Assessing safety margins and hydraulic strategies in Restionaceae: evaluating xylem hydraulic traits in two *Elegia* species.

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ABSTRACT

The movement of water through a plant is responsible for acquiring nutrients, avoiding desiccation and enabling gas exchange. Therefore, access to soil water is vital in determining vegetation structure in a landscape, which suggests that plant hydraulic strategies are an important ecological driver of responses to seasonality and intensity of drought events, especially in the face of climate and habitat change. Wide-spread mortality has been observed over recent decades as a result of drought stress. Many plant species have exhibited hydraulic niche segregation as a result of a trade-offs between stomatal regulation and xylem integrity, and the associated physiological adaptations. This is concerning in the face of climate change predictions of more frequent and intense drought and flooding events because predicted habitat water availability changes may exceed the physiological tolerance ranges of many species. The hydraulic safety margin (difference between xylem vulnerability (P_{50}; MPa) to cavitation and point at which stomata are considered closed (turgor loss point; MPa)) displays the tolerance margins based on plant physiological capacity. Restionaceae have shown extensive evidence of hydraulic niche segregation and are a key demographic of fynbos vegetation but not much is known about their hydraulic strategies. The optical vulnerability method was used to construct vulnerability curves and the turgor loss point (Ψ_{TLP}) was acquired from pressure-volume curves. E. fenestrata, a localized seep species, was more vulnerable (P_{12} = –0.52 MPa; P_{50} = –1.07 MPa; Ψ_{TLP} = –1.57 MPa; negative safety margin = –0.5 MPa) than E. tectorum, a widespread, dry habitat species (P_{12} = –0.99 MPa; P_{50} = –1.6 MPa; Ψ_{TLP} = –1.64 MPa; slightly negative safety margin = -0.04 MPa). The Cape Floristic region is predicted to receive less rainfall and become drier with ongoing climate change. We expect that this overall drying trend will have a profound impact on the Restionaceae, particularly E. fenestrata that does not have the physiological capacity to deal with severe drought stress.

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CHAPTER 1: Novel insights into the plant water relations in the Restionaceae and their role in understanding current and future distributions and habitat selection.

1.1. The study of plant hydraulics and its usefulness in assessing plant hydraulic strategies.

Plant hydraulics is the study of the movement of water from the soil to the atmosphere via the plant hydraulic transport system consisting of the root network, the xylem network, the mesophyll, and the stomatal pores on the surfaces of leaves, stems or culms (Dixon and Joly, 1895, McDowell et al., 2019). In the process of transpiration, water is drawn up through the plant largely because of evaporation from internal sites in the leaf mesophyll (Dixon and Joly, 1895, Silvertown et al., 2015, Lens et al., 2013). Although most water loss is unavoidable due to plants having a shared pore for carbon dioxide uptake and other gaseous exchange (Martin-Stpaul et al., 2017), land plants need significant amounts of water to function effectively (e.g. for growth, nutrient uptake from the soil (Silvertown et al., 2015), and evaporative cooling). Consequently, water availability plays an important role in determining the distribution and productivity of any given species (Volaire, 2018).

Given the fundamental links between plant and water transport and plant function and between plant water transport and climate and growing environment, understanding the mechanisms of water movement through the plant hydraulic transport system can offer a vital tool to predicting plant responses in the face of ongoing climate change (McDowell et al., 2019, Pittermann, 2010).

Evolved plant responses to water deficit are vital for their resistance to drought and persistence in many habitats (Volaire, 2018), and understanding these phenomena are critical in understanding which species are of concern in the context of future climate scenarios. In many ecosystems it has been predicted that the severity and frequency of extreme events (i.e. droughts and flooding) may increase as a symptom of changing climate (IPCC, 2014). In this context, it is important to generate knowledgeable predictions for plant and ecosystem response to novel conditions (i.e. extreme events) that are based on quantitative physiology.

1.1.1. The Soil-Plant-Atmosphere Continuum (SPAC) and the physics of water transport.

Land plants exist across a wide variety of habitat types that differ in their soil moisture availability and climate conditions (Martínez-Vilalta et al., 2014). In order to thrive in a particular habitat, a functional (evergreen) plant requires the uninterrupted movement of water from the soil to the atmosphere through the water transport system, termed the Soil-Plant-Atmosphere-Continuum (SPAC) (Cochard et al., 2013). Plants can physiologically adjust their water transport at (at least) two locations along the SPAC, namely the xylem hydraulic transport system (responsible for water movement from soil to leaves) and the stomata (Martínez-Vilalta et al., 2014, Sperry et al., 2002).

To avoid dangerous levels of desiccation, the water being lost via transpiration needs to be replaced by water from upstream (Venturas et al., 2019, Venturas et al., 2017). The mature, dead-celled xylem conduits transport water passively from roots to leaves by means of a negative pressure (tension) gradient (Dixon and Joly, 1895) where it is used for photosynthesis or exits the plant via transpiration (Choat et al., 2018, Dixon and Joly, 1895, Venturas et al., 2019).

1.1.1.1. The process of xylem embolism and its consequences for plant survival.

The amount of tension in the xylem at any given time (P_x) is dependent upon the water potential of the soil (P_{soil}), the hydraulic conductance of the plant (K_{plant}) and the rate of sap flow (F) in the following way (Van Den Honert, 1948):

$$P_x = P_{soil} - \frac{F}{K_{tree}} \qquad (1)$$

According to equation 1, increasing the transpiration rate (increase in F) and/or having P_{soil} decline will result in a drop in P_x (Cochard et al., 2013). An increased transpiration rate because of a higher demand from the atmosphere (when the air is hot and dry, more water is evaporated from stomata as a result of an increased vapour pressure deficit, or VPD) and a decrease in soil moisture, both being classical characteristics of drought, increases the tension acting on the liquid water column (Volaire, 2018, Gilbert and Medina, 2016). Water held under tension is metastable and consequently, vulnerable to vaporization after a specific threshold has been reached (Lens et al., 2013, Steudle, 2001, Dixon and Joly, 1895, Brodribb et al., 2017).

At this threshold, a rapid phase change occurs in which liquid water changes to water vapour (Lens et al., 2013). This change is triggered by the presence of small bubbles of air in xylem vessels (either dissolved gases from within the sap or from neighbouring conduits) or weaker adhesion forces at the xylem wall interface (Lens et al. 2013). Air can get aspirated into functional conduits from adjacent empty ones (these vessels are air-filled for a variety of reasons (e.g. herbivory and mechanical damage or developmental deformities), through selectively permeable pit membranes embedded in the xylem walls (Tyree and Sperry, 1989, Lens et al., 2013). The function of these pits is to allow for the adjacent flow of sap between adjacent vessels but simultaneously act as a barrier to foreign objects such as air, and in some cases pathogens or other solid particles (Lens et al., 2013, Pittermann, 2010, Choat et al., 2018). The pores have small enough diameters to hold the air-water meniscus between water-filled and air-filled vessels under normal conditions, however, under increasingly negative pressure during drought this ‘seal’ can collapse allowing air to spread between vessels throughout the plant (Lens et al., 2013). The process of air aspirating into functional vessels results in air blockages, called xylem embolism.

The threshold pressure at which air nucleates into the xylem sap is called the ‘air-seeding’ threshold and it is the tension at which embolism starts to occur (Tyree and Sperry, 1989, Venturas et al., 2017). If embolism is allowed to spread extensively the complete blockage of the water transport pathway would occur- often referred to as hydraulic failure- which would slow down and eventually halt the conductive capacity of the xylem network (Choat et al., 2018, Cochard et al., 2013, Tyree and Sperry, 1989). Complete embolism causes desiccation of above-ground, downstream tissues, and can ultimately induce whole plant mortality (Choat et al., 2012, Brodribb and Cochard, 2009, Martínez-Vilalta et al., 2014, Tyree and Sperry, 1989, Creek et al., 2018). Increasing frequency and severity of drought predicted for the future could lead to plants operating at or close to their ‘air-seeding’ threshold, increasing the chance that embolism propagation will occur.

Globally, plants tend to maintain positive margins between the *in situ* water potential and the ‘air-seeding’ threshold (Choat et al., 2012, Brodribb et al., 2016), and this safety margin is considered to have potential to predict species moisture stress and loss of function (Brodribb and Cochard, 2009, Brodribb et al., 2016), especially in light of climate projections.

1.1.1.2. Mechanisms of mortality: Hydraulic failure and carbon starvation.

Hydraulic failure as a result of rampant embolism has been highlighted as a primary mechanism of drought-induced mortality and provides crucial predictions about species mortality thresholds (Choat et al., 2012, Brodribb and Cochard, 2009, Urli et al., 2013). However, current research also highlights an additional physiological mechanism of drought-induced mortality, namely carbon starvation as a result of prolonged stomatal closure. Plants primarily control the resistance to water flow through xylem conduits by stomatal closure (Martin-Stpaul et al., 2017, Pittermann, 2010, Tyree and Sperry, 1989, Martínez-Vilalta et al., 2014). Stomatal function in the context of water transport acts like a pressure regulator that controls the changes in tension by controlling flow and limiting variation in xylem pressure with soil water and VPD to avoid risky drops in water potential (Sperry et al., 2002). Closing the stomata for prolonged periods of time may have detrimental effects with regards to carbon acquisition for photosynthesis (McDowell et al., 2008, Miyashita et al., 2005).

Stomatal closure and carbon limitation facilitates an imbalance between carbon supply and demand which has multiple implications associated with metabolism and defense (Venturas et al., 2017, Urli et al., 2013, Adams et al., 2017, Sala et al., 2010). In order to make reliable predictions about plant mortality, a comprehensive understanding of the physiology of plant death is needed (Choat et al., 2018).

Significant progress has been made in understanding the mechanisms involved in plant mortality, and it has become clear that drought-induced mortality is intricate and involves various interdependent physiological mechanisms. Importantly, the two mortality mechanisms are not mutually exclusive, and while carbon starvation occurs in many species, reductions in non-structural carbohydrate concentrations do not seem to be universally coupled to dehydration (Adams et al., 2017). Studies have shown that drought-induced mortality because of a high degree of embolism (usually $\geq$ 60 % PLC) is common to all species studied but not all species exhibited a reduction in non-structural carbohydrate concentration during drought induced mortality (Adams et al. 2017). Specific survival thresholds in non-structural carbohydrate concentrations are not well-represented in the literature (Adams et al., 2017) but Sapes & Sala (2020) demonstrated that reductions in non-structural carbohydrates may be more influential in plant water relations than previously thought.

Although carbon starvation is viewed as a secondary mechanism of plant mortality during drought, the degree of its influence is determined by drought length and microhabitat (ref?). For example, species residing in dry microhabitats would require larger NSC pools to maintain sufficient osmotic potentials while still being able to carry out metabolic functions (Sapes et al., 2020) and may run out of NSC reserves under prolonged drought. Despite the evidence that the role of carbon starvation is more crucial than previously thought, much research into the influence of carbon starvation in drought-induced plant mortality is needed (Sapes et al., 2020). This would be a step in the right direction in being able to understand the influence of carbon starvation on drought-induced mortality in relation to hydraulic failure, as well as, how the two mechanisms of mortality interact.

1.1.2. Trade-offs involved in the drought response of plants to drought: Safety-vs-efficiency and Carbon acquisition-vs-water conservation.

Land plants face important trade-offs, with regard to water transport, arising from the need to acquire carbon in a dry atmosphere. As we have seen, the most fundamental of these trade-offs is the need to balance carbon acquisition against water conservation at the stomatal level. This demand has resulted in the evolution of remarkable stomatal control of water loss, and it has also been posited to explain other aspects of mesophyll functionality (e.g. the evolution of CAM photosynthesis and night time transpiration, and leaf succulence).

The carbon acquisition-vs-water conservation trade-off influences how sensitive stomata of different species are to variation in soil and atmospheric moisture availability. Similarly, the xylem has also evolved, balancing the need to supply water to the mesophyll tissue (and therefore being hydraulically efficient) versus the need to maintain a continuous, intact water column. Such a demand is the basis of a proposed xylem safety-efficiency trade-off, where the capacity of a plant to transport water efficiently to the leaf mesophyll is challenged by the requirement to avoid propagating xylem embolism (Gleason et al., 2016). The hypothesis is that larger, more efficient venation is associated with lower capacity to withstand embolism formation and propagation (Gleason et al., 2016).

The safety-efficiency trade-off is centered around the observation that xylem traits that provide safety from desiccation also tend to decrease conductive capacity and vice versa (Venturas et al., 2017, Skelton et al., 2015). Conduit diameter (according to the Hagen-Poiseuille equation) and length have a significant effect on water transport efficiency (Pittermann, 2010). Wider and longer conduits increase transport efficiency, implying that angiosperms transport water more efficiently than short, narrow tracheids present in gymnosperms (Pittermann, 2010, Tyree and Zimmerman, 2002). These characteristics, however, reduces xylem conduit safety from damage as a result of desiccation (Hacke and Sperry, 2001, Hacke et al., 2001). Xylem conduits need to be sufficiently reinforced in order to withstand high tensions, and possibly collapse, when transpiring (Hacke & Sperry. 2001). In gymnosperms, the safety-efficiency trade-off is apparent and driven by changes in tracheid diameter (Pittermann, 2010). Cell wall thickness is inversely related to conduit diameter, and so, having narrower tracheids allows for greater investment in denser wood and increased mechanical strength.

Angiosperms have a similar trade-off, but it has to do with pit membrane anatomy rather than conduit diameter, despite frequently observing relationships between conduit width and xylem vulnerability to embolism (Pittermann, 2010). Longer, wider vessels characteristic of this group, have a larger pit pore area because intervessel pit area scales with conduit width (increased efficiency; Hacke et al, 2006, Pitterman et al. 2010). However, this larger area could possibly be to its detriment because larger pores are believed to be more vulnerable to xylem embolism because air seeding is believed to initiate through the largest pit membrane (Skelton, 2020, Sperry et al., 2006, Choat et al., 2008). Having extensive knowledge of xylem anatomy is useful to assess interactions between xylem form and function and pinning down the mechanism of the proposed safety-efficiency trade-off has potential to unravelling the complexity form and function.

A third, under-explored trade-off that land plants might face is a hydraulic failure-vs-oxygen starvation trade-off, particularly in the root tissues. Plants occurring in waterlogged soils run the risk of being starved of oxygen due to anoxic conditions. To counter this, plants found in mesic habitats have often developed aerenchyma tissue that promotes the availability of air to the otherwise waterlogged tissues, as is witnessed in some restios (Huber and Linder, 2012). However, evolving aerenchyma tissue may come at the cost of introducing air into the xylem network, which may promote embolism and hydraulic failure in plants occurring in mesic conditions. Consequently, one might anticipate that plants growing in waterlogged conditions possessing aerenchyma might be more vulnerable to xylem embolism than plants without aerenchyma might be more vulnerable to xylem embolism than plants without aerenchyma, such as those growing in either seasonally waterlogged soils or drier soils. Plants that do not possess the aerenchyma tissue are unlikely to experience embolism through internal gas movement in the absence of environmental water stress but might suffer from oxygen limitation if they occur in seasonally saturated environments.

In this study we aim to examine the hypothesis that species will align along these spectra of having safe-vs-efficient xylem, prioritizing conserving water-vs-acquiring carbon and being able to tolerate either aeration or drought stress in order to persist in the physical environment or niche they occur in.

1.1.3. Hydraulic strategies: Isohydry and anisohydry.

Two contrasting strategies have been proposed to exist in relation to avoidance or tolerance of drought: Isohydry (avoidance of low water potentials) and anisohydry (tolerance of low water potentials). Stomata of isohydric species are highly sensitive to changes in moisture availability, and close during drought stress to maintain hydraulic function and midday Ψ (Tardieu & Simmoneau. 1998; Martinez-Vilalta *et al.*, 2014; Farrell, Szota and Arndt, 2017). These species, however, run the risk of starvation because of the inability to acquire CO_2 for photosynthesis while the stomata are closed (Hochberg et al., 2017). Stomata of anisohydric species are less sensitive to changes in moisture availability and remain open during drought stress to allow photosynthesis to continue. These species run the risk of hydraulic failure due to continued loss of limited water supplies throughout the dry period (Martinez-Vilalta *et al.*, 2014). Isohydry and anisohydry have been demonstrated to be extreme ends of a spectrum of responses (Skelton et al. 2015).

Being either isohydric or anisohydric is not mutually exclusive but rather the strategy used by species to avoid/tolerate drought falls on a spectrum ranging from completely isohydric to completely anisohydric (Martinez-Vilalta *et al.*, 2014; Hochberg *et al.*, 2018). Species residing in arid or semi-arid habitats have also exhibited complex root patterns that are able to access water deep in the soil when the shallower levels of soil are dry (Nardini *et al.*, 2014). These overlapping processes makes it extremely difficult to characterize precise drought response strategies but have given enormous insight into how species deal with drought stress and which mechanisms play a role in mortality as a symptom of drought (Martinez-Vilalta *et al.*, 2014; Nardini *et al.*, 2014).

1.2. Quantifying the important parameters associated with the study of plant response to drought.

1.2.1. Using vulnerability curves and their parameters to determine the resilience of the water column against embolism.

To quantify xylem capacity to tolerate water deficit, species are typically compared by extracting parameters from xylem vulnerability curves, the relationship between xylem water potential (P_X; MPa) and loss of xylem function (e.g. percent loss of hydraulic conductance; PLC; %) (Skelton et al., 2015). The 'air-seeding' threshold, referred to as P_{12}, is the xylem pressure at which 12% of xylem conductivity is lost as a result of embolism. The xylem pressure at which 50% loss of hydraulic conductivity has occurred (P_{50}) is a metric of substantial loss of function, and has been shown to be correlated with habitat water availability (e.g. Li *et al*., 2018). The xylem pressure at which irreversible damage to the water transport system and mortality occurs has been shown to be P_{50} in gymnosperms and P_{88} in angiosperms (Brodribb and Cochard, 2009, Li et al., 2018) which could be due to the differences in their vessel anatomy and structure (Choat *et al.*, 2018).

Xylem vulnerability curves frequently show an initial phase in which no embolism happens despite a decrease in xylem water potential until P_{12} is reached, followed by a rapid phase of embolism propagation, followed by another flat portion indicating that 100% loss in xylem conductivity (Fig. 1). This relationship between PLC and water potential can be described using a sigmoidal equation, which is useful for calculating the P_{50} and slope of the curve (Pammenter and Van der Willigen, 1998) as follows:

$$PLC = \frac{100}{\left(1 + \exp\left(\frac{S}{25(P_i - P_{50})}\right)\right)} \qquad (2)$$

Where S is the slope of the curve at the inflection point (% MPa^{-1}) (Brodribb *et al.*, 2017; Lobo *et al.*, 2018).

The shape of vulnerability curves has been a controversial topic within the field of plant hydraulics. Some studies yielded exponential curves (also referred to as being 'r-shaped'); which indicate that embolism is a common occurrence under normal growing conditions and from which it is often inferred that plants are able to refill embolized conduits. Other studies yield sigmoidal curves ('s-shaped'), which indicate that embolism is a rare phenomenon occurring only under conditions of acute drought stress (Fig. 1) (Cochard et al., 2013). Although embolism refilling has been demonstrated in several plant species under specific circumstances (e.g. where sufficient positive root pressure acts to dissolve air bubbles, see Brodersen *et al*., 2010; or by the accumulation of non-structural carbohydrates during drought that lower the osmotic potential and increases the refilling capacity post stress, see Tomasella et al. 2019), many other species have not shown any indication of being able to refill (e.g. Choat et al. 2015). Understanding which species are capable of embolism refilling and under what circumstances is a step in the right direction to begin to understand its role in species response to drought (Bartlett et al., 2012), especially in the face of predicted changes in severity and frequency of such events. Regardless of whether refilling occurs or not, vulnerability curves can be used to determine how resilient the xylem is to air entry and water stress.

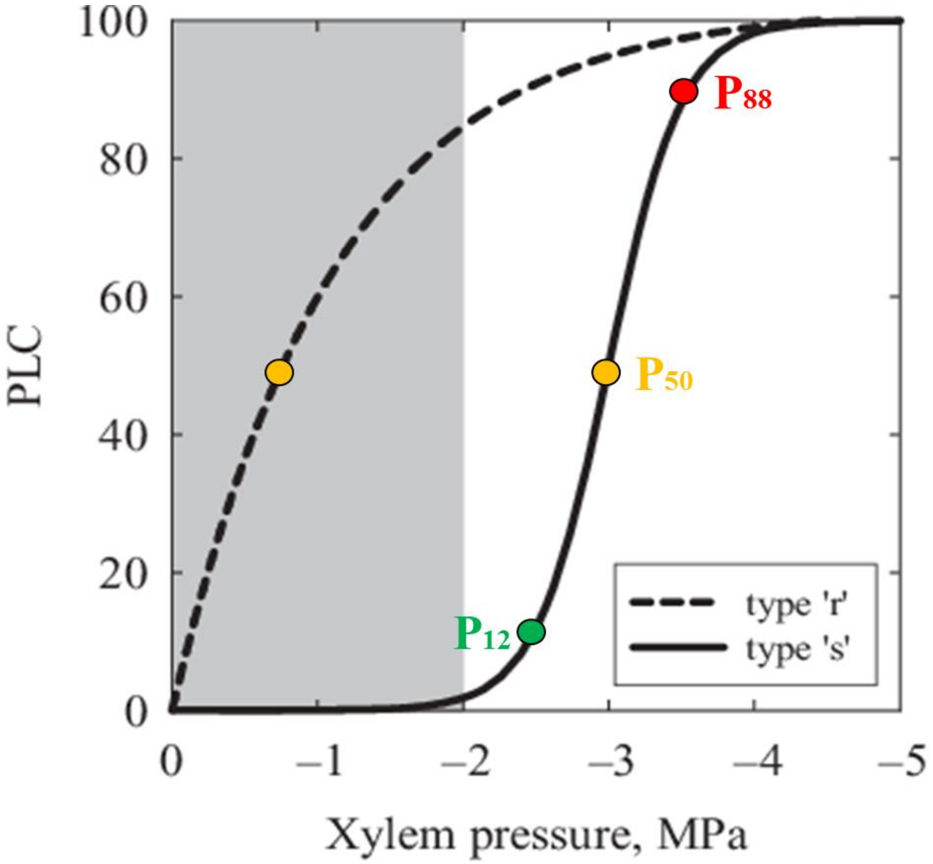


Figure 1: Schematic representation of xylem vulnerability curves depicting the relationship between percent loss conductivity (PLC) and xylem pressure (P_x). 'S-shaped' curves display a safe zone (grey shaded area) whereas 'R-shaped' curves do not. The important points are displayed in the figure in different colours (Adapted from Cochard et al. 2010).

1.2.2. Stomatal regulation as a strategy for conserving plant water status during unfavourable conditions.

A major feature of land plant drought tolerance is hypothesized to be the ability of different species to close stomata at differing water potentials. The turgor loss point (water potential at which cells lose turgor and collapse; Ψ_{TLP}) can be used as an important trait that determines how tolerant a species can be to drought (Bartlett, Scoffoni and Sack, 2012; Nardini *et al.*, 2014; Farrell, Szota and Arndt, 2017). When this pressure is reached, the guard cells responsible for keeping the stomatal pore open loses all turgor and collapse, thereby closing the stomata. A more negative Ψ_{TLP} (in conjunction with lower P_{50}) allows species to be more tolerant of drought conditions by allowing the stomata to remain open as long as physiologically possible to allow carbon acquisition to continue for as long as possible, before hydraulic failure occurs (Bartlett, Scoffoni and Sack, 2012; Nardini *et al.*, 2014). The Ψ_{TLP} is one of the many parameters that can be extracted from constructing a pressure-volume (PV) curve which depicts the relationship between the inverse of the water potential ($\frac{1}{\Psi}$; MPa^{-1}) and the relative water loss during the dry down period (100-RWC; %) (Figure 2).

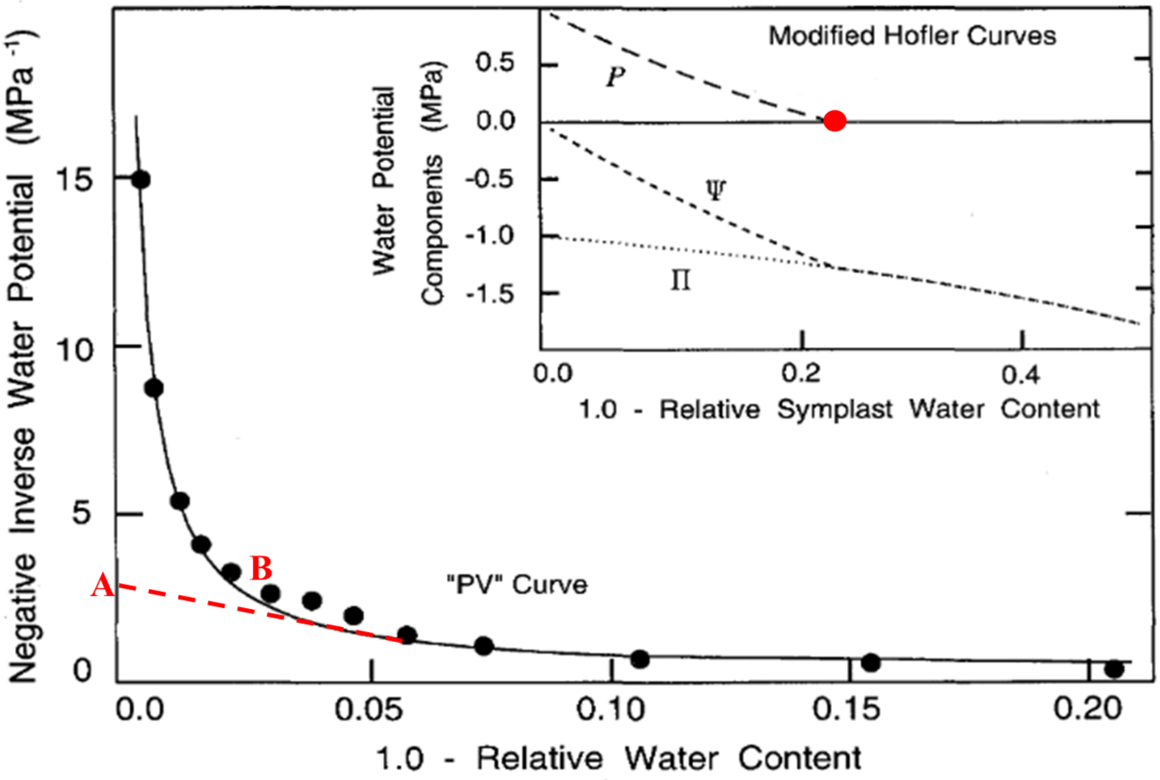


Figure 2: Schematic of an average Pressure-Volume (PV) curve adapted from Alm et al. (1994). (A) The point at which the osmotic potential at full turgor can be read from. (B) The turgor loss point can be read off the y-axis where the point of inflection occurs. (Inset) A general Höfler curve depicting the comprehensive way that leaf turgor potential (Ψ), osmotic potential (Π) and total potential (P) changes during dehydration. The red circle is where the turgor loss point occurs.

The other significant parameters are the osmotic potential at full turgor (salt concentration in cells when fully hydrated; π_0) and the modulus of elasticity (cell wall flexibility; ε) (Bartlett, Scoffoni and Sack, 2012). These two parameters have an indirect effect on the drought tolerance of species because of their influence on Ψ_{TLP} (Bartlett, Scoffoni and Sack, 2012). With that said, π_0 is the parameter that has more of a significant effect on Ψ_{TLP} by becoming more negative (increased solute concentration) and maintaining the ability of cells to take up more water via osmosis, called osmotic adjustment (Bartlett, Scoffoni and Sack, 2012; Nardini *et al.*, 2014). Both Ψ_{TLP} and π_0 exhibit strong correlations with habitat water status within and across biomes, which highlights their importance in being better indicators of physiological drought tolerance than ε (Bartlett, Scoffoni and Sack, 2012; Farrell, Szota and Arndt, 2017).

