Diel growth dynamics in tree stems: linking anatomy and ecophysiology

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Abstract

Impacts of climate on stem growth in trees are studied in anatomical, ecophysiologial, and ecological disciplines, but an integrative framework to assess those impacts is still lacking. In this opinion article, we argue that three research efforts are required to come up with that integration. First, we need to identify the missing links in diel patterns in stem diameter and stem growth and relate those patterns to the underlying mechanisms that control water and carbon balance. Second, we should focus on the understudied mechanisms responsible for seasonal impacts on such diel patterns. Third, information of stem anatomy and ecophysiology should be integrated in the same experiments and mechanistic plant growth models to catch diel and seasonal scales.

Tree stem growth has huge implications but is poorly understood

Forests cover 30% of the earth's land surface, store 45% of terrestrial carbon, and are responsible for 50% of the terrestrial net primary production [1, 2]. Forest productivity has increased globally over the past decades, which has been attributed to the positive effect of increasing CO_2 on tree growth, thus far offsetting negative impacts of warming and drought [3, 4]. However, the long-term impacts on trees and forests of increasing CO_2, rising temperatures, and drought remain highly uncertain [5-7]. Another uncertainty is the role of trees in mitigating rising ambient CO_2 [8] and global warming by sequestering carbon in stems [1, 2]. We argue that such ecological uncertainties can only be tackled by developing an understanding of stem growth of individual trees that is based on underlying anatomical and ecophysiological principles, which are currently represented by separate scientific domains.

In this opinion article, we briefly present an overview of the major fluxes and pools of water and carbon inside a stem segment of a tree. We then examine the diel dynamics in radial stem growth and underlying water and carbon mechanisms under wet and dry conditions. We also elucidate the possible processes affecting stem growth across a wet and dry growing season, integrating seasonal trends in stem anatomy and ecophysiology. We distinguish between major known patterns and processes, and more speculative ones. All these discussions are based on observations in the different research disciplines, but also result from mechanistic plant models aiming at integration. Based on this, we show the missing pieces that are critical to building an integrative theory to understand the causes and consequences of tree stem growth on diel and seasonal scales. Addressing the key missing pieces of information is very much needed in order to understand and predict the impacts of a changing climate on annual tree growth patterns and the future production and carbon sequestration potential of forests.

Carbon and water fluxes in stem segments

Water is transported upward in the sapwood, downward in the phloem, radially between sapwood and phloem, and is stored in both sapwood and phloem (Figure 1, fluxes/pools steps 1-4). Carbon is transported downward in the phloem in the form of sugars (Figure 1, step 2), and those sugars are used for maintenance of living cells in sapwood, cambium and phloem (Figure 1, step 6), for growth in the cambium and developing cells (Figure 1, step 5), or for storage in the form of starch (Figure 1, step 11). Some carbon released as CO_2 by respiring cells in the tree stem diffuses directly into the atmosphere (Figure 1, steps 7 a, b, and c), whereas another substantial portion of this respired CO_2 remains inside the stem (Figure 1, steps 8 a, b, and c) where it dissolves in xylem sap and is transported away from the site of origin (Figure 1, step 7d). Some CO_2 slowly diffuses in the axial direction (Figure 1, step 9). The amount of CO_2 escaping into the atmosphere (measured efflux, Figure 1) is further reduced when respired CO_2 is refixed in sugars through photosynthesis within the stem (Figure 1, step 10). Below we discuss diel patterns in these water and carbon fluxes and their consequences for stem growth (see also Figure 2), and we provide an overview of the current state of art technology and methods used to quantify these fluxes (Figure 1, Box 1 and 2).

Stem dynamics in water fluxes and storage

Large forest trees lose up to 98% of their acquired water through leaf transpiration, whereas less than 2% is used for photosynthesis [9]. On a sunny summer day, an adult tree may lose and acquire several hundred liters of water. Leaf transpiration typically starts minutes to hours earlier than water flow in stem and roots, because transpiration is also supported by water from internal water storage [10]. The daily amount of water withdrawn from storage contributes 5-22% to the total daily water loss [11-13], and its diel dynamics affect radial stem growth. The typical diel patterns in water relations at the stem level for a fully exposed canopy tree during a sunny day, after a wet period (unstressed conditions with ample soil water reserves), and a dry period are shown in Figure 2. We distinguish between well-established patterns and more speculative patterns in green and red, respectively.

