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1 **Threats to xylem hydraulic function of trees under ‘new climate normal’ conditions**

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11

12 **Abstract**

13

14 Climate models predict increases in frequency and intensity of extreme environmental
15 conditions, such as changes to minimum and maximum temperatures, duration of drought
16 periods, intensity of rainfall/snowfall events, and wind strength. These local extremes, rather
17 than average climatic conditions, are closely linked to woody plant survival, as trees cope with
18 such events over long lifespans. While xylem provides trees with structural strength and is
19 considered the most robust part of a tree’s structure, it is also the most physiologically vulnerable
20 since tree survival depends on its ability to sustain water supply to the tree crown under variable
21 environmental conditions. Many structural, functional, and biological tree properties evolved to
22 protect xylem from loss of transport function due to embolism or to restore xylem transport
23 capacity following embolism formation. How ‘the new climate normal’ conditions will affect
24 these evolved strategies is yet to be seen. Our understanding of xylem physiology and current
25 conceptual models describing embolism formation and plant recovery from water stress,
26 however, can provide insight into near future challenges that woody plants will face. In addition,
27 knowledge of species-specific properties of xylem function may help guide mitigation of climate
28 change impacts on woody plants in natural and agricultural tree communities.

29

30 **Introduction**

31

32 Trees are long-lived organisms, anchored in one place for tens, hundreds, or thousands of
33 years. They rely on a vascular transport system that, although continuously renewed via annual
34 xylem and phloem growth, must endure local climatic extremes including events of variable
35 frequency, intensity, and duration. Global climate models predict that the small changes in
36 recent climate observed across the planet are only a prelude to more dramatic changes in the near
37 future, such as prolonged drought periods and increased frequency of frost events (IPCC, 2013,
38 Bloom, 2010, Hayhoe *et al.*, 2004). Changing frequency or intensity of extreme events might
39 overwhelm the adaptive limits of vascular transport, resulting in continued periods of vascular
40 function loss and increased incidence of tree mortality. In fact, we already observe increased
41 mortality of trees in natural ecosystems stretching across multiple regions around the world that
42 are being linked to drought and carbohydrate starvation (Hartmann *et al.*, 2013, O'Grady *et al.*,
43 2013, Sevanto *et al.*, 2014). Not only are natural ecosystems in danger, the same fate might be
44 expected for the perennial woody crop plants in orchards. Despite increased agro-engineering
45 efforts, we are limited in our ability to protect orchard trees from global scale consequences of
46 climate change, including loss of access to fresh water, increased salinization of agricultural
47 soils, and rising atmospheric temperature. In addition, orchards are planted today with the
48 expectation of continued productivity for the next 20-40 years, an expectation that might be
49 increasingly difficult to achieve under predicted climate change scenarios (Lobell *et al.*, 2006).
50 To mediate potential damage to agricultural investments and protect natural forest ecosystems,
51 we should better understand how “new climate normal” conditions will influence critical aspects
52 of vascular physiology, namely vascular embolism formation and recovery.

53

54 **Embolism**

55

56 Long-distance water transport in vascular plants occurs in the xylem, a network of nonliving
57 cells connecting roots to leaves (Sperry *et al.*, 2003). Typically, the apoplastic water column in
58 functional xylem is under tension and considered to be in metastable state (Stroock *et al.*, 2014).
59 When internal tension reaches a critical threshold at which the conduit radius is larger than the
60 critical radius of the vacuum/water interface (critical radius r_{critical} being defined as equality
61 between meniscus force defined as water surface tension (γ) divided by air bubble radius) that
62 could oppose expansion due to water surface tension, the conduit water column can break
63 forming an embolus, a phenomenon referred by many plant biologists as ‘cavitation’ (Tyree &
64 Zimmermann, 2002). We note that cavitation (considered as spontaneous initiation of vapor
65 bubbles in pure metastable water) is a highly improbable event in xylem, as it occurs
66 spontaneously at pressures far more negative [i.e. below -140 MPa (Brenner, 1995)] than are
67 physiologically relevant (i.e. above -20 MPa). Thus, we will abstain from using word
68 ‘cavitation’ and only refer to the process as ‘embolization’ or ‘formation of embolism’. Despite
69 the very low probability of true spontaneous cavitation in stems of trees, it was shown using
70 multiple indirect and direct methods that xylem conduits (vessels and tracheids) can switch from
71 a water filled to an embolized state even under tension as low as 1 MPa (Brodersen *et al.*, 2010,
72 Holbrook *et al.*, 2001, Zwieniecki *et al.*, 2013). To reconcile the low probability of spontaneous
73 cavitation with observations of naturally occurring embolism levels, an “air-seeding” hypothesis
74 was proposed as a possible mechanism to explain formation of embolism in xylem under
75 relatively low tensions (Zimmermann, 1983).

76
77 “Air-seeding” is assumed to have several potential origins that are not mutually exclusive, all of
78 which can influence the level and threshold for xylem embolism formation (Capron *et al.*, 2014,
79 Zimmermann, 1983). All proposed explanations assume that somewhere along the conduit an
80 air-water interface exists with a radius that is smaller than the expansion threshold (Figure 1).
81 This interface can exist across the inter-vessel connection (bordered pit field) (Lens *et al.*, 2013),
82 in the crevices of the vessels or tracheid (Pickard, 1981, Zimmermann, 1983), or simply on the
83 wall surface where stable bubbles persist for a prolonged time (Weijs & Lohse, 2013).
84 Increasing tension would result in the change of the critical radius required to overcome water
85 surface tension such that the bubble slowly changes shape and expands into the conduit
86 eventually reaching a critical radius and ‘explodes’ into the conduit causing an embolism that
87 effectively ceases water transport as tension cannot be transmitted via gas. This type of
88 embolism formation might be considered a spatially and temporally unpredictable phenomenon
89 or it might be related to the degree of tension, thermal environment, xylem physical properties,
90 water chemical properties, and history of previous embolism activity (Hacke *et al.*, 2001,
91 Holbrook & Zwieniecki, 1999, Stiller & Sperry, 2002, Tyree & Zimmermann, 2002). In addition
92 to purely tension driven embolism, another origin of the embolism results from freeze-thaw
93 events, where ice formation may lead to release of air from water which form small gas bubbles
94 in the frozen liquid (Mayr *et al.*, 2007, Sperry & Sullivan, 1992). The fate of air bubbles during
95 thawing depends on their size and the amount of tension in the xylem during thawing. If the air
96 bubble radius is smaller than the critical radius for expansion and pressure differential between
97 air in the bubble and water ($2\gamma/r_{\text{critical}}=P_{\text{air}}-P_{\text{water}}$), the air will dissolve in water and the bubble will
98 either collapse or attach to the conduit wall forming a future “air-seeding” site. Alternatively, if

99 the air bubble radius is larger than the critical radius, the bubble will expand and an embolus will
100 form.

