Steudle and coworkers; Knipfer et al. (2007)). Unstirred layer effects could also be responsible for low values of radial root reflection coefficients. Most likely the significance of the apoplastic pathway is erroneously over-estimated when these effects are neglected.

The same conclusion was drawn, about a decade before, by Schneider et al. (1997a, b) from their osmotic experiments on intact seedlings of maize, barley and wheat using the xylem pressure probe. Schneider et al. observed that the radial reflection coefficient in roots challenged with an osmotic shock strongly depended on the transpiration rate. In accordance with Steudle (2000a, b), apparent σ values were significantly smaller than one in roots of non-transpiring plants and at low transpiration rates, but increased to unity when transpiration was stimulated by the light regime. Only at peak photon densities prevailing in greenhouses on Hawaii at noon, σ values passed through a maximum and tended to decrease again. Schneider et al. (1997a) argued that transpiration-driven flow would tend to abolish unstirred layer effects to some extent, leading to an increase of apparent σ values. Consistently, it could be shown that the transpiration rate remained unaffected by the osmotic challenges in those experiments, with the exception of seedlings exposed to extremely high irradiation rates. It was concluded that radial water flow was predominantly symplastic and that low σ values were due to unstirred layer effects, but not to an apoplastic bypass for water transport, at least in the cereals tested in those studies. The same conclusion was later drawn by Knipfer and Fricke (2010) when repeating earlier experiments on barley with the root pressure probe while stirring the external medium. Evidence against the composite transport model was also obtained by Fritz and Ehwald (2011). For maize, they investigated radial transport into the root xylem of mannitol and other test solutes that are known to be almost membrane-impermeable. Hence, radial root transport of these solutes is necessarily predominantly apoplastic. In contrast to the predictions of the composite transport model, no evidence for a solvent drag effect in the transport rates of these solutes was obtained and the radial root reflection coefficient was close to unity; transport into the xylem did occur, however, but it was largely diffusive, leading Fritz and Ehwald to the conclusion that the endodermis prevented radial water transport in the root, but retained some permeability to solutes like mannitol. The same conclusion may be true for Ca^{2+} (White 2001). Suberins and waxes are generally thought to form an effective diffusion barrier. Therefore, apoplastic diffusion of solutes across the endodermis is most likely restricted to the developing root zone where cell walls are not fully suberised, especially at passage cells, and at

sites of lateral root formation. However, no real quantitative information is available on the permeability of suberin layers to solutes as pointed out, e.g. by White (2001).

While radial reflections coefficients were apparently misinterpreted in the past, this is no reason to dismiss these results. On the contrary, apparent reflection coefficients provide insight into the response of the plant root to an osmotic challenge. Values significantly lower than one imply that the impact of soil osmotic pressure fluctuations on xylem pressure is damped. This is highly beneficial for the plant, since a drop in xylem pressure to very negative values could entail cavitation.

