Letters

Remaining uncertainties in the Pneumatic method

A reply to Brum *et al*. (2023) 'Reconciling discrepancies in measurements of vulnerability to xylem embolism with the Pneumatic method'

The integrity of the plant water transport system is crucial for plant performance and survival (Rowland *et al.*, 2015). To estimate the vulnerability of the water transport system to drought-induced embolism (blockage of water flow in conduits by air bubbles), a number of methods have been developed to generate vulnerability curves (VCs), that is, the relationship between xylem embolism level and water potential (Cochard *et al.*, 2013). However, since the report of the 'cutting-under-tension' artifact (Wheeler *et al.*, 2013), all published VCs may need to be re-examined, and researchers need to carefully avoid artifacts (Cochard *et al.*, 2013; Zhang & Holbrook, 2014). As a newly developed method, the Pneumatic method (the manual or the automated Pneumatron) measures air volume discharged from terminal shoots (Pereira *et al.*, 2016, 2020). This method assumes that the increase in air volume discharged during desiccation is mainly extracted from embolized conduits, and it is believed to be a non-hydraulic, economical, and less time-consuming method. This method also generates P_{50} (water potential causing 50% of embolism) values comparable to other methods in some studies (Pereira *et al.*, 2016; Bittencourt *et al.*, 2018; Zhang *et al.*, 2018; Pereira *et al.*, 2020; Paligi *et al.*, 2021; see summary in Brum *et al.*, 2023). All current methods have both advantages and limitations (Skelton & Diaz, 2020), and so does the Pneumatic method. We pointed out some potential uncertainties of the Pneumatic method in our early published study (Chen *et al.*, 2021) so that the method could be improved by solving these uncertainties. In an article published in this issue of *New Phytologist*, Brum *et al.* (2023; pp. 374–383), provided explanations on some issues we raised before, but we think some fundamental uncertainties remain and have not been well addressed.

Uncertainties in the maximum air volume discharged

The main criticism in the reply Letter by Brum *et al.* (2023) on our earlier study is that we incorrectly performed the experiment, that is we stopped the measurement too early, which resulted in an underestimated AD_{max} (maximum air volume discharged) and embolism resistance. Here, we claim that we presented both 'stop early' and 'stop late' curves in our previous paper, and we clearly pointed out that the stopping time would affect the AD_{max} estimation and the Pneumatic VCs, which we termed as the 'two-plateau' concern (see fig. 6 in Chen *et al.*, 2021, we define 'plateau' as the stage that extracted air volume maintains a stable value for a relatively long duration; see diagram in Fig. 1 here). Our concern is that using AD_{max} from plateau 1 would overestimate the vulnerability, whereas using AD_{max} from plateau 2 could underestimate the vulnerability due to uncertainties of air source in plateau 2. Furthermore, we believe that air volume discharged (AD) at plateau 2 not only represents the maximum dischargeable air volume from the vessels, but also includes other air sources, for example, tissue shrinkage, and cracks in the pith and xylem (see discussion). Therefore, it is not that we incorrectly measured the AD_{max}, but rather we separated the curves into two stages, pointed out the uncertainties, and provided some mechanistic explanations. Brum *et al.* (2023) misinterpreted the 'two-plateau' concern as 'incorrect implementation and an underestimation of AD_{max}' and they did not address it directly. Additionally, our new data obtained using the new automated Pneumatron (constructed following Pereira *et al.*, 2020; Trabi *et al.*, 2021; see Supporting Information Methods S1) agree with our previous data generated using the manual pneumatic device (Fig. S1), supporting our early concerns and showing that there were no technological biases in our earlier published study.

The 'two-plateau' pattern was first reported in our previous study (Chen *et al.*, 2021). Interestingly, now we found multi-plateaus (> 2) when the desiccation lasts long enough (*c.* > 8 d; Figs 2, S2–S6). The curves in our previous paper (fig. 6 in Chen *et al.*, 2021) clearly showed a 'two-plateau' pattern in AD during desiccation, that is, AD increased during desiccation, reached a stable value at a certain water potential (plateau 1), and then increased again and reached another stable value (plateau 2). Similarly, this 'two- or multi-plateau' pattern can also be found in Pneumatic VCs from other studies, including researchers who developed this method, for example, Pereira *et al.* (2020) and Paligi *et al.* (2021) (see reproduced Figs S7–S9). Notably, when combining different replicates in one figure (see Figs 1g, S7), the first plateau was easily masked due to the great variation among samples and relatively small AD values compared to the AD obtained from a completely dehydrated sample in the late stage. Brum *et al.* (2023) questioned the reliability of water potential measurements in our early paper as we used extremely low values estimated using the relationship between desiccation time and water potential. While these predicted extremely negative water potentials might not be as reliable as directly measured values, they would not change the 'multi-plateau' pattern (also see Methods S1).