Despite not having a direct role in drought tolerance, ε influences the maintenance of a high relative water content at Ψ_{TLP} (RWC_{TLP}; %) through the production of hardy, sclerophyllous leaves and elastic adjustments (changing the flexibility of cell walls), which maintains cell water content and therefore, metabolism is allowed to continue (Bartlett, Scoffoni and Sack, 2012; Li *et al.*, 2018).

1.2.3. Using stomatal and xylem plant hydraulic parameters to determine Hydraulic safety margins and Stomatal safety margins.

Unpacking plant response to changes in the availability of water within habitats is complex, as there are multiple interdependent processes at play when plants are experiencing water stress. A simple trait-based approach based on functional traits can assist in reducing this complexity and lead to an enhanced understanding of plant drought responses. This can be achieved by comparing hydraulic functional traits between species, especially closely related species which can be expected to share many other traits that are unrelated to water use. For example, Skelton et al. (2015) combined stomatal traits pertaining to water use and xylem hydraulic traits pertaining to water transport and vulnerability to embolism to develop a framework to quantify species drought tolerance (Skelton et al., 2015).

Key traits that have been identified are those involved in calculating the hydraulic and stomatal safety margins, the water potential difference between the water potential associated with 50% embolism and seasonal minimum water potential (HSM, in MPa) or the water potential associated with stomatal closure (SSM; in MPa). These safety margins illustrate the level of conservatism associated with species hydraulic strategies (Choat et al. 2012; Skelton et al. 2015). The utility of a safety margin is significant because neither stomatal regulation nor vulnerability to embolism can be used in isolation when attempting to assess response to drought (Skelton et al. 2015). For example, large positive stomatal safety margins imply that stomata close long before dangerous levels of hydraulic damage. Small or negative stomatal safety margins imply that stomata remain open during dehydration, but also that the water transport system is at risk of hydraulic failure.

Comparing safety margins between species provides insight into the tolerance margins and can highlight which species are at risk of extinction with changing habitat as a result of ongoing climate changes.

1.3. Using the Restionaceae as a target group.

The Cape Floristic Region (CFR) is a biodiversity hotspot holding up to 9500 plant species within an area of approximately 90 000 km^2 (Goldblatt, 1997, Allsopp et al., 2014). The CFR resides in a southern Mediterranean climate system (Born and Linder, 2018, Araya et al., 2011, West et al., 2012), and is characterized by predominantly winter rainfall and summer dry periods. Rainfall gradients across the CFR are the most influential parameter affecting vegetation growth patterns (e.g. winter vs aseasonal rainfall associated with highly seasonal or year round growth) (Born and Linder, 2018, Allsopp et al., 2014). In addition to being exceptionally rich in biodiversity, these hotspots have been identified as some of the most vulnerable ecosystems in the face of climate change. This region is unique in its diversity and is particularly susceptible to effects of reduced water availability, which implies that large-scale changes in the hydrological cycle as a result of changing climate is a significant threat to CFR ecology (Jacobsen *et al.*, 2009).

Strategies for coping with the heterogeneity of moisture availability across the CFR are plenty. The fynbos biome makes up the bulk of the area of the CFR and occurs all the way along the hydrological gradient (Araya *et al.*, 2011; Cordova, 2013; Born and Linder, 2018). Fynbos is fire-prone and consists of woody, evergreen shrubs persisting in porous, well-drained, oligotrophic soils made from quartz sand (Jacobsen *et al.*, 2009; Araya *et al.*, 2011; Cordova, 2013; Born and Linder, 2018). There are, however, some fynbos vegetation that occur in more swampy, waterlogged soil types (Born and Linder, 2018). It is increasingly clear that soil moisture availability is an important niche parameter in fynbos which is apparent when observing the way in which species segregates along fine-scale moisture gradients (Born and Linder, 2018).

A trade-off between adapting to drought stress and aeration stress (oxygen-poor, waterlogged conditions) may exist (Born and Linder, 2018). The western half of the CFR experiences seasonal droughts during summer because almost all its rain falls in winter (Araya *et al.*, 2011) and maintaining sufficient water transport during this time could become challenging if vegetation has not developed adaptations to dealing with being dry for half the year. The above traits and the proposed vulnerability of fynbos vegetation, especially to future drought scenarios, highlight the need to start evaluating the possible vegetation responses. Using a traits-based approach is suitable for this because hydraulic traits are highly coupled to the environment, especially habitat water availability.

1.3.1. Restionaceae diversity and ecological dominance in fynbos.

Plants of the family Restionaceae (known colloquially as restios) consist of approximately 350 species of evergreen, mostly shallow-rooted (Linder, 2020) grass-like monocots and make up the most of the graminoid layer in fynbos ecosystems (Cordova, 2013; Born and Linder, 2018). Restios physiologically differ from grasses by using culm surfaces (i.e. photosynthetic stems) instead of leaves as the primary sites of CO_2 uptake through stomata (Born and Linder, 2018). They are also dioecious, possess cluster roots, and appear to have no symbiotic relationship with root fungi and are exclusively wind pollinated (Huber and Linder, 2012; Born and Linder, 2018).

1.3.2. Restionaceae ecophysiology

Restios ecologically dominate fynbos vegetation and have been shown to exhibit hydraulic niche separation across moisture gradients (Araya *et al.*, 2011; Huber and Linder, 2012; Born and

Linder, 2018). This is significant because we observe restio presence across the entire moisture gradient of the Cape Floristic Region (CFR), from predominantly winter rainfall along the west coast to largely aseasonal along the south coast and from well-drained to waterlogged soil types (Huber and Linder, 2012; Born and Linder, 2018). In order to successfully navigate this highly heterogenous environment, species must have special adaptations (Born and Linder, 2018). In Restionaceae one such adaptation is the ability to tolerate anoxia in waterlogged soils via the production of root aerenchyma (Born and Linder, 2018).

Aerenchyma is a tissue type consisting of air-filled gas pockets that are connected to each other to facilitate ventilation to avoid anoxic stress in oxygen-poor, waterlogged soils (Huber and Linder, 2012). While many plant lineages do not possess the ability to make aerenchyma, some are only able to produce it when subjected to waterlogging and others express it as a permanent trait (Huber and Linder, 2012). The presence of root aerenchyma is common in multiple, distantly related angiosperm lineages and plays a part in determining where a species will lie along the moisture gradient (Huber and Linder, 2012). Non-aerenchymatous species are not usually found in waterlogged habitats but aerenchymatous species can persist in both waterlogged and well-drained habitats (Huber and Linder, 2012).

1.3.3. Hydraulic Niche Separation (HNS) in Restionaceae.

The concept of hydrological niches is fundamental to the range of species distribution, how vulnerable they are to changing conditions and how they co-exist in the same landscape (Huber & Linder. 2012, Born & Linder. 2018). The hydrological niche axis extends from permanently waterlogged to severely droughted soils and special adaptations are required to tolerate the various stress associated with each. Droughted soils are moisture limited and can induce water stress in plants which will have a profound impact on plant functionality. Waterlogged conditions provide enough water but not enough oxygen (anoxic soil conditions). Species occurring at this latter, mesic end of the spectrum need to invest in morphological adaptations that can aid against the anoxic stress (Born & Linder. 2018).

The Restionaceae have shown prominent hydraulic niche segregation, meaning that the species in the group tend to utilize different hydrological niches according to their physiological capacity for water uptake and transport (Figure 3; Araya et al. 2011). This ensures that there is minimal competition for resources and allows for the high diversity witnessed in the family. Fynbos soils have a niche space that is concentrated in the bottom left corner and no species occur in the top left because soils in the fynbos do not have properties that promote both aeration and drought stress simultaneously (Araya et al. 2011). Species possess adaptations in order to trade-off between aeration stress and drought stress. Restios are also believed to be capable of extracting cloud moisture which could be an advantage during drier conditions (West et al. 2012).

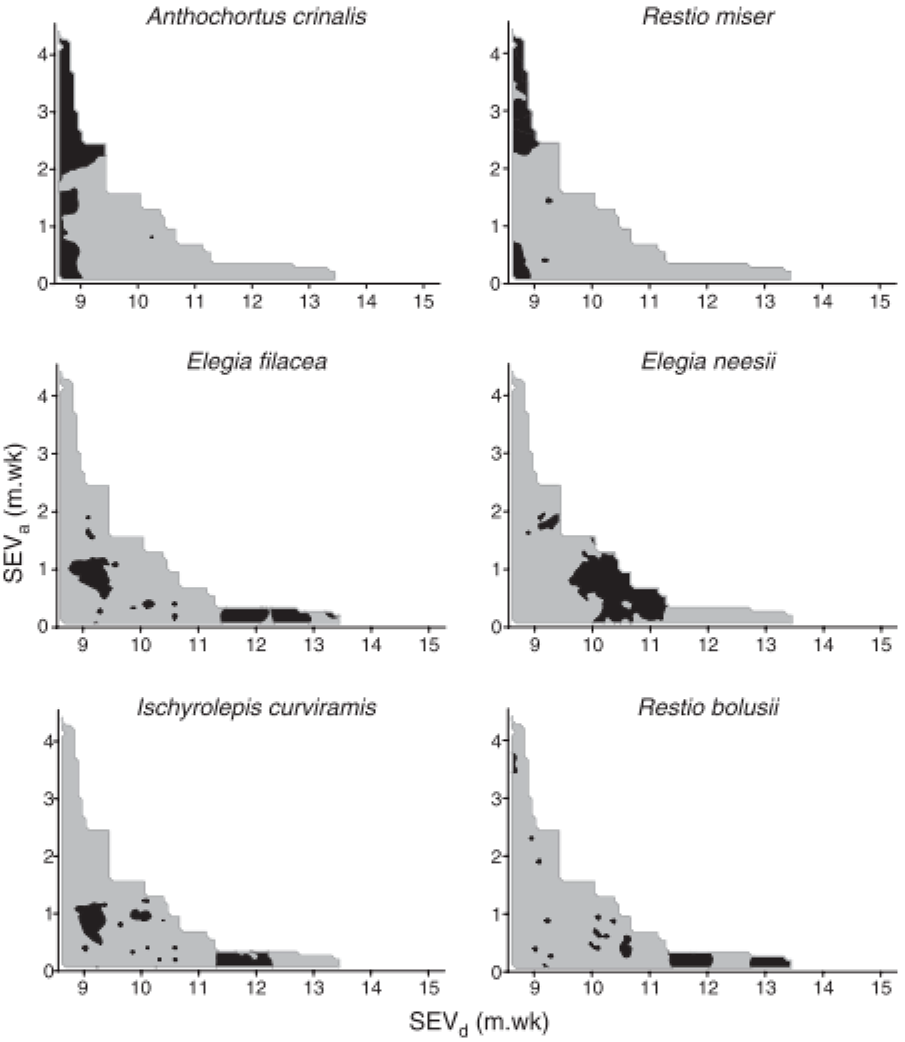


Figure 3: Diagram from Araya et al (2011) showing the hydrological niche space of 6 common restio species. Both axes represent a sum exceedance value with drying stress (SEV_d) on the x-axis and aeration stress (SEV_a) on the y-axis. The area shaded grey is the available niche space and black areas are where that particular species occurs. Occurring in the top right section of the graph means risk of aeration stress (wet end of spectrum) and the bottom left means risk of drought-stress (dry end of spectrum).

Although non-overlapping niches of different species have been identified, the basic mechanisms that determine these niche limits and the associated species distributions are not fully understood. In particular, vital gaps exist in the knowledge of how morphological and physiological adaptations determine the hydrological niche that a given species can occupy (Huber & Linder. 2012). My thesis attempts to address this gap in our knowledge by merging hydraulic trait frameworks of plant drought tolerance with hydraulic niche theory.

1.4. Outlining the study

1.4.1. Target species

The two target species of *Elegia* used in this species occur on opposite ends of the hydraulic niche axis. *Elegia fenestrata* (Pillans) is a localized species found in a small area along the Cape Peninsula. They grow along the coast at low altitude (0 – 100m) in marshy areas and on the banks of small streams (waterlogged soils). *Elegia tectorum* (Moline & Linder) on the other hand, is widespread and found across the Western and Eastern Cape. Individuals of *E. tectorum* reside in seasonally damp, but well-drained sandy soils as well as in seeps at a higher range of altitudes than *E. fenestrata* (10 - 600m). These species represent two closely related species that occupy extremes of the hydraulic niche axis in the Restionaceae.

1.4.2. Optical Vulnerability (OV) technique.

Methods used for constructing vulnerability curves and quantifying vulnerability to embolism are abundant and differ from each other in the way that water stress is imposed and how conductivity is measured (Cochard *et al.*, 2013).

Here I used the OV technique, which records changes in light transmission caused by a transition from water-filled to air-filled vessels (Brodribb *et al.*, 2016, 2017). The OV method/technique provides the best evidence to show how embolism behaves within the water transport system of plants (Brodribb *et al.*, 2016). The technique is based on early work showing that vessels that have undergone embolism have a higher reflectance value than fully functional ones (Brodribb *et al.*, 2016). Vulnerability parameters calculated using this technique has been shown to correspond closely with parameters calculated via other methods which confirms the accuracy of the OV technique in assessing hydraulic failure and predicting mortality thresholds (Brodribb *et al.*, 2016, 2017). The method uses a flatbed document scanner or camera to either scan or take images of stem segments being subjected to experimental drought (Brodribb *et al.*, 2017). The

OV technique evaluates the loss of function in xylem as a cumulative area of stem that has experienced embolism at any given time over the period of imposed drought (Brodribb *et al.*, 2017). Embolism area is used as a proxy for PLC because of the nearly 1:1 relationship between P_{50} and the point at which 50% of xylem area has experienced embolism (Brodribb *et al.*, 2016).

1.4.3. Aims, objectives and hypotheses.

The aim of this thesis is to develop the methodology and to evaluate the safety margins and hydraulic strategies of two closely-related species from contrasting habitats to explore possible mechanisms underlying species niche limits. I hypothesized that a species safety margin will correspond with its hydrological niche. To examine this hypothesis, I tested the prediction that species occurring in more mesic habitats should have smaller safety margins resulting from more vulnerable xylem (i.e. more anisohydric with a greater risk of hydraulic failure) in comparison to species occurring in drier habitats. The latter species is predicted to have safety margins that reflect a more conservative approach to water loss (i.e. more isohydric with a greater risk of carbon starvation). I quantified xylem vulnerability to embolism curves and pressure-volume curves to obtain both the vulnerability of the xylem (P_{50}) and the stomatal closure point (Ψ_{TLP}) in order to calculate the stomatal safety margins.

CHAPTER 2: Assessing the stomatal safety margins in two species of Restionaceae (*Elegia fenestrata* and *Elegia tectorum*) using xylem vulnerability to embolism and pressure-volume curves.

2.1. Introduction

2.1.1. Stomatal safety margins provide insight into the hydraulic niche.

Drought has been identified as one of the biggest contributors to plant mortality and is predicted to increase with ongoing climate change (IPCC, 2014). Yet, our understanding of the precise mechanisms underlying how plants respond to water deficit remains limited, hindering our ability to predict which species will die during drought. There exists an urgent need to better understand the mechanisms underlying how plants respond to water deficit if we are to accurately predict impacts on vegetation structure, distribution and species composition generated by differential plant mortality (Born and Linder, 2018, Powers et al., 2020).

Much recent progress has been made on trait-based frameworks, which can assist in predicting how plant species are likely to respond (see, for example, Skelton et al. 2015). A major challenge of such frameworks is to identify which species characteristics (i.e. traits) govern the process of drought-induced mortality, given that drought-induced mortality involves multiple processes, including xylem failure and subsequent desiccation of downstream tissues and/or an inability to move photosynthate/osmolyte around the plant leading to disruption of biochemical processes, and increased susceptibility to biotic and abiotic agents (i.e. pests and pathogens) (Mitchell et al., 2013, Choat et al., 2018). Recent studies indicate that hydraulic failure is the primary mechanism of drought-induced plant mortality (Urli *et al.*, 2013; Choat *et al.*, 2018; Powers *et al.*, 2020) and consequently trait frameworks for predicting plant response frequently include xylem capacity to withstand hydraulic failure (e.g. P_{50}).

Stomatal traits, also, are frequently incorporated because they are recognized as the “master controllers” of water loss: decreasing stomatal aperture provides a mechanism for controlling water loss, reducing the risk of atmospheric demand for water exceeding the supply through the xylem thereby avoiding hydraulic failure (Martínez-Vilalta et al., 2014, Martin-Stpaul et al., 2017, Creek et al., 2018). The water potential associated with stomatal closure co-varies with growing site aridity (Martin-Stpaul et al., 2017, Bartlett et al., 2012). However, stomatal closure as a protective mechanism against desiccation limits photosynthesis and growth and prevents evaporative cooling (Martínez-Vilalta et al., 2014), ensuring that the relationship between the water potential associated with stomatal closure and drought tolerance is non-linear (Martin-Stpaul et al., 2017). One way forward is to merge xylem vulnerability to embolism and stomatal dynamics to generate a so-called "stomatal safety margin" (the water potential difference between the water potential associated with stomatal closure and the water potential associated with 50% loss of function). Larger safety margins indicate species that are more conservative in their water use, closing stomata prior to experiencing disruption to the water transport network.

To the extent that habitats have filtered species composition and that species are in equilibrium with their growing environments, one might expect that the traits that species possess reflect the fundamental hydrological niche (Silvertown, 2004). This niche is described as the hydrological envelope that the species can physiologically tolerate, based on the amount of water available for use within their habitats (Huber and Linder, 2012; Born and Linder, 2018).

Hydrological niches range from severely dry to permanently waterlogged microhabitats and being able to persist at either end requires special adaptations in xylem and/or stomatal form and function (Araya *et al.*, 2011; Silvertown, Araya and Gowing, 2015; Born and Linder, 2018). Current evidence indicates that long-lived evergreen species occurring in drier environments must possess either resistant xylem (Pockman and Sperry, 2000) or large stomatal safety margins (Skelton et al., 2021). However, it is unclear why drier adapted species are filtered from mesic or waterlogged habitats. One possibility is that aerenchyma tissue is required to function in waterlogged, anoxic conditions (Silvertown et al., 2015, Araya et al., 2011), but that this tissue introduces air into the hydraulic network, rendering the xylem more vulnerable to embolism. Although this mechanism would restrict the capacity of mesic adapted species to enter into dry environments, it is yet to be investigated. Understanding how species are separated out along hydrologic niches are important particularly in the face of climate change since understanding the limits of species can provide insight into which species will not be able to persist under future drought conditions (Silvertown, Araya and Gowing, 2015).

Here I examine the connection between plant hydraulic traits and the physical environment testing the hypothesis that trade-offs in hydraulic function will lead to separation of species within different habitat types (or microenvironment). I predict that species occurring in dry environments that are water limited should possess less vulnerable xylem and larger positive safety margins (i.e. should close stomata prior to reaching water potentials associated with embolism). On the other hand, species occurring in wet or waterlogged habitats might require aerenchyma tissue that renders them more vulnerable to xylem embolism and causes them to display narrow, or even negative stomatal safety margins (stomata close after some level of hydraulic failure has occurred) (Fig. 4).

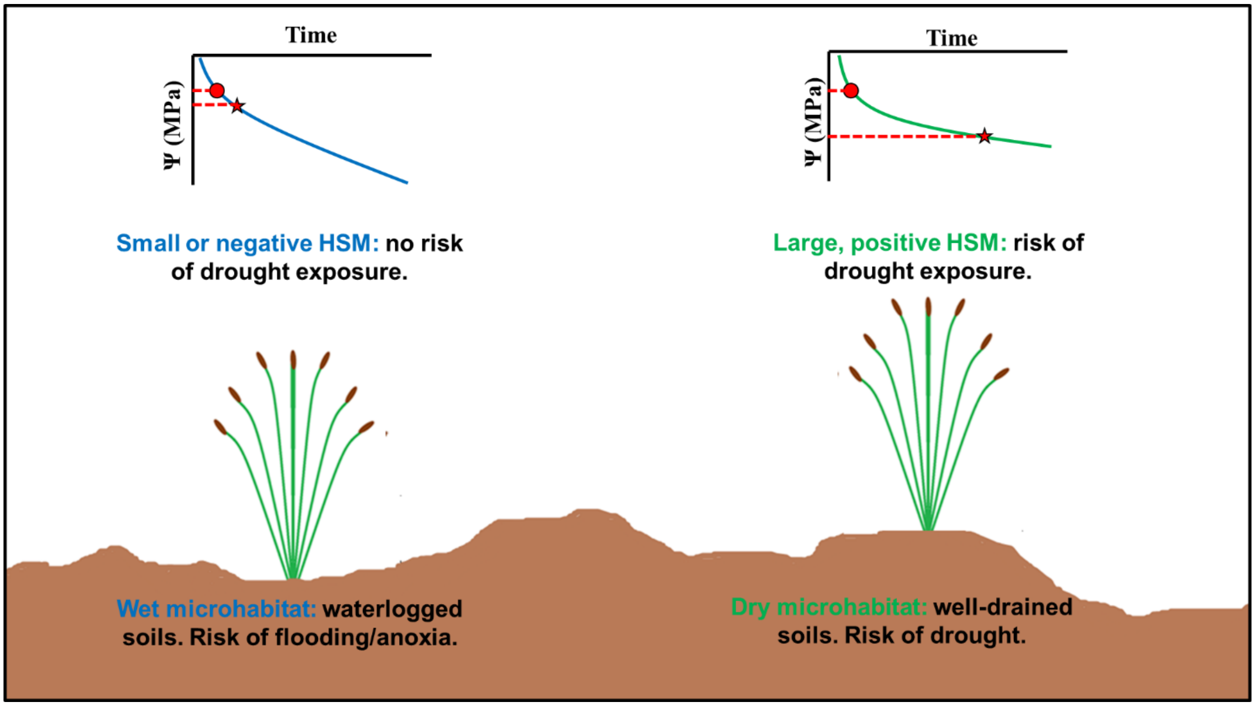


Figure 4: Diagram depicting the hydraulic safety margins for two hypothetical species occurring in different hydraulic niches. The red circles represent the turgor loss point (Ψ_{TLP}; MPa) and the stars represent the xylem pressure at which 50% of embolism has occurred (P_{50}; MPa). These two species represent the extremes of the hydraulic niche axis, but species segregate out into distinct hydrological niches across the entire axis between these two examples. The species present in the dry microhabitat have larger safety margins because the risk of being exposed to drought is high and protection is necessary. The species present in the wet microhabitat have lower safety margins because the chance of being exposed to drought is low and therefore these species are of more concern as they would be less prepared for drought and more vulnerable.

2.1.2. Using a trait-based approach to quantify hydraulic safety margins in Cape Floristic Region (CFR) Restionaceae.

The Cape Floristic Region – one of the world's most biodiverse regions – presents an interesting vegetation type in which to examine variation in hydraulic safety margins among diverse plants and its potential role in determining species' response to global change. Most of the CFR experiences seasonal droughts during the summer due to its Mediterranean-type climate system, but these patterns are likely to shift with climate and land use change (e.g. water abstraction).

Furthermore, the Restionaceae – a reed-like monocotyledonous group and one of the main functional types within the CFR – are an interesting candidate group to investigate hydraulic safety margins because their distribution has been shown to be coupled with accessibility to water because species segregate along fine-scale moisture gradients, exhibiting hydraulic niche segregation. Restionaceae species also occur across the entire precipitation gradient (winter-rainfall to aseasonal) as well as from well-drained to waterlogged soil types. Yet, relatively little is known about the hydraulic physiology of this group (Linder 1993).

A study by Skelton *et al* (2015) in the region, used the trait-based approach to define species hydraulic safety margins of various fynbos species. The two species of Restionaceae included in the study had negative stomatal safety margins (i.e. an anisohydric stomatal regulation strategy) (Skelton et al. 2015). These strategies are thought to be infrequent in long-lived woody trees which habitually experience highly negative water potentials because of the high risks of hydraulic failure during short, intense periods of drought. However, water potentials in the Restionaceae remain high (i.e. less negative) and it is unclear how likely these non-woody graminoids are to experience embolism.

Finally, if the Restionaceae are similarly prone to declines in function this may impact fynbos vegetation structure, functional dynamics and demographics substantially because restios are an ecologically significant component of fynbos vegetation (Rebelo et al. 2006; Huber & Linder. 2012; Born & Linder. 2018). If their hydraulic safety margins are finely tuned to their physical environment, then it would be reasonable to question how these species would fare with predicted changes in habitat water availability due to droughts and flooding.

Here, we hypothesized that species' hydraulic safety margins would reflect the habitat or microenvironment that they occur in (Fig. 4). We predicted that species from waterlogged/seep microhabitats might possess smaller or negative stomatal safety margins compared to species occurring in better drained sites. To test this hypothesis, we used a newly developed optical method to quantify the capacity of two congeneric species to withstand xylem embolism and the turgor loss point as a proxy for stomatal closure. One species, *Elegia fenestrata*, is a wetland endemic and the second, *E. tectorum*, occurs in more arid sites.

2.1.3. Using the Optical Vulnerability (OV) technique and the turgor loss point (Ψ_{TLP}) to calculate and assess Hydraulic Safety Margins (HSM) in Restionaceae.

Considerable uncertainty surrounds the capacity of plants to withstand embolism. Vulnerability curves have mostly been generated on woody tree and shrub species (99.5%), but very few graminoid species (0.5%) (Choat *et al.*, 2012) and not many restios (Skelton, West and Dawson, 2015). In addition, vulnerability curve data collection has been hindered by methodological artefacts associated with certain techniques (Cochard *et al.*, 2013). The Optical Vulnerability (OV) technique provides a way of efficiently sampling many species, facilitating studies in comparative physiology. This technique has been widely used to visually quantify embolism spatially and temporally in woody and herbaceous angiosperms (Brodribb et al., 2016, Brodribb et al., 2017, Skelton et al., 2021, Skelton et al., 2017), but it is unclear how successful it will be when used to quantify vulnerability in graminoid species. The OV method has been used successfully on a monocotyledonous graminoid (wheat leaves; (Johnson, Jordan and Brodribb, 2018)).

Instead of using Pg_{12} as a proxy for stomatal closure, this study used the water potential associated with turgor loss (Ψ_{TLP}, MPa). The Ψ_{TLP} is a proxy for stomatal closure that is influenced by multiple other metrics related to water use and storage. The Ψ_{TLP} is the xylem pressure at which leaf cell turgor drops to zero during drought stress causing cells to collapse (Bartlett et al., 2014, Choat et al., 2018). The stomata are closed due to the collapse of guard cells flanking the stomatal pore and both gas exchange and growth are halted (Bartlett et al., 2014). The turgor loss point also defines the 'permanent wilting point' which is the soil water potential threshold which if surpassed can severely impact the ability of plant to take up sufficient moisture to combat wilting (Bartlett, Scoffoni and Sack, 2012; Bartlett et al., 2014).

The turgor loss point is one of the metrics that can be quantified using a pressure-volume curve which defines the relationship between leaf water potential and the leaf water volume (RWC; %)

(Bartlett et al., 2014) and provides many parameters defining the elasticity and water storage capacity of plant cells. In addition to the turgor loss point, a few other significant parameters that can be calculated from this curve namely, solute concentration (Ψ_0; osmotic potential at full turgor), water content (RWC_{TLP}; relative water content of cells at the turgor loss point and a_f; extracellular water content) and cell elasticity ($\mathcal{E}$; modulus of elasticity).

The more negative the turgor loss point is, the longer plants are able to keep their stomata open and plants regulate other PV parameters in order to achieve the most negative turgor loss point suitable for their habitat water availability (Bartlett et al., 2014). The turgor loss point is considered a ‘higher-level’ drought tolerance trait because it influences structural integrity, metabolism and whole plant functionality (Bartlett, Scoffoni and Sack, 2012) and is mostly influenced by the osmotic potential at full turgor (accumulating solutes in existing leaves or producing new leaves with higher solute concentrations), with cell elasticity and extracellular water content having negligible effects (Bartlett et al., 2014).