The previous discussion of radial root transport at the organ level has revealed severe conceptual as well as methodological problems of techniques to measure interaction of water and nutrient transport at the organ level. Even though there are no "artefact-free" methods in physiology, it is important to remain aware of potential sources of error and to minimise them as far as possible. A major problem of the root pressure probe (and some other techniques) is that work is done on excised roots and relevance for intact, transpiring plants is questionable (Shabala et al. 2009). Moreover, transport parameters and flux data are extracted under rather non-physiological conditions. It was vigorously debated, e.g. whether pressure clamp or pressure pulse relaxation protocols would be more suitable to determine the hydraulic conductance of the root, but the reader is left with the conclusion that eventually both approaches are inadequate. Physiologically meaningful data on root hydraulic conductance of transpiring plants can only be obtained with steady-state volume flow and at a "free-running" xylem pressure under transpirational control. While volume flow can be assessed gravimetrically (Wegner and Zimmermann 2009), using the heat balance technique or by gas exchange measurements, xylem probes are the only instruments to provide us with relevant data on hydrostatic xylem pressure. Therefore, Wegner and Zimmermann (2009) revisited the problem, using a multifunctional xylem probe that allowed to record xylem K^+ in addition to xylem pressure (Fig. 3). With this approach, root hydraulic conductance could be calculated for intact maize seedlings according to Eq. (4) using steady-state data. All parameters were experimentally accessible, assuming the osmolarity of the sap to be four times the K^+ activity. Interestingly, both hydraulic conductance and radial K^+ transport depended in a non-linear way on radial volume flow (Fig. 4a–c), but not directly on hydrostatic xylem pressure, the main driving force for radial volume flow. However, in the light of the context described above this was not interpreted in terms of a solvent drag effect in the strict sense. Rather, it was argued that radial volume flow into the xylem leads to an accumulation of K^+ in xylem parenchyma cells, building up a steep K^+ concentration gradient across the membrane of these cells. This will lead to an enhanced rate of K^+ efflux because of an increase in the driving force; moreover, the open probability of the relevant ion channel, the K^+ outward rectifier SKOR (Liu et al. 2006), is enhanced by an increase in cytosolic K^+, providing an additional feedforward effect. Non-linear dependence of volume flow on the driving force is probably due to the regulation of aquaporins in inner cortex and endodermis cells that serve as a bottleneck for radial water transport (Wegner and Zimmermann 2009). Evidence for an enhancement of
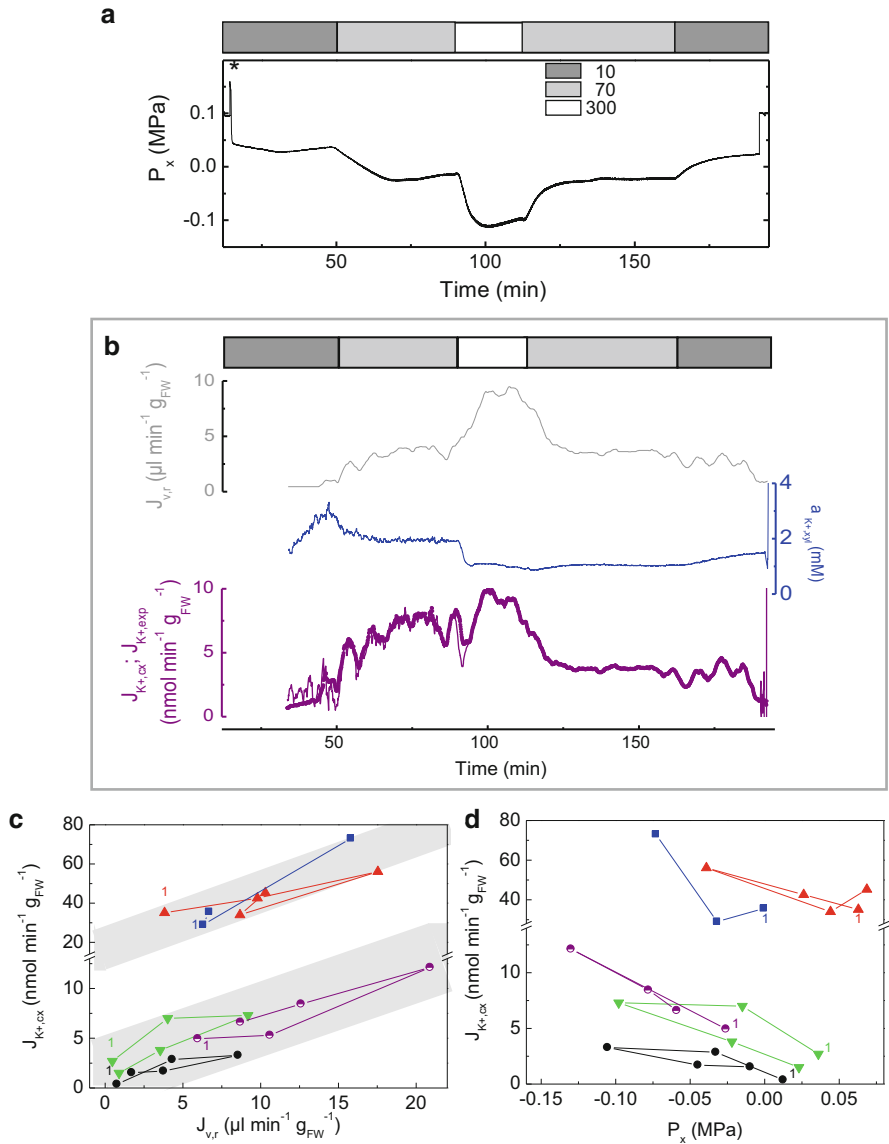
Fig. 3 Dependence of various water and K⁺ transport parameters on a varying light regime in a maize seedling. Response of xylem pressure (P_x), radial volume flow ($J_{v,r}$), xylem K⁺ activity ($a_{K+,xyl}$), K⁺ flux into the xylem ($J_{K+,cx}$) and K⁺ export to the shoot ($J_{K+,exp}$) to a stepwise increase and subsequent decrease of light irradiation was recorded with a multifunctional xylem probe (see Fig. 1a) and simultaneous gravimetrical recording of water uptake by the seedling. *Bars on top* of the figures indicate the time schedule of light regime changes (in μmol m⁻² s⁻¹); Fig. 1a shows the time course of xylem pressure. Impalement of a xylem vessel (indicated by *asterisk*) at laboratory light (10 μmol m² s⁻¹) was associated with a pressure drop from atmospheric to 0.44 MPa. In (**b**), the radial volume flow (*top trace*), the xylem K⁺ activity (*middle trace*) as well as the K⁺ flux into the root xylem (*bottom, thin line*) and, almost identically, the K⁺ export to the shoot (*bottom, thick line*) are plotted with time for the same experiment. Note that for technical reasons, recording of $J_{v,r}$ started with a delay of about 15 min with respect to impalement. For this (*filled inverted*
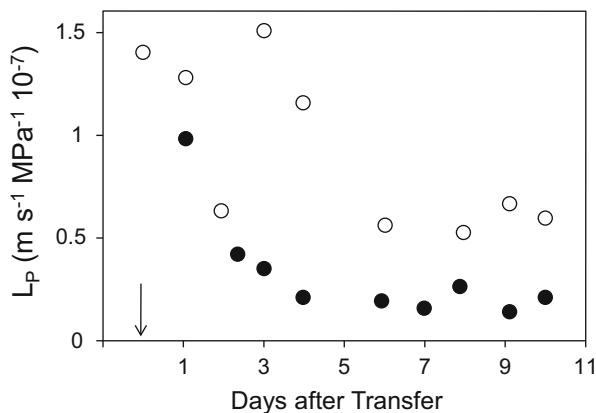
Fig. 4 The hydraulic conductance recorded on individual root cortex cells of cotton depends on the nutritional status of the plant. The experiment was performed with a cell pressure probe. Seeds were allowed to germinate on moist vermiculite and transferred at day 3 (*arrow*) either to a full nutrient solution (*open symbols*) or to a –N medium composed in the same way except for nitrate being replaced by chloride (*solid symbols*). Note that hydraulic conductance dropped on a daily basis in root cells deprived of N, whereas the drop in fully supplied roots was less pronounced. After Radin and Matthews (1989), with modifications