The 'two- or multi-plateau' patterns suggest some fundamental uncertainties in the Pneumatic method, which should not be ignored and need to be addressed carefully. We hypothesized that the 'multi-plateau' patterns in Pneumatic VCs could be explained by the isolation of the cut end and distal part of the measured branch, which can be visually explained by the illustration here (Fig. 1). As explained in the gas dynamics model in Jansen *et al.* (2020) and Yang *et al.* (2022), the pneumatic apparatus only discharges air from cut-

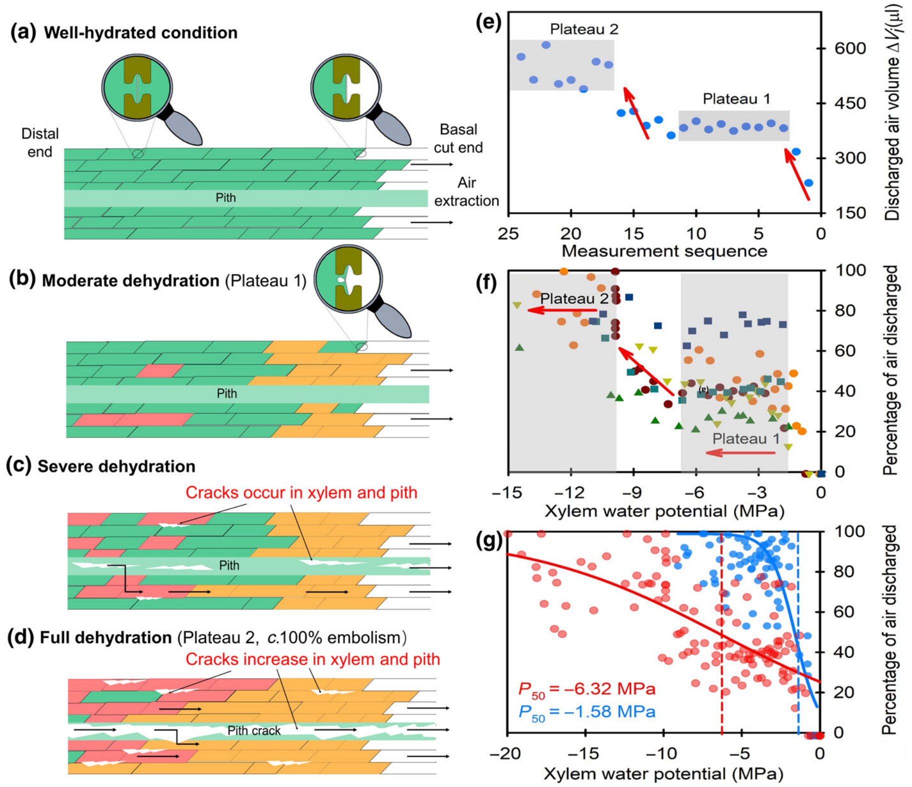

Fig. 1 A diagrammatic representation of air sources discharged from a stem and the spread of embolisms with the use of the Pneumatic method under conditions of well-hydrated (a), moderate dehydration (b), severe dehydration (c), and full dehydration (d) stages. The green color indicates functioning vessels and the red color indicates embolized vessels at the distal end of the stem, which are isolated from other embolized vessels by functioning vessels. The white and orange colors indicate cut-open vessels and intact embolized vessels neighboring the cut-open vessels at the basal end, respectively. Arrows indicate the direction of air extraction. The insets show the status of the pits connecting two adjacent vessels. This diagram refers to supporting information fig. S9 in Chen *et al*. (2021) and fig. S4 in Pereira *et al*. (2016). The right column (refers to fig. 6 in Chen *et al*., 2021) shows the kinetics of discharged air volume (ΔV_i, μl) (e) of one representative stem sample of *Lasiococca comberi* using the Pneumatic method; percentage of air discharged against xylem water potential for different stem segments (f); and comparison of the Pneumatic vulnerability curves using the maximum air volume discharged at plateaus 1 (blue) and 2 (red) (g), respectively, in *L. comberi*. See detailed descriptions of the method in Chen *et al*. (2021).