The leaf hydration at wilting (RWC_{TLP}) is also proposed to be an important drought tolerance parameter, but after extensive debate, it has been decided that while it is important, it is secondary to turgor loss point as a key drought-tolerance trait because it does not correlate with habitat moisture availability, but the turgor loss point does (Bartlett, Scoffoni and Sack, 2012). With that said the cell hydration at turgor loss point is important for plant survival during drought as a drop in hydration below approximately ~60% would negatively affect plant metabolism (Bartlett, Scoffoni and Sack, 2012). Both the turgor loss point and osmotic potential at full turgor have been shown to correlate with habitat water availability (Bartlett, Scoffoni and Sack, 2012) and further highlights their importance in determining species niches and safety margins.

This study aims to use the abovementioned hydraulic safety margins to develop a methodology for determining the hydraulic safety margins in Restionaceae. We hypothesized that the hydraulic safety margin should be coupled to hydraulic niche and that we would see smaller/more negative safety margins in *E. fenestrata* and larger ones in *E. tectorum*.

2.2. Materials and methods

2.2.1. Study species and plant material

The study was conducted on two species of Restionaceae from the *Elegia* genus. *Elegia tectorum* is a widespread, dry habitat species. They occur in seasonally dry, sandy soils at a wider range of altitudes (10 – 600m) (Linder et al. 2018). *Elegia fenestrata* is a wet habitat species occurring in mostly waterlogged soils at a low altitude (5 – 100m) in a localized area on the Cape Peninsula of South Africa (Linder et al. 2018). *E. fenestrata* is a vulnerable species (according to the IUCN) threatened by alien invasive species and habitat degradation in fynbos and Afromontane forests, which is where they are generally found. *E. tectorum* is of least concern and despite extensive habitat loss, is still able to persist successfully along their distribution range (IUCN).

Five individuals from each species were sourced from two nurseries. *E. fenestrata* individuals were obtained from the SANBI production nursery situated at Kirstenbosch Botanical Gardens. E. tectorum individuals were sourced from Starke Ayres, a commercial nursery situated in Rondebosch, Cape Town, because the individuals at SANBI's production nursery were too small and the culms were not yet big enough for measurement to be successful. An individual was selected for measurement if it had at least 8 culms that were approximately 5mm in diameter. The size of the culm was important because the culm needed to be robust enough to be able to expose the xylem without too much damage during preparation for scanning and to be able to fit into the pressure chamber comfortably.

Plants were kept in a glasshouse for 24-72 hours before measurement (because only one individual could be measured at a time, some individuals were kept longer than others) and thereafter, placed in the dark for approximately 16 hours (overnight) to halt transpiration and ensure that plant was fully hydrated before measurements commenced.

2.2.2. Xylem anatomy: Visualizing the cross-sectional and longitudinal view of xylem vessels.

To obtain a visual assessment of the xylem anatomy and architecture in these two species, the xylem of a representative culm of each species was stained with Toluidine Blue O. A 5 ml syringe was used to perfuse the dye through the culm sections. Culm sections of approximately 2cm in length were cut underwater using a single-edged carbon steel blade (GEM) and had one open end of the section secured into the nozzle of the syringe with Parafilm (Bemis Company, Inc., USA). The other open end was suspended over some absorbent tissue. Pressure was applied to the culm segment until a steady flow of dye was observed coming out the open end. Thereafter, the segments were flushed with reverse osmosis (RO) water in the same way that the dye was perfused, until water ran clear of excess dye.

A window of epidermis and chlorenchyma tissue was removed in the same way a culm would be prepped for scanning (see Section 2.2.3.1.) to expose stained xylem tissue. Images of the windows were taken using a phone camera over a dissecting microscope at a magnification in the range of 5-8X depending on culm diameter. To photograph the cross-sectional areas, a thin slice of the culm was sliced off using a single-edged razor blade and photographed in the same way as the lateral view.

2.2.3. Vulnerability curves: Determining the xylem P_{50} value for each species.

The following methods of determining vulnerability to embolism was adapted from methods found on opensourceOV.org website:

2.2.3.1. Culm preparation

Using a dissecting microscope and a single-edged carbon -steel blade, the epidermal and chlorenchymal layer of three culms of similar diameter and length was carefully scraped away to reveal the xylem tissue. During this procedure, care was taken to not cut into any xylem tissue and to ensure that the exposed vascular tissue remained moist by periodically dousing it in water to prevent any air entering the xylem before measurement.

Before culms were excised from the intact plant, they were attached to the bed of a desktop document scanner (Epson Perfection V850 scanner; Epson America) with the window of exposed xylem tissue facing down. Each window was surrounded by a rectangular putty (Prestik, Bostik SA) frame in order to create a containment well for ultrasound gel (Tensive ultrasound gel; Parker Labs, America). The ultrasound gel ensured that the window did not desiccate too rapidly without impeding the transmission of light to the xylem surface. Each Prestik encased well was covered by a glass microscope slide. Culms and microscope slides were secured to the scanner bed with duct tape to prevent any movement which may interfere with measurements during the dry down process. Culms were then cut from the plant immediately before measurements began to make sure that culms did not start dehydrating before scanning started.

Tests were conducted to ensure that cutting culms did not introduce embolism into the xylem in the scanning window, which was some distance from the cut end (> 30 cm and spanning several inter-nodes). The test involved applying pressurized air to one side of a culm and submerging the other side under water while cutting the culm back until air bubbles emerged in the water. The pressure applied was insufficient to force air through pit membranes, thus bubbles could only arise as a result of transport through pith/aerenchyma or open xylem vessels. Air bubbles were only witnessed when the culm had been cut back such that there was no node between the air source and the end of the culm, confirming that vessels do not span across nodes.

2.2.3.3. Culm water potential and mass

Culm xylem water potential (Ψ_{xylem} MPa) was measured on 5 additional culms during the scanning period. Water potential was not able to be directly measured on culms that were being scanned as this would disrupt the optical measurements. These 5 culms were excised from the plant at the same time as the culms on the scanner and kept in as similar conditions as possible. Their water potential was measured periodically using a Scholander pressure chamber (Model 1000; PMS instruments, USA) with a digital pressure gage (Model CPG500; Wika, SA) attached (for more accurate reading of measurement) and weighed (analytical balance; Mettler Toledo, International).

In order to fit ± 30cm culms into the pressure chamber, a longer chamber (model 600-EXP; 60 cm; PMS Instruments USA) was used that had been optimized for use on restios. Culms were measured approximately every 15 minutes in the beginning of the dry down period and gradually changes to approximately every 2-3 hours nearer to the end of the dry down period because the drop in water potential becomes slower as the water potential becomes more negative. Water potential measurements occurred for the first 3 days of the scan period, after which they had reached -4MPa, which was the lowest the pressure chamber could measure. At this water potential culms were visibly dehydrated and often became damaged, indicating that there was little point in continuing past this point for our measurements.

Water potential measurements and the time they were taken from excision were used to model a relationship from which water potential at time of scan could be calculated. Water potential decline in these restios happened in 3 phases; an initial rapid phase, a slower middle phase and an almost flat third phase. Segmented regressions were fit to these data using the time points at which water potential decline rate changed. The scanned images were then arranged into time bins reflecting the 3 distinct rates in water potential decline. Depending on which time bin an image ended up in, the modelled water potential at the time of scan could be calculated using the slope and intercept data of the corresponding section of the segmented regression. In this way, each scan can be related back to a water potential at time of scan.

Culm mass was also measured periodically during dry down in order to calculate relative water content (RWC; %) and construct Pressure-Volume curves (see Section 2.2.4).

2.2.3.4. Image processing

Once the scan period was complete, the images were downloaded and processed using Image J (NHI) (see http://www. opensourceov.org/ for specifics). The raw images are qualitative and have to be processed in order to have quantitative value. Each culm's image sequence was loaded into Image J and processed using the OSOV toolbox (see http://www. opensourceov.org/ for specifics). Using the image difference function in the OSOV toolbox, an image stack was generated by subtracting each image from the subsequent image. This image stack showed any changes between low reflectance (water-filled) and high reflectance (air-filled) at each scan interval. A change from water-filled vessels to air-filled vessels ("embolism" events) occurred as an area of white pixels on a dark grey background (APPENDIX A).

Images were processed manually, and all events were identified. Events were confirmed by looking at the same images in the original image stack and observing a colour change when moving between images. Considerable care was taken not to include colour changes that were that were due to movement and shrinkage as a consequence of non-xylem tissue collapse during the dry down period. Additionally, at the end of the scan, the total area cavitated was compared with the expected total area of xylem vessels for this species, based on staining (Figure 2) to ensure we were not capturing false events due to non-xylem tissue collapse.

The number of pixels in each image in the sequence containing embolism events was recorded by using the 'measure stack' function in the OSOV toolbox. Images without any events were recorded as having zero pixels. The cumulative number of cavitated pixels was calculated at each time point. This was converted to the percentage area of embolism by dividing by the maximum cumulative cavitated pixel area.

2.2.3.5. Constructing vulnerability curves.

The optical measurements were then plotted against the modelled water potentials at the time of scan to construct a vulnerability curve for each individual culm. The above was repeated for each replicate per individual.

A relationship was fitted to each individual curve defined as:

$$Embolism\ area\ (\%) = 100 - \frac{100}{(1 + e^{a(x-b)})}$$

The mean species P_{50}, P_{88}, and P_{12} were extracted from the curve using these fitted relationships by plugging the percentage area (12%, 50%, 88%) into the above equation. Individual culm curves were then averaged to obtain an average species curve for each species, provide average species vulnerability curve parameters and provide a degree of variability of vulnerability between and within species.

2.2.4. Pressure-volume (PV) curves: Determining the stomatal closure point.

Pressure-volume curves were created from the water potential and mass data gathered during the optical scanning dry down. Once the water potential measurements were completed, at the end of a scanning period, the culms were put in a drying oven at 60°C for 48 hours and then weighed in order to get the dry weight. The relative water content (RWC; %) was then calculated as:

$$RWC = \frac{m_w - m_d}{m_s - m_d} \times 100$$

Where m_w is the wet weight (g) at any given time, m_d is the dry weight measured after oven drying, and m_s is the saturated mass which is the first mass measurement taken on the culms when it was fully hydrated.

For each culm the pressure-volume curve was plotted as the inverse of the water potential ($\frac{1}{\Psi_C}$; MPa^{-1}) versus the relative percentage water loss calculated by subtracting the RWC from 100. The graph has two very distinct phases and the point of inflection between these two sections was calculated by fitting a segmented regression. The point where the two lines meet (breakpoint) is the point of inflection. The turgor loss point (Ψ_{TLP}; MPa) is calculated as the water potential at the point of inflection. Stomatal guard cells collapse at the point at which turgor pressure reaches zero. As a consequence, the stomatal pore closes, and the movement of water and carbon is partially halted. Therefore, the turgor loss point has been recognized as a reliable proxy for stomatal closure. The osmotic potential (Ψ_0) is calculated as the y-intercept of the second linear phase of the graph (i.e. it is extrapolated). The relative water content at turgor loss point (RWC_{TLP}) was calculated by subtracting the water loss (%) at Ψ_{TLP} from 100.

2.2.5. Statistics

In order to test for differences in the measured parameters between the two species, I used two-sample T-tests for normally distributed data. I tested normality using QQ-plots and Shapiro-Wilks normality tests. All data was normally distributed. All plots and tests were conducted in R (R Studio; v. 1.3.959).

2.3. Results

2.3.1. Xylem anatomy.

Culm anatomy in these two *Elegia* species is characteristic of monocotyledonous graminoids. The vascular bundles are flanked by non-xylary tissue (Fig. 5). There is an obvious physical difference between the two species: *E. fenestrata* has a lower vessel density than *E. tectorum* (Fig 5A & B). In both species, it is safe to assume that most, if not all, of the scan window should be filled with embolism events for the entire embolism pattern to be captured (Fig 5C & D).

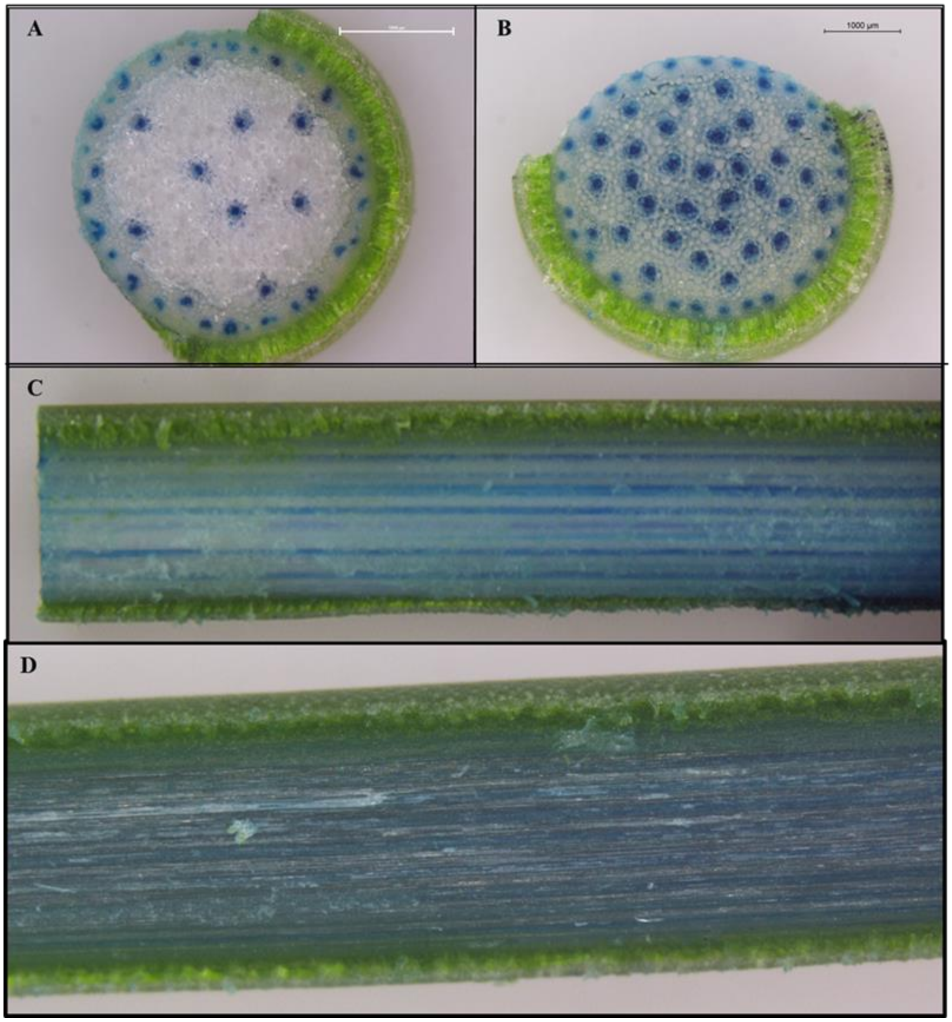


Figure 5: Xylem anatomy of two Restionaceae species. Cross-sectional area of (A) *E. fenestrata* and (B) *E. tectorum* shows different vessel densities. Scan window view of (C) *E. fenestrata* and (D) *E. tectorum* which gives an idea of what a completed scan window should look like if all xylem tissue has become embolized.

2.3.2. Culm water potential during dry-down.

The relationship between water potential and time in these species was non-linear (Fig. 6). Although there was some variation between culms within individuals and between species, the general pattern of dehydration was highly consistent. The variability appeared not to be measurement error, but rather reflected real differences in the rates of drying down between individuals or species, as indicated by the consistency of the PV curves generated from the same data (Fig. 7). Overall, all culms exhibited a rapid initial phase of dry down followed by a second slower phase and a third even slower phase. The initial rapid phase shows little variation, and the relationship is much tighter than the second phase. In the second and third phases, however, culm water potential diverged from each other significantly. Despite the variation, the segmented regression suitably captured trends in water potential over time during dry down (Fig. 6).

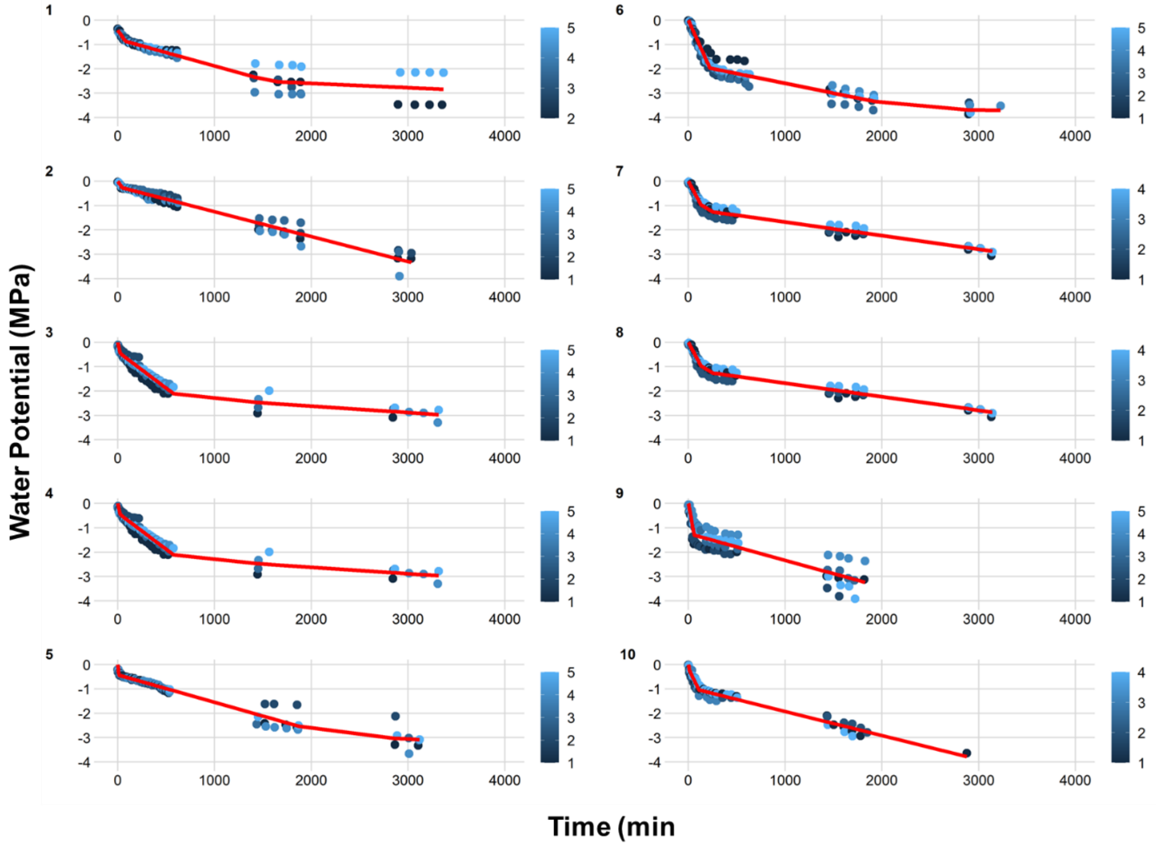


Figure 6: Water potential over time in multiple culms per individual. Some culms were excluded due to damage and breakage during measurement. All individuals of (1 – 5) *E. fenestrata* and (6 – 10) *E. tectorum* show the modelled segmented regression between time and water potential.

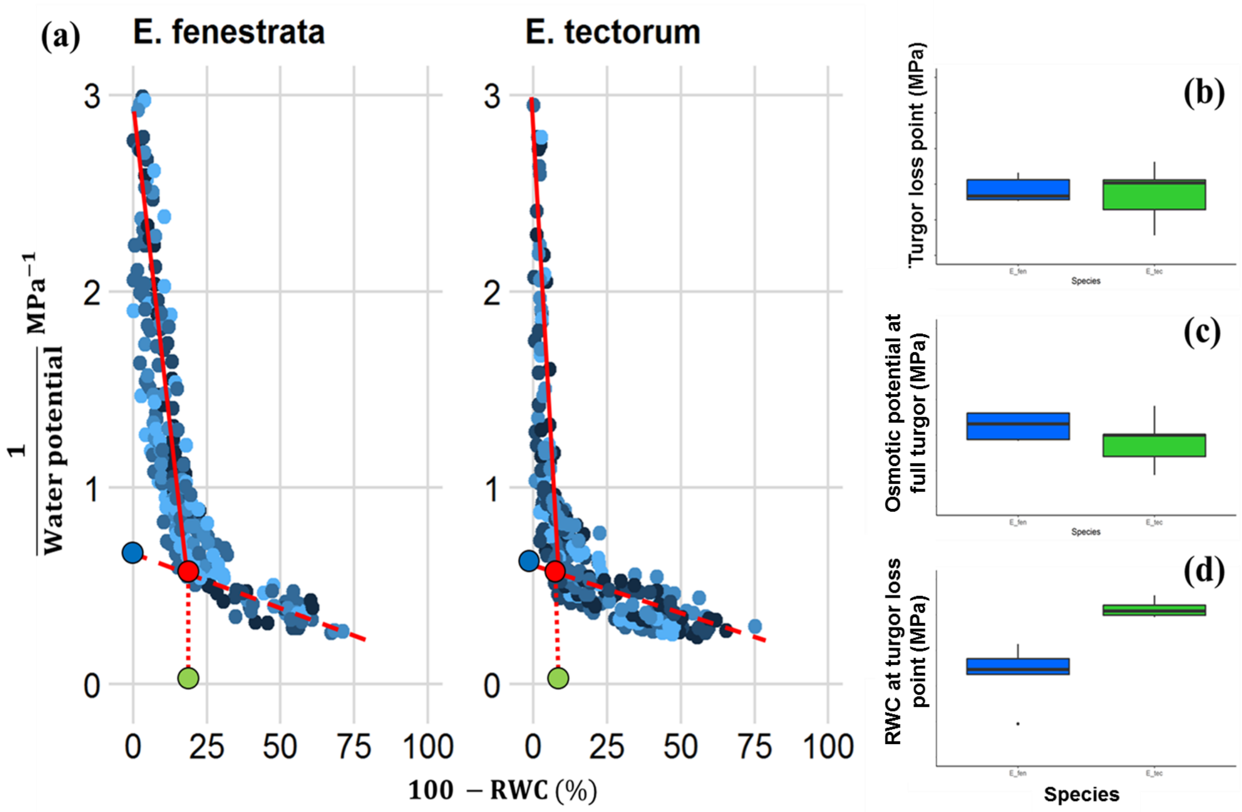


Figure 7: (a) Average species pressure-volume curves showing the point of inflection (red circle; where turgor loss point can be read off the graph), osmotic potential (blue circle) and RWC_{TLP} (green circle). The boxplots show the comparison between mean (b) turgor loss point, (c) osmotic potential, and (d) RWC_{TLP} between *E. fenestrata* (blue) and *E. tectorum* (green).

2.3.3. Pressure-volume curves.

Species lost turgor at similar water potentials (t = 0.29; df = 5.52; $p > 0.05$) and possessed similar osmotic potentials at full turgor (t = 1.18; df = 8, p = 0.27) (Fig. 7). The mean turgor loss point of *E. fenestrata* was -1.58 ± 0.12 MPa and *E. tectorum* -1.64 ± 0.11 MPa. The Ψ_0 of *E. fenestrata* was -1.37 ± 0.09 MPa and *E. tectorum* -1.59 ± 0.16 MPa. The only PV parameter that is significantly distinct between species is RWC_{TLP} (t = -4.65; df = 4.67; p = 0.007); *E. fenestrata* = 73.4 ± 3.8 % and *E. tectorum* = 91.7 ± 1.1%.

2.3.4. Xylem vulnerability curves.

2.3.4.1. Cumulative pixel counts.

In most cases, embolism increase (in cumulative pixel area) appeared to reach a plateau (maximum pixel area) before 4000 minutes of scan time, which corresponds with a minimum water potential of close to -4MPa (Fig. 8). A few culms have maximum pixel areas that are significantly lower than other culms of the same individual. This is mostly observed in *E. fenestrata* (2C, 3A, 3C, 5A and 5C) and only observed once in *E. tectorum* (1C) however, the maximum pixel area is consistent with culms from other individuals and both 1A and B have very high maximum pixel counts relative to all other culms (Fig. 8).

Scan windows for most culms are adequately full once -4MPa has been reached. The representative culms for both *E. fenestrata* and *E. tectorum* (Fig. 9) closely resemble the expected embolism pattern shown in Figure 5C & D. Culms that had an inadequately filled window by the time -4MPa was reached were excluded from the analysis based on the assumption that we had most likely missed embolism events (APPENDIX B). Considering the desiccated state of the culms by -4MPa, and the timing of plateaus seen in cumulative pixel area, it was considered extremely unlikely that substantial embolism events were still to occur.