K^+ and nitrate loading into the xylem by volume flow was also obtained by Schurr and Schulze (1995) on intact castor bean plants (but not on detopped root systems!). Xylem sap was sampled locally at a site of incision of the shoot while pressurising the root system. This approach was retrospectively justified by the observation of Wegner and Zimmermann (2009) that xylem pressure has no direct effect on the rate of xylem loading of K^+. Schurr and Schulze found little dependence of the K^+ and nitrate concentrations in the xylem sap on volume flow in the range tested experimentally (in contrast to the detopped root system that rendered a hyperbolic dependence). This pinpoints to an increase in the rate of xylem loading for both ions with the radial volume flow. Moreover, the importance of using intact plants for those studies is again highlighted by their study!

Fig. 3 (continued) triangle) as well as four other seedlings, dependence of $J_{K+,cx}$ on $J_{v,r}$ and P_x is shown in (**c**) and (**d**), respectively. The sequences of light regimes for these experiments were (irradiance in $\mu mol \ m^{-2} \ s^{-1}$): 10–70–300–70–10 (*filled circle*, *filled inverted triangle*, *filled triangle*); 10–70–300 (*filled square*). The index "1" indicates the starting value for the respective experiment. Despite some hysteresis, plants could be divided into two groups with respect to the dependence of $J_{K+,cx}$ on $J_{v,r}$ as indicated by shaded areas in (**c**): In three plants, K^+ flux was close to zero in the absence of volume flow, whereas in two further plants, extrapolation yielded a considerable rate of xylem loading at zero volume flow (about 15 nmol min^{-1} g_{FW}^{-1}). A weak correlation was also found between $J_{K+,cx}$ and P_x (**d**), but separate experiments revealed that varying P_x at a constant volume flow would not affect xylem loading of K^+ (not shown). For more details, see Wegner and Zimmermann (2009)

5 Radial Transport of Water and Nutrients in Roots: Root Pressure

The xylem pressure probe and its further developments proved to be very useful tools to study water and nutrient transport in transpiring plants. However, since a below -atmospheric pressure is required to locate the probe tip in a vessel with certainty, these probes are not suited to impale roots of non-transpiring plants building up root pressure. From a physiological point of view, root pressure is a special case; it dominates long distance water transport, e.g. in very young seedlings and at a water-saturated atmosphere. According to most textbooks, radial water flow is driven by an osmotic overpressure of the xylem sap with respect to the ambient medium. However, this explanation is at least insufficient to describe the phenomenon adequately; there are many reports in the literature that water secretion into the vessels prevails in the absence of an osmotic gradient between xylem sap and ambient medium, or even against such a gradient. The latter observation has puzzled researchers for decades (Oertli 1966; Enns et al. 2000). Recently Wegner (2014) has reviewed the available experimental evidence and suggested a new hypothesis to explain root pressure [and root pressure exudation; i.e. constant "bleeding" from the cut surface(s) of excised roots, root segments and even cortex sleeves after the removal of the stele (Volkov and Zholkevich 1993)] by a "non-osmotic" mechanism. It was suggested that water secretion across the plasma membrane of xylem parenchyma cells is driven by a cotransport of water and solutes as previously shown for mammalian epithelia; solute concentration gradients across the cellular membrane of xylem parenchyma cells are supposed to provide the free energy to drive water secretion into the xylem vessels, even against a gradient in the chemical potential of water (or "water potential"). For various mammalian epithelia, T. Zeuthen and his co-workers provided multiple evidence for the existence of membrane transporters that co-translocate solutes and water at a fixed stoichiometry (Zeuthen and MacAulay 2012; Zeuthen 2010). A key role is apparently played by transporters of the CCC type that transport either KCl, NaCl or both salts simultaneously together with a fixed number of 150–500 water molecules. In order to maintain the ionic gradient across the membrane that is dissipated by this transport step, ions have to be retrieved again from the extracellular compartment at the expense of metabolic energy.