open and the neighboring embolized vessels, while the embolized vessels isolated by water-filled vessels in the distal end are excluded. Thus, the initial stage only measures the part close to the cut end because the gas in cavitated vessels of the distal end could not be extracted by the pneumatic apparatus. The first plateau represents the full embolism status for the section close to the cut end (Fig. 1b), which could be more prone to embolism due to their proximity to cut-open vessels (see discussion about *open vessel artifact* later). Then, the air discharged starts to increase again when an embolized vessel connects the distal part to the cut end (Fig. 1e), and reaches the second (or third) plateau when most conduits in the distal part embolize (Fig. 1d). Importantly, the AD_{max} is hypothesized to include all vessels both in the cut end and in the distal end, and likely include other air sources (see discussion). Thus, at the initial stage, the calculated percentage increase in air discharged (PAD) value undoubtedly underestimates the embolism level (Fig. 1d). Although we used the first plateau to generate AD_{max}, we also clearly pointed out in our previous paper that using the second plateau would generate a lower and relatively more reasonable P_{50} if the air discharged was only from the embolized vessels (Fig. 1g, also see the Uncertainties in the air sources section).

Uncertainties in the criterion for stopping time

While we agree with Brum *et al.* (2023) that the stopping time is essential for the AD_{max} determination in the Pneumatic method, a definite, quantitative, and easy-to-follow criterion for the stopping time has not been established even now. The desiccation degree of leaves was an important indicator for stopping the experiment in most published studies using the Pneumatic method. For example, Pereira *et al.* (2016) initially described the stopping time as 'when the Ψ_x was lower than the maximum limit of the pressure chamber (−10 MPa) or when the branches had lost their leaves'. In our previous study, we followed the criterion that 'leaves were severely desiccated, crispy, and started to shed' and 'the AD value stopped increasing or started to decrease' (Pereira *et al.*, 2016; Zhang *et al.*, 2018; Trabi *et al.*, 2021), which occurred at water potentials of −2 to −7 MPa (plateau 1). However, when we let samples continue to dehydrate (> 8 d), we found occurrence of a second and third plateaus in many samples (Figs 2, S2–S6). Bittencourt *et al.* (2018) defined stopping time as 'the last data point for a branch sample should be one where it is fully embolized'. Paligi *et al.* (2021) defined stopping time as 'when discharged air volume maintain stable within 24 h' and Trabi *et al.* (2021) defined stopping time as 'measurements can be stopped when the branches are completely dehydrated'. Generally, these descriptions for stopping time have uncertainties and are hard to follow.

First, rare previous studies took long enough time, for example > 8 d as we did here (Figs 2, S2–S6), and plant water potentials at the stopping point were generally > −10 MPa in both the manual Pneumatic method and automated Pneumatron measurements (Pereira *et al.*, 2016; Bittencourt *et al.*, 2018; Zhang *et al.*, 2018; Paligi *et al.*, 2021; Trabi *et al.*, 2021; see fig. 3 in Brum *et al.*, 2023). These durations or water potentials used for AD_{max} determination generally are close to the second plateau but may not be enough for reaching the final stable stage of AD (Figs 2, S2–S6).

Second, the first (or second) plateau could be long or short and varies among species or samples (Figs 2, S2–S6), which is probably associated with plant anatomy and needs further study. In the case that the first plateau covers a wide range of water potentials and lasts for a long time (e.g. > 2 d or even longer in our study; see summary in Table S1 and Figs S2–S6), the first plateau can easily be interpreted as the final stage for AD_{max} as most pressure chambers could not measure accurately for water potentials lower than −7 MPa (range varies from −4 to −10 MPa for most commercial pressure chamber equipment). This uncertainty is easy to happen if we follow the initial protocol in Pereira *et al.* (2016), which was qualitatively described as 'AD_{max}, AD value obtained when the branch was at its most dehydrated status (lowest Ψ_x)'. This brought an essential uncertainty to the methodology. By following the recent standard protocol (Trabi *et al.*, 2021; and also Brum *et al.*, 2023), researchers could also stop doing further measurements when a stable AD_{max} has been reached and maintained stable. A plateau from −2 to −7 MPa (Fig. 1e) could be defined as being stable. Furthermore, we could not exclude the possibility that the VCs in other studies only reached plateau 1 due to difficulties in measuring low water potentials, especially for highly resistant species or those drying rather slowly. In other words, some published VCs may not reach the second plateau.