On a sunny day in unstressed conditions, a strong symmetric hump-shaped pattern of sap flow in the tree stem is observed (Figure 2B), leading to large day/night differences in water potential, and changes in internally stored water in xylem and phloem (Figure 2C). Embolisms in the xylem [14], which can be detected by acoustic emissions (Figure 2B), occur in concert with the decrease in stem water potential and reduce xylem hydraulic conductivity. The embolisms also release water into the transpiration stream, and can thus be considered as a source of storage water or capacitive discharge, damping the amplitude of diel fluctuations in xylem tension [15-18]. Recovery of hydraulic conductivity and overnight refilling of conduits under wet conditions is thought to be possible, but open questions remain [19, 20]. Although trees can utilize osmotic adjustment to maintain turgor in their living cells [21], mechanistic plant models and supporting observations [22, 23] indicate that water flows from living cells to xylem conduits when xylem water potential is reduced when leaf transpiration exceeds root water uptake. As a consequence, cell turgor (Figure 2D) follows the same decreasing trend as stem water potential, which results in stem shrinkage (Figure 2A) rather than cell expansion and growth during daytime hours [24]. Later in the afternoon, cell turgor, cell expansion and, hence, stem growth resume because of rising stem water potentials, which allows water to flow from xylem conduits into the living cells of the stem. These observations show that diel patterns in growth are not directly driven by carbon limitations – with photosynthesis and phloem loading peaking during daytime hours – but are rather influenced by the turgor pressure in living cells, which coincides with the availability of sugars for growth [25]. More precisely, the difference between the turgor pressure and a wall-yielding threshold value (Figure 2D) determines irreversible cell expansion [24, 26]. This threshold value is estimated to be around 0.9 MPa [26] for woody tissue, below which the cell cannot expand further [27, 28]. Because highest turgor values are established after sunset, highest rates of structural stem growth occur during the night (Figure 2A).

These patterns change during a moderately dry period (Figure 2) and even more dramatically during a persisting dry period (e.g. [29]). Because the soil is no longer fully hydrated, soil and stem water potentials are lower, and water storage pools in the phloem and the xylem are no longer fully replenished overnight (Figure 2C). Under these conditions, internal stem water storage pools are depleted, which causes an even more pronounced stem shrinkage during the day (Figure 2A) and more acoustic emissions linked to more embolism formation (Figure 2B). The predicted turgor (Figure 2D) is reduced, following the stem water potential. Once dropping below the wall-yielding threshold value, turgor limits cell expansion and growth (Figure 2A), even during the night. While soil drying continues, most patterns are asymmetric which implies a whole cascade of consequences: more embolism formation in the xylem, resulting in a decreased hydraulic conductivity, stem water potential, turgor pressure and storage of water. Under long persisting droughts, the trends may become irreversible: leaves may wilt and be dropped [29] and trees may eventually die [30, 31].

We conclude that the qualitative trends in diel water relationships are relatively well studied and understood. There are nevertheless several open questions about how to link embolism repair to underlying mechanisms, the role of turgor and internal water storage in diel trends of stem growth, and the generality of the turgor threshold value across tree species. Another challenge is to better understand why the presented qualitative trends shown in Figure 2, differ so much quantitatively across species.

Stem dynamics in carbon fluxes and storage

Diel patterns in fluxes, use and storage in tree stems are much less well understood for carbon than for water (Figure 2), even in unstressed conditions with ample soil water reserves. Besides water, radial stem growth depends on carbon as structural material for the formation of new tissue and as source for metabolic energy [32]. The carbon that is locally used for both processes may come from four sources, recently fixed sugars that are transported in the phloem, transitory leaf starch stored during the day and broken down during the night, local stem starch reserves, or locally refixed CO_2 in photosynthetic tissue of the stem (Figure 1). Unraveling the much-needed carbon-related mechanism underlying radial growth requires concurrent measurements of photosynthesis, and the fluxes of recently produced photosynthate to respiration, growth and storage as well as the flux of nonstructural carbohydrates out of storage pools. Tracking variations in stem diameter with dendrometers (Figure 1, Box 1) shows promise to quantify growth, but needs mechanistic plant models to unambiguously interpret the signal and separate structural stem growth from reversible stem diameter fluctuations (Figure 2A). In addition to the impact of turgor on cell wall expansion, as discussed above, turgor (rather than water potential) affects cell formation, and deposition and assembly of new wall material [33-36]. We therefore speculate that all growth processes (cell expansion, structural growth and its specific energy requirement) mainly occur during the nighttime, in concert with an improved water status and thus favorable turgor [32, 37, 38]. Turgor pressure thresholds for these different processes remain to be explored.

Another key challenge in understanding stem growth is to quantify diel patterns in respiration. Respiration is often estimated from measurements of CO_2 emitted to the atmosphere from the bark surface (Figure 1, Box 2), however this CO_2 efflux (Figure 2F) actually reflects the net result of multiple processes, including local growth and maintenance respiration, woody tissue photosynthesis, maintenance of ion transport over cell membranes, decomposition in heartwood, and CO_2 originating from respiration in lower stem or root tissues (Figure 1) [39, 40]. The measured diel pattern may follow the hump-shaped trend as expected from the exponential relationship between respiration and temperature [41], but daytime depressions in net CO_2 efflux may occur (Figure 2F), because of CO_2 transport in the xylem with the transpiration stream, or restricted growth during the daytime due to the loss of turgor in the living tissues [37]. A tight coupling has been observed between stem CO_2 efflux and CO_2 dissolved in xylem sap (Figure 2F) [39], and this relationship has been used to quantify the resistance to radial CO_2 diffusion [42]. Some indication exists that CO_2 does not only diffuse radially, but may also slowly diffuse axially along air-filled spaces in the wood [43]. Multiple processes, both locally in the considered stem section and remotely in other tree parts, thus drive the CO_2 emitted by a stem segment, but quantifying their relative importance remains a challenge. Stem anatomy likely influences the resistance to radial CO_2 diffusion, as well as local respiration and sap flow rates, in turn affecting net CO_2 efflux and the amount of CO_2 retained in the xylem [39, 40]. Investigating stem anatomy, including bark thickness and tree hydraulics, may help to explain the large variations observed in net CO_2 efflux, and contribute to a clearer understanding of stem respiration [40, 42].