Interestingly, homologues of the CCC family have also been discovered in plants. Kong et al. (2011) cloned a cation-chloride cotransporter in rice that seems to translocate K^+, but not Na^+. These authors tested subcellular localisation of a CCC–GFP fusion protein and could demonstrate that the protein was predominantly allocated to the plasma membrane. The only CCC-type transporter found in *Arabidopsis* showed also highest homology with the subfamiliy of KCl cotransporters (the KCCs), but reconstitution in oocytes showed that $^{86}Rb^+$ (as a tracer for K^+) was only translocated in the presence of Na^+, indicating that this transporter functions like cotransporters that transport Na^+ and K^+ together with two Cl^- ions (the NKCCs; Colmenero-Flores et al. (2007)). Interestingly, the

cotransporter found in *Arabidopsis* turned out to be prominently expressed in vascular tissue. It is well conceivable that these transporters are involved in the directed, radial transport of water into xylem vessels by which root pressure is built up, although availability of Na^+ and Cl^- and the gradients of these ions are a critical factor. It is unknown whether the plant transporters can also work with other anions like nitrate that are of no relevance for the animal system, nor is any information on water permeability of the plant transporters available yet. A major challenge to the "water cotransport hypothesis" both in mammalian and plant tissues is also provided by the presence of aquaporins in the cellular membranes of the cells that tend to dissipate any water potential gradient; however, it could be shown by model calculations (Wegner 2014) that cotransporters could operate against a moderate hydraulic conductance of the membrane. Re-absorption of K^+ and Na^+ (required for "keeping the battery charged") would be brought about by inward-rectifying K^+ channels in xylem parenchyma cells (Wegner and Raschke 1994; Wegner et al. 1994) and HKT transporters, respectively. For Cl^-, this role could be played by $Cl^-/2H^+$ symporters. Note that salt release by cotransporters is an electroneutral process (Zeuthen and MacAulay 2012) and would not interfere with K^+ re-uptake by ion channels that requires a membrane potential more negative than the Nernst potential of K^+, which is maintained by proton pump activity. Evidence for "simultaneous" uptake and release of K^+ has indeed been obtained for root tissue, using refined radioactive tracer techniques (Britto and Kronzucker 2006). Rapid, seemingly "futile cycling" of ions is apparently a common phenomenon at root membranes that was found for K^+, Na^+ and Cl^- and becomes more prominent at elevated concentrations of these ions.

Co-transport of water together with one or more substrates is not a unique property for the CCC transporters. The Na^+-glucose co-transporter SGLT1 and the glucose transporter GLUT1 also translocate water at a fixed stoichiometry (Loo et al. 1999; Zeuthen 2010), and evidence was obtained that the same is true for a range of amino acid transporters, including those from plants. Even ion channels, that co-transport 4–12 water molecules together with one ion, could be involved in water secretion (Wegner 2014), provided that ion release into the xylem and re-absorption occur via different pathways and differ in ion/water stoichiometry, and that the membrane potential oscillates continuously. Further research into this direction is required in the near future. For more details on this matter, the reader is referred to the original publication (Wegner 2014).

6 Long-Distance Transport of Water and Nutrients in the Xylem

Axial transport of water and nutrients from roots to shoot occurs via the xylem, more precisely—in angiosperms—via both xylem vessels (trachees) and tracheids that form a continuum of highly interconnected pipelines extending from the fine

roots to the leaves. Nutrients dissolved in the xylem sap are transported upwards by the transpiration-driven mass flow, i.e. nutrient transport is proportional to volume flow. Volume flow depends on vessel anatomy, i.e. on the geometric properties of the conduits, and on local pressure gradients and is, to a first approximation, well described by Hagen–Poiseuille's law (Nobel 1991). Mechanisms of mass flow in the xylem against gravity have been debated violently during the last decades; the more than 100-year-old cohesion-tension theory has been questioned repeatedly and obviously needs at least modifications and extension (Zimmermann et al. 2004; Wegner 2014), but this will not be discussed in more detail here.

While coupling of nutrient and water transport in the xylem conduits follows quite simple physical principles, some aspects require special attention. Those are (1) the ion exchanger properties of the matrix of the xylem walls that can buffer changes in the ionic composition of the xylem sap by selective de- and resorption of ions; (2) the effect of K^+ and other cations dissolved in the xylem sap on the hydraulic conductance of the interconnecting pits and, hence, the entire xylem and (3) the role of adjacent cells in changing the composition of the xylem sap.

The "chromatographic effect" of the xylem walls for ions results mainly from fixed negative charges of polygalacturonic acids that are part of the pectic matrix; this effect is highly dependent on the protonation of carboxylic groups and, hence, on xylem sap pH (Wolterbeek 1987). The interaction, preferentially of divalent cations with xylem walls and the role of these processes in translocation in the xylem, was investigated in a range of classical papers (Bell and Biddulph 1963; van Ieperen et al. 2000; de Geijn and Petit 1979; Wolterbeek 1987). Xylem walls were found to have a fixed cation exchange capacity (CEC) and tend to bind divalent cations (Ca^{2+}, Cd^{2+}) tightly (but less than chelators like EDTA). Hence, an increase in divalent cation concentration in the sap would be buffered by the xylem wall. This buffering effect would be even more pronounced with respect to ion exchange between xylem and adjacent cells and the phloem.