Third, because no current technique can accurately quantify the embolism level in whole branch samples during the experiment *in vivo*, it is likely that researchers have to arbitrarily decide the timing for stopping the measurement. Obviously, it is unknown whether samples have reached 100% embolism or not in previous studies following these criteria, suggesting the possibility of insufficient air discharged and thus underestimated AD_{max} in these studies as well. We believe that it is very likely that a third plateau would occur in most plants in their studies as long as the desiccation durations are maintained for long enough. This can be tested using a visualization technique (e.g. micro-CT) to check whether vessels are fully embolized before stopping the measurement.

Brum *et al.* (2023) used a criterion that 'the total cumulative volume of air discharged reached a saturation level and air

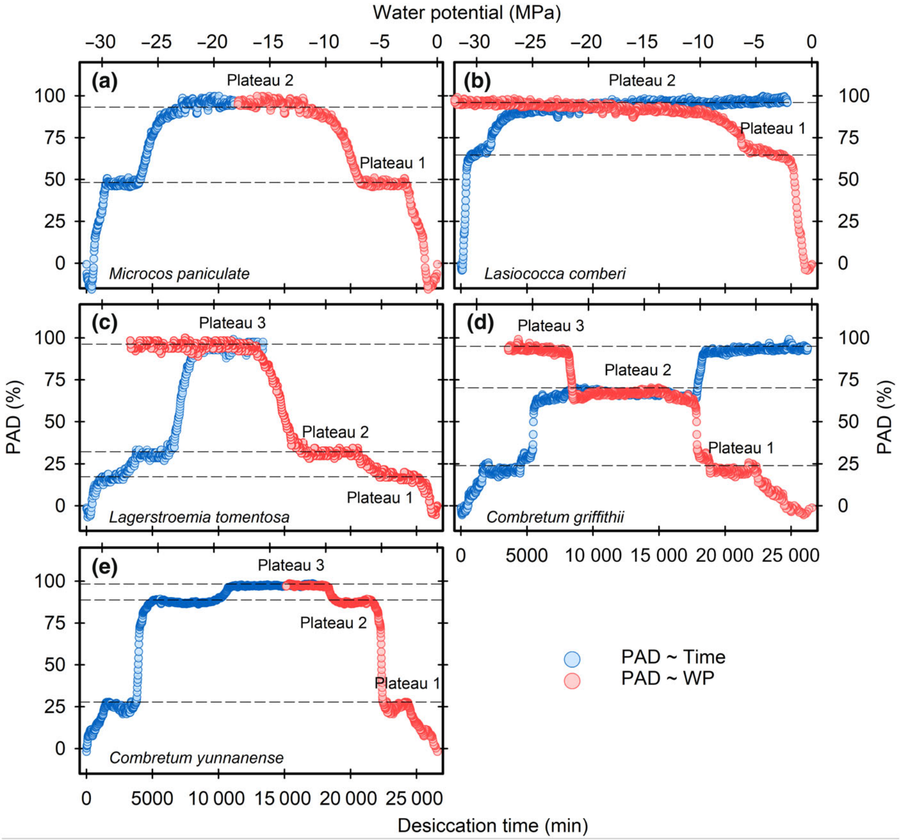

Fig. 2 The dynamic changes in the percentage increase in air discharged (PAD, %) during the desiccation process for five different tropical species. One representative sample per species is shown here (see data for all replicates in Supporting Information Figs S2–S6). Species names are listed in the figure. Blue and red symbols represent changes in PAD value against the desiccation time and water potentials (WP), respectively. Different plateaus are indicated by dashed lines.

discharges no longer increased with further reductions in branch water potential' and 'reach the limit of water potential measurement device'. However, our data clearly showed that the AD value in many samples started to increase again after a relatively long period of stable AD (plateau 2), which typically took several days and much longer than in their previous studies (Figs S2–S6). At this stage, the plant water potential already declined below −10 MPa, and the plant showed obvious shrinkage and severe dehydration in appearance. This should be defined as the stopping point following their stopping rules. An increasing AD in the stopping point was also found in Pereira *et al.* (2020) (Fig. S7). Based on a model, Brum *et al.* (2023) provided a paradoxical suggestion as 'the measurement should continue to the water potential of twice of the P_{50}'. Because users may not have reliable VCs from other methods, it is impractical to follow this suggestion. On the contrary, given that there are reliable VCs from other methods, there would be no need to construct new curves using the Pneumatic method.

Uncertainties in the air sources

Brum *et al.* (2023) claimed that woody plants would not have shrinkage, cracks, or leaky pith tissue, and even if cracks occur, they would not be connected to xylem conduits. Thus, they concluded that these issues would not be common problems for woody tree species. We disagree with this point as our micro-CT images clearly showed that they occurred in many woody plants and could connect xylem conduits.

