New Phytologist

Meetings

A crucial phase in plants – it's a gas, gas, gas!

International online meeting on 'Plant Pneumatics', Ulm University and the University of Natural Resources and Life Sciences (BOKU), Ulm (Germany) and Vienna (Austria), 22 April and 29 September 2021

Like all organisms, plants are heterogeneous porous media, which means that they are a collection of solid matter, with plenty of open spaces in and around the solids. The spaces vary from nano- to macro-scale openings, and enable liquids and gasses to pass through or around them. Most plants also grow in a porous medium (the soil), and are embedded in a free flow domain (the atmosphere), representing a soil–plant–atmosphere continuum (Fig. 1). Transport processes within such unsaturated media are difficult to examine due to the complex and nonlinear nature of multiphase interactions between the gas, liquid, and solid phases. While the liquid phase has received considerable attention in research on plant water relations, especially long-distance transport of water through xylem (Venturas *et al.*, 2017), the gas phase is at least equally important and omnipresent along the entire hydraulic pathway (Gartner *et al.*, 2004). Yet, phase changes between liquid, water vapour, and gas media are not only challenging to the understanding of transport of xylem sap, but are also crucial for fundamental processes related to photosynthesis and respiration. Indeed, plants have been known for many years to show pneumatic properties, with gas flow between internal structures and the atmosphere determining key aspects of plant fitness and evolution (e.g. Hales, 1727; MacDougal, 1932, 1936).

A new series of online meetings has been initiated to address questions related to plant pneumatics, with the aim of bringing together a diverse group of plant scientists world-wide. The first online meeting, which was hosted by Steven Jansen on 22 April 2021, explored potential interest in xylem pneumatic measurements, while Norbert Kunert chaired the second meeting on 29 September 2021, with the aim of broadening the scope to gas-related research in plant biology. Here, we briefly report why the gas phase needs more attention, and how this may facilitate collaboration, discussion and communication across many subdisciplines of plant biology, aiming to broaden researchers' views on the multiple aspects of gases in plants.

Pneumatics in research on plant water transport

The recent meetings were motivated by the implementation of Pneumatron devices, which are automated tools for extracting and quantifying gas from plants, and their invention has created new ways for studying the gas phase in plants. These instruments were initially designed to take manual discharge measurements of gas created by embolism in conduits of dehydrating xylem tissue (Pereira *et al.*, 2016; Bittencourt *et al.*, 2018). By making measuring steps fast and automated, Pneumatrons have been shown to provide a fast and easy approach to directly measuring embolism resistance in xylem (Pereira *et al.*, 2020a; Trabi *et al.*, 2021). A first important point that was discussed in detail by Roman Link (University of Würzburg, Germany) highlighted the need for a robust metrological framework for comparing mismatches between methods, including pneumatic, hydraulic, visual, or acoustic quantification of embolism. A second crucial topic includes the normalisation of pneumatic data for vulnerability curves, which requires stable values of the minimum and maximum gas amount (Pereira *et al.*, 2021), as also suggested but not achieved

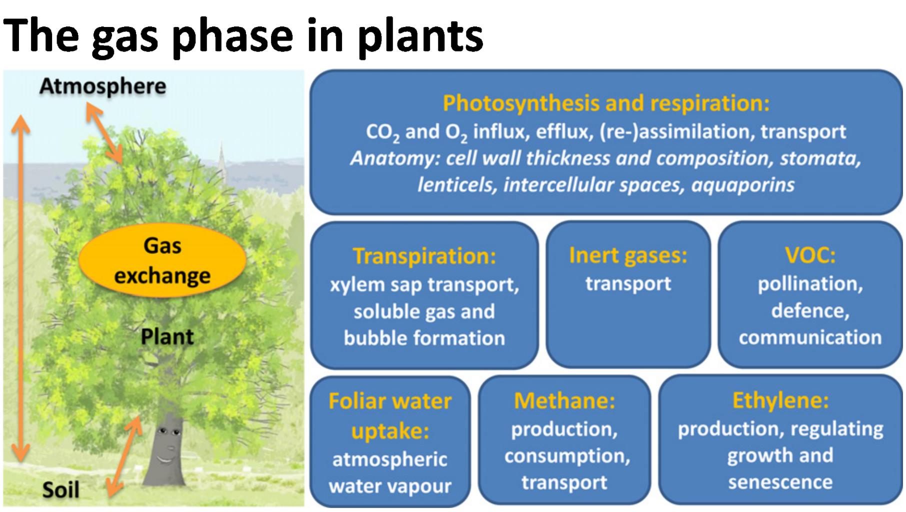

Fig. 1 Overview of the gas phase in plants, summarizing gas exchange within a soil–plant–atmosphere continuum. VOC, volatile organic compounds.