While in the past the xylem conduits were considered to have a fixed hydraulic conductivity mostly resulting from the length and diameter of the vessels and being invariant to short-term adjustment, this view started to change gradually during the last decade. Ion-mediated regulation of xylem conductivity (frequently short-termed the "the ionic effect") was originally demonstrated by M. Zimmermann (1978) and later revisited by van Ieperen et al. (2000) and Zwieniecki et al. (2001). These reports caused much excitement and since then numerous follow-up studies have dealt with this topic (summarised, e.g. by Nardini et al. 2011) even though the physiological significance of the effect is still under debate (van Ieperen 2007) and may be highly species-dependent (Herbette and Cochard 2010). In short, it was observed that the axial hydraulic conductance of shoot segments and individual conduits increased with an increase in the electrolyte concentration in the xylem fluid (mostly monovalent and divalent cations). Other osmolytes had no comparative effects. Conductivity increased by 1.9 up to 58 % in 35 species tested (Nardini et al. 2011). However, these results were, in the vast majority, obtained with artificial perfusion media that frequently did not reflect the natural composition of the xylem sap (van Ieperen 2007). As pointed out by this author, it is inadequate to

use distilled water as a reference solution, since this medium is non-physiological and will affect the mechanical properties of cell walls and in particular the pectin structure of inter-conduit pit membranes that were identified as the major axial resistance for longitudinal water flow. These are highly susceptible to the ionic composition of the ambient medium. Swelling and shrinking of pectin matrix, and concomitant changes in pit membrane porosity, or rather membrane thickness (Lee et al. 2012), was identified as the most likely molecular mechanism underlying the ionic effect (Zwieniecki et al. 2001; Nardini et al. 2011). Van Ieperen has argued that in the presence of Ca^{2+}-free media not matching the natural composition of the xylem sap properties of the pectin gel matrix may change in a non-physiological way. Moreover, the ionic effect tends to saturate with increasing Ca^{2+} and K^+ concentration in the xylem. The most dramatic effect occurs when distilled water is exchanged for (artificial) xylem sap containing the usual background ionic concentrations (but see also Nardini et al. (2007)). Despite this ambiguity with respect to the physiological relevance of the ionic effect, it was discussed as an important mechanism of short-term adjustment of xylem conductivity at sites where a large fraction of vessels is blocked by embolisms (Trifilò et al. 2008, 2011), for water allocation to branches receiving sunlight (Sellin et al. 2010; Nardini et al. 2011), for seasonal adjustments in xylem conductivity, or at fluctuating environmental conditions (Nardini et al. 2012). Maximum increase in xylem hydraulic conductivity was observed when adjacent vessels became dysfunctional by embolism (Trifilò et al. 2008); at those sites, increase in xylem K^+ under natural conditions appears to be most pronounced (Trifilò et al. 2011), and evidence for a physiological relevance is most compelling. Increased local K^+ concentration may also reflect an elevated osmotic pressure that may be part of mechanisms to repair embolism (Wegner 2014) and to circumvent embolised vessels with water passing through ray cells (Zimmermann et al. 2004). More research on this "ionic effect", its variability and its molecular basis are required to establish its physiological significance unambiguously.

This discussion on the interplay of xylem ionic composition and volume flow in the xylem would be incomplete without a few words on the role of xylem paren-chyma cells in controlling the composition of the xylem sap. This topic would merit a separate review paper because of tremendous recent progress on this issue. Unfortunately, it can only be covered in the form of case studies here. Since the role of xylem parenchyma ion channels in controlling composition of the xylem sap was highlighted for the first time 20 years ago (Wegner and Raschke 1994), a great number of transport proteins located at the plasma membrane of cells bordering on xylem vessels have been identified and characterised with respect to their role in long-distance transport. In the methodological and conceptual context discussed here, the elegant work of Metzner et al. (2010b) on lateral water and nutrient exchange between the xylem and adjacent cells in *Phaseolus vulgaris* deserves particular attention. These authors used cryo-mircroscopy in combination with time-of-flight secondary ion mass spectrometry (SIMS) to trace the distribution of stable isotopes in tissues (Metzner et al. 2008) that were fed via the xylem conduits across the cut surface of the excised stem. A very high spatial resolution could be

realised (<1 μm in some of the images), allowing to separate apoplastic and symplastic transport. First of all, this work highlighted again the importance of the exchange of water and nutrients (K, Mg, Ca) between xylem vessels and their environment. Complete equilibration of labelled water between lumens of the vessel and adjacent tissues occurred very fast [with the exception of lignified cell walls in the vicinity of the vessels Metzner et al. (2010b)]. Unexpectedly, however, the investigated rare isotopes of K^+ and even Ca^{2+} and Mg^{2+} supplied via the transpirational stream also equilibrated rapidly with both apoplastic and symplastic pools of xylem parenchyma, and to a lesser extent also with other tissues, indicating that these ions were highly mobile and permeability of cellular and vacuolar membrane were comparatively high. In a separate study (Metzner et al. 2010a) evidence was presented that ion exchange occurred by diffusion and that solvent drag was not likely to play a major role. A modelling approach to these datasets would probably be rewarding, since it potentially offers the possibility to quantify individual fluxes at the single-cell level and possibly relate them, e.g. to ion channel activity.

The control of relative ion concentrations seems to be of major importance for the plant, rather than adjusting absolute xylem sap concentrations that are subject to perpetual fluctuations. A good example is maintenance of the K^+/Na^+ ratio that is optimised in salt-tolerant barley cultivars when exposed to mild salt stress, whereas absolute concentrations seem to be less important (Shabala et al. 2010). Much progress has recently been made in unraveling the mechanisms of how xylem sap Na^+ load is adjusted and Na^+ accumulation in the shoot is reduced, e.g. by retrieval of Na^+ from the xylem sap by xylem parenchyma cells at root and shoot base (Jacoby 1979), and by re-circulation via the phloem (Davenport et al. 2007; for more details, see Sect. 8). Circulation of ions between xylem and phloem seems to be a general mechanism in higher plants contributing to ion homeostasis (Lüttge 2013).