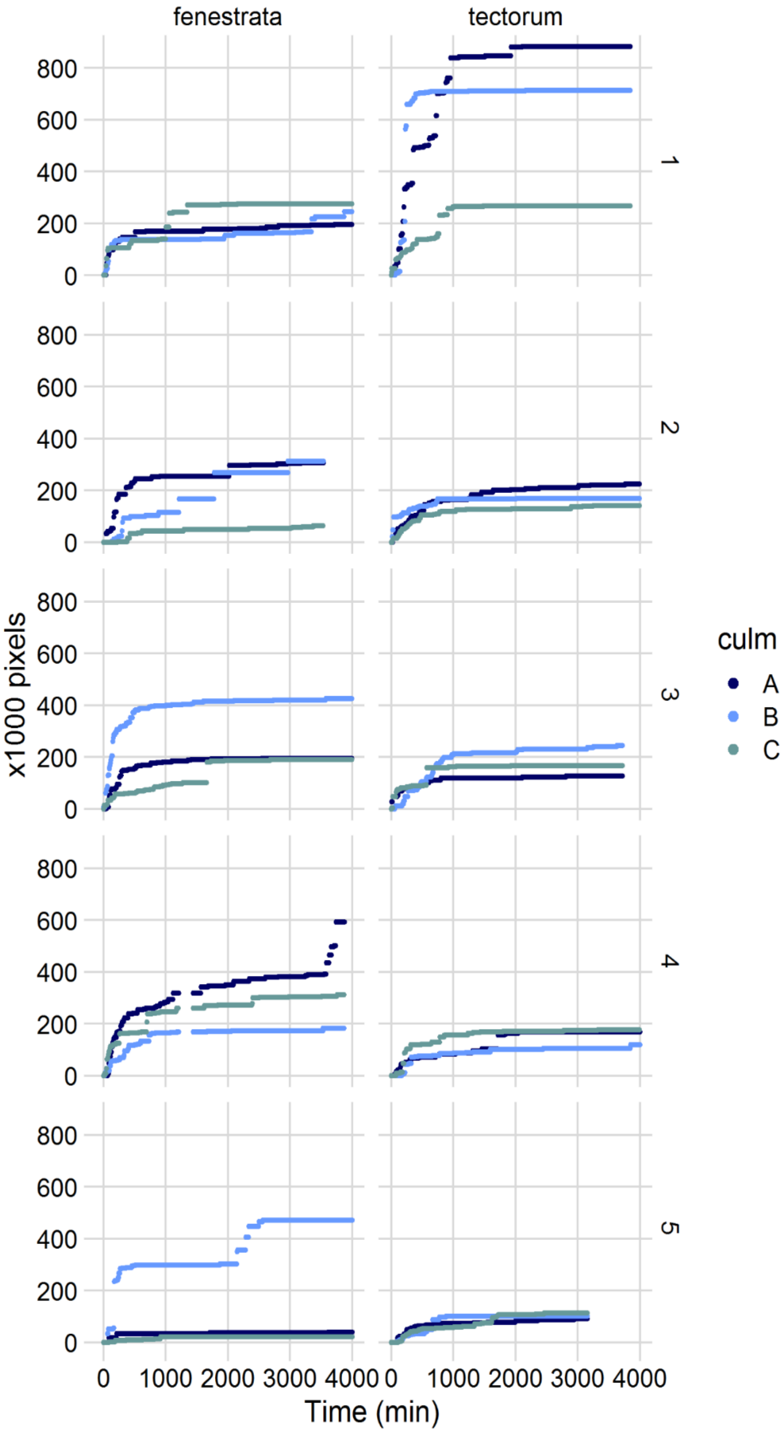
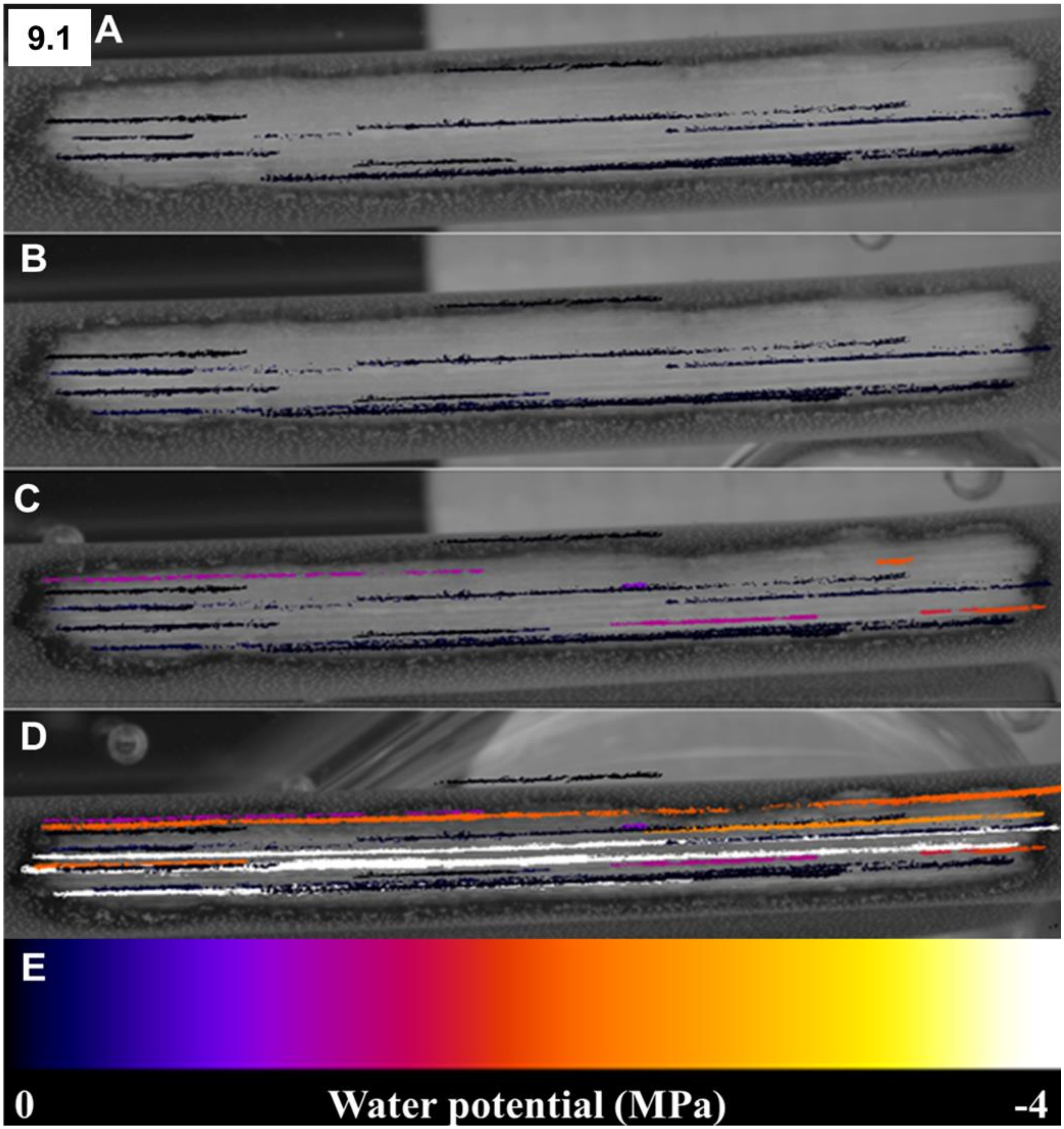


Figure 8: Cumulative embolism area (in pixels) over time for 5 individuals of each species. With the exception of a few culms, pixel area counts are consistent within individuals and species and a plateau is reached before 4000 min (which equates to approximately -4MPa) in almost all culms.

9.1



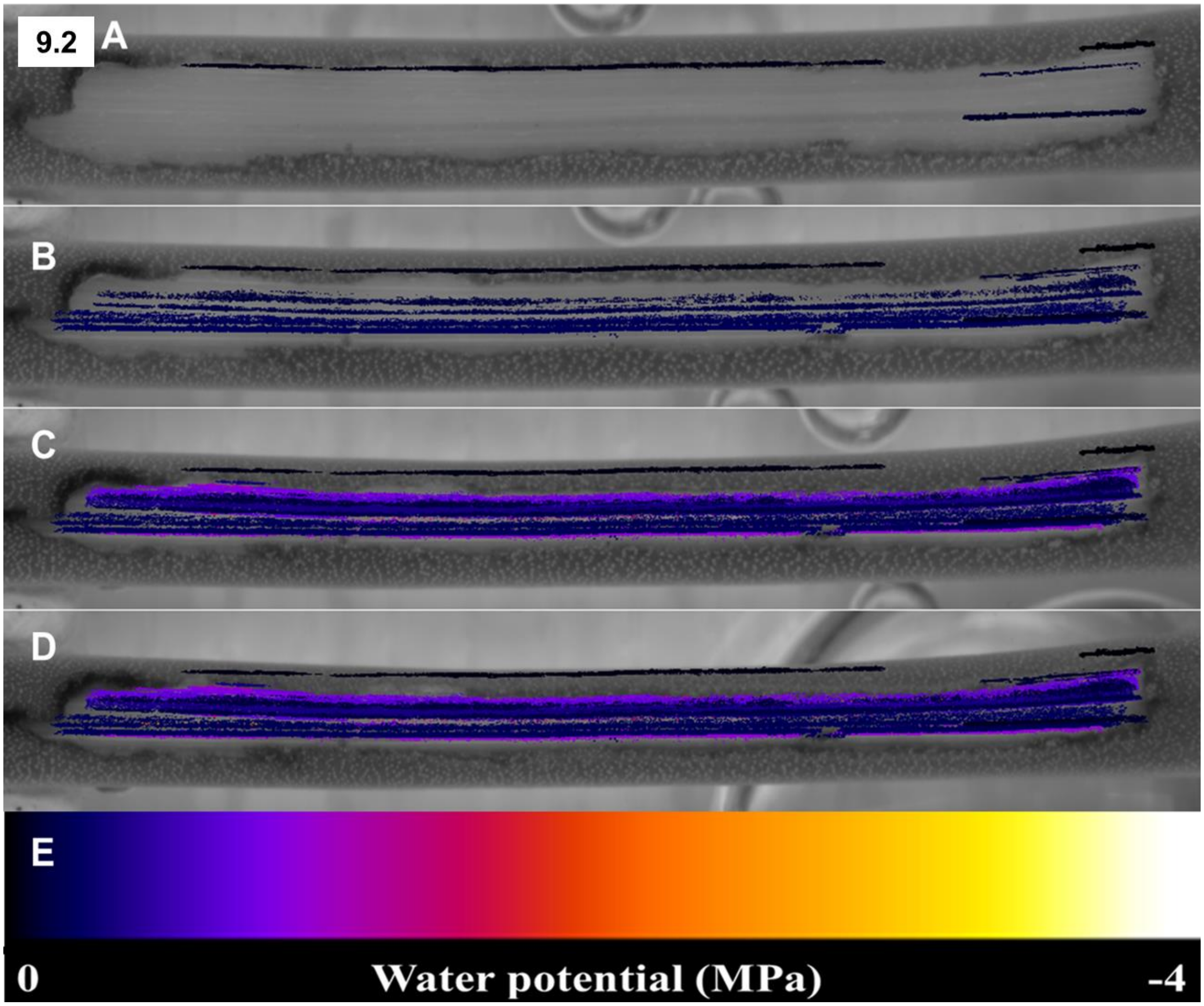


Figure 9: Scanned images of representative (9.1) *E. fenestrata* 1B and (9.2) *E. tectorum* culms at (A) -1MPa; (B) -2MPa; (C) -3MPa; and (D) -4MPa. Embolism events shown in colour which corresponds to the water potential at which it occurred (E).

2.3.4.2. Construction of xylem vulnerability curves.

The preliminary vulnerability curves terminated at -4MPa are sigmoidal with a few exceptions (Fig. 10). The culms that were excluded based on the abovementioned criteria (sections 2.3.1, 2.3.2 & 2.3.3.1) are shown in red or orange. After removing the culms mentioned above, the patterns of embolism were still variable between some culms from the same individual (Fig. 10). Considering the variability in water potential data (Figs. 6 & 7), it is unlikely to be due to methodological artefact but is rather as a result of within species physiological variability. Mean individual embolism area over time curves shows consistent patterns within species indicating that while there is variation in embolism propagation between culms for each individual, the average embolism propagation pattern is similar (Fig. 11). Most individuals plateau by -4MPa (except *E. fenestrata* 4; could be due to culm 4A), lending further justification for not including events after this water potential in the analyses.

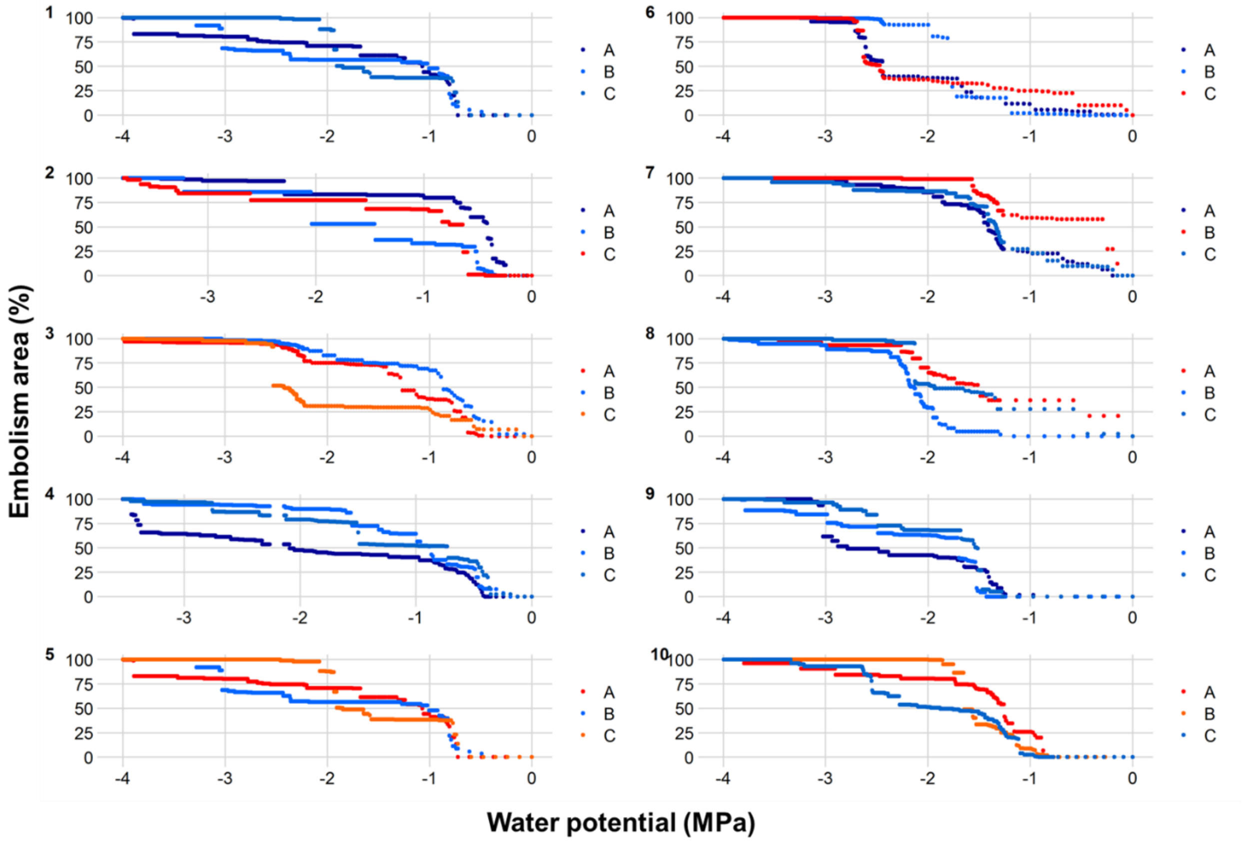


Figure 10: Vulnerability curves for each culm included in the experiment. Culms depicted in blue are the culms that were included to fit an average species vulnerability curves and analyses. The red/orange curves were removed before the curve fitting process due to various criteria that made them unfit for analysis. The criteria were (1) inconsistent maximum pixel number (Fig 8); (2) insufficiently filled scan window (Fig. 9) and (3) inconsistent sigmoidal distribution.

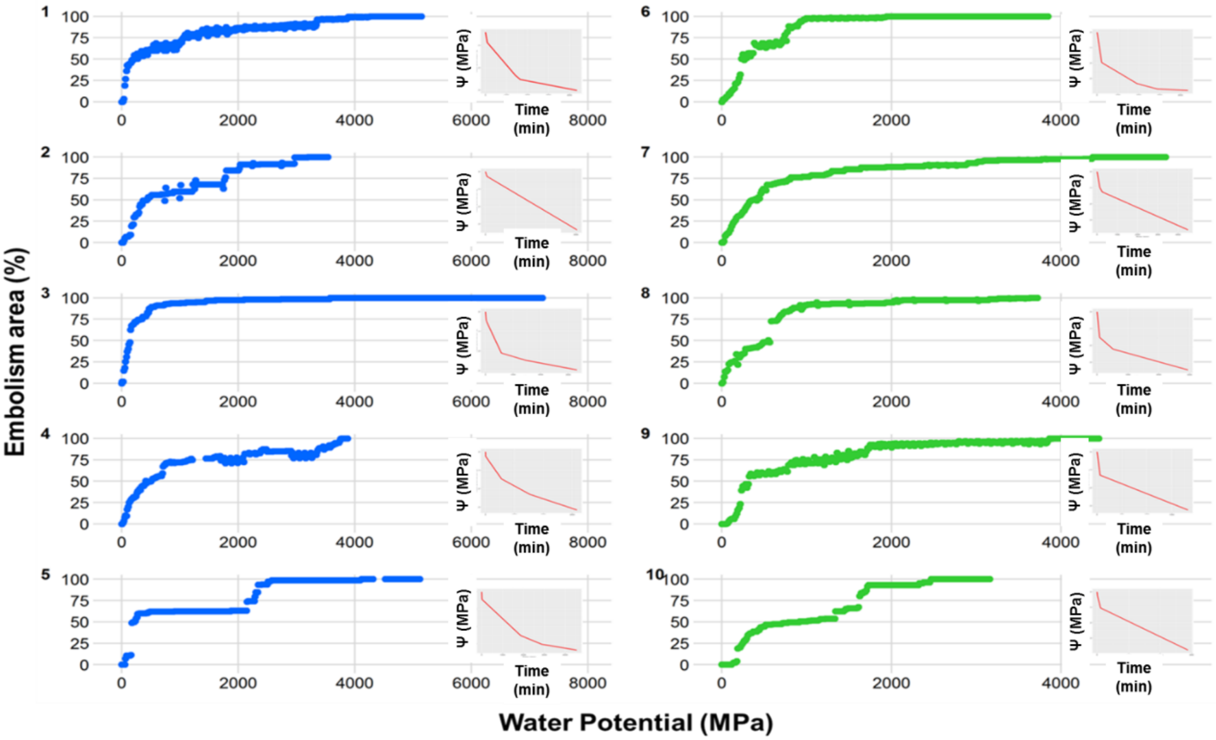


Figure 11: Average embolism area over time for each individual. Each individual of (1 – 5) *E. fenestrata* (blue) and (6 – 10) *E. tectorum* (green) have their modelled water potential (Ψ; MPa) over time (minutes) displayed (inset).

The average species vulnerability curves show a stark difference in vulnerability between the two species (Fig. 12). Most of the variability between individuals is observed between the P_{12} and P_{50} values but both species exhibit sigmoidal curves. The P_{50} values are significantly different between species (t = 5.89; df = 8; p = 0.0004; Fig. 9). *E. tectorum* appears to be less vulnerable to embolism (P_{50} = -1.6 ± 0.3 MPa) than *E. fenestrata* (P_{50} = -1.07 ± 0.06 MPa). The onset of embolism is also significantly different from one another with the average P_{12} of *E. fenestrata* occurring at less a negative water potential (–0.52 ± 0.07 MPa) than *E. tectorum* (-0.99 ± MPa). The P_{88}, however, was almost identical in both species (t = 3.095; df = 8; p = 0.01); *E. fenestrata* = –2.57 ± 0.2 MPa and *E. tectorum* = –2.56 ± 0.15 MPa. *E. tectorum* exhibited a slightly negative safety margin of –0.04 ± 0.18 MPa and *E fenestrata* a negative safety margin of -0.5 ± 0.16 MPa (Fig 7 & 12).

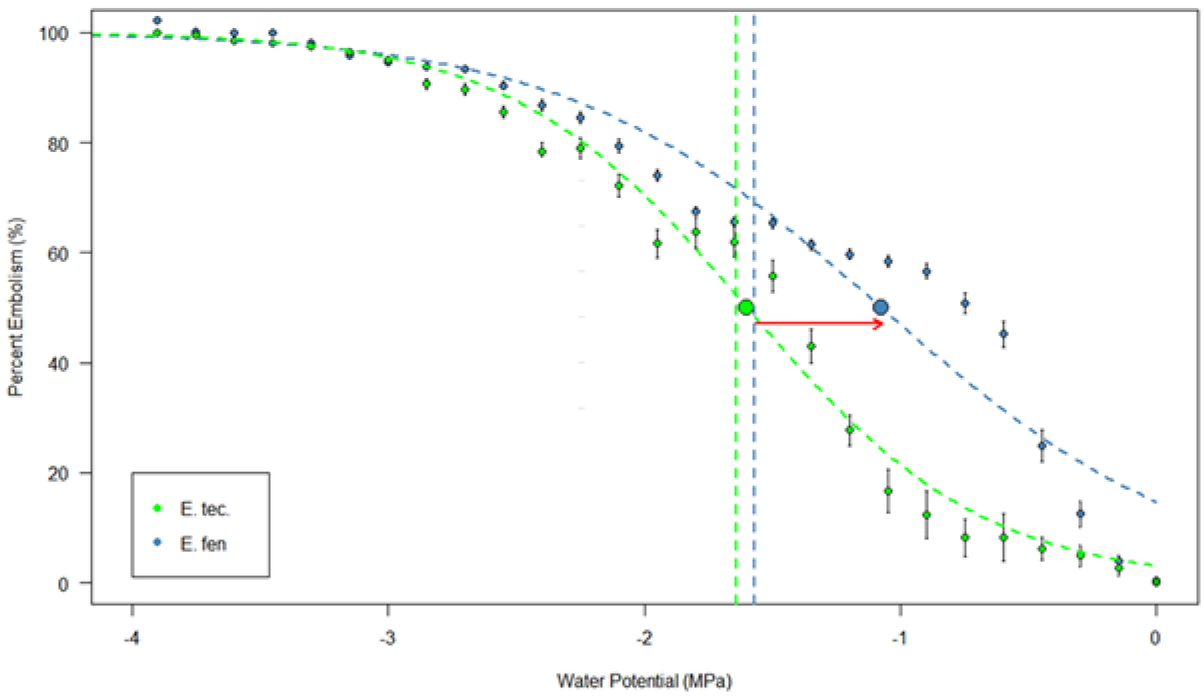


Figure 12: Average vulnerability curves for both *E. fenestrata* and *E. tectorum*. The point of stomatal closure (Turgor loss point; MPa) is shown by dashed lines. Average P_{50} values from the PLC model shown as blue and green circles for *E. fenestrata* and *E. tectorum*, respectively. The red arrow (→) depicts the large negative safety margin for *E. fenestrata*. The safety margin for *E. tectorum* was omitted because it was too small in magnitude.

2.4. Discussion

Our results show that closely related *Elegia* species can exhibit dissimilar hydraulic strategies. One species exhibited an anisohydric strategy (*E. fenestrata*; safety margin = –0.5 ± 0.16 MPa), while a congeneric species exhibits a slight anisohydric strategy (*E. tectorum*; safety margin = –0.04 ± 0.18 MPa). These results add to the previous findings of Skelton et al. (2015), which showed that two species of restio from different genera exhibited negative safety margins, indicating that they employ an anisohydric stomatal regulation strategy. By associating the trait data with microhabitat of the two *Elegia* species I provide support for the hypothesis that safety margins are coupled to microhabitat: *E. fenestrata,* a seep habitat species, has a negative safety margin indicative of leaving stomata open relatively late during dry down and E. tectorum, a dry habitat species, has a slightly negative safety margin and closes stomata before lethal levels of embolism (P_{50}) are allowed to occur.

2.4.1. Using the Optical Vulnerability Technique on monocotyledonous graminoids (Restionaceae).

Using the OV technique has given tremendous insight into the hydraulics of these species. However, the hydraulic anatomy of these species did pose certain challenges when using the OV technique. The technique was developed on woody angiosperms and the monocotyledonous nature of xylem anatomy in the restios (Fig. 4) gave rise to an issue with distinguishing between embolism in xylem and colour change of non-xylem tissue.

My study has shown that this can be effectively dealt with by identifying a water potential after which point additional changes in optical properties are most likely due to culm shrinkage, and/or tissue collapse. This was done by the combination of 1) physical observation of the culms during the drydown, 2) observation of the size and shape of events in the cleared portion of the culm, 3) identifying the water potential at which the cleared window was full of events.

Additionally, for practical purposes, we were limited to water potentials > −4.1MPa due to the safety valve of our pressure chamber. However, this was more than sufficient for these species, as physically, culms were severely dried out and appeared ‘crispy’ by the time the culms reached –4MPa. As such, it was highly unlikely that the xylem was functional beyond this point, and any additional ‘events’ observed thereafter would be due to culm shrinkage or non-xylem tissue collapse. Lastly, the scan window for most culms was adequately filled by the time −4MPa was reached.

2.4.2. Unpacking the Optical Vulnerability (OV) and Pressure-volume (PV) traits involved in drought response.

The two species in this study showed opposing hydraulic strategies according to their safety margins which we can attribute to their very different habitats. *E. fenestrata* is a localized species found in a small area along the Cape Peninsula. They grow along the coast at low altitude (0 – 100m) in marshy areas and on the banks of small streams (waterlogged soils). Having relatively higher (less negative) P_{50} (−1.07 ± 0.095 MPa) and P_{12} (−0.52 ± 0.07 MPa) values provides evidence of a link between microhabitat and vulnerability to drought-induced embolism (Fig. 12). Due to the negative safety margin (−0.5 MPa), we conclude that this species does not close their stomata before dangerous levels of drought stress in the form of percentage of the vascular system inundated with embolism.

E. tectorum is widespread and found across the Western and Eastern Cape. They reside in seasonally damp, well-drained sandy soils as well as in seeps at a higher range of altitudes than *E. fenestrata* (10 - 600m). According to our curves, this species has lower (more negative) P_{50} (-1.78 ± 0.12 MPa) and P_{12} (−0.99 ± MPa) indicating that it is less vulnerable than *E. fenestrata* solidifying the hypothesis that vulnerability to drought is coupled with microhabitat (Fig. 1). The stomata of this species close at approximately the same time that P_{50} is reached (Safety margin = -0.04 MPa). While *E. fenestrata* is significantly more vulnerable than *E. tectorum*, both species are highly vulnerable relative to woody angiosperms (Skelton et al., 2015).

The Ψ_{TLP} does not differ between species, which leads to the conclusion that the xylem is the aspect that is causing the difference in safety margins observed (Fig. 7). *E. tectorum* coordinates stomatal closure to avoid the effects of excessive cavitation in the xylem but *E. fenestrata* does not. This species is not invested in conserving water (RWC_{TLP} = 73.4 ± 3.8 % in *E. fenestrata*; almost 20 % lower than *E. tectorum*) or protecting xylem from embolism (P_{50} = −1.07 ± 0.095 MPa in *E. fenestrata*; almost 1 MPa earlier than *E.tectorum*) because it occurs in a wet microhabitat and therefore does not need to because there is no threat of drought or embolism formation.

2.4.2.1. Hydraulic and physical strategies involved in determining a species niche and the response to change.

We would expect that species such as *E. tectorum*, that resides in well-drained, dry habitats may fare better during drought than *E. fenestrata* (wetland species). Usually, when woody species display an anisohydric stomatal regulation strategy, they can protect their water transport system by producing rigid, embolism resistant xylem, but judging by the vulnerability of the species, this is not the case in these graminoid monocots. It is likely that the risky drought strategy observed in *E. fenestrata* is a flood-tolerating strategy. Maintaining high transpiration rates and hydraulic conductivity are the strategies that aid in minimizing the anoxic effect of waterlogged soils in mostly wet habitats, however, these strategies could be detrimental in increasingly drying climates being observed worldwide. Species such as this, that experience drought are less likely to have the physiological capacity to cope when faced with a reduction in water available for use and, surprisingly, we might expect higher rates of drought-induced die-back as a result of hydraulic failure.

A physical mechanism by which these species deal with the anoxic conditions of permanently waterlogged soils is by the development of aerenchyma in their root tissue. This air-filled tissue allows for oxygen to be present in the roots to counteract the anoxic environment of the soil. Although, one may then expect to find that *E. tectorum* does not have aerenchyma since it is predominantly found in seasonally damp and dry habitats, this is not the case. Non-aerenchymatous species cannot survive in waterlogged habitats and will succumb to anoxic stress but aerenchymatous species can reside in both waterlogged and well-drained soil types (Huber & Linder. 2012). Huber & Linder (2012) showed that *E. tectorum* does have aerenchyma tissue in their roots and that the absence of aerenchyma limits certain species to only residing in non-waterlogged habitats but that the presence of aerenchyma does not limit species to only residing in waterlogged habitats.

If this is, in fact, true then solely based on root anatomy, *E. fenestrata* should be able to cope in drier soils, however, survival during drought stress is dependent on a suite of hydraulic factors and not just the presence of aerenchyma. In this case, *E. fenestrata* has vulnerable xylem which excludes it from being able to survive in dry habitats. The fact that both species can cope with waterlogged, anoxic conditions due to the presence of aerenchyma may imply that extreme flooding may not cause as much concern in the CFR as much as extreme drought might. With that said, the presence of aerenchyma may prove to cause more harm than good during extreme drought. Having air-filled tissue in roots during a soil drought could introduce a source of embolism should drought conditions become more severe.

Despite the presence of aerenchyma in *E. tectorum,* this species does not often co-exist in wet habitats where *E. fenestrata* can be found. *E. fenestrata* most likely outcompetes *E. tectorum* in wet habitats due to their efficient (albeit unsafe) xylem. *E. tectorum* has relatively safer xylem which is hypothesized to come at the cost of efficiency. If the above is, in fact, the case then we can attribute the presence of aerenchyma in *E. tectorum* to its widespread distribution and the necessity to be prepared for any microhabitat type to improve the chance of survival.

This study attempted to remedy the lack of information about xylem vulnerability to embolism propagation by producing the first ever optical VCs for two species with contrasting habitat types. Consequently, our data has started to unpack the links between both. This study has begun to tease apart the hydraulic strategies and how they are linked to microhabitat in the family of Restionaceae. The Optical vulnerability technique has given us an effective way to quantify vulnerability in these species and can be utilized to quantify safety margins for more species of Restionaceae. We can also utilize it to quantify vulnerability and safety margins of other fynbos species to give us an idea of the response of threatened fynbos vegetation as a whole.

CHAPTER 3: Synthesis of Restionaceae response to changing habitat water regime and the way forward.

Water availability is a key parameter in land plant radiation and evolution. Transporting water from the environment (soil) to the atmosphere is a vital process for plant function and facilitates photosynthesis and evaporative cooling. Failure of the hydraulic transport system is considered a lethal process and the interactions of the underlying traits associated with stomatal behaviour and xylem and root anatomy are an active area of plant hydraulic research. What is clear is that the inter-specific variation in these hydraulic traits has resulted in the wide array of hydraulic responses to desiccation stress.