A mechanism of structural growth is dependent on incorporation of new carbon skeletons in the cell structure, and thus requires information on sugar concentrations in the stem. Although critical, predictions of dynamics in sugar concentration in xylem, phloem and cambium are highly uncertain (Figure 2E), and so are the relative contributions of the different sugar sources (from leaves, stem starch reserves, or local woody tissue photosynthesis) driving those dynamics. Sugars originating from recent leaf photosynthesis [32, 44], woody tissue photosynthesis refixing respired CO_2 in stem chloroplasts during daytime [45], and transitory leaf starch storage during nighttime [46], may contribute significantly to diel growth dynamics, whereas local stem carbon reserves appear to contribute only marginally to growth [32, 44]. Despite our knowledge of such carbon-based processes, diel patterns in stem growth are predictable by tree water status only [24, 47]. However, the water-growth model predictions are only valid over short time periods, and we need a better understanding of the described carbon processes to understand and predict seasonal growth. This also highlights that much remains to be learned about the relative contribution of the co-occurring and interconnected growth processes. Another ambiguity is the predicted diel dynamics in phloem water flow (Figure 2E) [48, 49], which are contradicted by the constant phloem flow observed in MRI studies [50]. Despite the continuous attempts to refine the mechanism of turgor-driven transport of sugars from leaves to sink tissues with active and passive loading strategies and a leakage-retrieval process along the pathway [51], phloem transport in trees still remains poorly understood, with little connection between theory, research data, and the actual behavior observed for trees [52]. Thus we advocate the use of new experiments for deciphering phloem transport mechanisms by combining a focus on water and carbon relationships and adding measurements of sugar concentrations (Figure 1). Very few data exists, because measuring the pressurized living phloem is a daunting task, often hindered by induced wound reactions. Therefore, new promising techniques and methods for measuring phloem sap, turgor pressure, and labeled CO_2 should be further explored (Box 2).

When the soil is dry, trees reduce leaf water loss through stomatal regulation [29], which is directly at the cost of leaf photosynthesis and sugar loading, resulting in lower sugar concentrations in the phloem (Figure 2E). The lower level of sugar loading and sugar concentrations, the lower turgor, and the buffered dynamics in phloem water flow on drier soil are mainly hypothetical, as are the carbon dynamics on days with well-watered soil. Experimental studies have produced a number of relevant observations about these carbon balance interactions within the stem on dry soils. For example, lower cell turgor affects growth and maintenance respiration, resulting in lower stem CO_2 effluxes and dissolved CO_2 in the xylem sap [37] (Figure 2F), and the formation of fewer and narrower cells (Figure 3) because of the sensitivity of cell expansion and, to a lesser extent, cell division to turgor [53, 54]; woody tissue photosynthesis in xylary chloroplasts likely fulfills local energetic and carbohydrate demands for repair of embolised conduits [55, 56]; and local stem carbon reserves, only marginally contributing to stem growth under non-stressed conditions, may become more important with drought [25]. These examples highlight the inherent tight coupling of water and carbon interactions between phloem and xylem but also illustrate the need for more comprehensive studies of these processes.

In conclusion, the fluxes, use and storage dynamics of carbon in stems remain largely hypothetical, despite some understanding of the qualitative patterns. Remarkably, mechanistic plant models can predict diel trends in stem diameter and, through optimization of model parameters, provide hypotheses for diel trends in sugar concentrations and phloem water flow which remain to be tested. It is clear that respiration rates of tree stems remain highly uncertain, as does the contribution of woody tissue photosynthesis to the observed fluxes. Finally, we lack information on this shorter timescale on fluxes from starch to soluble sugars, and their contribution to diel dynamics in stem growth. We propose using new experiments that combine the more classic measurements of water and carbon relationships with new ones (Figure 1, Box 1 and 2), and emphasize the value of the integrative information obtained with diel stem diameter variation dynamics, which is particularly important for providing parameter values for the models. Despite their speculative nature, we consider the simulations by mechanistic plant models as a promising way to develop new hypotheses. However, this approach will require testing and validation with the more extensive and comprehensive data framework that needs to be built for different tree species with different wood anatomical properties under different water conditions.

Seasonal impacts on diel stem growth

Overall, mechanistic plant models capture the water dynamics and diel stem growth variation [24, 29, 49], but most of the emerging dynamics in carbon remain hypothetical. A second complication is that the models cannot yet capture the gradual changes in those dynamics across the growing season because of rudimentary knowledge of the coordination between stem tissue formation and whole tree function (Figure 3). Seasonal stem growth as measured by dendrometers reflects the formation of xylem and phloem tissue. Both tissues originate from cambium cell division, but xylem mother cells divide more compared with phloem mother cells, which explains the narrower phloem than xylem ring [57]. In addition to a slower growth rate, the timing of differentiation also differs, with phloem growth peaking before xylem growth [58]. Although longer-term phloem information is absent because sieve cells are functional for only 1-2 years and collapse afterwards, the phloem to xylem ratio increases with decreasing tree vitality [59] and with the level of environmental stress [60], indicating that higher priority is given to phloem tissue formation. As phloem growth becomes an important fraction of total stem growth under stress conditions, which affects the interpretation of stem diameter measurements, simultaneous investigation of xylem and phloem is imperative for us to understand seasonal stem growth.