7 Xylem Unloading and Water and Nutrient Transport in Leaf Tissues

While nutrients allocated to leaves remain there serving various physiological functions, or are, to a varying extent, recirculated via the phloem, the water is mostly lost to the atmosphere by evaporation via stomatal pores and the epidermis. This implies a constant mass flow from the vascular tissue to substomatal cavities and to the epidermis, passing through mesophyll tissue. Water could move either apoplastically via cell walls, or through the symplast. Like in the root, this is of relevance for the coupling of flows. Fluorescent dyes have been used as tracers to explore pathways of water and solutes in leaves, but it was not before the landmarking review of Canny (1990) that a firm basis for the correct interpretation of these data was established. Canny argued that local apoplastic accumulation of a

dye (called "swamps") marks sites where partitioning of water and solutes occurs, and water passes cellular membranes to enter the symplast (termed "flumes"). Visible traces of the dye extending through cell walls from the vasculature to the epidermis result from diffusive transport starting at swamps. From the evaluation of a large series of micrographs, he concluded that water transport in the leaf is predominantly symplastic. This was later confirmed by other techniques, e.g. measurements of turgor pressure (Ye et al. 2008). It is well known that nutrients are unevenly distributed among different cell types (and sites) in the leaf, but little attention was paid so far to the impact of water flow. An exception is the work of Fricke (2004), who observed for barley leaves that Cl and Ca were preferentially accumulated in the epidermis cells, while P was primarily found in the mesophyll. Changes in the transpiration rate affected this distribution significantly and increased Ca levels close to the substomatal cavity. Basic patterns were, however, not affected.

8 Phloem Transport

When discussing the interplay of water and nutrient transport, the phloem seems, at first glance, to be the least obvious candidate. Nutrient transport is usually not associated with phloem function nor with the transport mechanism. According to a general consensus, volume flow in the phloem is thought to be driven by a pressure gradient supported by phloem loading of mainly sucrose at source tissues (and subsequent passive water uptake by sieve tubes) and phloem unloading (and, in turn, water release) at the sinks. This so-called pressure flow ("Druckstrom") hypothesis first introduced by (Münch 1930) is based on tight coupling of water and solute flow, but corresponding to the main function of the phloem associated with assimilate transport, major osmolytes are supposed to be sugars rather than salts. However, this textbook scenario may oversimplify the real situation; K^+ seems to play a previously undervalued role in phloem transport, particularly when photosynthesis is reduced and sucrose loading at the sink is limiting, or when the H^+ ATPase activity is insufficient to energise transport across the sieve tube membrane. First evidence for this was obtained by Hartt (1970), and later a detailed model was worked out by Lang (1983). K^+ gradients in phloem sap tapped along the shoot provided direct evidence for K^+ loading at the source and K^+ release associated with the sink (Vreugdenhil and Koot-Gronsveld 1989). Consistently, Deeken et al. (2002) demonstrated that in mutants that lacked the phloem-located K^+ channel AKT2, phloem transport was strongly affected. More recently, Gajdanowicz et al. (2011) undertook a comprehensive study on the role of this channel in phloem transport. Mutants expressing an inward-rectifying version of AKT2 (in the wild type, the channel mediated both K^+ influx and efflux across the sieve tube membrane) were deficient with respect to phloem transport, especially at low rates of photosynthesis. Combining experimental work with extensive modelling, the authors came to the conclusion that maintenance of a K^+ gradient across

the sieve tube membrane serves as an energy source for loading assimilates into the phloem even at low H^+ ATPase activity.

A final remark on the phloem refers to its role in re-circulation of nutrients from the shoot to the root that was already discussed in detail by Lüttge (2013). Particularly excess K^+ is transported back to the root via the phloem and may serve as a shoot-to-root signal on the K^+ status of the shoot (Wegner and De Boer 1997).

9 The Whole-Plant Perspective: Macronutrients and Transpirational Flow

While the previous paragraphs deal with coupling of water and nutrient transport in plant organs, such as the root, and in vascular tissues representing "functional units" within the plant, the remaining part of this essay is dedicated to the main macronutrients K, N and Ca, and their interaction with long-distance volume flow (that is to a great extent identical with transpirational flow). Interaction is truly mutual, since, on the one hand, nutrients (and nutrient availability) regulate hydraulic properties of plant tissue, e.g. via gating of aquaporins and by affecting stomatal function. On the other hand, evidence has been presented for transpirational flow having impact on the allocation of nutrients in the shoot and among parts of it (e.g. younger and older leaves) in a nutrient-specific way.