We agree with Brum *et al.* (2023) that the $\mathrm{AD}_{\max}$ is extremely important in the Pneumatic method. Meanwhile, users should be cautious because the total gas that can be extracted when samples are completely dry or reach 100% embolism does not necessarily equal to the total amount of gas in xylem conduits. In our previous study, we provided evidence that non-vessel air sources are likely to cause uncertainties in AD value. First, cracks in the xylem and connections between cracks and vessels did happen in some species in our study (Fig. 3; Video S1), challenging the assumption of Brum *et al.* (2023) and Trabi *et al.* (2021) that 'if cracks due to tissue dehydration do not happen in the xylem area or are not connected to cavitated vessels, it would not influence the gas extraction'. We found cracks in one sample of *Combretum griffithii*, a liana species with big pith area, only after 24-h dehydration, and in all three samples both in the pith and xylem after 48 h (Fig. S10). This was also supported by our recent experiment, which found that 10 out of 20 studied species (Table S2) showed obvious cracks in xylems and piths in entire long branches after drying for over 60 h (water potentials declined to −5.51 to −9.41 MPa; Figs 3, S11; Video S1). Thus, we think cracks are very likely to happen in the xylem and pith under severe dehydration, especially for plants with large pith and abundant parenchyma tissues. The gas in these cracks would contaminate the discharged gas because they would be treated as air discharged from embolized vessels (Fig. 1d).

Brum *et al.* (2023) argued that the cracks detected (fig. 7 in Chen *et al.*, 2021) could be because short stem sections were used for micro-CT. However, long branches (> 2 m) were used for dehydration in our previous study. After dehydration, all micro-CT samples were cut from the long branches underwater, and then connected with water-filled tubing before micro-CT scanning. Meanwhile, the whole short segment was wrapped with parafilm to reduce dehydration during scanning (Chen *et al.*, 2021). Therefore, there is no reason to expect artificial cracks in sample preparation for micro-CT. Furthermore, both the pith and xylem at the cut end directly connect to the pneumatic apparatus, and are likely to be the first place to see cracks as they are exposed to dry air and thus dehydrate most quickly. Further experimental evidence to support that air sources from other parts contributed to the air discharge is that, when fully embolized stems (by quick air-

