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## Fermentation-Mediated Growth, Signaling, and Defense in Plants

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**Social media of K. Jardine (Facebook: Kolby Jardine, Twitter: @CarbonKolby)**

**Summary:** While traditionally considered important mainly in hypoxic roots during flooding, upregulation of fermentation pathways in plants has recently been described as an evolutionarily conserved drought survival strategy, with acetate signaling mediating reprogramming of transcription and cellular carbon and energy metabolism from roots to leaves. The amount of acetate produced directly correlates to survival through potential mechanisms including defense gene activation, biosynthesis of primary and secondary metabolites, and aerobic respiration. Here, we review root ethanolic fermentation responses to hypoxia during saturated soil conditions and summarize studies highlighting acetate fermentation under aerobic conditions coupled with respiration during growth and drought responses. Recent work is discussed demonstrating long distance transport of acetate via the transpiration stream as a respiratory substrate. While maintenance and growth respiration are often modeled separately in terrestrial models, here we propose the concept of “Defense Respiration” fueled by acetate fermentation in which upregulation of acetate fermentation contributes acetate substrate for alternative energy production via aerobic respiration, biosynthesis of primary and secondary metabolites, and the acetylation of proteins involved in defense gene regulation. Finally, we highlight new frontiers in leaf-atmosphere emission measurements as a potential way to study acetate fermentation responses of individual leaves, branches, ecosystems and regions.

32 **Keywords:** Abiotic Stress, Drought, Flooding, Growth, Defense Respiration, Aerobic  
33 Fermentation, Acetic Acid

34  
35 **Plain language summary:** While traditionally considered only important in oxygen poor tissues  
36 like roots during flooding, here we summarize recent work that demonstrates fermentation plays  
37 important roles during aerobic processes including respiration, growth, and as a warning signal  
38 during drought.

39

## 40 **Introduction**

### 41 *Ethanolic fermentation and flood tolerance*

42 In higher plants, molecular oxygen (O<sub>2</sub>) deficiency can dramatically alter cellular metabolism  
43 resulting in reductions in productivity (Fukao & Bailey-Serres, 2004). Understanding and  
44 predicting plant metabolic responses to O<sub>2</sub> deficiency through changes in respiration and  
45 fermentation, and physiological processes like growth, has become an increasingly active area of  
46 research (Bailey-Serres & Freeling, 1990; Gibbs *et al.*, 2011). The capacity to ferment  
47 carbohydrates in higher plants derives from an ancient biochemical pathway that can be traced  
48 back to green algae that ferment starch under anaerobic conditions to numerous products  
49 including acetate, ethanol, formate, glycerol, lactate, H<sub>2</sub> and CO<sub>2</sub> (Gaffron & Rubin, 1942; Davies  
50 *et al.*, 1974; Catalanotti *et al.*, 2013; Xia *et al.*, 2016). The enhanced production of acetaldehyde  
51 and ethanol in higher plants by pyruvate decarboxylase (PDC) and alcohol dehydrogenase  
52 (ADH) during fermentation induced by hypoxia is well established (Kimmerer & MacDonald,  
53 1987; Vartapetian *et al.*, 1997). During hypoxic conditions in roots associated with saturated  
54 soils and flooding, the suppression of ATP production by mitochondrial respiration may be  
55 partially compensated by ethanolic fermentation (Tadege *et al.*, 1998). Ethanolic fermentation  
56 capacity under hypoxia has been identified as a highly conserved characteristic of modern land

57 plants (Bui *et al.*, 2019), generally considered a critical adaptation to hypoxic environments such  
58 as those encountered by roots in saturated or flooded soils (Vartapetian & Jackson, 1997; Tadege  
59 *et al.*, 1998).