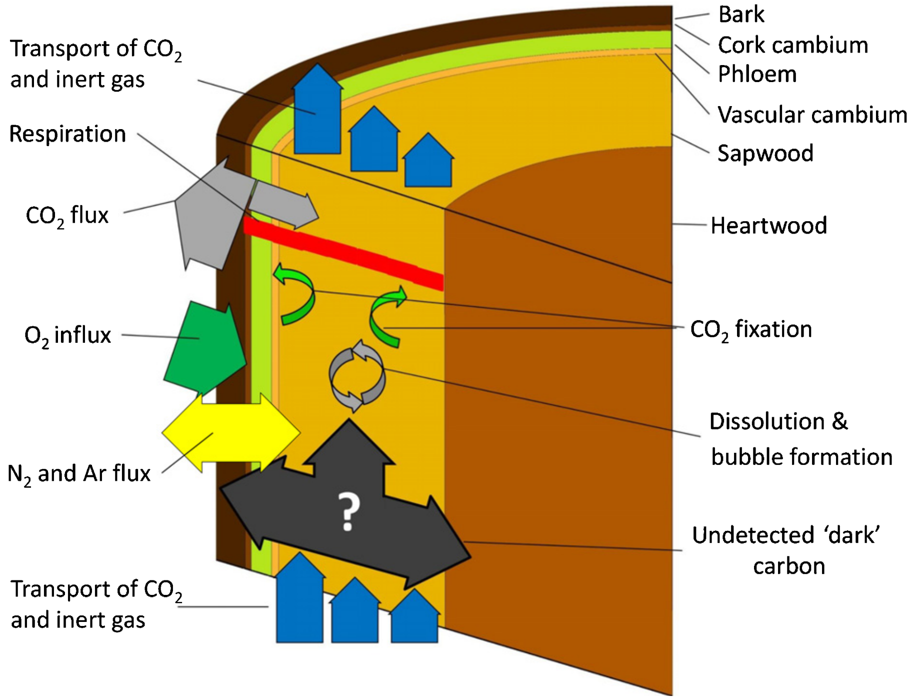

Fig. 2 Simplified drawing of CO_2, O_2, and inert gas sources, sinks, and transport processes in a tree stem. The red stripe indicates the region in which tree autotrophic respiration (i.e. growth respiration or maintenance respiration) occurs. CO_2 fixation can be through bark photosynthesis, or through enzymes in the sap wood, and CO_2 can be transported acropetally (blue arrows) in gas form or dissolved in xylem sap. The black arrows with a question mark represent the 'dark carbon', which could not be detected in the labelling experiment of Salomón *et al*. (2021). Image adapted from Trumbore *et al*. (2013).

by Chen *et al.* (2021). Hydraulic vulnerability curves face similar challenges of data normalisation, and the main difference is that the reference points are measured in a reverse order, with the maximum amount of gas extracted from embolised conduits at the end, and the maximum hydraulic conductivity measured at the beginning of a dehydration experiment. Therefore, the R package PNEUMA, which will soon be made available by Paulo Bittencourt, will be most useful. This script will allow Pneumatron users to test, in an objective way, the stability of the minimum and maximum gas volumes extracted in dehydration experiments.

The two online meetings made clear that there is a serious need to connect experimental data with anatomical characteristics and pneumatic models of gas flow through conduits, gas diffusion kinetics through interconduit pit membranes, and conduit walls, in addition to other cells and organs such as bark tissue and leaves. Modelling, for instance, may allow us to estimate which gas resources contribute to gas extraction measurements, and how their flow contribution changes over time (Yang *et al.*, 2021). Sharath Paligi (University of Göttingen, Germany) and Roman Link demonstrated in their presentation high agreement between xylem embolism resistance data obtained using the Pneumatron and flow-centrifuge methods, especially when gas extraction was limited to 16 s, which is in line with a Unit Pipe Pneumatic model (Paligi *et al.*, 2021; Yang *et al.*, 2021). Moreover, Luciano Pereira (Ulm University, Germany) demonstrated that gas extraction experiments over longer time intervals of 2.5 min were useful for the prediction of the amount of gas dissolved in xylem sap of well-irrigated plants of *Citrus sinensis.* The results presented by him suggested that gas concentrations in sap were frequently higher than what would be predicted based on Henry's law, and that soluble gas concentrations showed diurnal patterns associated with changes in xylem water potential. Gas saturation and oversaturation of xylem sap has been shown previously (Schenk *et al.*, 2016), and raises the puzzling question of how exactly plants are able to transport oversaturated sap under negative pressure without the constant formation of embolism. Up to now, large-scale evaporation-driven transport devices such as synthetic trees are unable to accomplish this achievement without using completely degassed, pure water. More research attention should also be paid to the transport of gas and water across the mesoporous pit membranes (i.e. with pore diameter between 5 and 50 nm) between water conducting cells. The likelihood that conduits show pit membranes with relatively large pore constrictions was shown to decrease exponentially with increasing pit membrane thickness (Kaack *et al.*, 2021).