Both water and carbon will shape the seasonal growth trend as well as the resulting stem anatomy and density, which, in turn, will influence xylem hydraulic conductivity and cavitation vulnerability [61]. The sub-processes shaping these anatomical variations within a tree ring are the rate and duration of cell enlargement and cell wall thickening [62]. Interestingly, however, individual model simulations for different days predict an exponential decrease in cell wall extensibility over time during the growing season [63-65], which is supported by some observations [66] and is expected to result in a lower growth rate potential. Here we present two alternative – but not mutually exclusive – reasons for this decrease in cell wall extensibility. First, the decrease in cell wall extensibility matches with the reduction in duration of cell enlargement during the growing season: from 10-20 days early in the growth season to less than five days later in the season [62, 67, 68]. It also agrees with the production of smaller cells near the end of growth (Figure 3C). Because cell size contributes more than rate of wall deposition to cell wall thickness and density [62], the reduction in cell wall extensibility and, thus, cell enlargement duration, determines a great part of the ring's morphology. Under stress conditions, this becomes critical: smaller cells with thicker walls and higher density will be formed, representing a compromise between efficiency and safety of the conducting system (Figure 3). Under chronic stress conditions [69] as well as in declining trees [70], wood with lower density and thus reduced hydraulic safety has been observed, which can be explained by carbon limitation on top of water limitation during cell wall formation [61]. Second, cell wall extensibility decreases with increasing auxin concentrations in the cambium [71]. With the onset of shoot development in spring, the production of auxin in shoots and transport downward through the cambium [72] increases auxin concentrations with time and also creates auxin gradients within trees [73]. It seems that high auxin concentrations accelerate cell differentiation and thus reduce the period available for cell enlargement [74, 75], as indicated by the smaller sizes of the conduit cells close to the source of auxin (in the shoots) [76], or in branch junctions where two auxin flows unite [77]. The gradually increasing auxin concentrations in the cambium during the growing season may thus accelerate cell differentiation and reduce cell wall extensibility over time, finally resulting in the cessation of growth. Here, we speculate that implementation of a mechanism of cell wall extensibility versus cell age trends, which originates from the fundamental interdependencies between ecophysiology and anatomy, may greatly improve plant models to simulate seasonal variation in stem growth during the season. Hormonal regulation might be required for simulating wood anatomy and stem growth patterns over branches [78] and whole tree bodies [79].

Concluding remarks

Radial stem growth, and its ecological implications, has been studied by scientists from rather separate scientific domains (anatomy, ecophysiology, dendrochronology, ecology), and we still lack an integrative and tested theory to understand the causes and consequences of diel stem growth patterns. Such a theory is required to understand diel and seasonal growth patterns in trees and, in turn, the long-term trends in stem growth as impacted by climate. One major gap in our knowledge is the quantification of diel dynamics in carbon within the stem. A second major gap is how those carbon dynamics interact with dynamics in water, for example, how stems coordinate embolism repair and cell turgor and, in turn, stem growth. A third major gap is the poor understanding of the seasonal variation in stem growth patterns. We therefore propose to combine the methodologies for studying water dynamics, carbon dynamics and (anatomical) stem growth within the same study. This will allow us for the first time to monitor the dynamics in carbon, water, and stem anatomy and diameter simultaneously. In turn, this will enable setting crucial parameter values and testing of mechanistic plant models for their emergent patterns in those dynamics. We are confident that such a joint effort from separate scientific domains will contribute to building and testing an integrated theory on causes of diel and seasonal patterns in stem growth. Such insights are much needed for predicting the impact of a changing climate on stem growth, with major implications for the well-being of trees and forests under global change.

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Box 1. Material list for quantifying water dynamics within stems

Integrative experiments with new technology and methods (Figure 1) can capture diel water dynamics within tree stem across the season under field conditions.

Sap flow sensor

Sap flow sensors measure sap flow rate (g h^{-1}) or sap flux density (cm^3 cm^{-2} h^{-1}), and allows quantification of whole-tree water use without altering the transpiration conditions. Many methods have been developed (see reviews by [80, 81]), and these use heat to sense sap movement in the stem xylem. Accurate estimates of sap flow are critical when assessing water transport and storage dynamics, but also when estimating xylem CO_2 transport in trees, or for understanding diel dynamics in stem CO_2 concentration and stem CO_2 efflux.

Sapwood water content sensor

Sapwood water content sensors measure sapwood water content (m^3 m^{-3} or kg water (kg dry weight)$^{-1}$), which is considered as critical component in the whole-tree water balance because of its direct link to changes in internal water storage. These techniques for assessing sapwood water content, such as frequency domain reflectometry [82] and Sapflow+ sensors [83] are new and promising, but still under further development.