The Restionaceae constitutes an ecologically significant group within fynbos vegetation. Restionaceae, and along with Proteaceae and Ericaeae are the characteristic families within fynbos. Restionaceae also make up most of the fuel load, which is important in fire-driven systems. Due to the abovementioned reasons, it is important for us to understand how these plants are responding to drought, especially since it has been predicted that drought events would become more frequent and severe in the region due to ongoing climate change. The proposed changes in frequency and severity of extreme events poses a question of which restio species would dominate during severe drought.

While much is known about how this group segregates into different hydrological niches, information about their hydraulic physiology is lacking. Considering that they segregate along fine-scale moisture gradients, I hypothesized that the hydraulic traits and consequently, the safety margins will be tightly coupled with the microhabitat. This thesis aimed to unpack the response of two Restionaceae species to changes in habitat water availability. The two species occurred on the extremes of the hydrological niche axis (*E. fenestrata* = wet and *E. tectorum* = dry) and their safety margins were consistent with their microhabitat.

E. fenestrata displayed a negative safety margin which implies that it is not common for the species to experience water stress and leaving the stomata open relatively late in the dry down period may be advantageous in a habitat that is permanently waterlogged. *E. tectorum,* on the other hand, displayed a slightly positive safety margin which implies that this species may experience water stress from time to time and that the ability to close stomata before dangerous levels of embolism occur is an adaptation to prevent desiccation. With that said, both species close their stomata at similar degrees of water stress, and therefore the vulnerability of the xylem network to damage via embolism is the characteristic that is determining the survivability of each species in their respective moisture regimes.

Aside from these two characteristics, there are many physiological traits that determine a species response to water deficit that are species, microhabitat and climate specific. The results in this thesis do not give a complete picture of how Restionaceae are going to fare with the changes in climate and habitat destruction in the form of groundwater abstraction but it is a foundation on which to build. What is evident is that at least in these two species, microhabitat determines hydraulic strategy.

3.1. OV method and Rehydration Kinetics.

The sensitivity of the xylem network to air invasion is the cause for extensive debate about the vulnerability of various species. The techniques used to quantify species vulnerability are varied and carry a high risk of methodological artefacts caused by the need to excise sample material and working with xylem water columns under tension. This introduces the risk of embolism forming in the xylem before data can be collected. Limited access to expensive equipment and laborious methodology are some of the other disadvantages of some of the more recent methods (e.g. centrifuge and X-ray microCT). The abovementioned disadvantages limit the number of species that we can quantify vulnerability for as well as, increase the risk of overestimating the species vulnerability.

Vessel length is also an important parameter in determining the reliability of some methods. Ensuring that measurements are being taken on intact xylem vessels is important because open vessels are naturally more vulnerable than closed, intact vessels. This concept has led to the discovery of an ‘open-vessel’ artefact which highlights the risk of overestimating vulnerability when samples have to be excised from the plant. This artefact can be accounted for if maximum vessel length is taken to account and samples are cut in such a way that all vessels being measured are intact.

The Optical Vulnerability technique is a perfect candidate to remedy the above methodological inconsistencies. It is cost effective and efficient, and there is no need for expensive equipment and can therefore, easily be utilized on a larger number of species. This allows for effective comparative physiological studies. An additional appealing feature of the technique is that it provides good quality spatial and temporal information on the propagation of embolism within an intact plant, eliminating the effects of native embolism before measurement and the ‘open-vessel’ artefact. While this method was developed on woody angiosperms, it has been shown to be successful when measuring vulnerability on monocot graminoids (Johnson, Jordan and Brodribb, 2018).

A typical vulnerability curve plots the loss in conductivity (PLC; %) against the water potential, and the P_{50} is the water potential at 50% loss in conductivity. Vulnerability curves generated by the Optical vulnerability method depicts the relationship between the embolism area (%) and the water potential, where the P_{50} is the water potential at 50% embolism propagation. In order to verify the P_{50} generated with the Optical Vulnerability technique, previous studies used the Rehydration Kinetics method. This technique generates a typical vulnerability curve by measuring the hydraulic conductance and water potential simultaneously over time during the dry down period. Usually, the P_{50} from both versions of the vulnerability curve are consistent with each other, highlighting the precision of the OV method.

3.2. The way forward.

The data in this thesis has taken a few more steps to understanding the hydraulic physiology of Restionaceae. The Optical Vulnerability technique provides the potential to become a high-throughput method to quantify vulnerability in a variety of species. Now that the method has been successful in unpacking the vulnerability data of the two restio species included in this study, it can be used more extensively so that we can begin to unpack vulnerability to drought not only in the Restionaceae but possibly in the fynbos as a whole.

The CFR is a highly diverse area that has an important ecological role for the area. The region also generates a fair amount of revenue in terms of ecosystem services (i.e. groundwater abstraction), but more importantly its utility as a tourist destination and as a supplier of the cut flower industry. The ecological and economic potential of the area highlights a need to conserve a vegetation region that is under threat. Fynbos vegetation is predicted to be highly susceptible to changes in climate and is therefore a concern in the face of predicted changes in climate.

Widespread consensus surrounding the increase in frequency and severity of drought predicted for the Fynbos raises the concern of the survivability of fynbos vegetation. The Restionaceae is a group of particular concern because they are characteristic of Fynbos vegetation and have key ecological roles in the ecosystem. They also exhibit niche segregation along fine-scale moisture gradients. Threatening water supply to species that are so physiologically coupled to water within their habitats increases the risk of dangerous embolism and possibly result in a higher frequency of mortality.

Utilizing the cost-effective and efficient Optical Vulnerability technique, we can begin to fill in the gaps that exist in understanding the hydraulic physiology of the ecologically dominant Restionaceae which would be an amazing feat considering that there are approximately 350 species. With this knowledge we can start to unpack their response to drought and predict their vulnerability to climate change. Having this information will be important for the conservation of these charismatic and important species.

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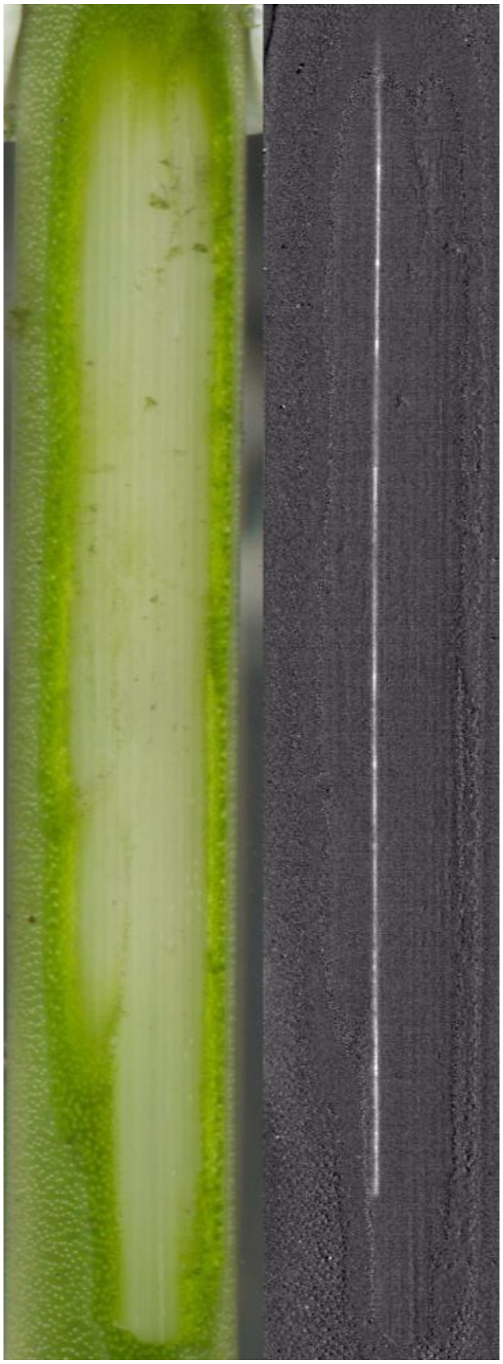
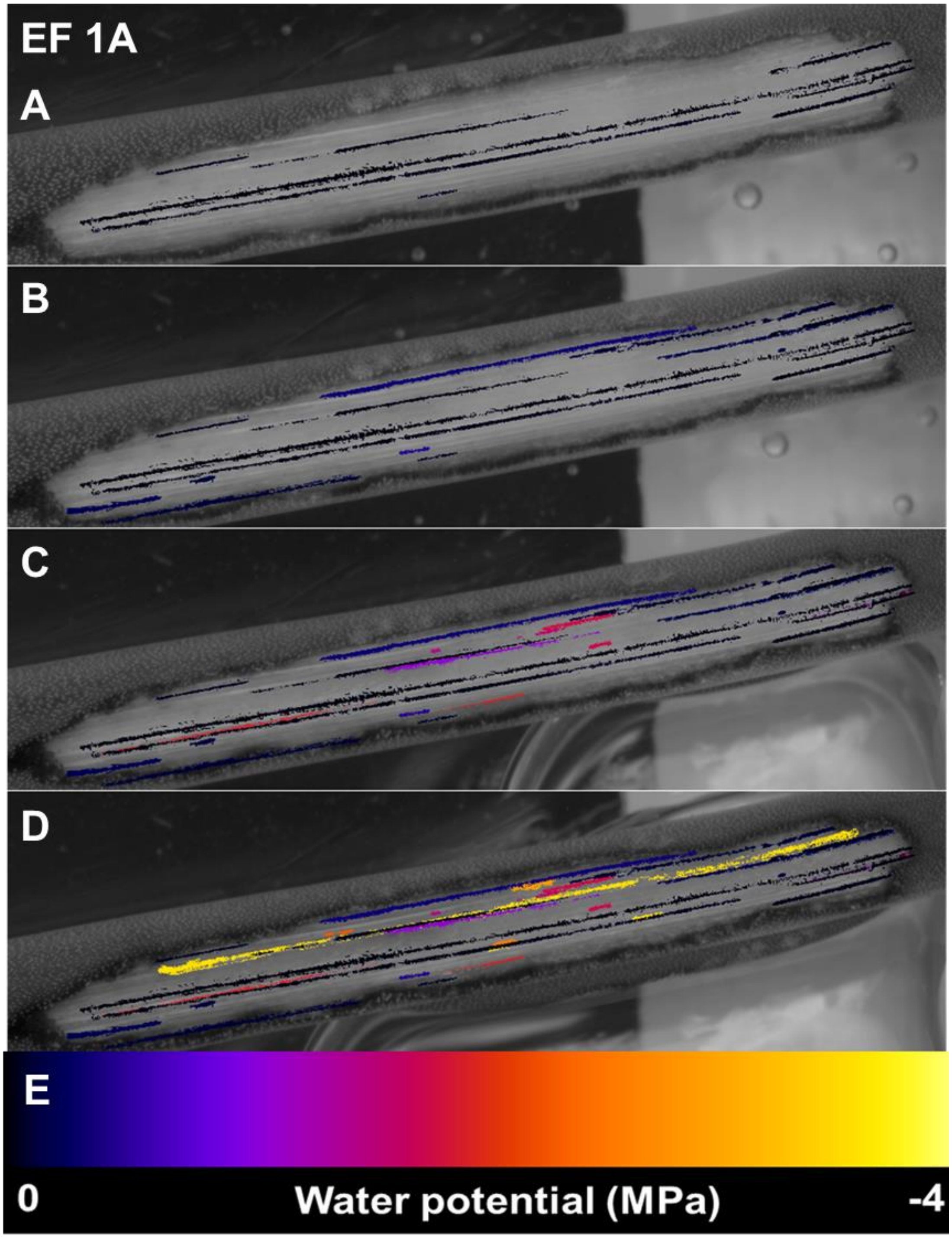
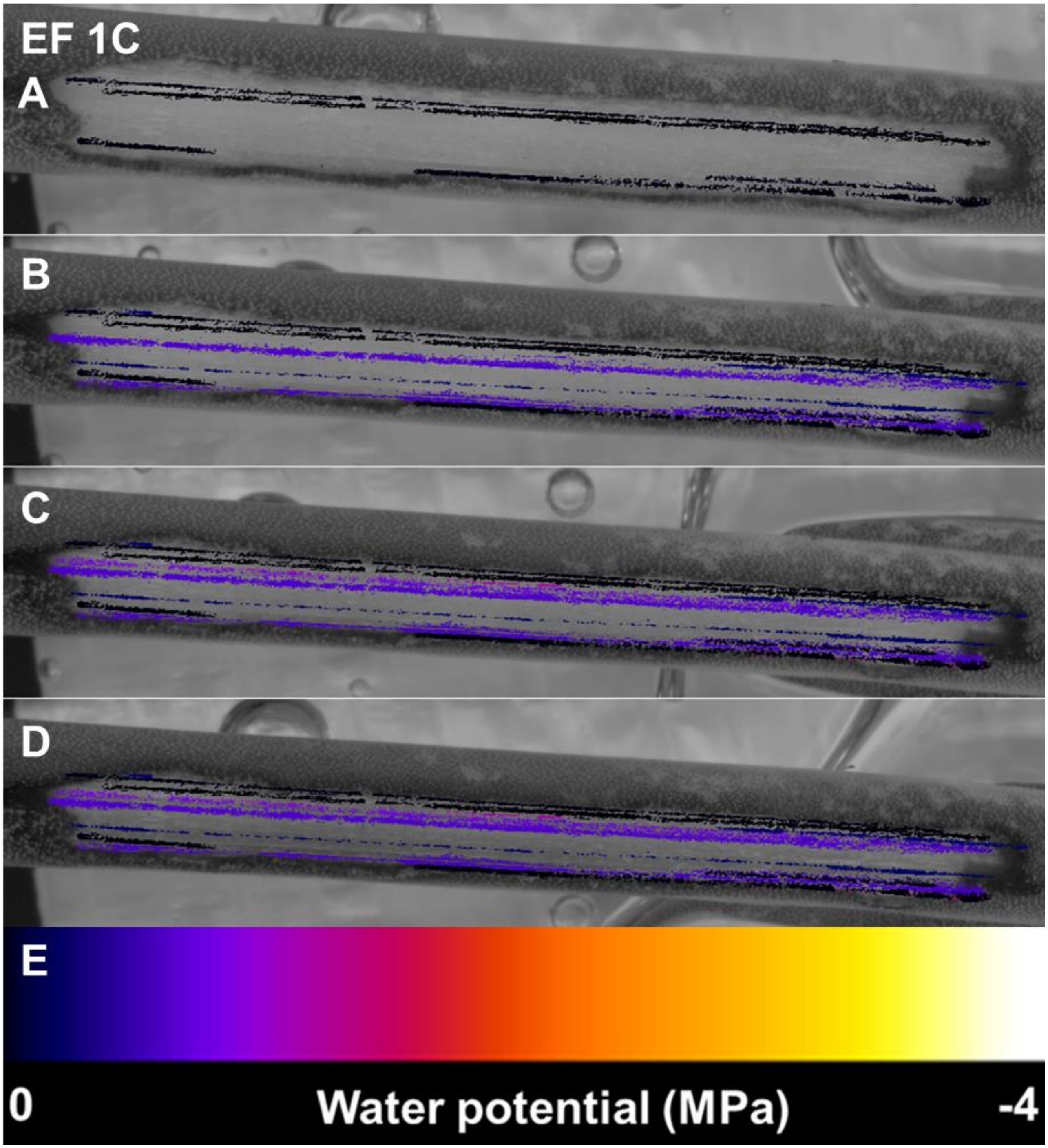
APPENDIX A