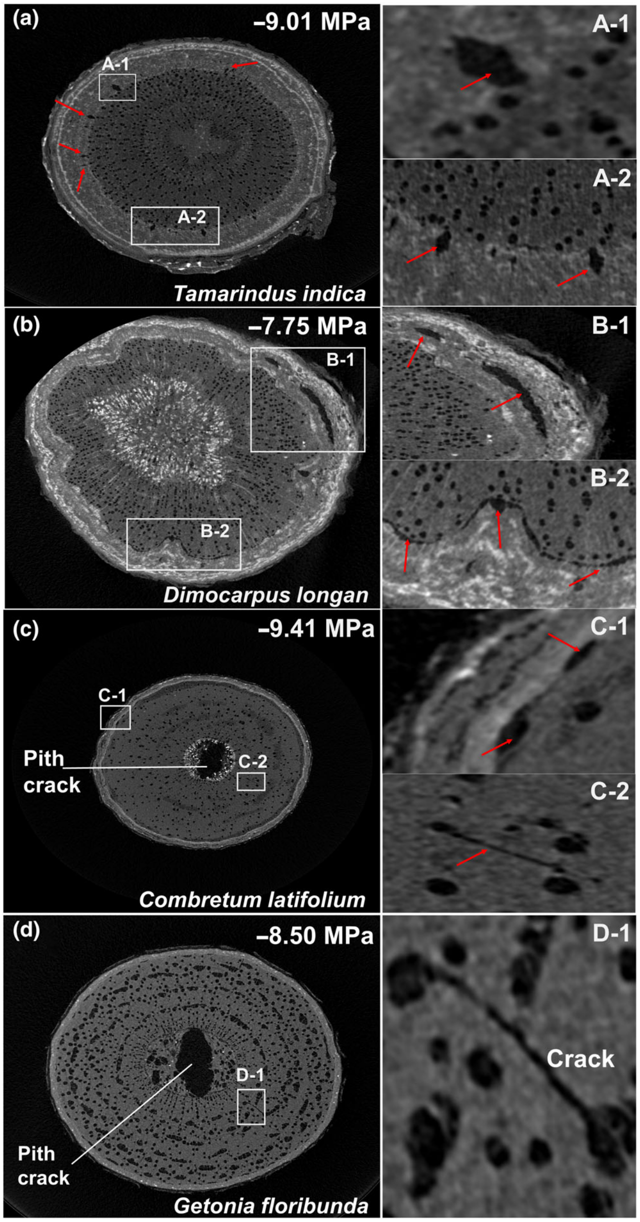

Fig. 3 The micro-CT images of stem cross-sections of four species (a–d) after drying for *c.* 60 h under laboratory conditions. Water potentials of the samples are shown in the figure. The subpanels in the right column (A1–D1) are magnified images cropped from the areas framed with white lines in the left column. Pith and xylem cracks are indicated with red arrows. See Supporting Information Table S1 and Methods S3 for a detailed description of species and methodology. See Fig. S11 for images of all 20 species.

injection) were exposed to desiccation, the AD can increase by 28–33% over time (Chen *et al.*, 2021). It was suggested to use fast glue to block the pith (Zhang *et al.*, 2018) to avoid leaking from the pith, but whether this treatment could stop the air leakage from the pith to the xylem at other positions rather than the cut end still needs to be tested.

Additionally, most published Pneumatic VCs showed declines in AD value during desiccation, which strongly supported our concerns about shrinkage. Therefore, the defined 'stable plateau of AD_{max}' is not very stable in many cases, and sometimes, an obvious decrease could happen (Figs S2–S6, S7, also see fig. 6 in Trabi *et al.*, 2021, and fig. 6a in Pereira *et al.*, 2020). This suggests that the AD value could be affected not only by gas in the embolized vessels, but also by other factors, such as tissue shrinkage as reported in our previous study. In our new experiment, we found that the xylem areas shrunk by 4.5–14.0%, and pith areas by 2.9–12.1%, while the change in the vessel lumen area varied among samples (Fig. S12). Furthermore, the contribution of different air sources to AD_{max} may vary across species. The extent to which this uncertainty may affect the generated VC parameters remains unclear. These uncertainties need to be solved and could not be avoided by simply paying 'careful attention to methods practices' as suggested by Brum *et al.* (2023).

Do cut-open vessels matter?

It is claimed that the Pneumatic method is not affected by the cut-open-vessel effect like other methods, and this is believed to be one of the advantages (e.g. 'Unlike sampling for hydraulic measurements, plant material can be cut in air because the conduits that are cut open need to be filled with air on purpose'; Trabi *et al.*, 2021). However, we think additional tests are still needed as many Pneumatic VCs are '*r*-shaped' and show a high increase in embolism level at high water potentials, in many cases > -1 MPa (see Figs S2–S6 and also in Pereira *et al.*, 2016; Zhang *et al.*, 2018; Brum *et al.*, 2023). When performing the pneumatic measurement, the vessels close to the cut-open vessels are directly in contact with outside air sources, and it is not clear whether they are more prone to air seeding and embolism. However, vessels in proximity to air sources (e.g. pre-existing embolism, cut-open vessels) are reported to be more prone to embolism (Brodersen *et al.*, 2013; Guan *et al.*, 2021). If this is true, cut-open vessels could result in an overestimation of the vulnerability, at least in the initial stage. For example, three of the four *Citrus sinensis* samples showed highly vulnerable curves at the initial stage in Pereira *et al.* (2020) (Fig. S8) and also in many samples in our study (Figs S2–S6).

Other methods, for example hydraulic measurement or micro-CT scanning after dehydration treatment, may also be prone to the same artifact as the cut end includes numerous cut-open vessels. However, these two methods could minimize the influence of this artifact using stem sections far away from the cut end (Torres-Ruiz *et al.*, 2015). By contrast, the Pneumatic method can only extract air from embolized vessels adjunct to the cut end initially (Jansen *et al.*, 2020; Pereira *et al.*, 2021; Yang *et al.*, 2022). Also, in some of the VCs, this artifact could be underestimated due to the calculation because the air discharged initially is from the cut end only (Fig. 1b), while the embolized vessels in the distal end are excluded. However, the AD_{max} includes all vessels both in the cut end and in the distal end (Fig. 1d). Thus, at the initial stage, the calculated PAD value undoubtedly underestimates the embolism level. Furthermore, because the diffusion speed of air through pit membranes is by orders of magnitude lower than the speed of bulk water flow, the relative importance of farther away vessels in the distal end depends on stem anatomy, such as pit membrane thickness, vessel connectivity, and so on. These traits are likely to differ considerably among species and thus may introduce species-specific biases in one or the other direction.