101 Regardless of embolism origin, the result is a reduction in the plant's capacity to transport water
102 and a loss of plant productivity or even plant death (Domec *et al.*, 2006b, Holtta *et al.*, 2009,
103 Tyree & Sperry, 1989, Zwieniecki & Holbrook, 2009). Therefore, a plant's capacity to reduce
104 the detrimental effects of embolism is an important adaptive trait for growth and survival
105 (Barigah *et al.*, 2013, Choat *et al.*, 2012, Jacobsen *et al.*, 2007, Pockman *et al.*, 1995, Tyree &
106 Ewers, 1991) and can be affected by climatic properties, especially temperature and moisture.

107 Resistance to drought and frost-induced embolism are important adaptive traits for defining the
108 limits of embolism tolerance across woody species and for predicting drought-induced forest
109 decline at regional and global scales (Choat *et al.*, 2012, Lens *et al.*, 2013). To better understand
110 the role of climate in this system we need to look at the anatomy and associated physicochemical
111 properties of xylem including (i) the structure of the pit membranes, (ii) diameter and length of
112 conduits and (iii) wood density within the context of major climate variables.

113

114 *Pit membranes.*

115

116 Bordered pits are cavities in the lignified cell walls of adjacent vessel or tracheid conduits. They
117 are a pivotal component in the water-transport system of seed plants. Bordered pits are not
118 simple holes that allow water passage between cells. They are highly engineered structures that
119 are strongly conserved among species and that are responsible for more than 50% of total xylem
120 hydraulic resistance (Choat *et al.*, 2008, Hacke *et al.*, 2006, Wheeler *et al.*, 2005) indicating that
121 they are an important factor in the overall hydraulic efficiency of plants and may be fundamental

122 to species survival. In general, bordered pits are formed as circular openings with species-
123 specific diameters ranging from 1 to 20 μm , protected on both ends by a bowl shaped wall,
124 typically with a smaller opening leading to the pit. The pit membrane is located at the center
125 where it separates adjacent conduits and mediates two seemingly contradictory functions: (1)
126 allowing water to flow between adjacent conduits and (2) protecting conduits against the spread
127 of embolism (Choat *et al.*, 2008, Tyree & Sperry, 1989).

128 The structure of pits and pit membranes varies among species, with differences in pit diameter,
129 pit depth, wall smoothness, membrane porosity, membrane thickness, and membrane total
130 surface area. For example, among 26 angiosperm species collected from a range of provenances
131 including riparian, temperate, and Mediterranean environments, the pit membrane thickness
132 range from 70 to 1900 nm and pore sizes within the membrane from 10 to 225 nm (Jansen *et al.*,
133 2008). Species characterized by thicker pit membranes usually also have smaller pores and thus
134 are better adapted to protection from embolism spread as radius of the pores is inversely related
135 to critical tension sustained at the air/water interface. Jansen *et al.* (2008) also found a negative
136 relationship between pit membrane thickness and maximum pore diameter. In addition, pore
137 size in a given species is variable with species specific probability of large pores. As larger pores
138 allow embolism to spread more easily from conduit to conduit, the species with bigger pores or
139 with higher probability of their presence due to a larger membrane area between conduits will be
140 more vulnerable to vascular dysfunction (Choat *et al.*, 2008). Interestingly, there was no
141 correlation between hydraulic resistance of the pit membrane and vulnerability to embolism
142 across a wide range of species. Species with lower hydraulic resistance and higher average
143 porosity were not necessarily more vulnerable to embolism (Hacke *et al.*, 2006). This
144 discrepancy could be explained by the fact that it takes only one large pore in one membrane to

145 spread embolism from conduit to conduit. As such big pores are relatively rare and might be
146 considered a membrane developmental error or could be a result of damage (Choat, 2013).
147 Increased cavitation risk may therefore be linked to total pitted area of xylem conduits, making
148 vessels with greater total area of pits more vulnerable to embolism (Jarbeau *et al.*, 1995). In
149 most gymnosperms, the bordered pit membrane is characterized by a porous margo and central
150 thickening (torus), which is considered to function as an impermeable safety valve against air-
151 seeding. Embolism resistance of conifer pits is correlated to the ratio of torus size versus pit
152 aperture diameter, suggesting a dependency on the torus' ability to seal the pit aperture (Domec
153 *et al.*, 2006a). Embolism resistant species showed higher margo flexibility and larger overlap
154 between the torus and pit aperture, suggesting that torus adhesion to the pit border may be a
155 determinant of cavitation resistance (Delzon *et al.*, 2010). In some conifer species the presence
156 of plasmodesmatal pores fields in the tori could contribute to air-seeding through them (Jansen *et*
157 *al.*, 2012) reducing effectiveness of the pore seal.

158
159 Overall, bordered pit membranes whether in gymnosperms or angiosperm trees seem to be the
160 first line of defense against conduit-to-conduit spread of embolism. A plasticity of bordered pit
161 morphology related to embolism formation was reported for Douglas-fir trees. It was shown that
162 in the branches and trunks the pit aperture diameter of tracheids decreases significantly with
163 increasing height, whereas torus diameter remains unchanged. The resulting increase in the ratio
164 of torus to pit aperture diameter provides better overlap of torus with pit aperture, resulting in the
165 capacity to sustain higher tensions before air-seeding (Domec *et al.*, 2008). There is also a
166 general pattern of smaller pits with thicker, less porous pit membranes in trees growing in drier
167 climates. However, almost nothing is known regarding the plasticity of bordered pit

168 morphological properties over the lifespan of a tree and whether the plant can respond to climatic
169 change. Specifically, it is not known if drier years trigger development of thicker pit membranes
170 and/or smaller pit areas within the same tree across multiple rings, ultimately leading to reduced
171 membrane porosity. This lack of knowledge leaves us unable to predict if trees growing today
172 can acclimate to changes in climate via developmental adjustment of bordered pit structure.
173 However, assuming that bordered pits evolved to protect xylem from embolism spread during
174 extreme events in the current climate, it is conceivable that increased frequency of extreme
175 weather events with more adverse conditions might lead to higher frequency of bordered pit
176 failures and greater incidence of embolism formation.