APPENDIX B

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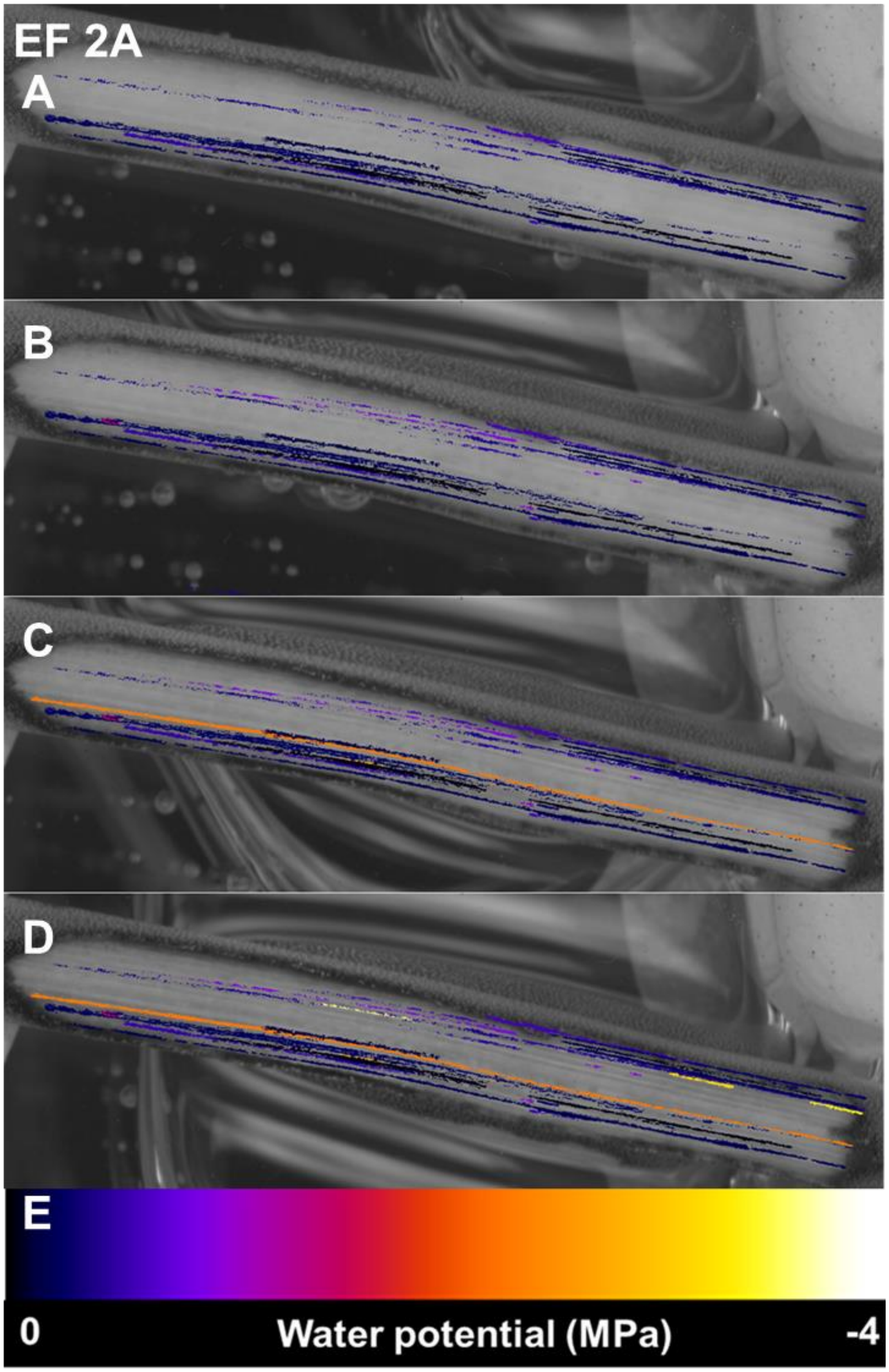
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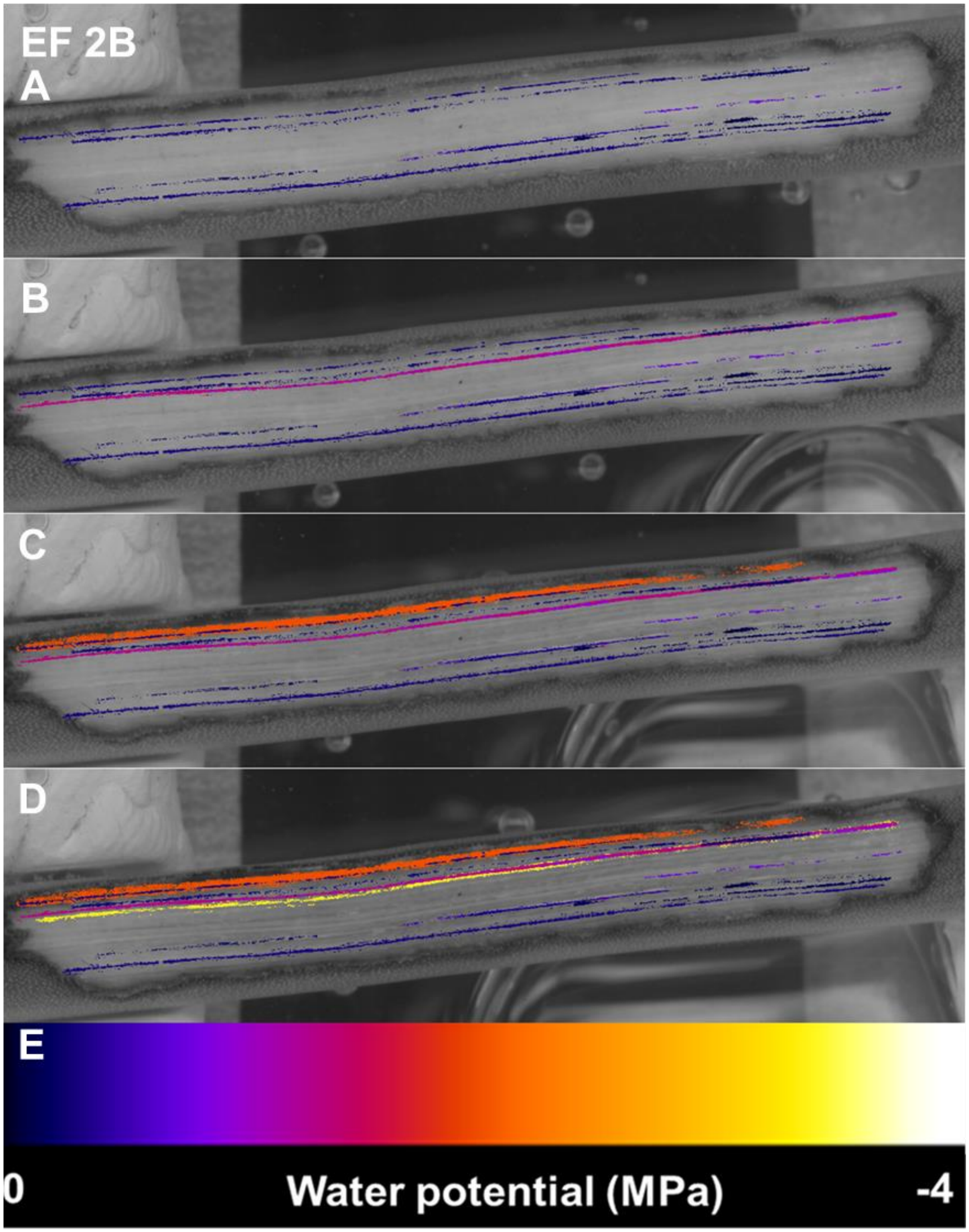
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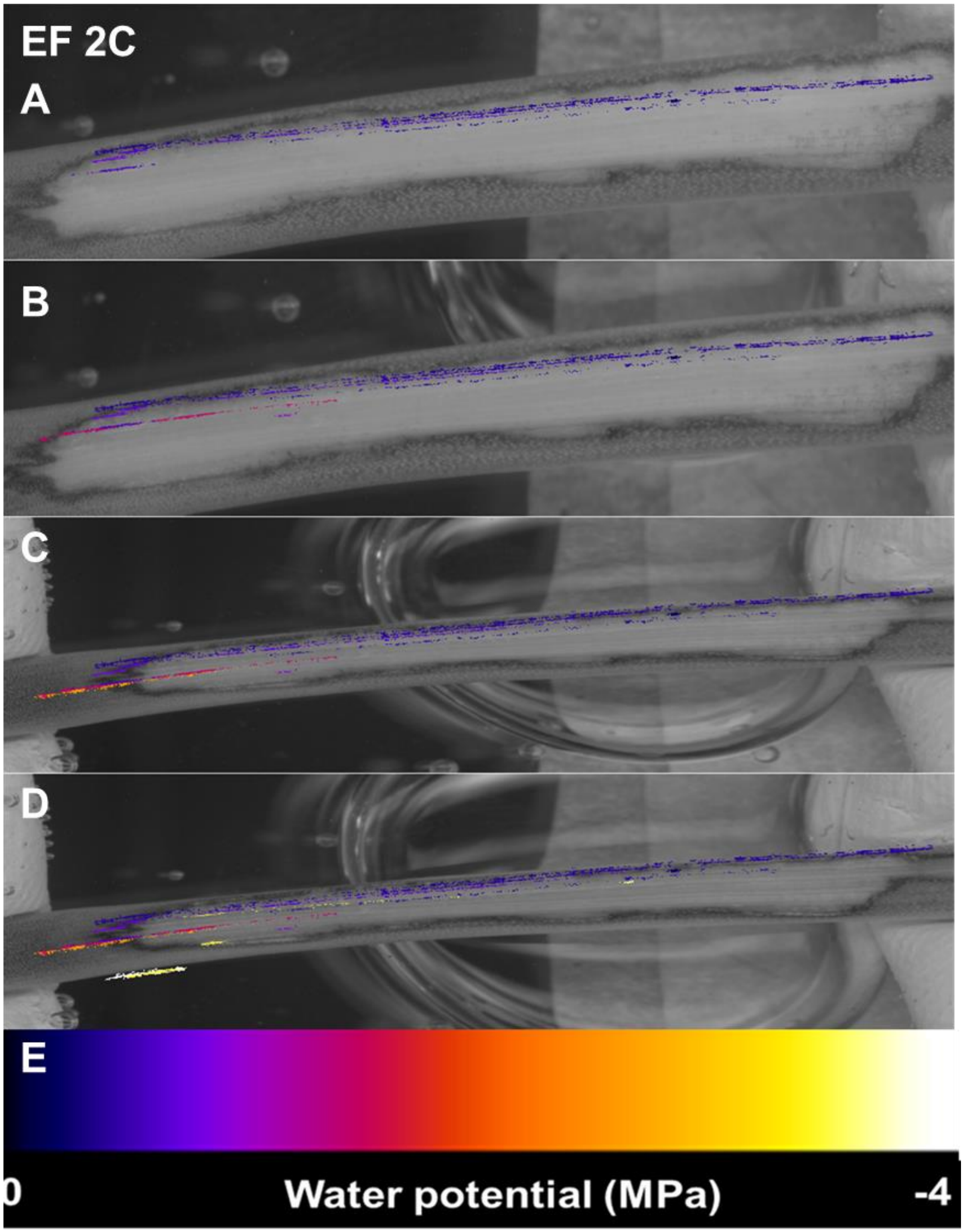
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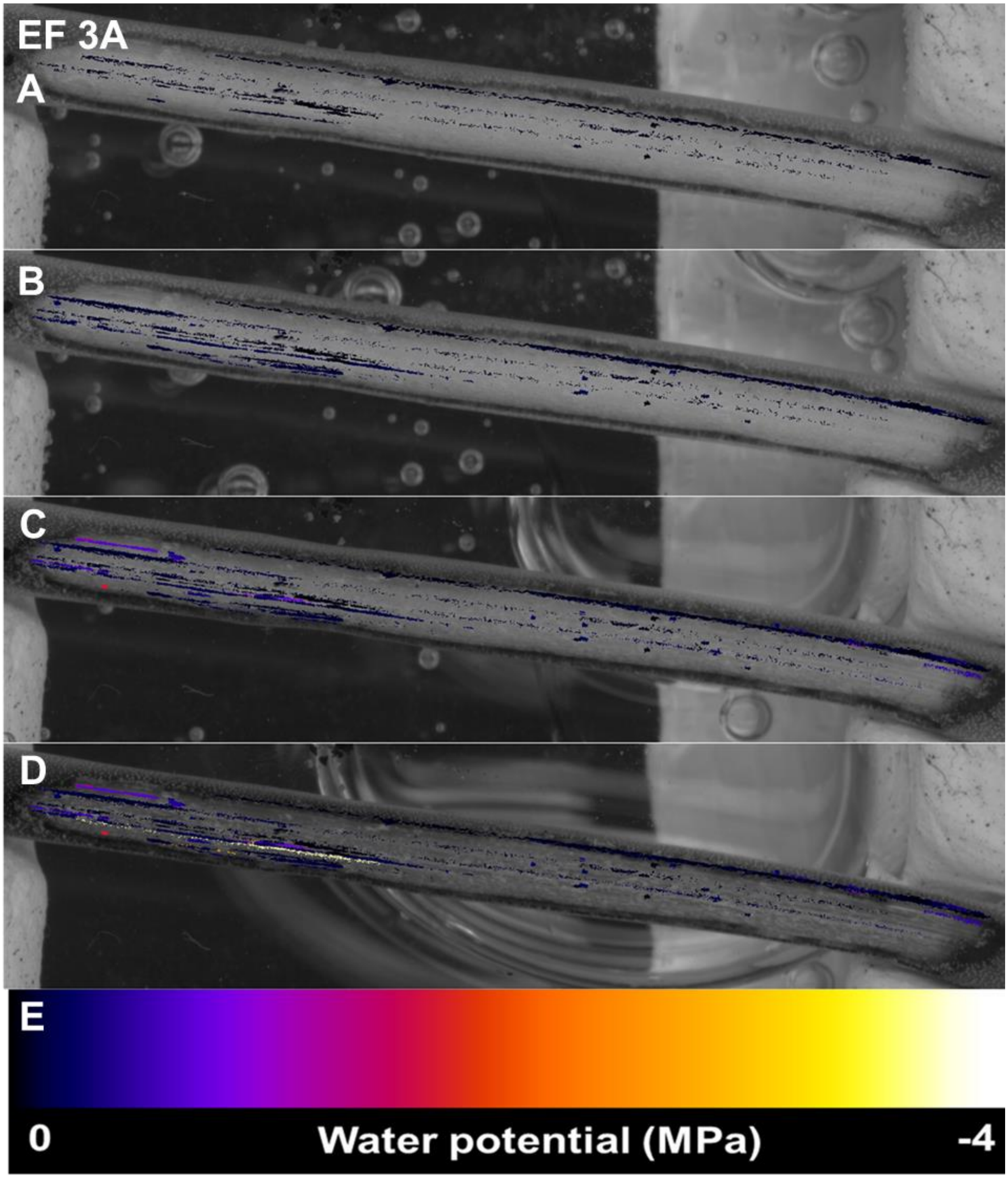
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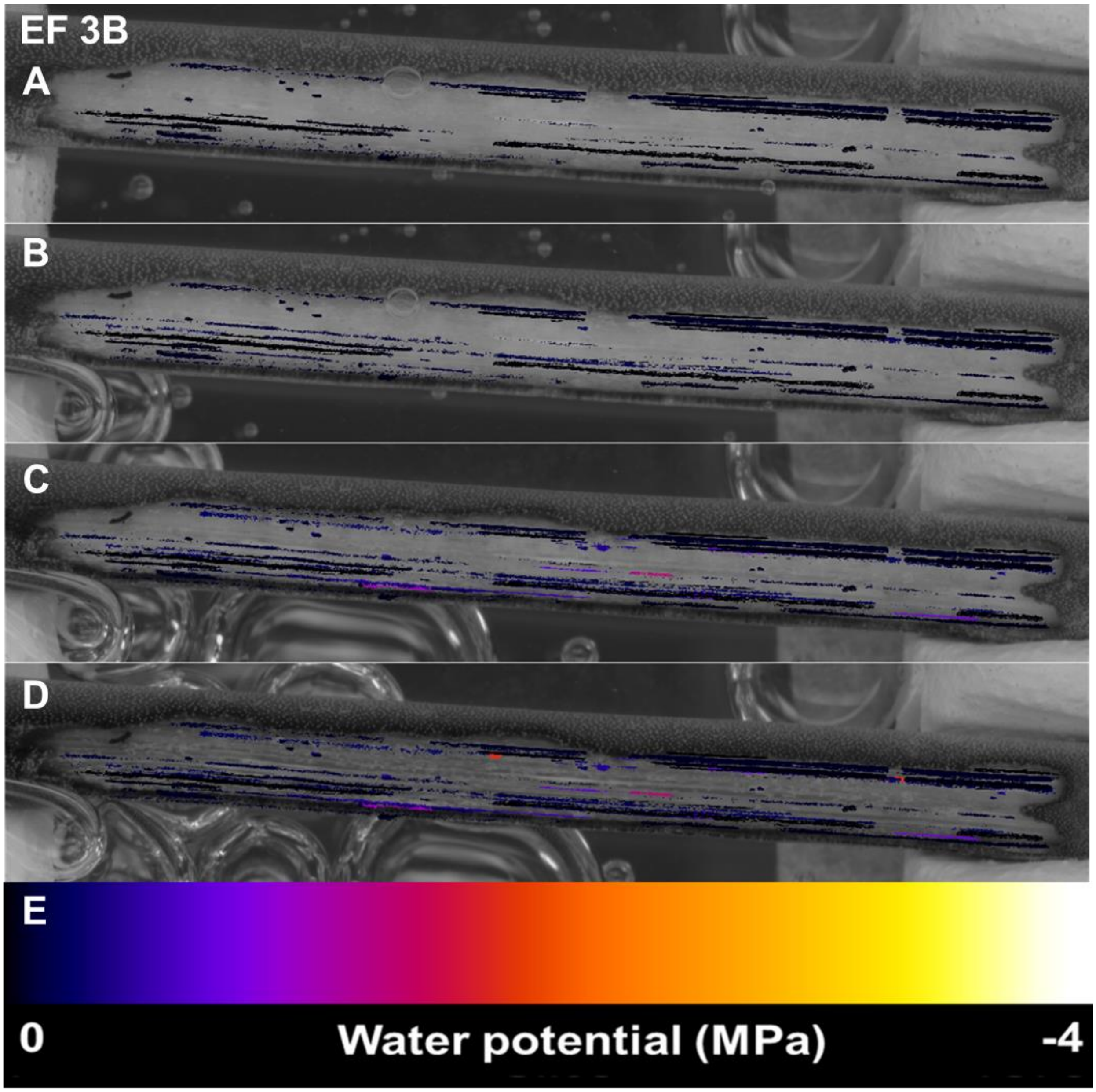
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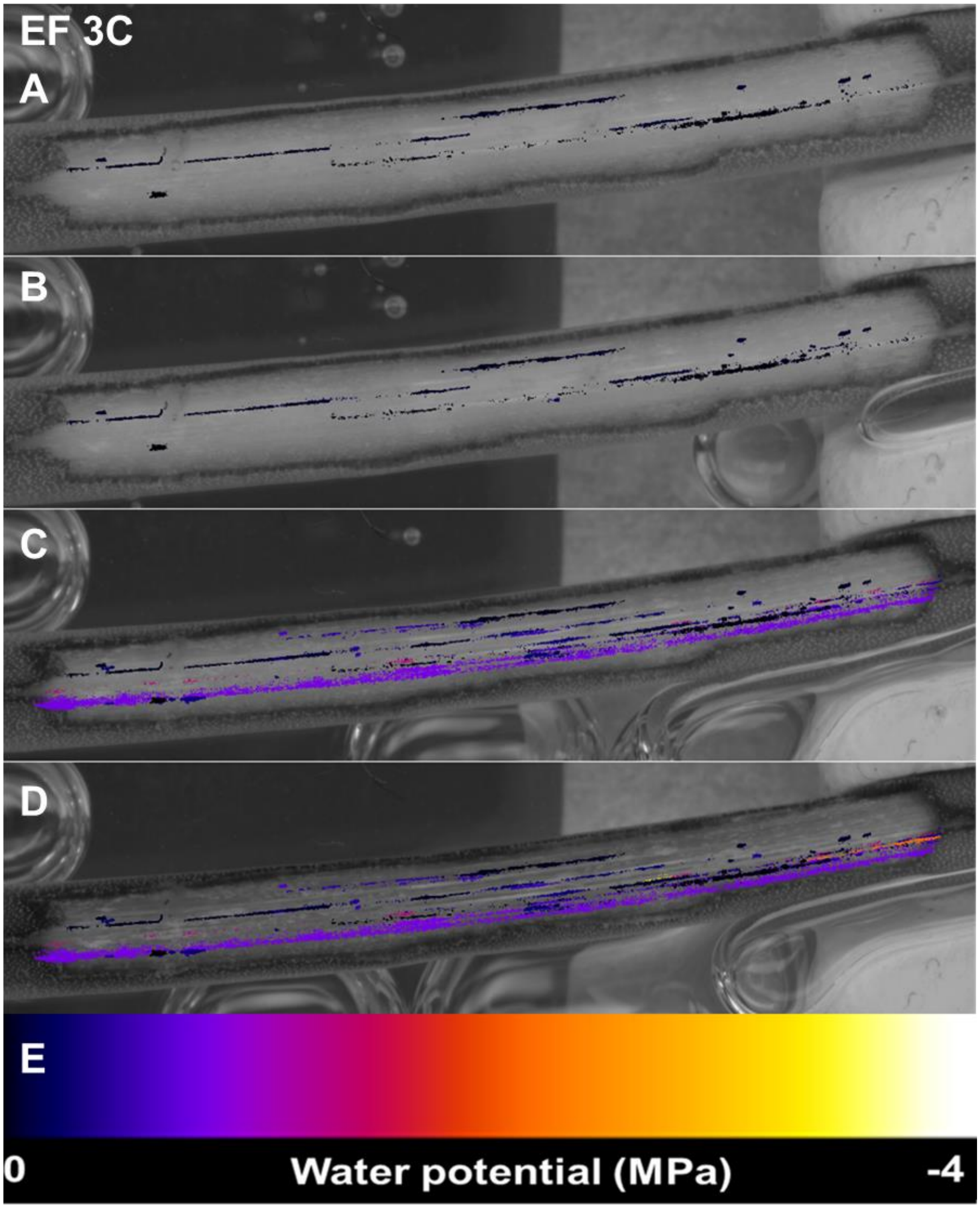
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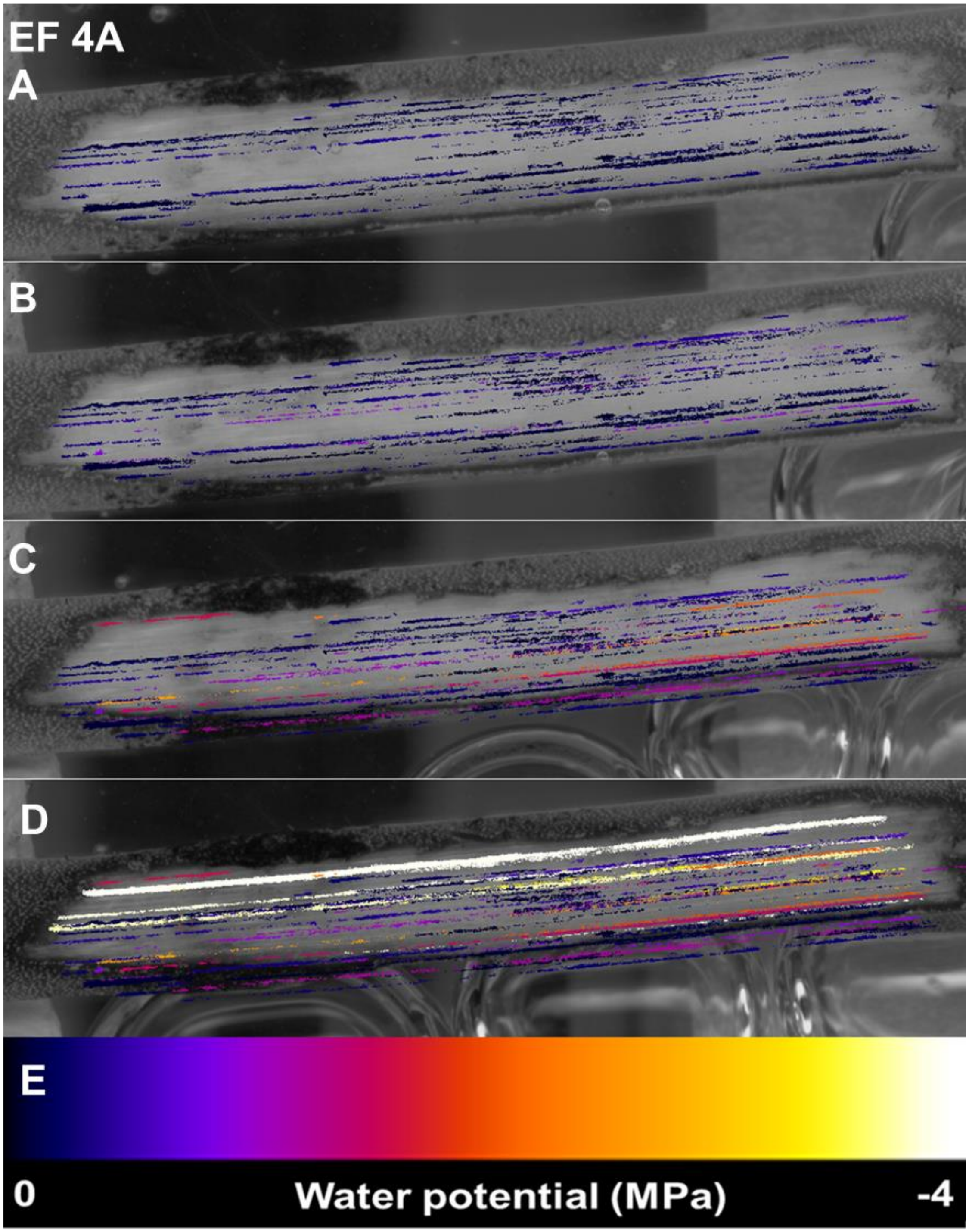
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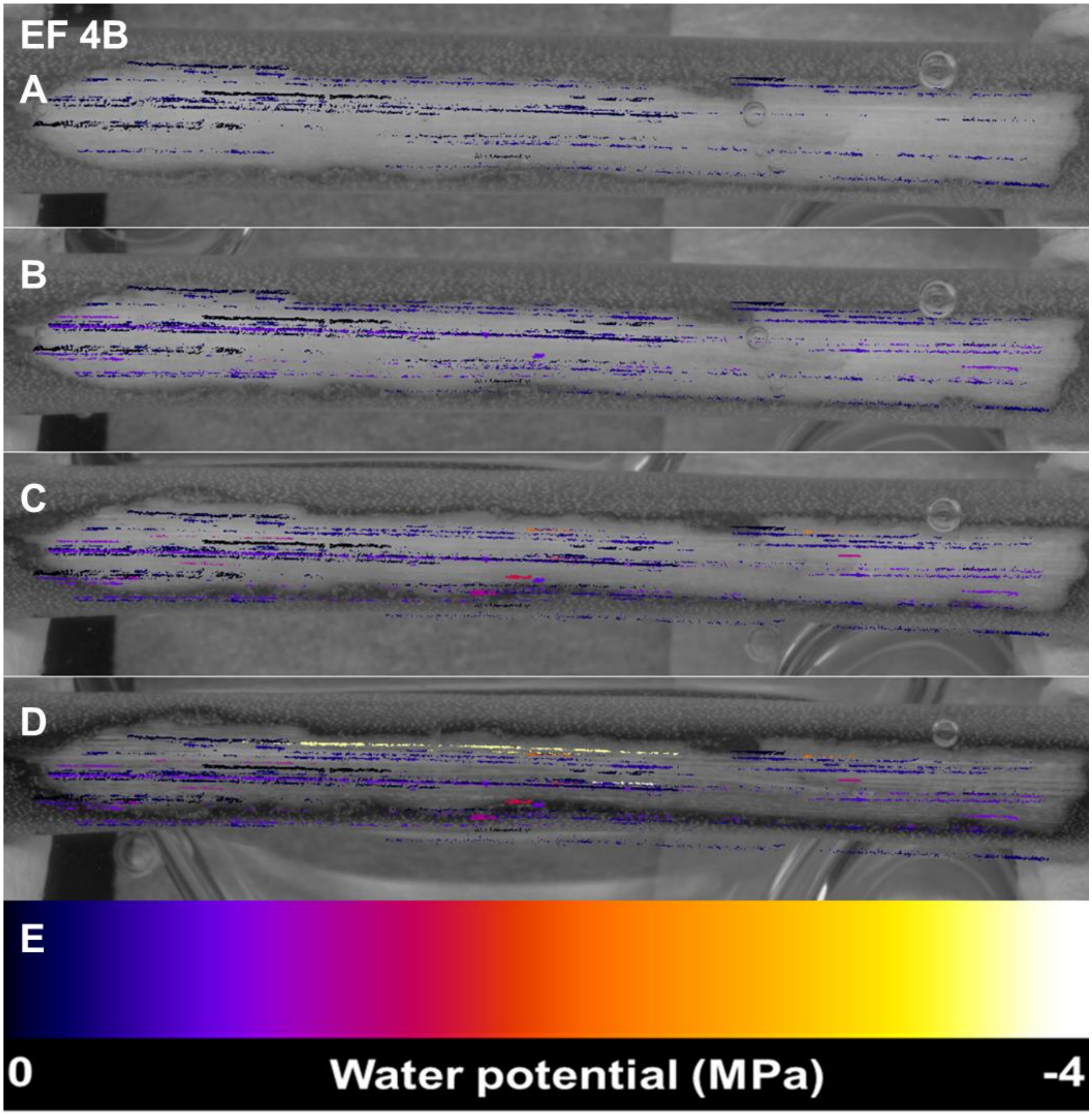
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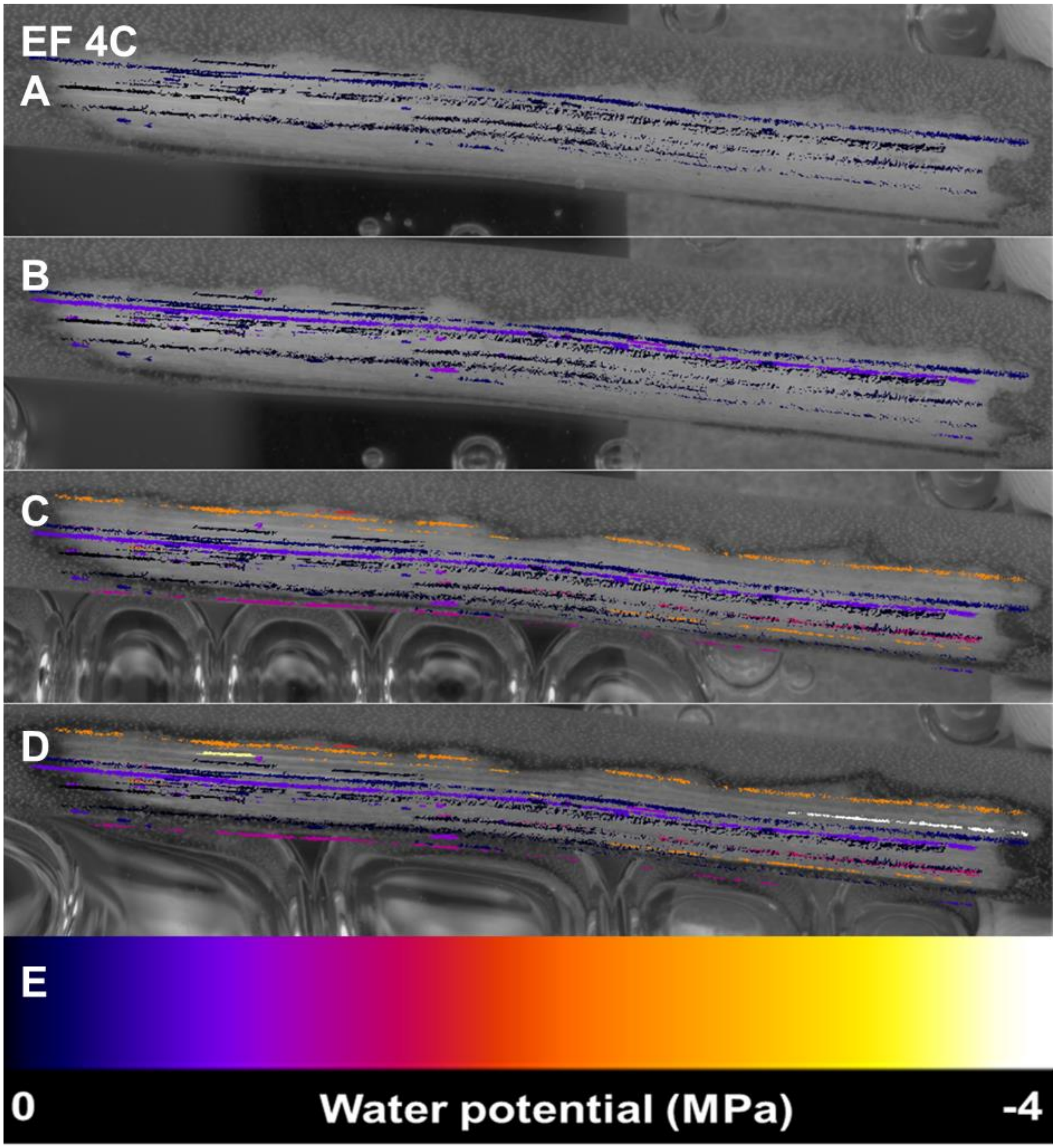