Stem psychrometer

Stem psychrometers measure stem water potential (MPa), which is a robust and direct indicator of the plant water status, and expresses the tension (negative values) along the continuous water column in the xylem, typically pulling the water upwards in the tree. Concurrent measurements of stem water potential and sapwood water content would allow us to explore, for the first time, *in situ* the diel dynamics in hydraulic capacitance (C [kg m^{-3}

MPa^{-1}), quantified as the amount of water that can be released from living tissues into the transpiration stream for a unit decrease in water potential [84].

Acoustic emission sensor

Acoustic emission sensors measure acoustic emissions (AE), which are linked to cavitation events, when gas nanobubbles expand and form embolisms [14], typically triggered by high xylem water tensions. Cumulative AEs may be used to estimate relative loss of hydraulic conductivity in the xylem [18].

Point dendrometer and linear variable displacement transducer

Point dendrometers and linear variable displacement transducers measure variations in stem diameter (μm) at high temporal resolution (e.g. 10 minute intervals). The sensor signal simultaneously displays the integrative result of: (1) irreversible radial xylem and phloem growth, (2) reversible shrinking and swelling of the living stem cells due to changes in internally stored water, (3) contraction and expansion of dead conducting xylem elements due to the increase and relaxation of internal tensions, and (4) thermal expansion and contraction of the stem [32]. When interpreting stem diameter measurements, it is important to consider that phloem tissue degrades over time with the inherent information disappearing over time. Tracking variations in stem diameter with and without phloem tissue has been suggested as a promising approach to study xylem-phloem interactions and phloem turgor without damage [22].

Box 2. Material list for quantifying carbon dynamics within stems

Solid state non dispersive infrared (NDIR) sensor

Solid state non dispersive infrared (NDIR) sensors measure the CO_2 concentration of gas (%) in holes drilled into the stem (high concentrations, range <1 to over 26%; [39]). Using Henry's law, it is possible to convert measured CO_2 concentrations in the gaseous phase ([CO_2], %) to the amount of CO_2 dissolved in xylem sap ([CO_2*], mol l^{-1}, [85]).

Stem cuvette connected to infrared gas analyzer (IRGA)

A stem cuvette connected to an infrared gas analyzer (IRGA) measures the net flux of CO_2 diffusing out of the stem section into the atmosphere (μmol m^{-2} s^{-1}). The CO_2 is produced by the living cells of xylem, cambium and phloem, or is imported in the transpiration stream (Figure 2). Stem cuvettes are typically dark to exclude local woody tissue photosynthesis.

Aphid stylet

Aphid stylets are used to collect phloem sap for analysis of osmotic pressure and sap sugar concentration, and when a cell pressure probe is glued to an exuding stylet of an aphid feeding from the phloem, turgor pressure can be monitored [86, 87].

Pulse labeling of trees with stable or radioactive carbon isotopes

The carbon isotope (atoms of an element with the same atomic number, but with different atomic masses) content of assimilated carbon is artificially altered using stable (^{13}C) or radioactive (^{14}C and ^{11}C) CO_2 as short pulses over longer periods, and the fate of labeled CO_2 into the tree and its release to the atmosphere is traced to quantify carbon allocation in trees and assess its role in stem growth. Methods and associated challenges are reviewed in [88].

Micro-borer

The micro-borer is a tool used to extract stem micro-cores from the tree stem to investigate cambial activity, which cannot be directly observed or measured from outside the stem. Repeated sampling of newly formed xylem and phloem allows quantifying the temporal dynamics of stem anatomy and formation during the growing season (Figure 3). To minimize damage, stem samples are extracted as small cores with a Trephor micro-borer [89]. (For instruction movie, see: http://www.wageningenur.nl/en/Expertise-Services/Chair-groups/Environmental-Sciences/Forest-Ecology-and-Forest-Management-Group/Show/Microcore-Processing-instruction-film-launched.htm)