The latter aspect touches a long-standing, more fundamental debate on the significance of transpiration for nutrient supply to the shoot. Tanner and Beevers (1990, 2001) provided evidence that transpiration is essentially not required to provide the shoot of sunflower plants with the full spectrum of nutrients. No evidence for nutrient deficiencies were found in sunflower plants that had been grown on hydroponics in a climate chamber, and that received mineral nutrients only during the dark period when the shoot was exposed to nearly 100 % humidity. Tanner and Beevers argued that water circulating between xylem and phloem and growth water would induce xylem flow sufficient for supplying the shoot with nutrients. It should be noted, though, that some residual transpiration was retained (about 7 % compared to the rate of control plants), that still contributed about 50 % to the total water flow from root to shoot in the humidity-exposed plants. Hence, a complete uncoupling of nutrient supply to the shoot from transpirational flow could not be achieved by their experimental approach. The case of Tanner and Beevers is supported by findings on aquatic higher plants growing in a submerged state. These plants maintain acropetal xylem water flow in the complete absence of transpiration, most likely to supply leaves with mineral nutrients like P, Fe and Mn that are hardly available from the ambient water at the leaf surface. These nutrients have to be taken up by the roots from sediments and are transported to the shoot by mass flow via xylem conduits (Pedersen and Sand-Jensen 1993, 1997). Independence of nutrient transport on transpiration as advocated by Tanner and Beevers contrasts,

however, with other reports that established a link between down-regulation of transpiration (e.g. as a consequence of elevated ambient CO_2 partial pressures) and reduced nutrient supply to the shoot (Conroy and Hocking 1993). Considerable transpiration rates at night were also hypothesised to serve the function of supplying the shoot with nutrients. The issue may be solved by stating that transpiration is, strictly speaking, not required to provide the shoot with nutrients, but since it is there and unavoidable under most conditions, plants have "learned" to make use of it. In habitats that do not require strict optimisation of water use efficiency, part of the transpirational flow being in excess of water requirements of the shoot may serve other purposes, such as optimising nutrient supply (Cramer et al. 2009).

Interactions of nutrient and water at the whole-plant level cannot exclusively be described in a mechanistic way, since various indirect effects have to be taken into account, e.g. regulation of stomatal conductance and photosynthesis (Cramer et al. 2009). Therefore, it is more adequate in some cases to talk rather about trade-offs.

9.1 Nitrogen (Nitrate and Ammonium)

The prime candidate for considering interactions and trade-offs of transport of water and inorganic ions in plants is certainly nitrate. Transport of water and nitrate interacts in various ways that have been a matter of extensive research since the 1980s of the last century. Radin and Boyer (1982) were among the first to detect that root hydraulic conductance was strongly affected by the availability of nitrate. In low nitrate medium, hydraulic conductance would be about half that of roots well supplied with nitrate. Transpiration was affected in the same way. Consistently, turgor pressure probe experiments revealed that the hydraulic conductance of cortex cells in roots deprived of nitrate was significantly lower compared to roots grown in full medium (Fig. 4; Radin and Matthews 1989). Later, this effect could be ascribed to a regulation of aquaporin activity in those cells. Nitrate complementation to roots grown in N free medium was shown to induce an up-regulation of aquaporin expression in fava bean roots (Guo et al. 2007), and N deprivation would suppress aquaporin expression (Clarkson et al. 2000). Moreover, regulation of aquaporin gating by (intracellular) nitrate independent of aquaporin expression was reported for maize (Gorska et al. 2008a, b). Both effects would contribute to an improved water supply to the shoot of plants well provided with nitrate and, in turn, an increase in transpiration and up-regulation of photosynthesis. Gorska et al. (2008a) and Cramer et al. (2009) have argued that an increased water flow would facilitate nitrate acquisition in soil by solvent drag. This may be particularly important in soils with local differences in nitrate availability, a situation that appears to be quite common in natural soils. The effect would favour effective exploitation of local nitrate resources over uptake of ammonium that does not induce a similar effect. However, when linking this effect to the response of the whole plant, complexity increases. In split-root experiments on bean with part of

the root supplied with nitrate and the other provided with ammonium, Schulze-Till et al. (2009) using the MRT technology, observed higher rates of water flow in the nitrate-fed roots due to a larger number of vessels per root contributing to flow than in those provided with ammonium. Flow velocity and xylem pressure of conducting xylem elements did not differ much, though, and anatomical properties were also unaffected by the N-form. Schulze-Till et al. hypothesised that part of the vessels remained non-functional in the ammonium-fed roots and thus were "switched off" to prevent cavitation.

Consistent with a facilitated water supply in the presence of nitrate, stomatal conductance (g_s) was found to increase when N-deficient plants received nitrate (Wilkinson et al. 2007). However, the dependence of g_s on soil nitrate was found to follow an optimum curve. High nitrate concentrations would tend to induce partial stomatal closure. Cramer et al. (2009) hypothesised that high nitrate delivery to the shoot (that would not only depend on nitrate uptake, but also on the nitrate assimilation rate in the root tissues) would lead to an increased NO production in the leaf and, in turn, to stomatal closure. Note that CO_2 uptake is also coupled to nitrate reduction in the leaves for adjustment of malate synthesis. Malate produced in the leaves neutralises OH^- formed as a by-product of nitrate reduction; moreover, malate replaces NO_3^- as a counter-ion for excess K^+ that is transported from root to shoot in the xylem conduits and is subsequently recirculated back to the roots via the phloem [see also Sect. 9.3 and Lüttge (2013)].

From this survey of various tight interactions of N fluxes and transpirational flow, it is not surprising that N supply was found to have strong impact on transport and accumulation of other nutrients, including K^+ and Ca^{2+} (Matimati et al. 2014).