Because the Pneumatic method quantifies air from the intact and embolized conduits neighboring to the cut-open vessels at the sample end (Jansen *et al.*, 2020; Pereira *et al.*, 2021; Yang *et al.*, 2022), in theory, it can only generate a correct curve when embolism initiates from the cut end and spreads toward the distal end. Otherwise, it could underestimate the embolism by excluding embolism events at the distal end. However, our micro-CT images showed heterogeneity of embolism along the branch sample under given dehydration stages (See Methods S2), and the embolism level did not consistently decrease from the cut end to the distal end (Fig. S13).

Agreements between Pneumatic and other methods: correlation or causality?

Brum *et al.* (2023) did a synthesis and claimed that the P_{50} derived by the Pneumatic method and traditional reference methods showed general agreements (but diverged from the 1 : 1 line in some cases, see fig. S1 in Brum *et al.*, 2023). We still think that caution should be paid before we apply it to large-scale studies (also see Sergent *et al.*, 2021). The correlation in Brum *et al.* (2023) does not solve the uncertainties mentioned earlier, and some of the Pneumatic VCs could be misleading in terms of plant eco-physiological performance. For example, because of the overestimation of embolism at the early stage, some Pneumatic VCs behaved '*r*-shaped' and indicate cavitation initiation at rather high (less negative) water potentials (in many cases, less negative than -0.5 MPa, e.g. fig. 7 in Pereira *et al.*, 2016; figs 1, 2 in Zhang *et al.*, 2018; fig. S1 in Brum *et al.*, 2023). This overestimation of embolism in the early stage is also common in most samples in our recent data (Figs 2, S2–S6). Thus, it could not give us correct information on the initiation of the embolism, which suggests frequent embolism occurrence and recovery on a diurnal basis. By contrast, our new data confirmed the 'multi-plateau' and 'non-*s*-shaped' curves when the desiccation lasts long enough, which is rather misleading regarding how plants perform in the natural world.

Interestingly, both the VCs generated by the Pneumatic and the reference methods behaved as '*r*-shaped' curves for some species in Brum *et al.* (2023) and Pereira *et al.* (2016). Although it is possible that the plants studied are rather vulnerable to embolism, it is very unlikely that cavitation initiated at water potential less negative than -0.5 MPa. Thus, the reliability of the data used to validate the Pneumatic method needs to be re-verified as well. We also noticed

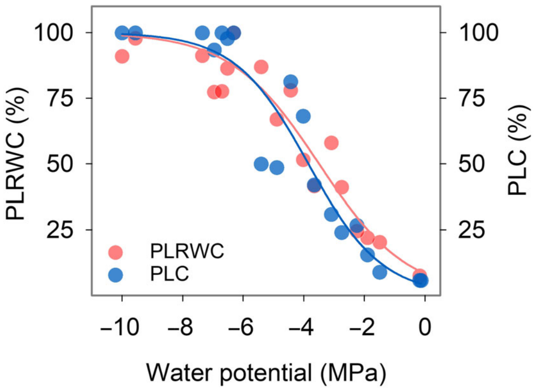

Fig. 4 The stem percentage loss of hydraulic conductivity (PLC; blue) and percentage loss of relative water content (PLRWC; red) in relation to water potentials of *Shorea assamica*. See Supporting Information Methods S3 for a detailed description of the method.

that when validating the Pneumatic method with other methods, data from different methods are often mixed, and some are collected from literature rather than comparing different methods using the same samples at the same time (e.g. Pereira *et al*., 2016; Zhang *et al*., 2018). This is important because our recent study revealed great variation among branch samples even within an individual (Shen *et al*., 2021). Therefore, we strongly recommend to use a more careful approach especially when the controversies on the VC methodology have not been completely settled, that is, comparing different methods using samples with less intraspecific variation, following the same protocol in a single study, and with artifacts carefully avoided.

Additionally, while discharging gas from the section in proximity to the cut end at the early stages overestimates the vulnerability, excluding the gas from distal embolized vessels due to a lack of gas diffusion pathway has the opposite effect. Furthermore, the contribution of other air sources at the late stage could underestimate the vulnerability. It is possible that when combining the two stages, and with both underestimation and overestimation effects merged, the generated P_{50} values could be close to that generated by other methods.