60 Numerous genetic studies have demonstrated that PDC and ADH play an important and often  
61 critical role in low-oxygen tolerance in plants (Ventura *et al.*, 2020). For example, in maize the  
62 *Adh1* null mutant, which fail to synthesize ADH1 isozymes, are very susceptible to hypoxia  
63 (Johnson *et al.*, 1994). In Arabidopsis, the *Adh1* null mutant also has reduced tolerance to  
64 hypoxia while in contrast, overexpressing *PDC1* or *PDC2* increases survival (Ismond *et al.*,  
65 2003). Under hypoxia, pyruvate generated by glycolysis in roots cannot be effectively oxidized  
66 by aerobic respiration in mitochondria and instead is decarboxylated to acetaldehyde by PDC. To  
67 help regenerate NAD<sup>+</sup> required to maintain high rates of glycolysis, a fraction of the  
68 acetaldehyde is reduced to ethanol, followed by transport of acetaldehyde and ethanol from roots  
69 to the canopy via the transpiration stream (Atkinson *et al.*, 2008). <sup>14</sup>C-ethanol labeling of excised  
70 leaves and shoots revealed that ethanol in the xylem in poplar trees is rapidly assimilated into  
71 plant biomass, with 95% remaining in plant tissues after 24 hr (MacDonald & Kimmerer, 1993).  
72 Despite its volatile nature, < 5% of the label was lost to the atmosphere via leaf emissions of <sup>14</sup>C-  
73 ethanol and/or <sup>14</sup>C-acetaldehyde while <1% was lost as <sup>14</sup>CO<sub>2</sub>, suggesting the involvement of  
74 mitochondrial respiration. Likewise, while strong increases in leaf acetaldehyde emission have  
75 been reported from several species following root flooding, the majority of acetaldehyde is  
76 thought to be further oxidized to acetic acid which is much less volatile with a higher water  
77 solubility than ethanol or acetaldehyde (Kreuzwieser & Rennenberg, 2013).

78 In addition to roots, aerial tissues like stems and leaves of trees constitutively express ADH  
79 isozymes that can convert ethanol produced in hypoxic roots back to acetaldehyde (Kimmerer &  
80 Stringer, 1988; Harry & Kimmerer, 1991; Strommer, 2011). Ethanol oxidation and assimilation  
81 in aerobic stem and leaf tissues is therefore mediated by the reversible ADH reaction where  
82 ethanol is oxidized back to acetaldehyde (Kreuzwieser *et al.*, 1999) which is subsequently  
83 oxidized to acetic acid by an aldehyde dehydrogenase (ALDH: EC 1.2.1) (Wei *et al.*, 2009).  
84 Following acetate activation to acetyl-CoA by an acetyl-coenzyme A synthetase (ACS) (Lin &  
85 Oliver, 2008), this central C<sub>2</sub> metabolite is rapidly utilized in both anabolic (e.g. lipid  
86 biosynthesis) and catabolic (e.g. mitochondrial respiration) metabolism (**Figure 1**), thereby  
87 explaining the rapid incorporation of root derived ethanol into stem and leaf biomass  
88 (MacDonald & Kimmerer, 1993). Subsequent studies provided additional experimental evidence  
89 that enhanced leaf emissions of acetaldehyde and ethanol during root hypoxia involves root  
90 ethanol production, its transport to aerobic tissues via the transpiration stream, and its oxidation  
91 and assimilation in aerobic tissues (MacDonald & Kimmerer, 1993; Kreuzwieser *et al.*, 1999;  
92 Holzinger *et al.*, 2000; Kreuzwieser *et al.*, 2001; Kreuzwieser *et al.*, 2004; Rottenberger *et al.*,  
93 2008; Kreuzwieser & Rennenberg, 2013; Kreuzwieser & Rennenberg, 2014). While these studies  
94 generally did not report acetic acid emissions, significant foliar emissions of acetic acid were  
95 observed from the tropical tree species that had the highest ethanol and acetaldehyde emission  
96 responses to flooding (Rottenberger *et al.*, 2008). Thus, these coupled below- and above-ground  
97 plant processes were proposed as a key physiological mechanism of flood tolerance  
98 (Kreuzwieser *et al.*, 2004).