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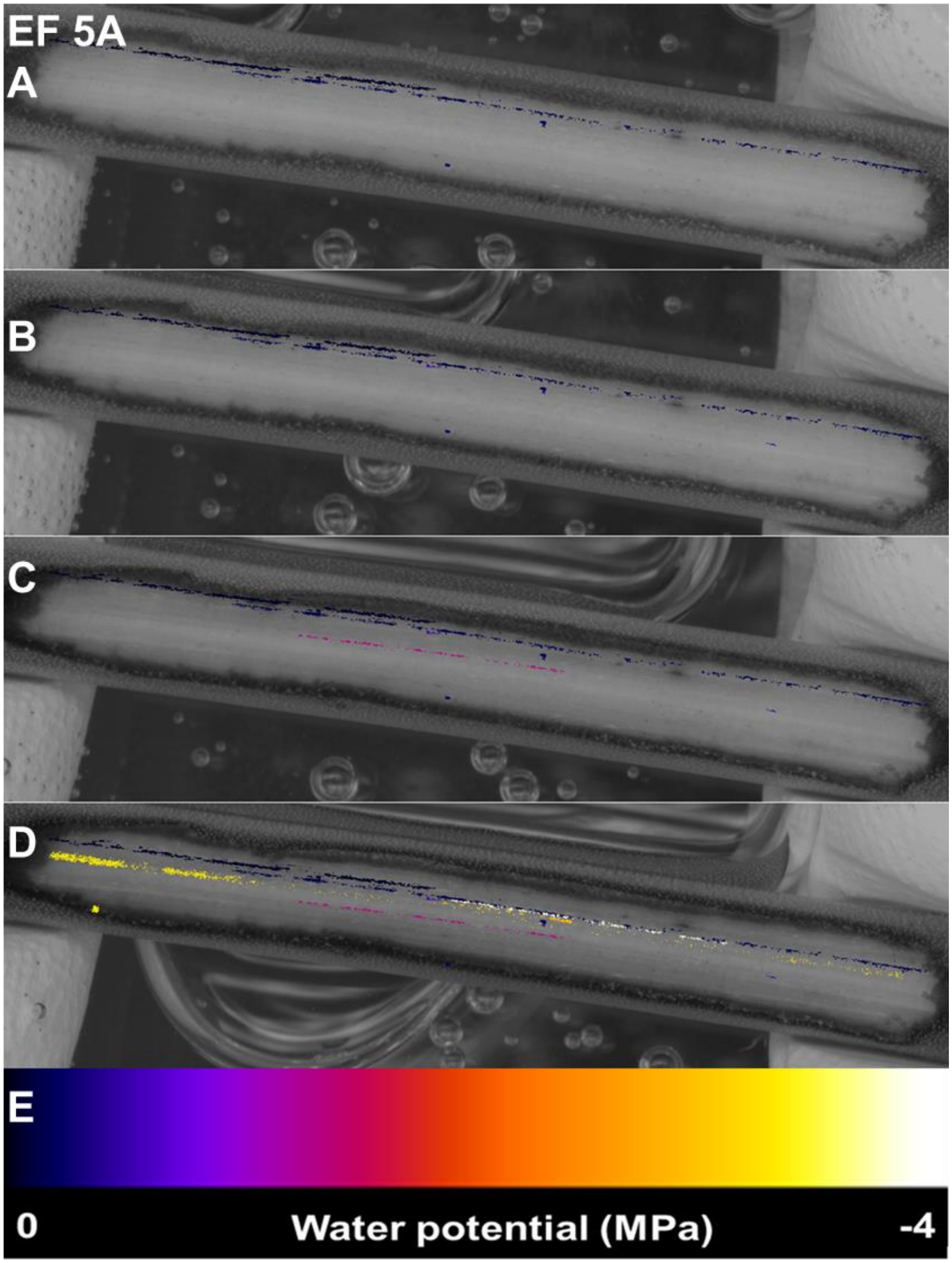
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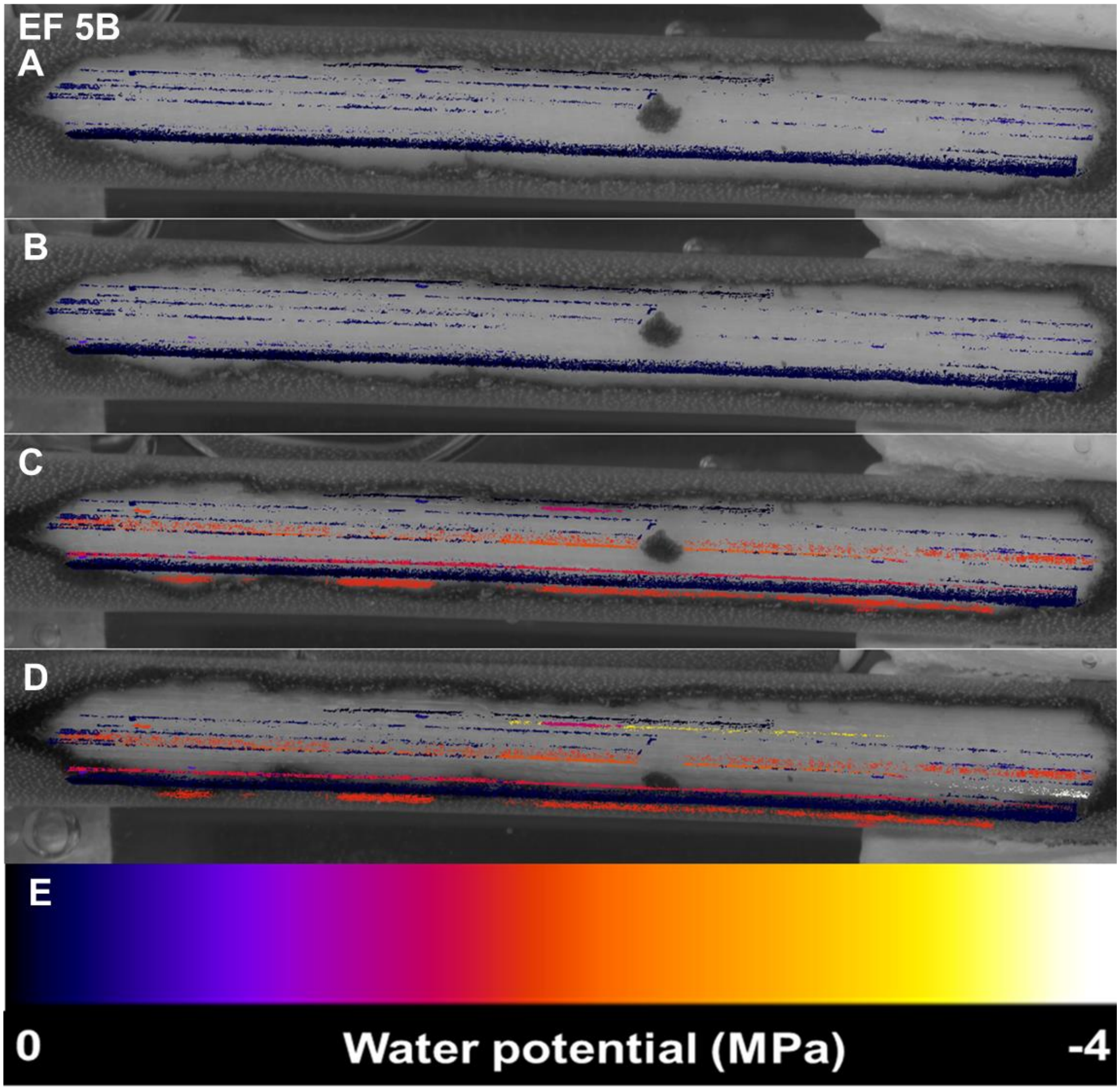
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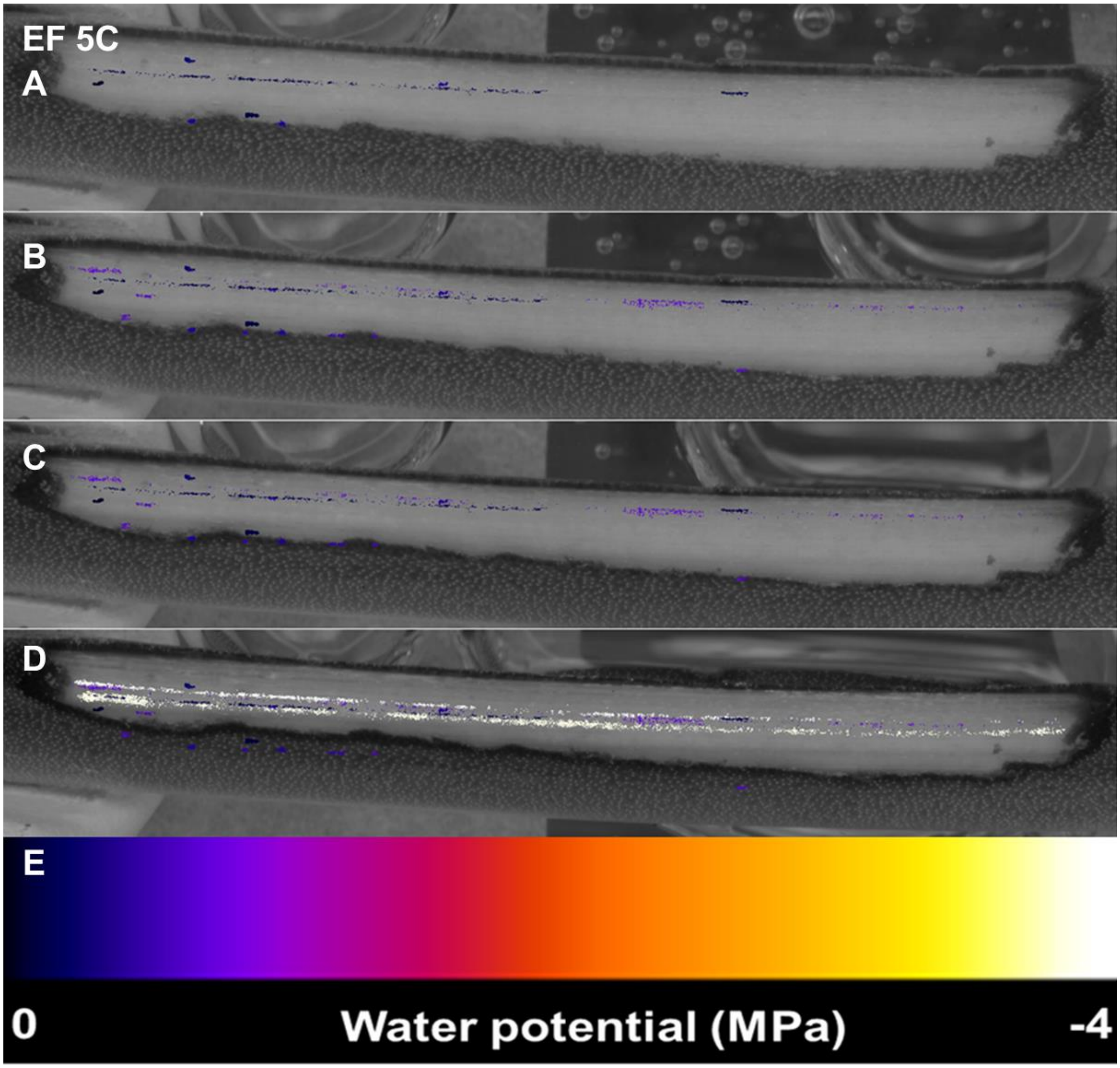
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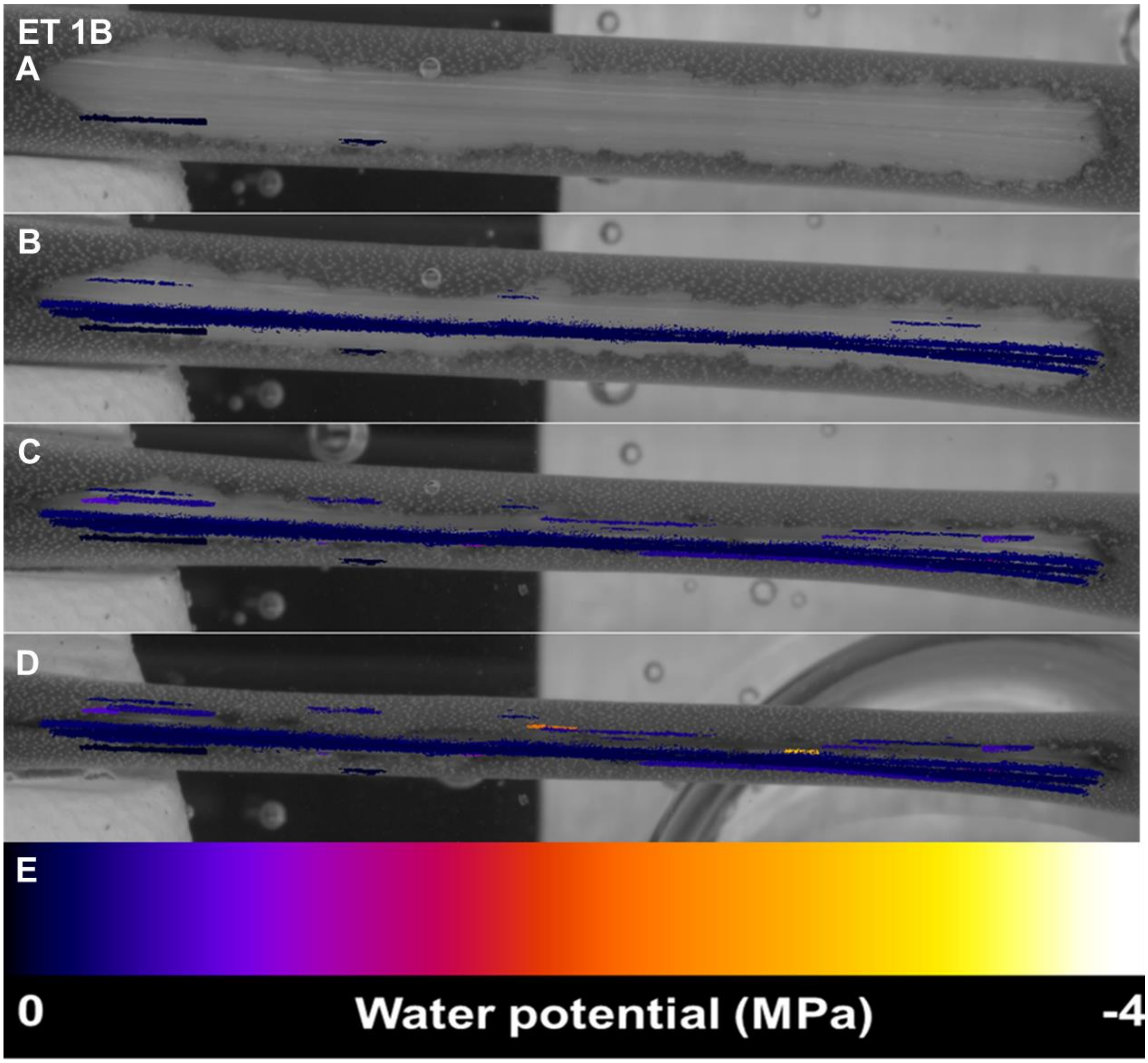
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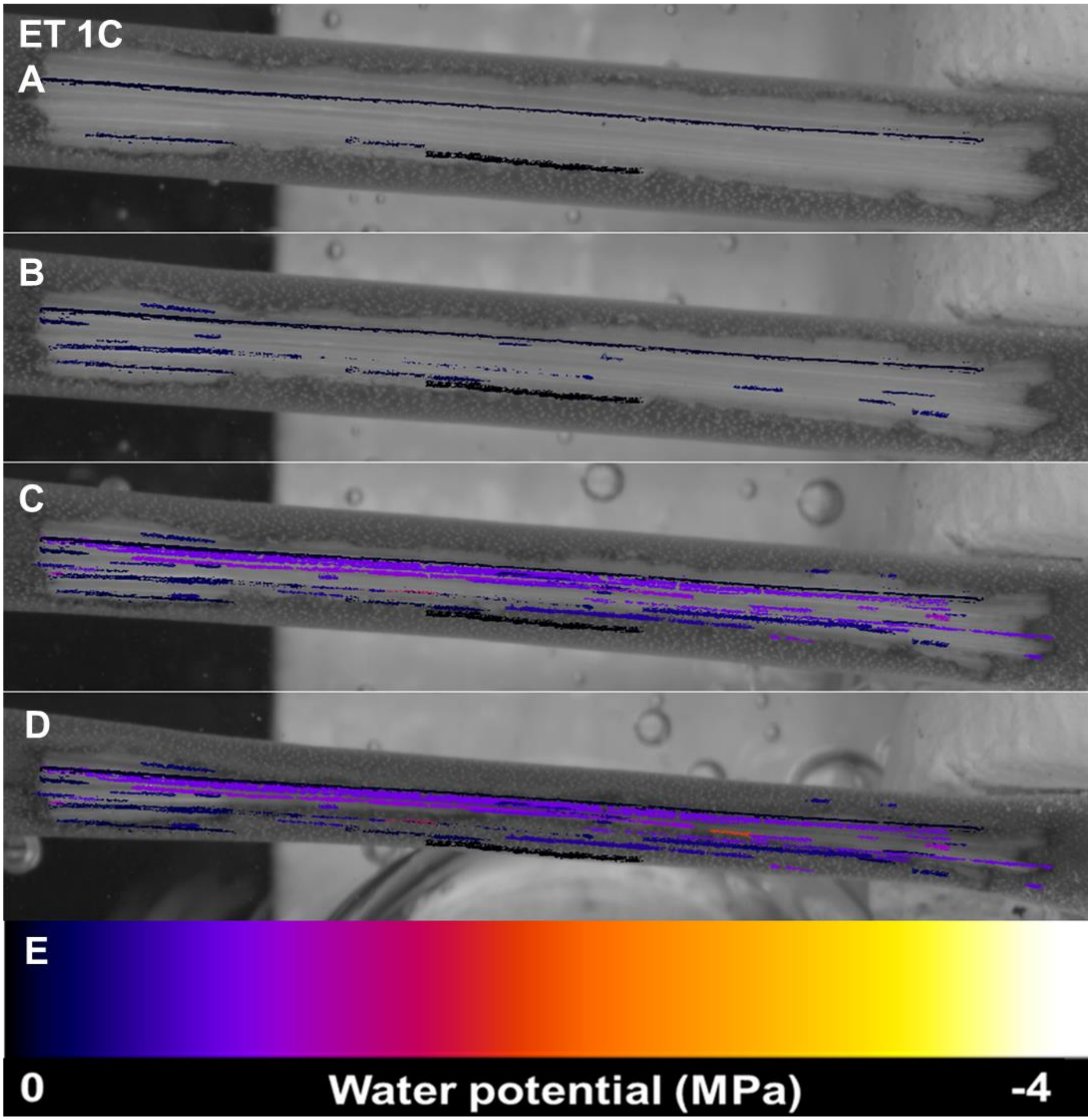
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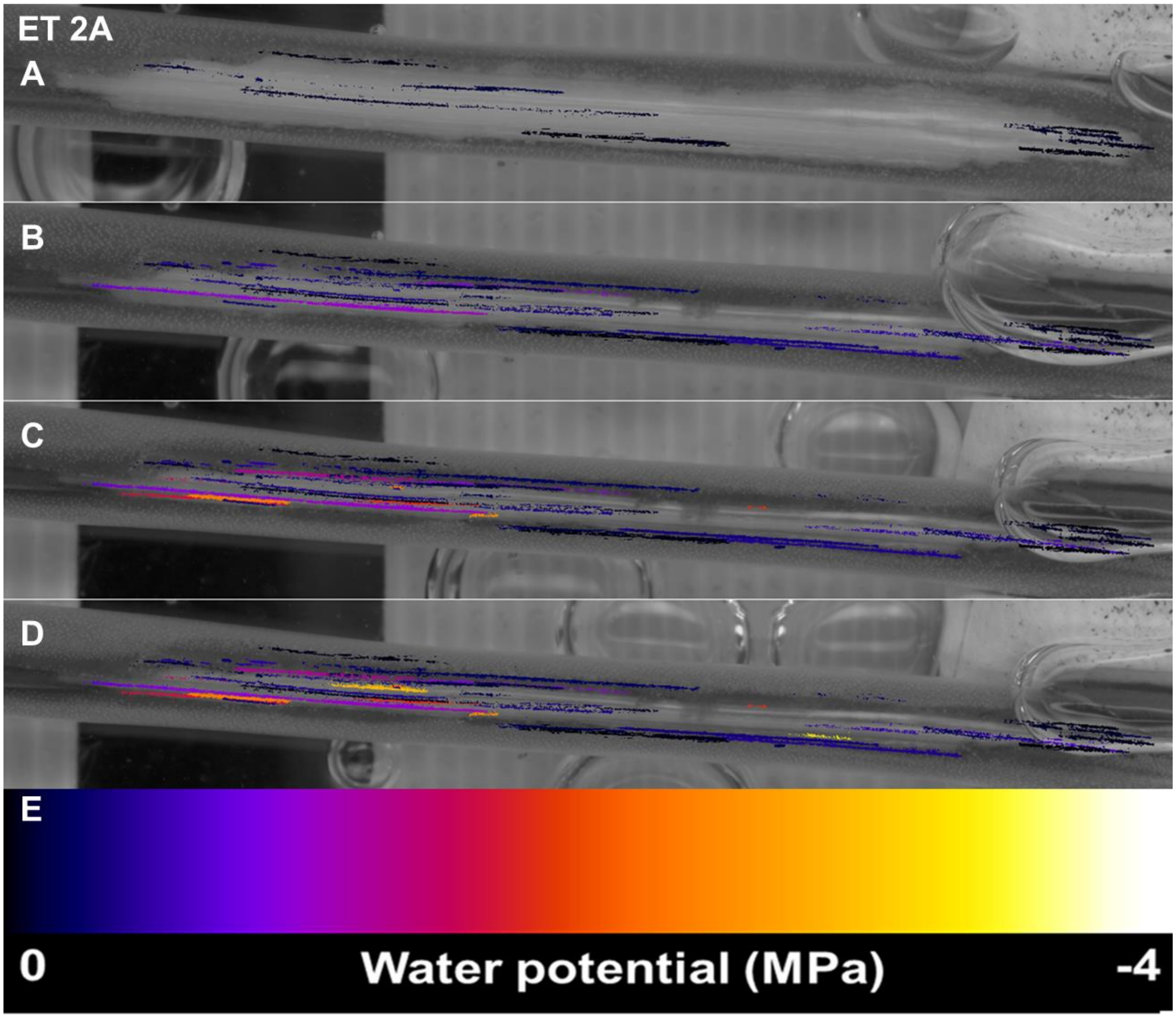
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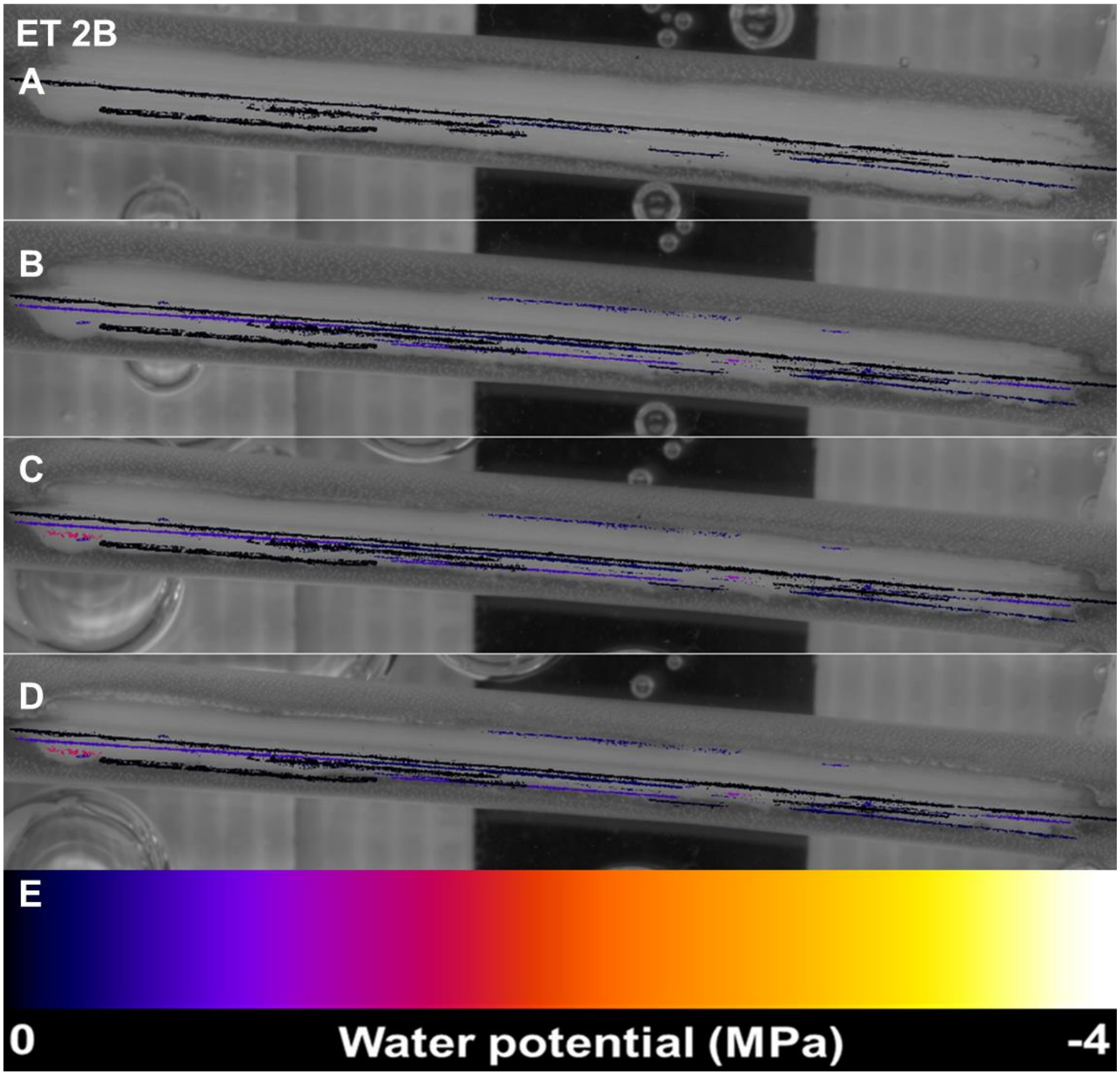
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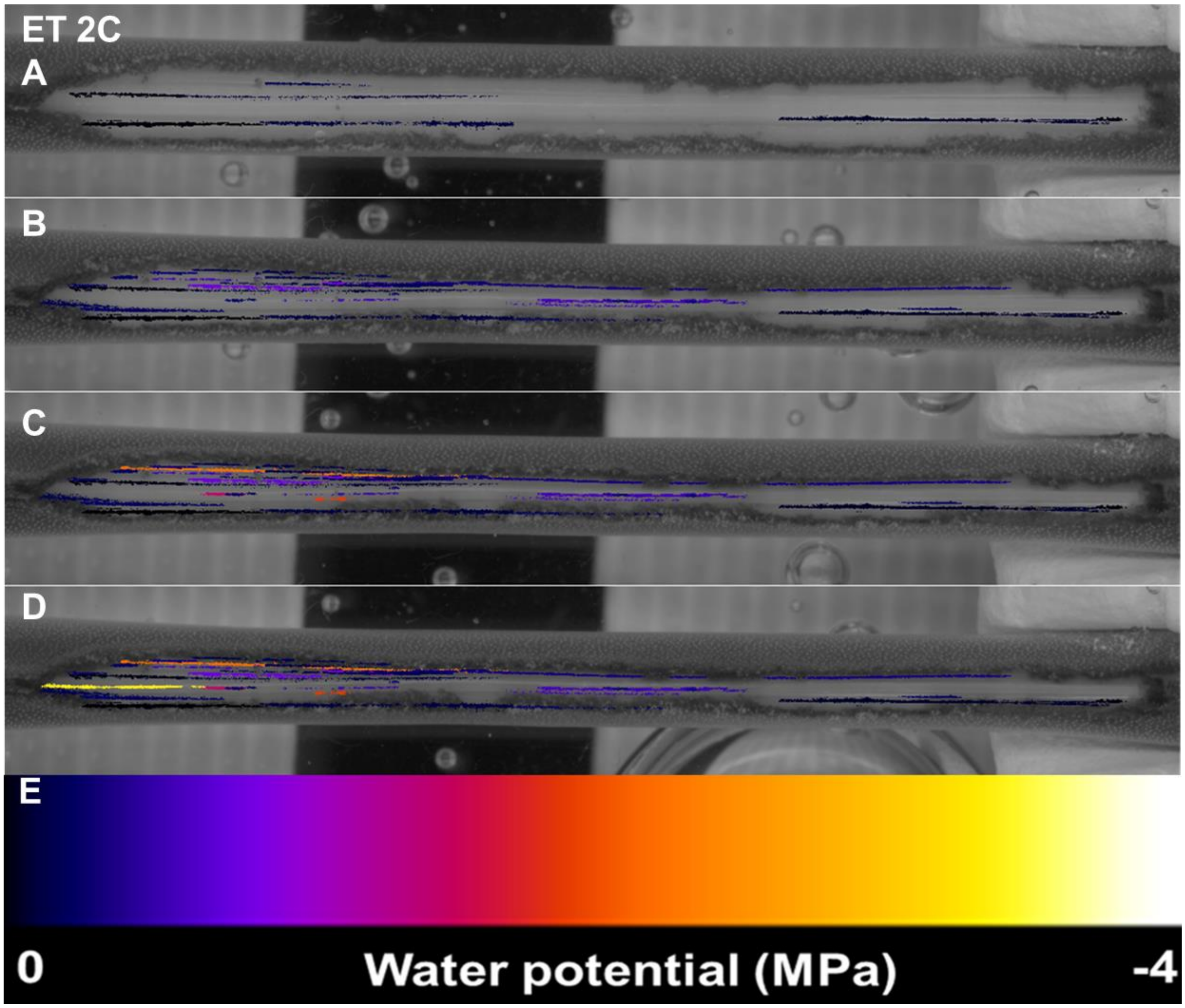
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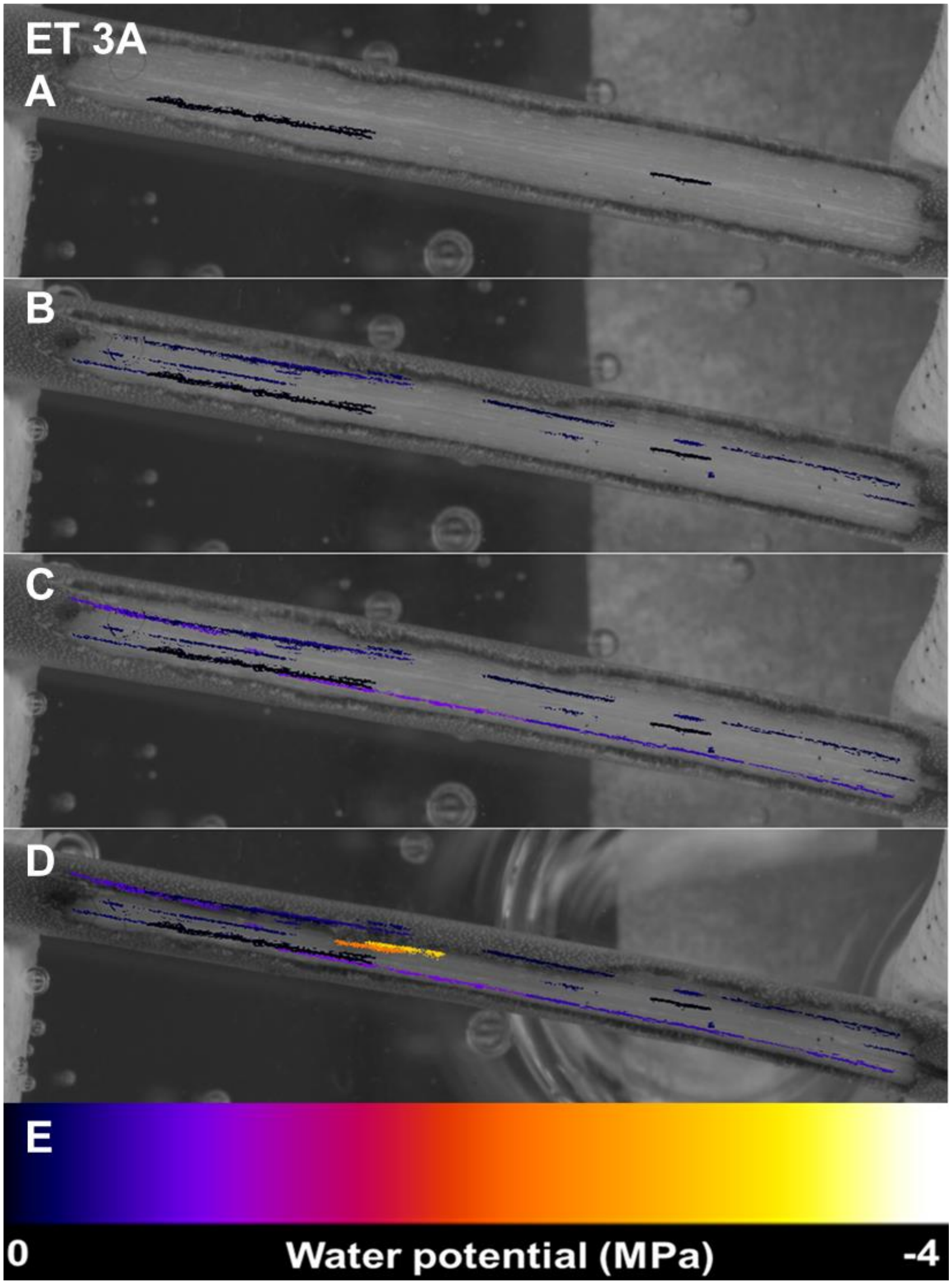