As analogous to the decrease in water content, the increase in gas volume in a stem during desiccation is a correlated process with embolism spreading and tissue desiccation. Simply measuring the relative water content of the stem, which is easier, and plotting it against the water potentials can also produce an '*s*-shaped' curve similar to VC curves (See Methods S4), and generate a value at 50% decline similar to P_{50} (Hao *et al*., 2013; and Fig. 4 here). However, that is more like a correlation, because many ongoing structural and physiological processes could be related to xylem embolism spreading during the desiccation process. While getting P_{50} values could be helpful, the shape of the curve and underlying mechanisms also matter.

Conclusion

In conclusion, while we agree that the Pneumatic method could potentially be an efficient and time-saving method to generate VCs, and in some cases can generate similar P_{50} values compared to other methods, some fundamental uncertainties remain. Before it can be widely used to generate a massive dataset, these uncertainties including those discussed here and in our published study, especially the 'two- or multi-plateau' pattern, uncertainties in air sources, the effect of cut-open vessels, and the '*r*-shaped' curves need to be carefully verified. Surely, novel methods could advance scientific knowledge, and no current VC methods are perfect (Skelton & Diaz, 2020). Meanwhile, uncertainties still need to be solved to generate solid data. We, therefore, suggest a careful test of these uncertainties first rather than getting more data to generate correlations, especially when the controversies of other methods for generating VCs are still up in the air as well. Some tests could be done to solve these uncertainties, for example, using micro-CT to track the embolism spreading of the part close to the Pneumatic apparatus, and the part far away from the cut ends used for air discharge. While the Pneumatic method could give us insights into the gas dynamics in the stem (Jansen *et al*., 2022), we believe that pointing out evidence-based uncertainties can promote the improvement of the method and the advancement of scientific understanding on this central topic in plant physiology and ecology. Rather than invalidating the Pneumatic method, we strongly wish this mechanistic discussion could help to improve the method.

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Author contributions

Y-JC, Y-JZ and PM conceived and designed this study. Y-JC performed the experiments. Y-JC and PM analyzed the data. Y-JC and Y-JZ wrote and revised the manuscript with input from PM and J-LZ. Y-JC and PM contributed equally to this work.

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Data availability

The data that support the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Comparison on vulnerability curves using the manual Pneumatic data in Chen *et al.* (2021) and new data within the same water potential range using the automated Pneumatron device.

Fig. S2 The dynamic changes in the percentage increase in air discharged (PAD, %) for *Microcos paniculate* during the desiccation process.

Fig. S3 The dynamic changes in the percentage increase in air discharged (PAD, %) for *Lasiococca comberi* during the desiccation process.

Fig. S4 The dynamic changes in the percentage increase in air discharged (PAD, %) for *Lagerstroemia tomentosa* during the desiccation process.

Fig. S5 The dynamic changes in the percentage increase in air discharged (PAD, %) for *Combretum griffithii* during the desiccation process.

Fig. S6 The dynamic changes in the percentage increase in air discharged (PAD, %) for *Combretum yunnanense* during the desiccation process.

Fig. S7 Reproduction of fig. 5 from Pereira *et al.* (2020), showing the occurrence of 'two Plateaus', shrinkage, and non-stable late stage.

Fig. S8 Reproduction of fig. 3 from Pereira *et al.* (2020), showing the occurrence of 'two Plateaus' and shrinkage in species *Citrus sinensis.*

Fig. S9 Reproduction of fig. 1 from Paligi *et al.* (2021), showing the occurrence of 'two Plateaus'.

Fig. S10 micro-CT images (side and cross-section directions) of three stem segments of *Combretum griffithii* at different stages of desiccation.

Fig. S11 micro-CT images of stem cross-section for 10 tropical liana and 10 tree species after drying for *c.* 60 h under laboratory conditions to low water potentials.

Fig. S12 Comparison on xylem area, pith area, and vessel lumen area for three stem segments of *Combretum griffithii* at different stages of desiccation.

Fig. S13 A test to determine the heterogeneity of embolism along the branch.

Methods S1 Methods for air discharge measurement using the Pneumatron apparatus for the same five species in Chen *et al.* (2021).

Methods S2 Methods for determining the heterogeneity of embolism along the branch.

Methods S3 Tissue cracks and shrinkage monitoring.

Methods S4 Vulnerability curve measurements.

Table S1 A summary of information about air-discharging experiment for five species using an automated Pneumatron device.

Table S2 Species information of 20 species that were used for observation of tissue crack and shrinkage during dehydration.

Video S1 Movie of three-dimensional cracks in pith of four representative species under severe dehydration.

Key words: climate change, drought resistance, embolism, micro-CT, plant hydraulics, vulnerability curves.

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