177

178 *Diameter and length of conduits*

179

180 Angiosperm and gymnosperm conducting elements differ in both diameter and length. Vessels
181 are bigger (length: 1-1000+ cm, diameter: 15-500 μm) than tracheids (length: 0.1-1.0 cm,
182 diameter: 5-80 μm) although the specific distribution of vessel lengths is not known (Choat *et*
183 *al.*, 2008, Zimmermann, 1983). Multiple lines of evidence suggest that wide and long conduits
184 tend to embolize before narrow conduits (Lo Gullo & Salleo, 1991, Lo Gullo *et al.*, 1995, Sperry
185 & Tyree, 1990). This may simply be related to the fact that conduits may contain more bordered
186 pit pores with larger total pit membrane area and thus higher probability of embolism spread due
187 to damage, malfunction, or developmental error (pit area hypothesis) (Sperry *et al.*, 2005, Tyree
188 *et al.*, 1994, Wheeler *et al.*, 2005). Indeed a strong correlation was found between conduit
189 diameter and embolism caused by freeze-thaw cycles (Davis *et al.*, 1999). However, analysis of
190 anatomical traits of xylem plasticity to water stress is more complex (Tyree *et al.*, 1994) as trees

191 were shown to plastically adjust their xylem anatomical traits (diameter and length of the
192 conduits) to drought. These adjustments are not straightforward as plants have to accommodate
193 contradicting demands, namely transport capacity and resistance to embolism formation. For
194 example, *Pinus sylvestris* trees grown in dry regions were characterized by large tracheid
195 lumens, thicker cell walls and high ray tracheid frequency. All of these anatomical features are
196 suggested to facilitate efficient water transport and stem water storage (Martin *et al.*, 2010), but
197 potentially lead to reduced protection from embolism spread between tracheids (pit area
198 hypothesis). In addition, the simple notion that conduit diameter relates to embolism formation
199 is further complicated by the fundamental difference between gymnosperms and angiosperms.
200 Gymnosperms have unicellular tracheids that are a few millimeters long, whereas angiosperms
201 have multicellular vessels with lengths measured in centimeters to meters. This anatomical
202 diversity is most likely responsible for the lack of a simple relationship between conduit diameter
203 and embolism pressure across tracheids and vessels (Sperry *et al.*, 2006).

204

205 These anatomical trade-offs can be considered in light of expected climatic change, although
206 specific predictions are hard to make. In general, drier weather patterns would promote
207 formation of narrower vessels in angiosperm species, a tendency that would limit the probability
208 of embolism formation. This, however, would lead to the reduction of xylem transport capacity,
209 potentially increasing hydraulic resistance and internal tension, thus, eventually leading to zero
210 or negative net effect of this strategy to avoid embolism. Although xylem structure can
211 acclimate to environmental variation during growth and development (Maherali & DeLucia,
212 2000a, Maherali & DeLucia, 2000b, Thomas *et al.*, 2007), the subsequent acclimation of
213 embolism resistance to stress is not possible because xylem conduits are dead at maturity (Choat

214 *et al.*, 2012). Thus, the presence of extreme weather events may further undermine this
215 anatomical adaptation strategy leading to embolism related dieback of already established trees.
216 At the very least, the tendency to grow narrower xylem conduits would most likely result in less
217 efficient transport system and would lead to overall reduction in photosynthetic capacity and
218 reduced growth rates.

219

220 *Wood density*

221

222 Wood density is another morphological parameter often related to a tree's ability to withstand
223 embolism. It depends on cellular wall thicknesses of tracheid and vessels, lumen diameters, and
224 percentage of latewood (part of the wood in a growth ring of a tree that is produced later in the
225 growing season). It was suggested that wood density and specifically wall thickness is related to
226 the risk of cell wall collapse under tension and embolism due to mechanical cell damage
227 (Jacobsen *et al.*, 2005, Sperry *et al.*, 2003, Wagner *et al.*, 1998). Indeed, species characterized
228 by a high wood density and a high thickness-to-span ratio [thickness:span represents the ratio of
229 conduit double wall thickness to lumen diameter, (Pittermann *et al.*, 2006)] of water conducting
230 cells are more resistant to embolism formation (Hacke *et al.*, 2001) and trees producing low-
231 density wood under favorable water availability are more stressed by sudden drought (Rosner *et*
232 *al.*, 2014).

233

234 It is very unlikely that cellular walls directly contribute to embolism spread between conduits.
235 Wall forming cellulose fibers are impregnated with lignin, hemicelluloses and pectin limiting
236 potential radius of pores to be smaller than those existing in bordered pit membranes. However,

237 under changing climatic conditions, induction of drought response might lead to changes in wall
238 surface chemistry (Kostiainen *et al.*, 2006). Lignin is a hydrophobic component of the xylem
239 conduits walls. Thus one can speculate that changes in lignin wall content in response to water
240 stress would be responsible for the increase of wall hydrophobicity. Consequently, the surface
241 would be more likely to nest persistent air pockets responsible for ‘air seeding’, thus limiting the
242 effectiveness of this response as an embolism avoidance strategy. Similarly, genetic
243 manipulation aiming at decreased lignin xylem content for fuel production might also have
244 unintended consequences, as a reduction in lignin content generally leads to thinner walls and
245 increases the potential of conduit collapse under tension (Donaldson, 2002) and higher
246 vulnerability to embolism (Voelker *et al.*, 2011). Again, we are left with highly limited
247 knowledge of natural potential for acclimation of trees to changing climate via adaptation of
248 wood structure.

249

250 *Tree mortality - hydraulic failure*

251

252 In the past 20 years an increased frequency of widespread forest mortality due to drought or
253 temperature stress has been observed in many different tree-dominated ecosystems, such as
254 tropical rainforest (Phillips *et al.*, 2009), temperate mountainous forests, Mediterranean forests,
255 and boreal forests (Carnicer *et al.*, 2011, Peng *et al.*, 2011, van Mantgem *et al.*, 2009). Also, in
256 temperate North American forests some mortality events were associated with “global change-
257 type droughts”, defined as severe drought coupled with elevated summer temperatures
258 (Breshears *et al.*, 2005, Shaw *et al.*, 2005, Worrall *et al.*, 2010). Therefore, to make predictions
259 of how future climate change scenarios will affect different biomes it is critical to understand the

260 physiological mechanisms responsible for tree mortality. In this context, we have to consider (i)
261 hydraulic failure and (ii) carbon starvation (McDowell *et al.*, 2008, Nikinmaa *et al.*, 2013,
262 Sevanto *et al.*, 2014).

263
264 Overall, evolution of xylem conduits can be described as an increase in transport efficiency at the
265 expense of embolism protection and vice versa. Trees dominate multiple ecosystems that are
266 characterized by high moisture availability. However, despite over 350 million years of
267 evolution, trees have a limited presence in many drier biomes. Woody plants mostly exist as
268 shrubs that deal with stress related injuries by re-growing lost stems. The absence of trees in dry
269 habitats suggests their limited ability to form a transport system that is perfectly protected from
270 embolism under extreme drought conditions. In areas occupied by trees, they may experience
271 ‘hydraulic failure’ when water loss from transpiration is greater than water uptake by roots,
272 creating high negative xylem tension that may result in embolism (Sevanto *et al.*, 2014, Sperry *et*
273 *al.*, 1998).