9.2   Calcium

Calcium distribution in the plant is predominantly shaped by apoplastic water flow, since symplastic mobility of this divalent ion is low. Cytosolic Ca^{2+} concentrations are kept at extremely low levels of up to about 500 nM—values exceeding this low regime are sensed as a stress signal. Vacuolar Ca^{2+} concentrations are much higher, but this Ca^{2+} pool is rather immobile and does not contribute to Ca^{2+} transport. Clarkson (1993) reported that radial transport of Ca^{2+} into the xylem was restricted to apical parts of the root and correlated with the maturation of the endodermis; he concluded that root Ca^{2+} transport was mainly apoplastic and only for circumventing the suberin barrier of the Casparian strip, Ca^{2+} was taken up and subsequently released into the stelar apoplast. By contrast, White (2001) hypothesised that Ca^{2+} may be transported into xylem vessels by a purely apoplastic pathway, and that Ca^{2+} in a complexed form may also be mobile in the symplast. Ca^{2+} transport in the phloem is also supposed to be negligible (and hence, root-to-shoot net Ca^{2+} transport is supposed to equal total Ca^{2+} transport in apical direction). Note that his view has also been questioned repeatedly (Biddulph et al. 1959; Ringoet et al. 1968). But apart from these uncertainties, Ca^{2+} predominantly moves

in the apoplast (interacting with fixed negative charges of the cell wall, see above). Local apoplastic Ca^{2+} accumulation allows to identify sites at which increased cellular water uptake by adjacent cells takes place. Ca^{2+} and water flow interact mutually, though, since Ca^{2+} can also exert feedback on water flow, e.g. by a regulation of aquaporins (Gilliham et al. 2011), or by its effect on stomatal aperture (Atkinson et al. 1992). An elegant model on this interaction was proposed by Gilliham et al. (2011). At elevated apoplastic Ca^{2+} levels, cytosolic Ca^{2+} will also increase with time, leading to a down-regulation of aquaporin activity; as a consequence, transcellular water flow is down-regulated and translocation of water will be restricted to the apoplast, contributing to a wash out of local apoplastic Ca^{2+} accumulation. The scenario can be extended and refined by taking Ca^{2+} secretion via the plasma membrane by Ca^{2+} ATPases as well as Ca^{2+} exchange between cytosol and vacuole into account. Physiological models with this degree of complexity will require quantitative modelling to identify strategies for experimental validation.

In his study on Ca^{2+} transport in maize, Engels (1999) came to the conclusion that root-to-shoot translocation correlates to some extent with transpiration; additionally, radial transport in the root is adjusted to shoot demand.

9.3   Potassium

Despite its abundance in the plant and its importance for various physiological processes, the link of K^+ transport to water flow (and vice versa) seems to be less "spectacular" than for NO_3^- and Ca^{2+}. K^+ transport from roots to the shoot is under tight control of shoot demand (Engels 1999), and excess K^+ in leaves is circulated back to the roots via the phloem (White 1997; Lüttge 2013). Radial translocation of K^+ in the root is strongly enhanced by volume flow, as stated previously (Wegner and Zimmermann 2009; Schurr and Schulze 1995, see also above), most likely due to K^+ accumulation in stelar cells. It was also shown previously that xylem K^+ is buffered at short-term changes in the external K^+ concentration (Wegner and Zimmermann 2002).

Rather than the presence of K^+, its deprivation seems to have a marked effect on plant water relations, though. At low K^+ availability in the soil solution, root hydraulic conductance and transpirational water flow are increased with respect to values at normal K^+ supply, and water use efficiency is reduced (Quintero et al. 1998; Fournier et al. 2005). Low K^+ prevents stomatal closure under mild drought stress conditions (Benlloch-González et al. 2008, 2010). These symptoms may be part of a mechanism to enhance K^+ retrieval from the soil by solvent drag. Only at a severe K^+ starvation, stomatal function is compromised by reduced availability of K^+ as an osmoticum in guard cells required to maintain stomatal conductance (Humble and Raschke 1971). As a consequence, stomata tend to close under these conditions (Hsiao and Lauchli 1986).

10 Conclusion

Far from being comprehensive, this overview of nutrient and water transport in plants was meant to provide the reader with some insight into the complexity of their interactions. Information on these phenomena is ever increasing, e.g. by the advent of new techniques like the ZIM probe. Moreover, interactions among ion fluxes were hardly considered here, but obvious constraints like electro-neutrality of transport processes in steady state imply tight coupling of nutrient fluxes.

It is clear from these considerations that plant nutrition needs a conversion to become a quantitative science in the near future, making use of the fast progress in computer modelling of complex systems that is currently taking place. The uprise of meteorology and climate sciences provides a good example and can be seen as an encouragement. However, these environmental sciences also demonstrate that a firm physico-chemical basis is mandatory for such an approach to be successful. It is an advantage of transport physiology over other disciplines of plant sciences that its subject is readily treated in a quantitative way in the form of fluxes, and that well-established concepts like the thermodynamics of irreversible processes are available to describe coupling of these fluxes in a comprehensive way. More efforts are required in the future to extend and adjust these concepts, e.g. to include non-linearities and regulatory processes in the quantitative treatment of transport processes.

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