Pneumatron devices can easily be modified, depending on the experimental requirements, and may serve various purposes. Instead of injecting gas into conduits under positive pressure (e.g. Cohen *et al.*, 2003), fast and easy extraction of gas from cut-open vessels (i.e. vessels that have no end wall within a stem segment of given length) has resulted in a pneumatic method for the study of hydraulically weighted vessel length distributions (Pereira *et al.*, 2020b). The main assumption is that gas flow is much slower across hydrated pit membranes than vessel lumina. Although the modelling approach relies on random distribution of vessel ends, it would be interesting to see if the pneumatic method could improve our insights into intra-tree variation of vessel length, and nonrandom vessel distribution near nodes, side branches, or stem–leaf transitions. Such nonrandom vessel distribution has also been related to compartmentalisation, segmentation, and sectoriality (Guan *et al.*, 2021). We expect that Pneumatrons will open up new possibilities to examine flow pathways and resistances, both within and outside xylem. An interesting example of how micro electro-mechanical sensors (MEMS) can be used to detect embolism in plant xylem acoustically was presented by Dinko Oletić (Zagreb University, Croatia). By working on crops in the field, he uses low power data acquisition systems and *in situ* machine learning processing to evaluate acoustic emissions induced by drought stress, and is currently seeking collaboration to test and validate new acoustic emission instrumentation.

The gas phase in plants: beyond pneumatics

Pneumatics is the field of study that addresses the mechanical properties of gases, but there are many other exciting research questions about the gas phase in plants. One of the presentations in our online meetings that explored the gas phase beyond pneumatics was provided by Roberto Salomón (Universidad Politécnica de Madrid, Spain), who discussed the transport and the role of CO_2 in tree stems (Fig. 2). Based on $^{13}CO_2$ labelling in three species (maple, oak, and cedar), he showed that interspecific differences in efflux and assimilation of xylem-transported CO_2 could partly be explained by different radial CO_2 diffusivity, in addition to xylem anatomy (Salomón *et al.*, 2021). Despite the advances made in understanding the fate of xylem-transported CO_2, it is not known to what extent re-fixation of respired CO_2 might contribute to enhanced drought resistance of various species. Moreover, there was a large fraction of CO_2 labelled, the sink of which could not be detected ('dark carbon'), highlighting that the fate of CO_2 in stems remains puzzling.

Obviously, there is scope and growing interest in the gas phase in plants. Various exciting topics, which have not been discussed yet within the two online meetings, include, for instance (Fig. 1): the production, consumption, and transport of greenhouse gases such as methane in trees (Putkinen *et al.*, 2021); CO_2 conductance in leaves, and the functional link between conductance and cell wall anatomy (Flexas *et al.*, 2021); gas exchange in stems via lenticels; and the fate of gases in plant tissues and their relation to respiration and woody tissue photosynthesis (Teskey *et al.*, 2008). Moreover, oxygenation of plant tissues is especially relevant for aquatic plants, in which leaves are periodically or permanently immersed in water. Internal gas pressures may also play a role in tree biomechanics (Gartner *et al.*, 2004), while volatile organic compounds produced and emitted by plants in gaseous form may serve multiple functions (e.g. pollinator attraction, defence against herbivory, and communication with neighbouring plants) and regulate aerosol production. Bringing together these highly diverse research topics and fields may seem challenging and ambitious, but it could certainly be rewarding and will hopefully stimulate multidisciplinary collaboration.

Future meetings

Future online meetings on plant pneumatics and the gas phase in plants will be hosted on a voluntary basis. Anyone interested in gases in plant biology is welcome to attend these sessions, and we especially encourage early-career scientists to join. Presentations recorded during the second meeting are available online (https://short.boku.ac.at/kzrgd9). We are also pleased to note that a future conference on gas in plants at the Hyytiälä Forest Field Station in Finland is envisaged, with a preliminary date scheduled for 2023. Readers are welcome to contact the authors for more information or feedback. For now, we would like to remind them about the lyrics of The Rolling Stones' *Jumpin' Jack Flash*: 'but it's alright now, in fact it's a gas!'

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