274
275 The ability of a plant to protect itself from hydraulic failure is often described in terms of its
276 ‘hydraulic safety margin’ (Meinzer *et al.*, 2009). This trait is often analyzed using ‘vulnerability
277 curves’ that plot how xylem hydraulic conductivity (Kh) declines as xylem pressure becomes
278 more negative. Declines in Kh are typically expressed relative to the maximum sample Kh as the
279 percentage loss of conductivity (PLC). Such curves are typically sigmoidal in shape with low
280 PLC at xylem pressure near zero and high PLC at large negative pressures. Embolism resistance
281 has been widely documented across species and varies greatly (Maherali *et al.*, 2004). There is
282 also evidence that embolism resistance can vary between populations of the same species (Alder

283 *et al.*, 1996, Mencuccini & Comstock, 1997, Sparks & Black, 1999) or between closely related
284 genotypes (Barnard *et al.*, 2011, Choat *et al.*, 2007, Neufeld *et al.*, 1992, Pita *et al.*, 2003,
285 Sangsing *et al.*, 2004). Usually, comparisons of vulnerability to embolism among species are
286 represented by the xylem pressure at which a 50% loss of conductivity (P50) occurs. The
287 hydraulic safety margin is defined as the difference between naturally occurring minimum xylem
288 pressures and pressures that would cause 50% of hydraulic dysfunction. The naturally occurring
289 minimum xylem pressure is usually related to stomatal activity and pressure at which stomata
290 start to regulate water loss. Once a plant has reached its P50, it is on the steepest part of the
291 vulnerability curve, which means that even a small decrease in xylem pressure will produce a
292 substantial reduction of K_h (Johnson *et al.*, 2012), increasing the risk of runaway embolism
293 (Tyree & Sperry, 1988). Usually, plants with narrow safety margins experience large amounts of
294 embolism in their respective environments and, therefore, show a high risk of hydraulic failure
295 (Choat *et al.*, 2012).

296

297 Differences in hydraulic safety strategies are reported between angiosperms and conifers.
298 Conifers tend to have greater safety margins than angiosperms (Choat *et al.*, 2012, Meinzer *et*
299 *al.*, 2009) and also experience smaller levels of embolism in the stem while being more
300 permissive of embolism formation in distal organs (i.e. leaves and roots), (Johnson *et al.*, 2012).
301 This strategy can be considered as a safety valve to protect the integrity of the stem hydraulic
302 pathway as it is impossible to replace the main stem while branches and roots can be regenerated
303 or potentially refilled (McCulloh *et al.*, 2011). Angiosperms are characterized by narrow or even
304 negative safety margins making them vulnerable to extensive levels of embolism formation
305 across branches and stems. Plants have evolved mechanisms to restore xylem function (i.e.

306 embolism recovery) following events of drought (Johnson *et al.*, 2012). Safety margins of 226
307 woody species among angiosperms and gymnosperms sampled from sites with a wide range of
308 mean annual precipitation and temperature were recently compared in a meta-analysis (Choat *et*
309 *al.*, 2012). Of all species analyzed, 70% were considered to operate at narrow safety margins
310 regardless of climate preferences. However, it is important to mention here that this analysis was
311 exclusively done on small branches that are known to be more vulnerable to embolism than large
312 brunches and trunks (McCulloh *et al.*, 2014). Such a large number of species operating with
313 narrow safety margins may suggest that all biomes will be vulnerable to drought-induced decline
314 with the increases of aridity and temperature that have been predicted for many regions with
315 expected global climate change. Moreover, if extreme weather events increase in frequency and
316 severity, then drought-induced forest decline might occur not only in arid regions but also in wet
317 environments since safety margins are independent of mean annual precipitation. Thus, general
318 trends in the ‘hydraulic safety margin’ of trees suggest that drought-induced decline and
319 mortality at regional and global scales has the potential to occur (Choat *et al.*, 2012). Yet the
320 magnitude of this effect will depend on the ability of different species to recover from xylem
321 conductivity losses and acclimate via stomata.

322

323 Stomata provide dynamic protection from cavitation by limiting the level of negative water
324 potential that a plant experiences (Meinzer *et al.*, 1992, Sperry & Pockman, 1993). Trees adjust
325 their water use and hydraulic safety through the coordination of hydraulic and stomatal
326 regulations (Brodribb & McAdam, 2011, Choat *et al.*, 2012, Sperry, 2000). Such an association
327 between xylem water potential at the onset of xylem cavitation and leaf water potential triggering
328 stomatal closure are well documented (Brodribb & Holbrook, 2003, Brodribb *et al.*, 2003,

329 Cochard *et al.*, 2002, Hubbard *et al.*, 2001, Klein, 2014, Nardini *et al.*, 2001). Without stomatal
330 control excessive embolism formation would eventually lead to ‘runaway embolism’, in which
331 embolism decreases hydraulic conductance and xylem water potential, leading to a cycle of
332 embolism that precipitously reduces water conducting capacity until it is completely lost (Tyree
333 & Sperry, 1988). The balance between the temporal dynamics of stomatal closure and stem
334 water potential is species-specific and is often linked to environmental properties. Under
335 changing climatic conditions species might experience a shift in the rate of water stress
336 enactment and, as a result, may be unable to track environmental change with stomatal closure.
337 In fact, a sudden increase in vapor pressure deficit may lead to transient ‘wrong way’ stomatal
338 response i.e. stomatal opening despite sudden drop in leaf water potential (Buckley, 2005,
339 Buckley *et al.*, 2011) and ultimately cause a runaway embolism. However, even when stomata
340 are capable of reducing transpiration rates, effectively protecting xylem from embolism, doing so
341 comes at the cost of reduced photosynthetic activity and overall loss of carbohydrate reserves,
342 which will likely be detrimental to tree health.

343

344 *Tree mortality – carbohydrate starvation*

345

346 Carbon starvation occurs when stomata are closed to prevent water loss and avoid hydraulic
347 failure. During shorter timeframes, species have strategies that account for the imbalance
348 between carbon uptake by photosynthesis and carbon loss due to respiration and growth.
349 However, prolonged periods of stomatal closure might lead to a negative total carbon balance
350 that depletes carbohydrates reserves and ends in tissue-level carbohydrate starvation (McDowell
351 *et al.*, 2008, McDowell *et al.*, 2013). For some temperate angiosperm species exposed to

352 extreme drought conditions hydraulic failure is the first cause of tree mortality (Barigah *et al.*,
353 2013, Urli *et al.*, 2013). In these species the lethal xylem water potential (xylem pressure
354 correspondent to 50% of mortality) occurred when drought had caused > 88-90% loss of stem
355 hydraulic conductivity (Barigah *et al.*, 2013, Urli *et al.*, 2013). Thus, processes involving
356 hydraulic impairments rather than carbohydrate impairments are considered to be more
357 important for predicting the effects of drought on forests (Anderegg *et al.*, 2012). However,
358 carbon and hydraulic properties cannot be considered in isolation as both are interrelated and
359 changed during mortality of aspen during a widespread die-off event (Anderegg *et al.*, 2012).
360 Evidence of carbon starvation-induced tree mortality is scarce, maybe because it can cause trees
361 to die only after long exposure to relatively mild water stress conditions characterized by
362 extended periods without positive net photosynthesis (Adams *et al.*, 2013), presumably due to
363 the reduction of carbon uptake accompanying prolonged stomatal closure (Mitchell *et al.*, 2013,
364 Ogasawara *et al.*, 2013).