Figure captions

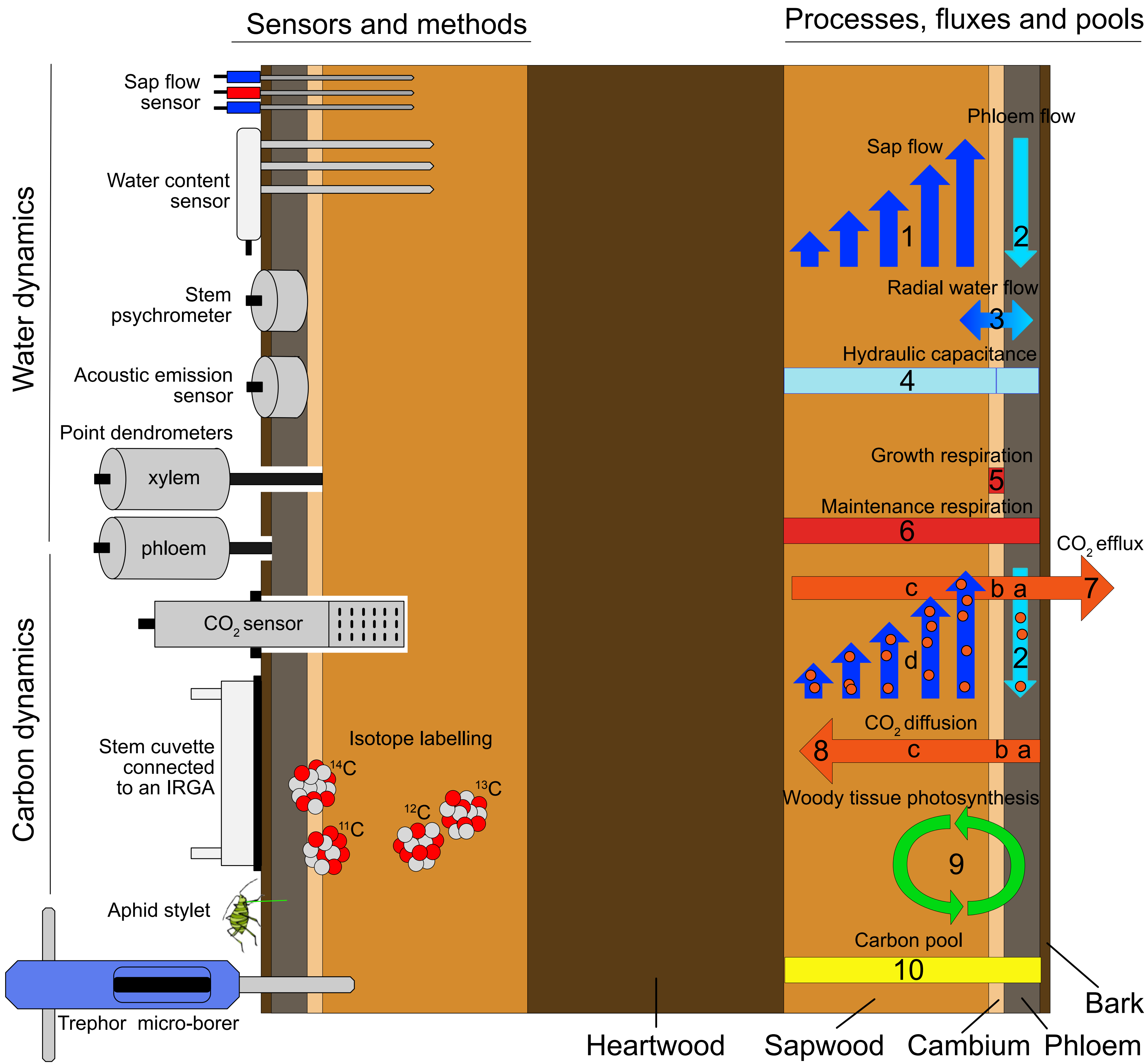
Figure 1. Schematic of important processes, fluxes and pools of water and carbon inside a stem segment of a tree (right): (1) sap flow in the xylem (or transpiration stream), transporting part of the dissolved CO_2 (7d); (2) phloem sap flow, transporting sugars and dissolved CO_2; (3) radial exchange of internally stored water between living cells in xylem and phloem, and the transpiration stream; (4) hydraulic capacitance, defining the capacity of living cells to store water and release it into the transpiration stream; (5) growth respiration; (6) maintenance respiration; (7) diffusion of CO_2 out of the stem from phloem (a), cambium (b) and xylem ray cells (c); or imported in xylem sap (d); (8) CO_2 diffusing into the transpiration stream from phloem (a), cambium (b) or xylem ray cells (c); (9) axial CO_2 diffusion along air-filled spaces in the wood; (10) CO_2 fixation by woody tissue photosynthesis, which can utilize CO_2 from all four sources (a, b, c, d) above; (11) carbon pool, which consists of recently assimilated sugars transported in the phloem (2), locally refixed CO_2 in photosynthetic tissue (10), and local starch reserves (modified after [39, 40]). Details of the technology and methods used for quantifying the diel dynamics in water, carbon and stem growth (left) can be found in Box 1 and 2.