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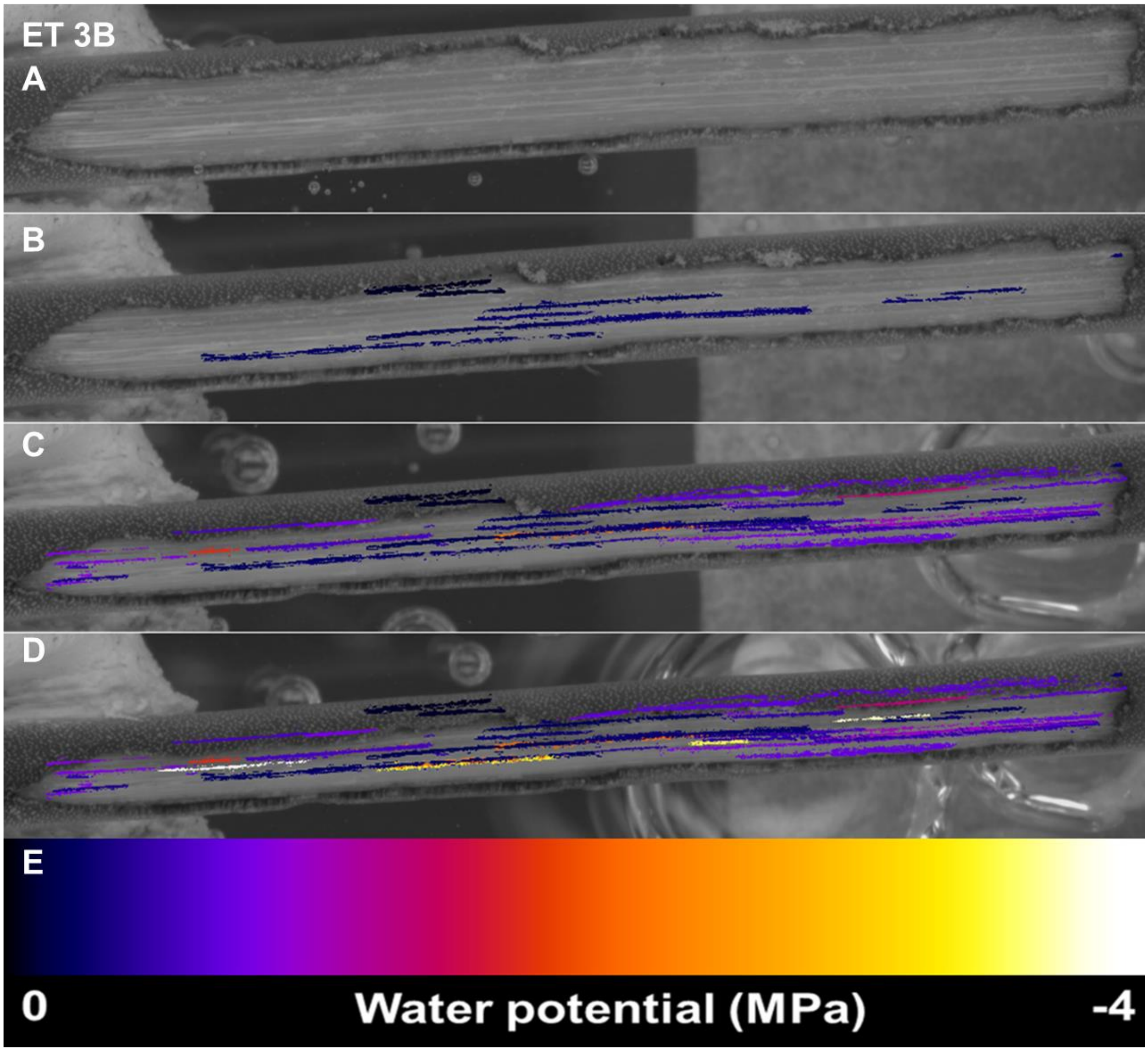
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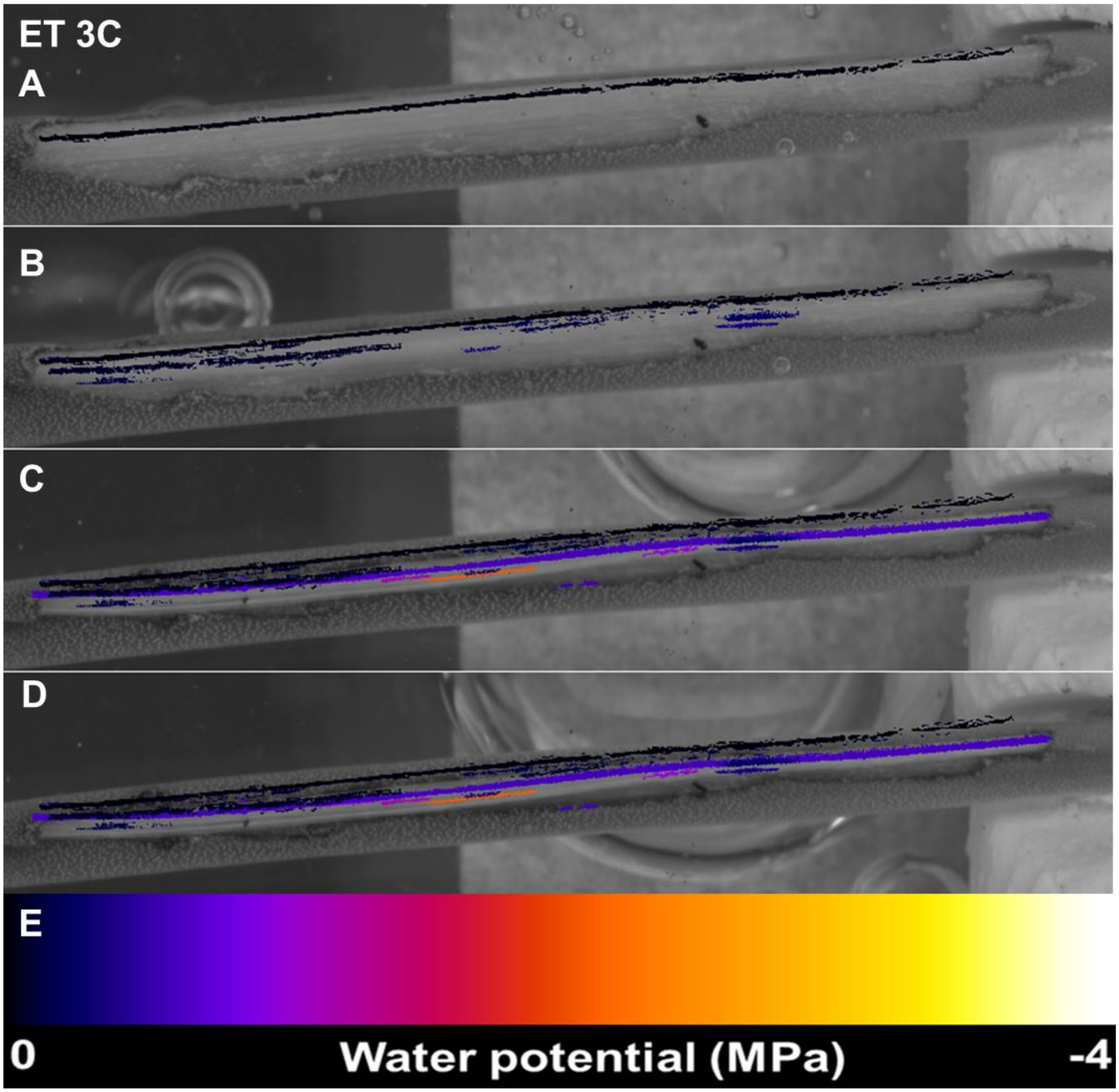
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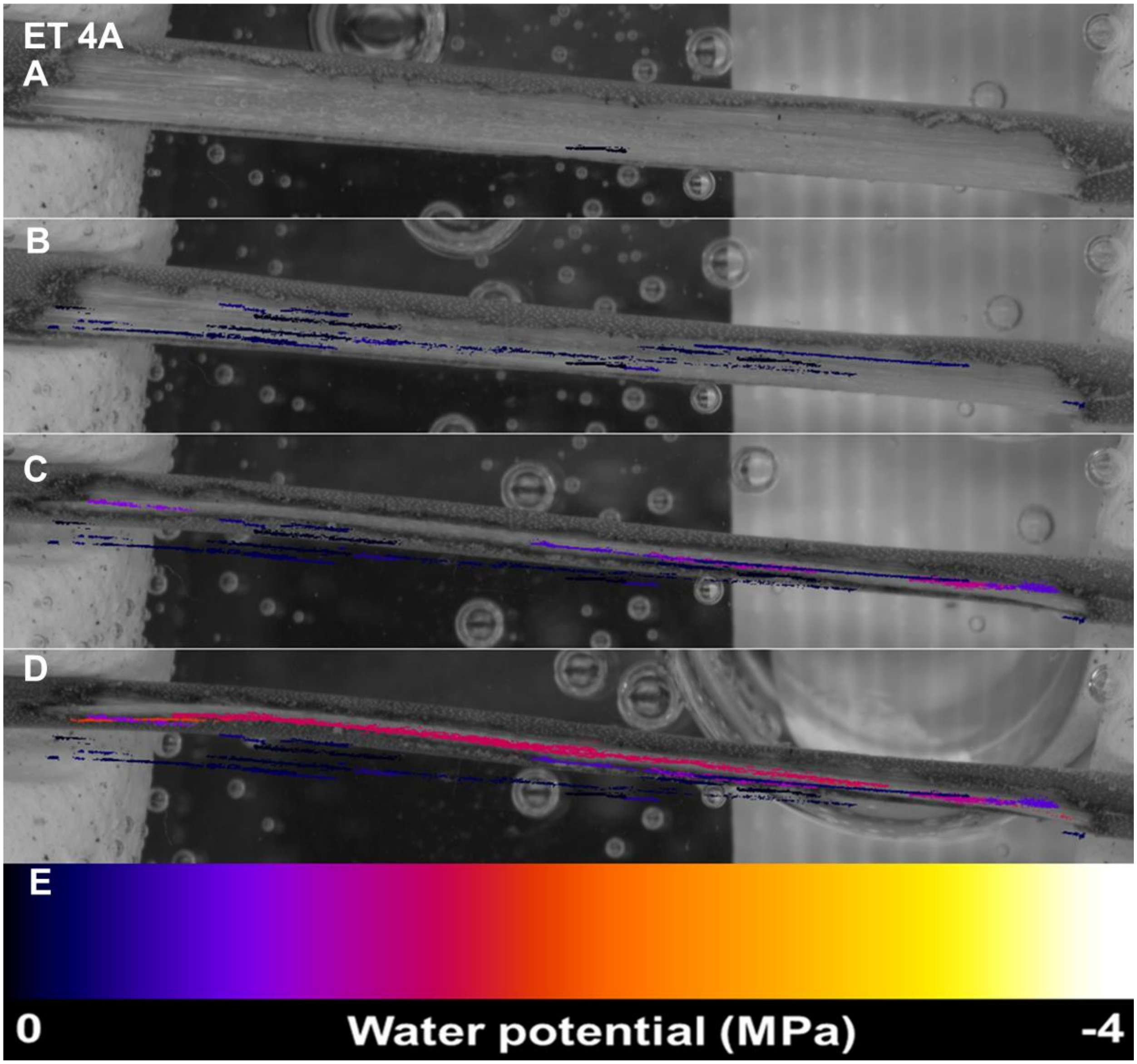
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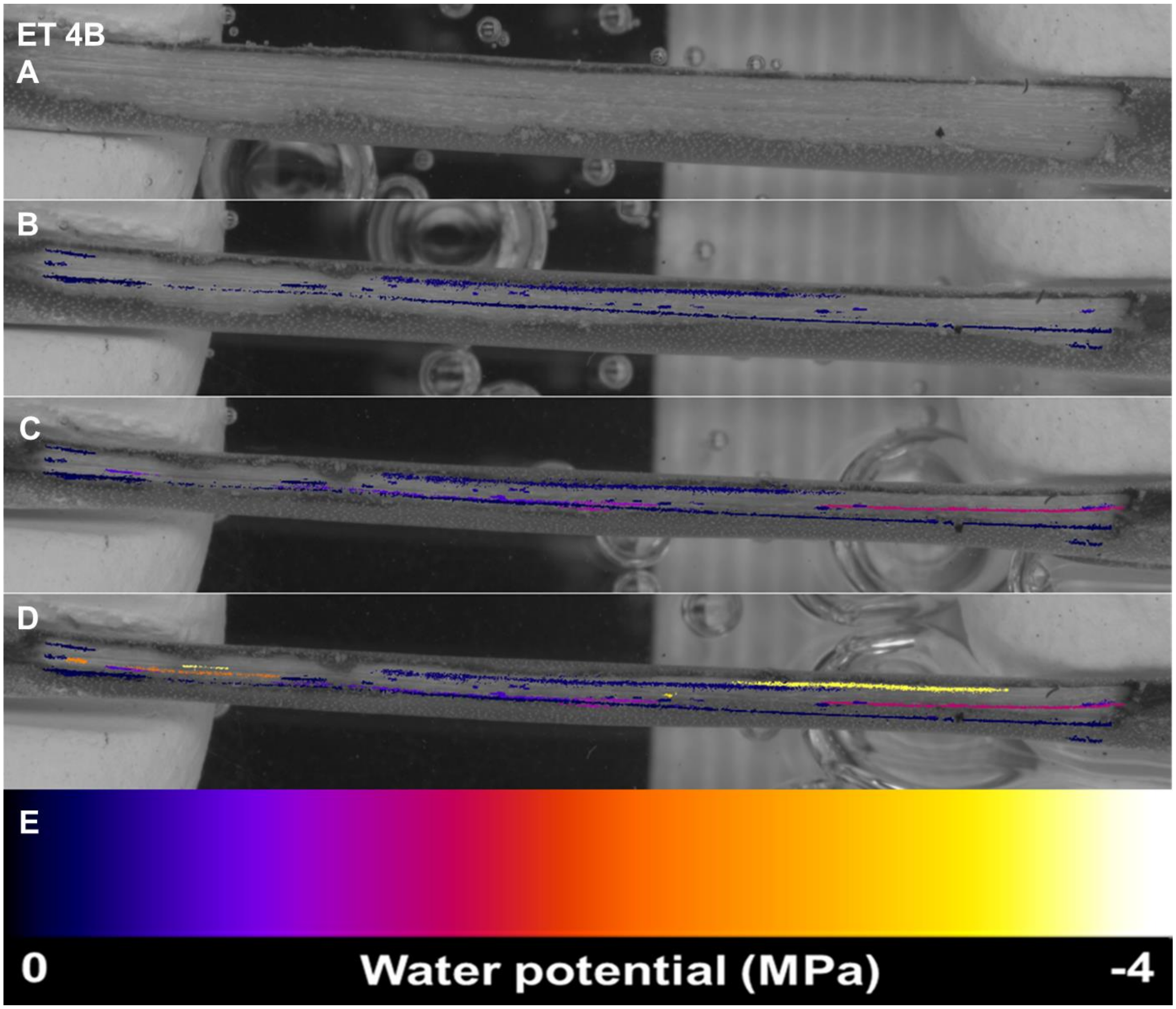
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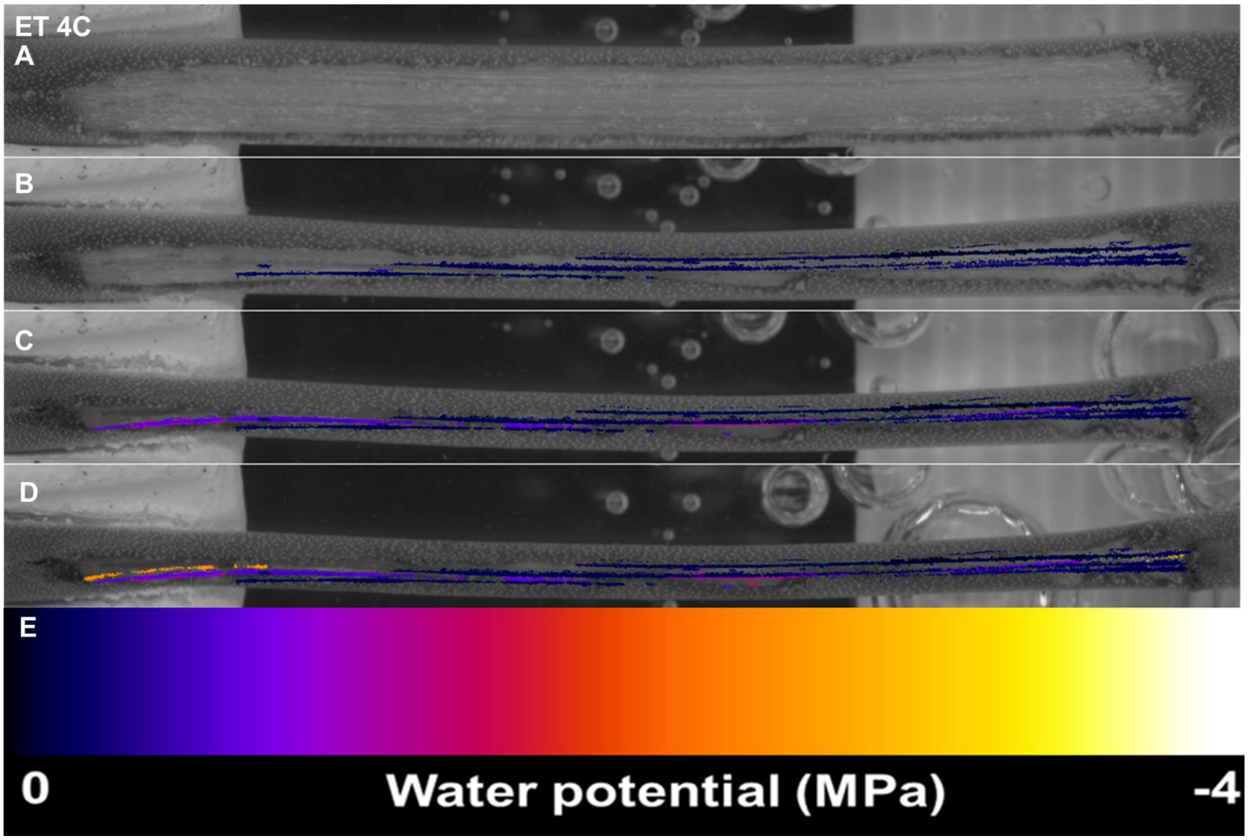
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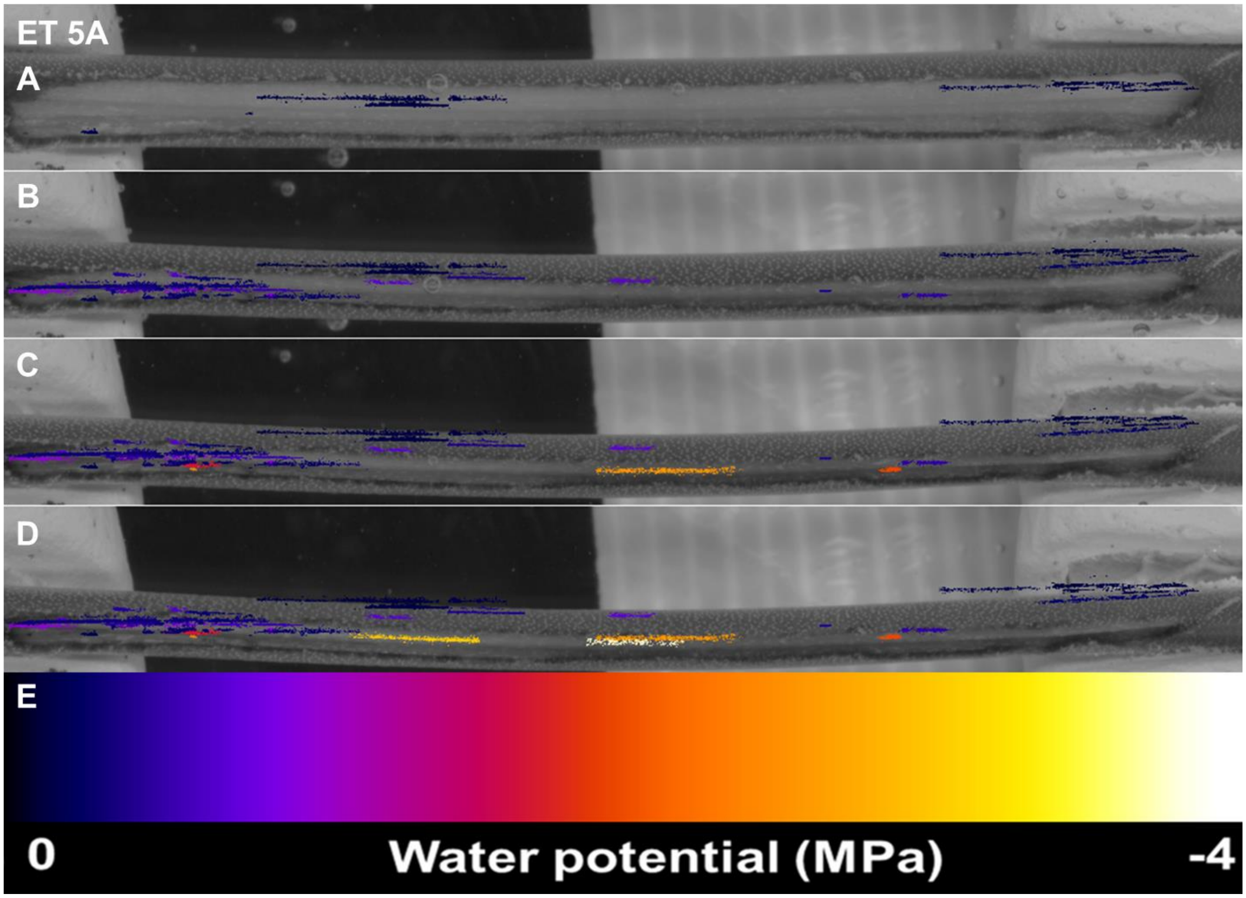
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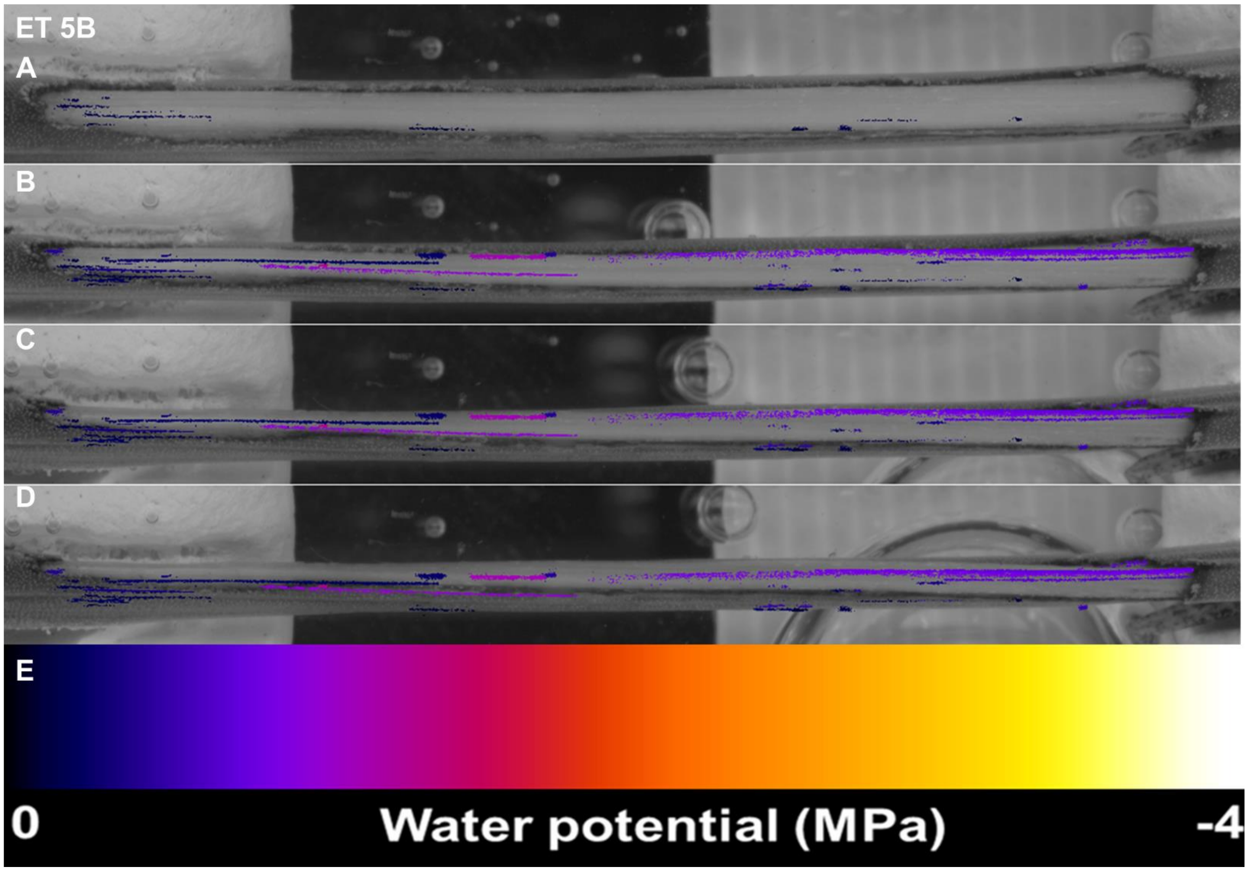
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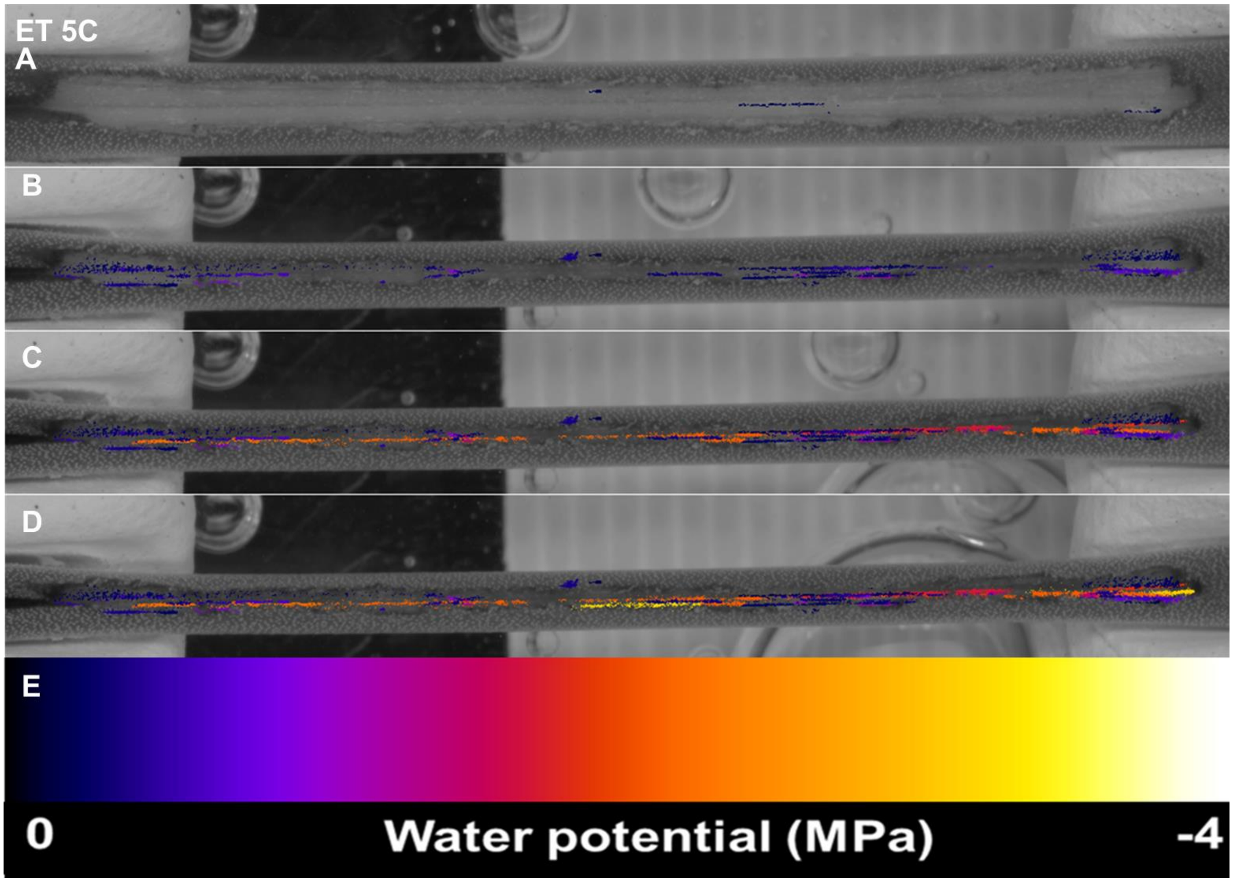
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