365
366 Across gymnosperms it was shown that the major cause of tree mortality is related to drought
367 and occurs when drought causes > 50% loss of stem hydraulic conductivity (P50) (Brodribb *et*
368 *al.*, 2010, Brodribb & Cochard, 2009). However, in *Pinus edulis* both hydraulic failure and
369 carbon starvation can be independent causal factors of tree mortality. Hydraulic failure causes
370 relatively fast tree death that is characterized by rapid decline in leaf water potential, high level
371 loss of hydraulic conductivity, and no changes in carbohydrate reserves at death. Carbon
372 starvation resulting from prolonged shading causes slow tree death characterized by no
373 significant change in leaf water potential and small variations in hydraulic conductivity but
374 significant reduction in carbohydrates reserves. Changing climatic conditions may lead to co-

375 occurrence of both processes, water stress and carbohydrate starvation. Thus mortality in trees
376 currently experiencing reduction in carbohydrate availability due to shading would be
377 accelerated by hydraulic failure and additional reduction of photosynthetic capacity due to
378 stomatal closure (Sevanto *et al.*, 2014).

379

380 While carbohydrate starvation under drought might be a problem in current conditions, the
381 carbohydrate balance of trees growing in elevated CO₂ and temperature, is not clear. In general,
382 both CO₂ and temperature alter tree xylem anatomy (Domec *et al.*, 2010, Maherali & DeLucia,
383 2000a) and wood hydraulic properties (Phillips *et al.*, 2011) such that observed anatomical and
384 physiological changes result in lower drought tolerance (Way *et al.*, 2013). Deciduous boreal
385 trees, for example, showed a greater vulnerability to embolism in leaves exposed to elevated
386 temperature compared to those grown at ambient temperatures, even though vulnerability in
387 stems did not change between treatments (Way *et al.*, 2013). Eucalypt seedlings exposed to
388 moderate drought stress and elevated temperatures showed reduced plant carbon accumulation
389 and increased leaf respiration. When the same seedlings were exposed to moderate drought
390 stress and high CO₂ concentrations, they exhibited increased carbon content and dry mass
391 accumulation. These beneficial effects vanished, however, when drought became more severe
392 (Duan *et al.*, 2013). Unfortunately, only a few studies have analyzed how plants respond to
393 elevated CO₂ concentration and temperature combined (Duan *et al.*, 2013, Maherali & DeLucia,
394 2000a, Phillips *et al.*, 2011) and more information is needed to understand how trees will
395 respond to drought under global change conditions.

396

397

398 **Recovery from embolism**

399
400 Plants have evolved several strategies to prevent and/or mitigate the effects of hydraulic failure
401 due to embolism and to restore xylem transport capacity once embolism occurs (Secchi &
402 Zwieniecki, 2012, Stiller & Sperry, 2002). Restoration of xylem capacity can be divided into two
403 sets of strategies: (1) growth and (2) maintenance. The first group of strategies includes passive,
404 often long-term, responses like shedding leaves or small branches followed by the growth of new
405 shoots (shrubs). They might also include growth of new xylem and the formation of new conduits
406 to assure continuity and capacity of the transport system under adverse conditions (Sperry *et al.*,
407 1987, Stiller & Sperry, 2002). The second group includes active and often relatively fast
408 physiological responses that result in the restoration of hydraulic capacity by generating positive
409 root pressure (often only found in small herbaceous plants) (Cochard *et al.*, 1994, Ewers *et al.*,
410 1997, Yang *et al.*, 2012), by stem parenchyma cell activity that results in localized embolism
411 removal in woody plants (Brodersen & McElrone, 2013, Nardini *et al.*, 2011, Salleo *et al.*, 2004),
412 and by strategies that require access to external water (rain, fog or snow) via leaves or buds in
413 order to soak the xylem. The last process has been observed primarily in coniferous species (Laur
414 & Hacke, 2014, Mayr *et al.*, 2014).

415
416 The impact of climate change on the first group of strategies would be related to changes in plant
417 growth and would depend on relative changes in carbon fixation and respiration rates and
418 acclimation pattern observed for specific species. Specifically, it is expected that small increases
419 in carbon assimilation due to higher CO₂ concentrations might be offset by increased respiration
420 due to rising average temperatures, suggesting that carbohydrates available for growth

421 (construction) might not change or could even decrease. Thus, the potential to restore hydraulic
422 capacity by conduit replacement, or growing new branches might not keep up with expected losses
423 due to increased rates of embolism formation, effectively leading to increased stress experienced
424 by plants, along with a reduction in gas exchange. Lower gas exchange would further impact a
425 plant's ability to maintain an adequate pool of carbohydrates to replace lost conduits and branches,
426 effectively creating a feed-forward loop that would result in progressive loss of tree vitality,
427 growth rate and a potential yield reduction, as a higher fraction of available resources will be used
428 for construction and maintenance. As the recovery strategies from this first group depend on plant
429 growth and are slow, the effects of climate change in plants relying on these strategies would be
430 expected to occur over extended periods of time (years). The effects of climate change will most
431 likely be reflected in reduced tree crown size due to die-back of branches and in reduced total leaf
432 area resulting in less overall transpiration. This reduction in crown size would match transport
433 capacity of the stem but it would reduce tree vitality and might be related to increased mortality.
434 Although, studies of the long term effect of increased temperature on respiration suggest that some
435 plants can acclimate and respiration can be greatly reduced in higher temperatures (Atkin *et al.*,
436 2005, Atkin & Tjoelker, 2003). This may still lead to higher respiration rates in warmer
437 environments than in cooler ones, but not to the degree implied by short-term temperature
438 changes.

439
440
441 The second group of strategies that might allow trees to recover from xylem hydraulic capacity
442 losses depends on the use of energy to dynamically repair embolized conduits under the presence
443 of adverse conditions, like tension or transpiration. Such strategies potentially allow plants greater

444 flexibility in response to periods of drought and mediate temporal losses to photosynthetic
445 capacity. Reconciliation of embolism recovery with continued xylem tension has proven to be
446 difficult to understand (Holbrook & Zwieniecki, 1999, Tyree *et al.*, 1999), and only recently has *in*
447 *vivo* imaging provided strong support for the notion that plants can recover embolized vessels,
448 even under low or moderate stress levels (Brodersen *et al.*, 2010, Clearwater & Goldstein, 2005,
449 Zwieniecki *et al.*, 2013). However, despite significant scientific efforts (Nardini *et al.*, 2011,
450 Salleo *et al.*, 1996, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011, Zwieniecki &
451 Holbrook, 2009), the mechanisms responsible for embolism recovery under negative pressure is
452 still not well understood.