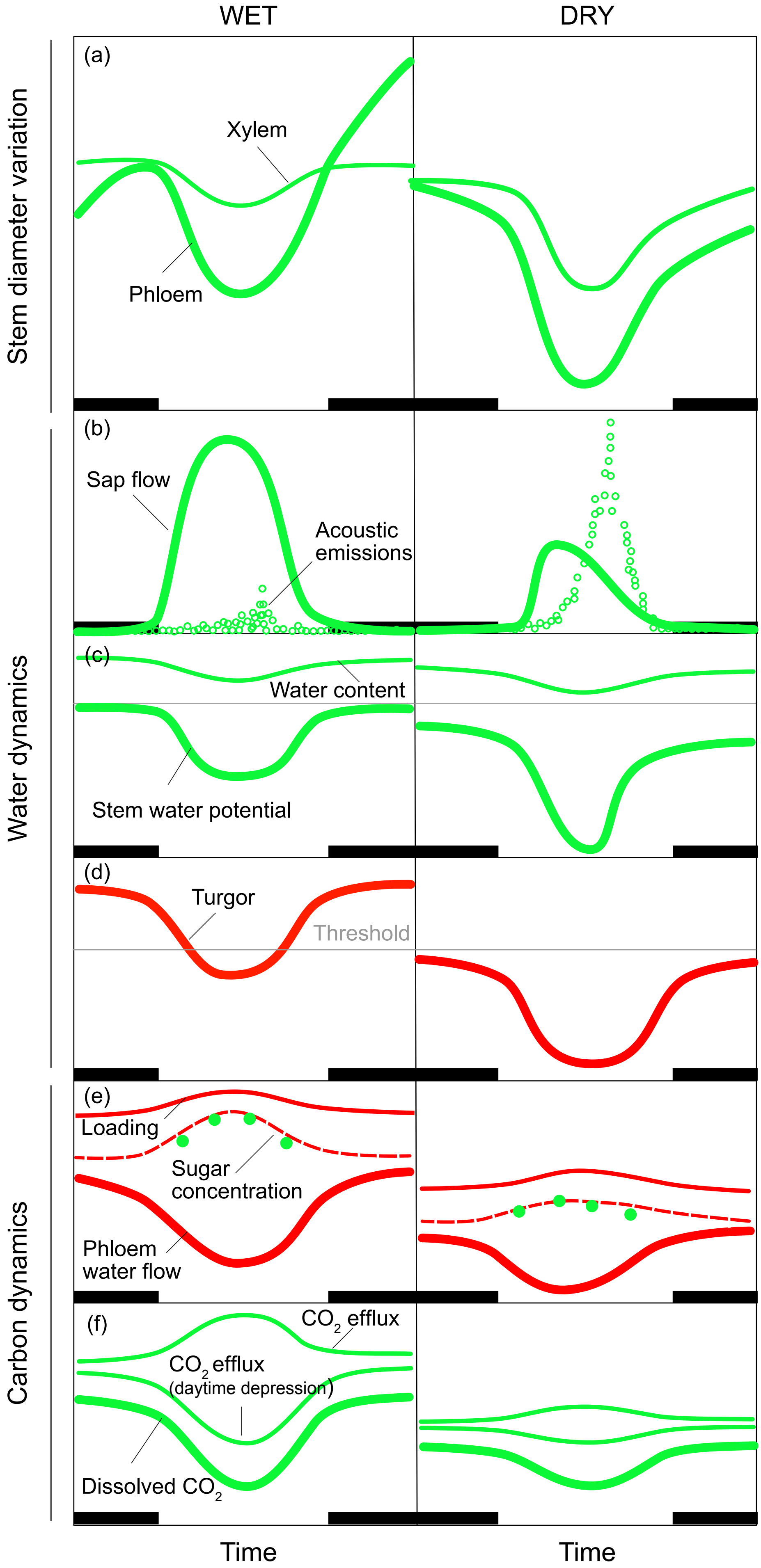
Figure 2. Diel patterns in water and carbon dynamics for a fully exposed canopy tree during a sunny day in unstressed conditions with ample soil water reserves (left), and a sunny day in dry soil conditions (right). We distinguish between well-established patterns and more speculative patterns in green and red, respectively. Measured variations in stem diameter (A) integrate diel dynamics in water (B, C, D) and carbon (E, F). (a) Stem diameter variation measured on xylem and phloem (see Figure 1). (B) The line shows the sap flow in