453 Predicting future climate impact on the embolism recovery process is difficult because the specific
454 physiological mechanisms involved are still unknown, however, a more general look at the impact
455 of climate change on embolism recovery is possible.

456

457 While embolism formation is a spontaneous, purely physical process related to the degree
458 of tension in the water column and to the physicochemical and anatomical properties of the wood
459 (Brenner, 1995, Tyree & Zimmermann, 2002), embolism removal requires that empty vessels fill
460 with water against existing energy gradients. Thus, recovery from embolism cannot happen
461 spontaneously and requires physiological activity that promotes water flow into embolized vessels
462 (Holbrook & Zwieniecki, 1999, Salleo *et al.*, 2004, Secchi *et al.*, 2011, Tyree *et al.*, 1999,
463 Zwieniecki & Holbrook, 2009). Visual evidence from cryo-SEM studies, magnetic resonance
464 imaging (MRI) observations, and computed tomography (CT)-scans showed that water (xylem
465 sap) can return to empty vessels, suggesting that plants have the ability to restore functionality in
466 the xylem (Clearwater & Goldstein, 2005, Holbrook *et al.*, 2001, Scheenen *et al.*, 2007). A more

467 recent study showed that in grapevine vessels, where the bulk xylem tissue was still under tension,
468 water droplets preferentially formed on the vessel walls adjacent to parenchyma cells and that
469 these droplets grow until the lumen was completely refilled (Brodersen *et al.*, 2010), although it
470 was not proven that these refilled lumens returned to full function i.e. being able to transport water
471 under tension. Droplet formation on the walls of empty vessels that are in contact with
472 parenchyma cells support predictions that these living cells play a critical role in embolism
473 removal by supplying energy, and possibly water, to drive the restoration of xylem hydraulic
474 function (Brodersen & McElrone, 2013). If xylem parenchyma cells supply a significant fraction
475 of water required for filling embolized vessels, water must pass a cellular membrane and, thus, the
476 flow must be facilitated by aquaporins (membrane proteins that facilitate water transport across
477 the cellular membrane). Studies on walnut (*Juglans regia*) showed that higher expression of two
478 aquaporin proteins (JrPIP2.1 and JrPIP2.2) was observed in vessel-associated parenchyma cells at
479 the same time that recovery from embolism took place (Sakr *et al.*, 2003). Moreover, expression
480 levels of several PIP1 and PIP2 genes were shown to increase during the recovery process in two
481 other species *Populus trichocarpa* and *Vitis vinifera* (Kaldenhoff *et al.*, 2008, Perrone *et al.*, 2012,
482 Secchi *et al.*, 2011, Secchi & Zwieniecki, 2010). Detailed analysis of how the transcriptome
483 responds to the presence of embolism in *P. trichocarpa* and *V. vinifera* petioles revealed that
484 several aquaporin subfamilies were strongly up-regulated during refilling (Perrone *et al.*, 2012,
485 Secchi *et al.*, 2011) especially PIP1, PIP2, and TIP.

486
487 Recovery from embolism requires a source of water to fill the empty vessels and a source of
488 energy to overcome existing energy gradients acting against water flow. Living parenchyma cells
489 can, parsimoniously, provide both. This assumption is supported by the inhibition of recovery in

490 response to either physical damage to phloem or metabolic inhibition of living cells in stems
491 (Bucci *et al.*, 2003, Salleo *et al.*, 2004, Zwieniecki *et al.*, 2004). A series of models have proposed
492 that sugars needed as osmoticum for refilling can be unloaded into the embolized vessels from ray
493 parenchyma cells. Sugars can either be transported from the phloem or be released from starch
494 stored in the stem. Once in the apoplast they can create an osmotic gradient that drives water from
495 the parenchyma to the empty vessels (Nardini *et al.*, 2011, Secchi & Zwieniecki, 2011, Zwieniecki
496 & Holbrook, 2009). For this process to work, the presence of embolism should alter carbohydrate
497 metabolism and carbon partitioning between starch and soluble sugars in the xylem parenchyma
498 and potentially influence both enzyme activities and gene expression. Indeed, both visualization
499 techniques and enzymatic analysis of non-structural carbohydrates levels in xylem of *L. nobilis*
500 and *P. trichocarpa* experiencing high levels of embolism demonstrated that starch content in stem
501 parenchyma cells decreased and that soluble sugar levels increased (Nardini *et al.*, 2011, Regier *et*
502 *al.*, 2009, Salleo *et al.*, 2009, Secchi & Zwieniecki, 2011). Furthermore, a drop in starch content
503 was associated with changes in gene expression, especially down-regulation of the
504 monosaccharide metabolic pathway (including enzymes involved in starch synthesis) and strong
505 up-regulation of the disaccharide metabolic pathway that also includes starch degradation enzymes
506 like alpha- and beta-amylases in the stems of *P. trichocarpa* (Secchi & Zwieniecki, 2011). The
507 same study also revealed an up-regulation of genes from ion transport and carbon metabolism
508 ontology groups. Similarly, an up-regulation of carbon metabolism was exhibited during petiole
509 recovery in grapevine (Perrone *et al.*, 2012). This transcription level response matched results
510 obtained from chemical analysis of liquid collected from non-functional (embolized) vessels,
511 where elevated levels of sugars and ions were found. However, the total osmotic concentration
512 increase was relatively small and could only account for recovery under low tension levels (Secchi

513 & Zwieniecki, 2012), thus, a significant relief from stress is most likely required before functional
514 recovery from embolism and restoration of hydraulic activity can occur.

515
516 A recent evaluation of the role of aquaporins in recovery of trees from embolism using transgenic
517 plants with down-regulated expression of the PIP1 subfamily (Secchi & Zwieniecki, 2014) in
518 combination with visual observation of the recovery process (Brodersen *et al.*, 2010, Zwieniecki *et*
519 *al.*, 2013) may suggest a new view of the embolism-recovery cycle. It is possible that embolism
520 level is a net effect of embolism formation (with a rate that is positively related to stress level) and
521 recovery (refilling, with a rate that is inversely related to stress level). This notion would explain
522 the observed higher vulnerability to embolism in the transformed plants even though the plants
523 show no anatomical changes that might affect embolism rate (Secchi & Zwieniecki, 2014): the
524 shift in vulnerability would be achieved not by any change in the rate of embolism initiation, but
525 rather by a differential rate of embolism recovery due to a lower membrane permeability of xylem
526 parenchyma cells. This view of the embolism-recovery cycle might suggest that a vulnerability
527 curve estimated from fresh material collected in the field reflects the current balance between
528 embolism and recovery processes, rather than a true measure of xylem embolism vulnerability.

529
530 Identifying how xylem recovers after embolism formation is fundamental to understanding the
531 impact of climate change on water transport and tree survival. As described above, xylem
532 recovery is highly variable among tree species and even among individual plants of the same
533 species, as recovery might depend on the current level of embolism and health status of the plant
534 (Hacke & Sperry, 2003, Holbrook *et al.*, 2001). For example, angiosperms are shown to recover
535 from embolism within days or even hours if provided relief from stress (Bucci *et al.*, 2003, Ogasa

536 *et al.*, 2013, Salleo *et al.*, 2004) while conifers tend to show very slow recovery (days or even
537 months). Such slow recovery would suggest that embolized tracheids either are refilling very
538 slowly or must be replaced by new tracheid in order to restore xylem hydraulic capacity (Brodribb
539 *et al.*, 2010, Brodribb & Cochard, 2009). However, some evidence of the fast recovery process in
540 gymnosperms has been recently shown in Norway spruce trees (*Picea abies*) that were able to
541 recover embolized xylem in later winter using melting snow as the source of water (Mayr *et al.*,
542 2014). This process was shown to be related to starch metabolism and an increase of aquaporin
543 abundance. Similarly, the role of aquaporins in facilitating radial water movement from the needle
544 epidermis towards the vascular tissue was also found in *Picea glauca* suggesting that if exposed to
545 reduced stress and foliar availability of water even conifers may have evolved a physiological
546 strategy to increase rate of recovery from embolism with refilling occurring in a matter of hours
547 (Laur & Hacke, 2014). When compared with angiosperms, gymnosperms tend to have less
548 parenchyma cells in the xylem and lower non-structural carbohydrate abundance in their wood.
549 As the ability to rapidly remove embolism relies on the proximity of parenchyma cells to xylem
550 conduits as a source of energy and water, this might explain the need for larger safety margin in
551 conifers relative to angiosperms at comparable levels of embolism (Johnson *et al.*, 2012). The
552 role of xylem parenchyma in embolism recovery is further underlined by a study showing that
553 among 47 plant species, including angiosperms and gymnosperms, axial parenchyma was
554 commonly present in most woody angiosperm species able to recover from embolism formation.
555 The few cases where parenchyma was absent were mostly in herbaceous annual plants without
556 secondary xylem where the recovery process could only be accounted by the presence of root
557 pressure (Brodersen & McElrone, 2013).

558

559 **Xylem function in the ‘new climate normal’**

560

561 We are just beginning to understand fully the complexity of water transport function in the
562 xylem--including its structure, rheology, chemistry, and physiology. Our knowledge still has
563 many gaps and only a superficial knowledge exists regarding both embolism formation and the
564 process of recovery from embolism in woody plant xylem. Yet, we face the prospect of rapid
565 changes to climatic conditions that are shorter than the lifespan of a single tree. Orchards and
566 forests growing today will experience a transition period to the ‘new climate normal’ while we
567 have very few tools to predict climate impact on tree growth, yield and survival, and even less
568 knowledge to inform breeding efforts aimed at mediating the negative impact of changing
569 climate. However, by scrutinizing available knowledge we can filter out a few important aspects
570 of how climate change may impact xylem function and try to predict the most likely scenarios
571 describing the effect of increased temperature, reduced water availability, and increased
572 frequency and duration of extreme weather events on tree hydraulic system capacity.

573

574 We can assume that reduced water availability due to a drop in precipitation abundance,
575 frequency, or both, would lead to extended periods of time during which trees would experience
576 xylem tension levels near their ‘safety margin’, i.e. the level of tension when stomatal
577 conductance is reduced to protect xylem from excessive embolism formation (Figure 2). “Safety
578 margin” is an evolved trait that likely is a deeply embedded property of species-specific survival
579 strategies and is not easily changed. Thus, prolonged periods of drought that force stomata to
580 close should result in loss of photosynthetic capacity and a reduction in non-structural
581 carbohydrate availability needed by a tree to maintain transport capacity under drought and/or

582 restore it during periods of stress recovery following the relief provided by a rainfall or fog event
583 (Figure 2). Thus, under drought conditions, trees might experience an accumulation of
584 embolized conduits in stems over time, leading to reduced benefits from their evolved ‘safety
585 margins’. We can expect that in the near future the potential for ‘run-away cavitation’ will
586 increase, especially in species operating at or near their physiological thresholds and not having a
587 strong stomatal control. In addition, if embolism level in a plant reflects the balance between
588 embolism formation rate and the capacity to refill embolized vessels using available energy, then
589 prolonged periods of drought would shift that balance toward trees capable of withstanding
590 higher levels of embolism. Under low water potential, the rate of new embolism formation will
591 exceed the rate of recovery. As frequency and length of stress relief periods are expected to
592 diminish, the recovery process will be shortened as well, forcing trees to operate at continuously
593 higher level of embolism. Operating at higher level of embolism will reduce the capacity of
594 trees to photosynthesize, as higher stress levels will force stomatal closure due to higher stem
595 hydraulic resistance. This general reduction in carbon assimilation will reduce the total
596 carbohydrate pool that could affect all aspects of plant growth, protection, and yield. This
597 reduction in production will be magnified by rising average temperatures that would increase
598 plant respiration which will further decrease the size of the total carbohydrate pool and the
599 ability of trees to maintain xylem transport capacity. Such significant reductions in the stem
600 non-structural carbohydrate pool would also lead to reduced growth, higher rates of mortality,
601 and may reduce the yield of commodity trees.

602 In conclusion, mediating future climate impacts and guiding breeding programs will require
603 better knowledge of tree stress physiology. Specifically, we should invest in improving our
604 understanding of xylem physiology under variable levels of stress. We are missing basic

605 information regarding daily carbohydrate balance, structure of carbohydrate use, and plant
606 flexibility to shift energy among multiple competing demands like maintenance, growth, stress
607 response, and recovery reserves. We are also missing fundamental knowledge regarding the
608 biological significance of how observed embolism/recovery relates to basic phenomena at both
609 cellular and whole plant levels. Without this basic understanding, we cannot confidently predict
610 how trees will respond to the 'new climate normal'.

611

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615

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1001

1002

1003 **Figure legends**

1004

1005 **Figure 1**

1006 Embolism formation in a vessel may originate from multiple sources including (1) breaking of
1007 the air/water interface at the bordered pit membrane if an adjacent vessel was already embolized,
1008 (2) expansion of air pockets from vessel crevices or (3) expansion of persistent air bubbles on the
1009 wall surface that could be remnants of previous embolism. The air-water interface might be in
1010 equilibrium if its surface tension is sufficient to oppose the tension from water in the
1011 transpiration stream. If tension from water in the transpiration stream exceeds water surface
1012 tension the bubble will expand. Such an expanding bubble will continuously decrease its radius
1013 if water/wall contact is fixed in one position. If tension in the vessel exceeds the maximum
1014 tension sustained by the bubble (at critical radius), the air-water interface breaks and the bubble
1015 expands forming an embolism.