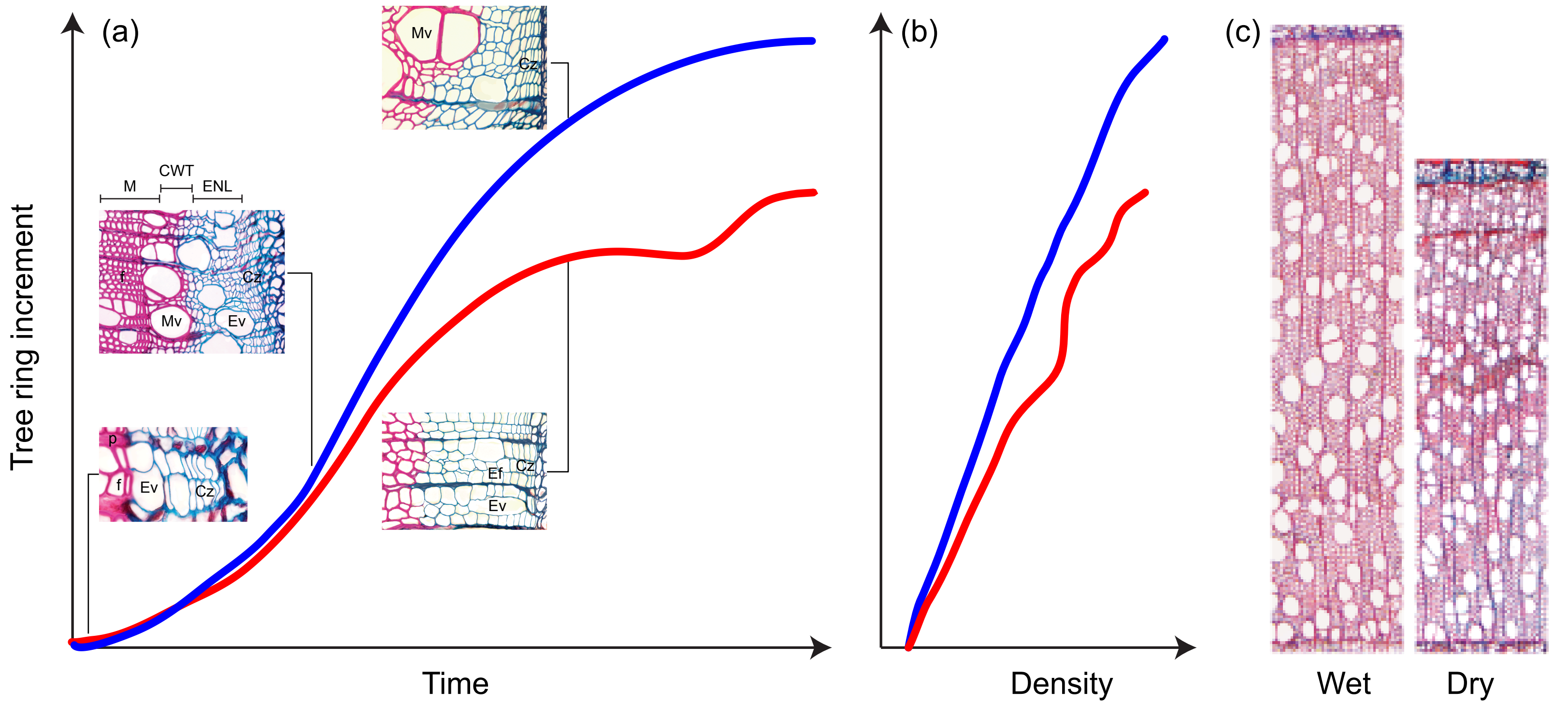
the sapwood, and the dots show the acoustic emission signals reflecting cavitation events, whenever gas nanobubbles expand and form embolisms. (C) Water content (positive values) and stem water potential (negative values). (D) Turgor pressure in the dividing cambium and expanding cells, and wall-yielding threshold value. (E) Sugar loading in the phloem, water flow rate in the phloem, and sugar concentration (dashed line indicates model simulation and green dots indicate measurements). (F) Two possible patterns for stem CO_2 efflux (see explanation in the text), and dissolved CO_2 in the xylem sap. Details of the technology and methods used to measure the diel patterns in water, carbon and stem growth (green) can be found in Box 1 and 2 and are illustrated in Figure 1. The scale of the y-axis is virtual, covering the range of values of each variable.

Figure 3. Seasonal growth patterns in expanding tree rings of *Populus × canadensis* trees in a temperate environment, for a tree on a soil with ample water reserves during the whole growing season (blue lines in A, B) and another tree exposed to dry soil conditions in the middle of the growing season (red lines in A, B). Stem samples of the micro-sections color reddish (after adding safranin) when cell walls with lignin had established, or color blue (after adding astrablue) in the absence of lignin and when still expanding. (A) Seasonal patterns in radial stem growth as measured with point dendrometers (without the diel fluctuation). Snapshots of possible co-occurring wood formation processes at some key moments during the growing season are shown. Wood formation resulted from cell division in the cambial zone (Cz) and cell enlargement (ENL), whereas cell wall thickening and lignification (CWT) happened afterwards to form secondary cell walls, ultimately shaping tree-ring width and anatomy. At the same time, the cambial zone (Cz) also produces sieve

cells of phloem (Ph). During spring, when cambium division just started, the first rows of enlarging cells appeared which eventually differentiated into vessels (Mv), fibers (f) or parenchyma (p). Finally, the cambium stopped producing new cells in both trees. During dry conditions, a lower number of smaller fibers (Ef) and smaller vessels (Ev) were formed due to the impacts of a lower turgor on cell division and cell expansion. As a result, tree-ring width and anatomy reflect plastics adjustments to best fit the environmental conditions when the stem was formed (B, C): the drought-exposed tree showed a narrower tree ring with higher wood density during drought, which generally reflects a lower hydraulic conductivity but a safer transport system.


Sensors and methods
Processes, fluxes and pools
Water dynamics
Carbon dynamics
Sap flow sensor
Water content sensor
Stem psychrometer
Acoustic emission sensor
Point dendrometers
xylem
phloem
CO_2 sensor
Stem cuvette connected to an IRGA
Aphid stylet
Trephor micro-borer
Isotope labelling
^{14}C
^{13}C
^{12}C
^{11}C
Phloem flow
Sap flow
1
2
Radial water flow
3
Hydraulic capacitance
4
Growth respiration
5
Maintenance respiration
6
CO_2 efflux
c
b a
7
d
2
CO_2 diffusion
8
c
b a
Woody tissue photosynthesis
9
Carbon pool
10
Bark
Heartwood
Sapwood
Cambium
Phloem


WET
DRY
Stem diameter variation
(a)
Xylem
Phloem
Water dynamics
(b)
Sap flow
Acoustic emissions
(c)
Water content
Stem water potential
(d)
Turgor
Threshold
Carbon dynamics
(e)
Loading
Sugar concentration
Phloem water flow
(f)
CO_2 efflux
CO_2 efflux (daytime depression)
Dissolved CO_2
Time
Time


(a)
(b)
(c)
Tree ring increment
Time
Density
Wet
Dry
M
CWT
ENL
Mv
Cz
Ev
f
p
Ef