1016

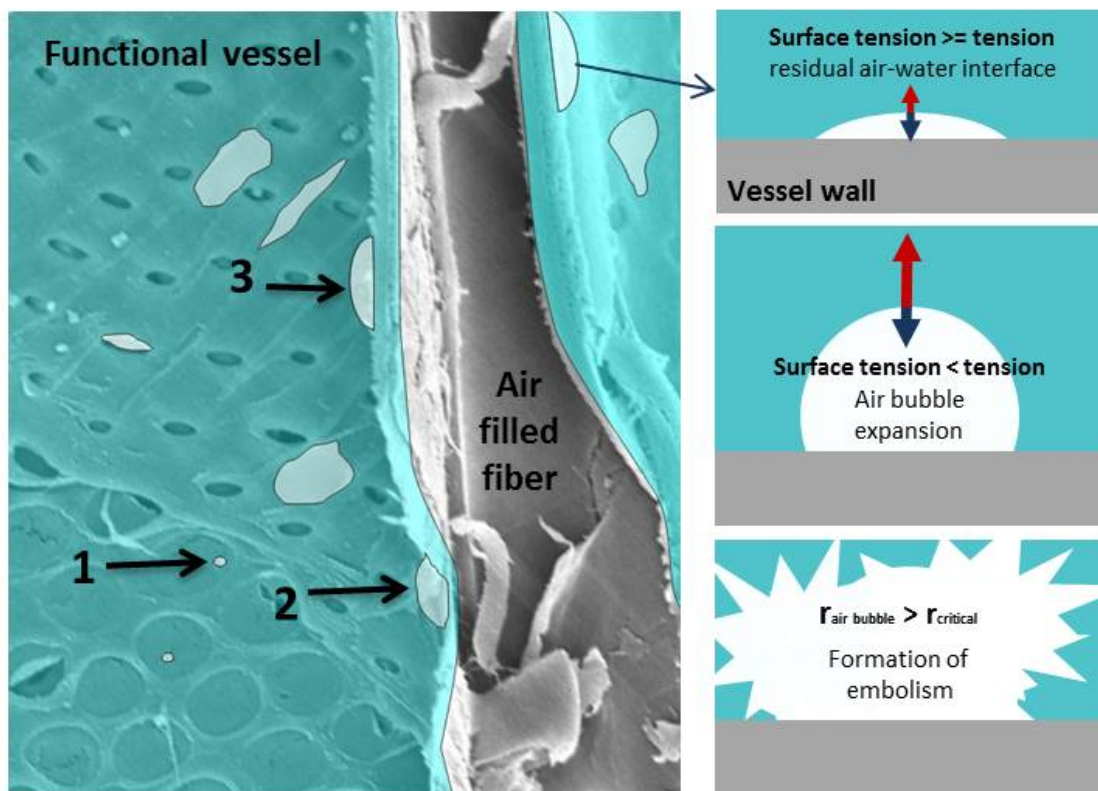
1017 **Figure 2**

1018 Figure 2

1019 Xylem transport capacity depends on the number of functional vessels (I) that can transport
1020 water under tension. The number of functional vessels is the total number of vessels in the stem
1021 minus embolized vessels and those refilled but not yet functionally linked to vessels conducting
1022 water under tension. Rates of embolism formation (II) and refilling (III) determine the number
1023 of non-functional vessels while the rate of returning vessels to functional state (IV) determine
1024 number of vessel capable of sustaining tension (V). These rates are under physiological control
1025 that will be impacted by climate change. Specifically, increasing atmospheric vapor pressure

1026 deficit (VPD) may result in increasing transpiration rates (a) that in turn would increase water
1027 tension in the xylem (b). Increasing xylem water tension would increase the rate of embolism
1028 formation (c) and effectively the number of embolized vessels. High tension would also
1029 negatively impact both potential for refilling of vessels (d) and rate of returning vessels to a
1030 functional state (e). In addition, climatic changes are expected to affect solar radiation and
1031 temperature which would have a major impact on both photosynthesis and respiration rates (f).
1032 Respiration rates are expected to increase with increasing temperature while photosynthesis rates
1033 are expected decrease due to both higher temperature and stomatal closure resulting from higher
1034 tension levels (g). Together such an impact would reduce the level of non-structural
1035 carbohydrates stored in the stem that would be available to provide energy required for vessel
1036 refilling (h), thus reducing the rate of refilling (i) and returning vessels to a functional state (k).

1037 Figure 1

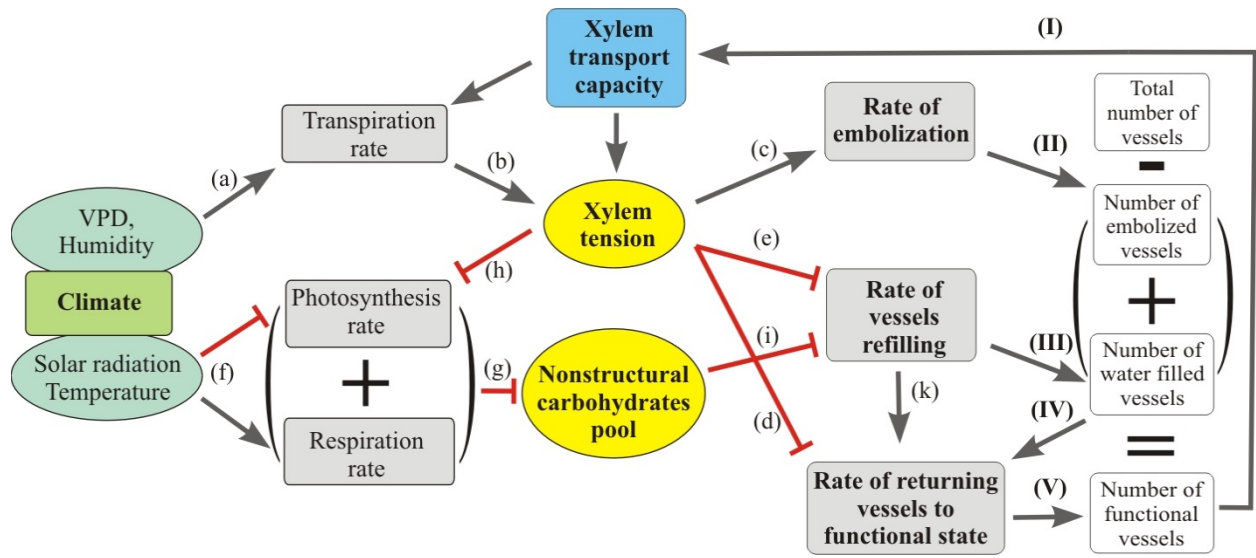


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1041 Figure 2



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