99 Curiously, survey studies revealed that in addition to roots, fermentation metabolism capacity  
100 under hypoxia is also widespread in leaves of both C3 and C4 species (Kimmerer & MacDonald,  
101 1987). However, the relationship between site flooding occurrence and leaf ADH activity in  
102 remains unclear. In roots, positive correlation between ADH activity, ethanol production, and  
103 flood tolerance has been observed in flood tolerant species like rice (Quimio *et al.*, 2000). While  
104 it was hypothesized that ethanol and acetaldehyde emission rates correlate with flood tolerance  
105 of trees (Kreuzwieser *et al.*, 2004), root ethanol production and flood tolerance appear to be  
106 poorly correlated (Raymond *et al.*, 1985). In contrast, flood sensitive species appear to emit far  
107 more ethanol and acetaldehyde than flood tolerant species (Copolovici & Niinemets, 2010). For  
108 example, a flood intolerant species *Quercus rubra* showed much higher stimulation of leaf  
109 ethanol and acetaldehyde emissions than the flood tolerant species *Alnus glutinosa*. Agricultural  
110 plants growing in drier semi-arid climates are well documented to be highly susceptible to soil  
111 saturation and flooding (Awala *et al.*, 2016). While soil saturation of a highly flood-sensitive  
112 Mediterranean species *H. halimifolium* resulted in high foliar emission of ethanol, acetaldehyde,  
113 and acetic acid (K. Jardine, unpublished), under normal aerated soils, this same species showed  
114 high temperature simulated diurnal leaf emissions of ethanol, acetaldehyde, acetic acid, and  
115 methyl acetate (Jardine *et al.*, 2014). Daytime leaf emissions of the volatile fermentation  
116 products were associated with high rates of net photosynthesis and transpiration, indicating non-  
117 stressed conditions for net carbon assimilation. These observations suggest the capacity of plants  
118 to produce and emit fermentation volatiles from leaves and other aerial tissues under aerobic  
119 conditions, not necessarily related to soil oxygen levels and instead suggests local production in  
120 tissues under aerobic conditions.

121 However, it should be acknowledged that root ethanolic fermentation could be activated in  
122 hypoxic microsites, which may exist in aerobic well drained soils (Keiluweit *et al.*, 2017). In  
123 addition, even under aerobic soil conditions, sub-ambient oxygen concentrations can occur in  
124 roots (Zabalza *et al.*, 2009) and aerial tissues like stems with high respiratory activities (Spicer &  
125 Holbrook, 2007). The existence of hypoxic niches in otherwise fully aerobic tissues is well  
126 established (Ventura *et al.*, 2020) including during shoot meristem (Weits *et al.*, 2019) and  
127 lateral root primordia (Shukla *et al.*, 2019) development. Thus, environmental stress-associated  
128 acute hypoxia during flooding is distinguished from metabolically generated hypoxia under non-  
129 stress conditions where lower oxygen concentrations than the rest of the plant are maintained by  
130 high respiration and/or barriers to oxygen diffusion (Weits *et al.*, 2021).

131 Poplar leaves placed under hypoxia produce more ethanol than roots (Kimmerer & MacDonald,  
132 1987). An apparent paradox was presented that the plant organ (leaf) least likely to be exposed to  
133 hypoxia due to efficient air exchange with the atmosphere and photosynthetic oxygen production,  
134 is rich in enzymes necessary for fermentation metabolism including PDC, ADH, and ALDH  
135 (Kimmerer & MacDonald, 1987). More recent studies with *Arabidopsis* highlighted the  
136 importance of cellular energy status in activating ethanolic fermentation in plant tissues (Zabalza  
137 *et al.*, 2009). Following 1 day of hypoxia, ethanolic fermentation in roots was activated together  
138 with a decline in internal tissue oxygen concentrations, and cellular energy status (ATP/ADP).  
139 While roots treated with pyruvate under aerobic conditions also activated ethanolic fermentation  
140 together with a decline in internal tissue oxygen concentrations and cellular energy status, it was  
141 delayed by 2 days. A strong decline in tissue oxygen concentrations in roots treated with  
142 pyruvate was already established after 1 d. These results suggest that ethanolic fermentation, and

143 its sensitivity to low oxygen availability, is activated via a mechanism linked to the decline in  
144 cellular energy status (Zabalza *et al.*, 2009).

145

146 In *Arabidopsis*, enhanced ethanolic fermentation metabolism under anoxia have been described  
147 to be directly regulated by group VII Ethylene Response Factor (ERF) transcription factors  
148 (Bailey-Serres *et al.*, 2012). Under aerobic conditions, ERFs are degraded which prevents  
149 transcription of hypoxia-responsive genes. In contrast, under hypoxia, proteolytic degradation of  
150 ERFs is inhibited resulting in transcription of genes that enhance anaerobic metabolism such as  
151 PDC and ADH (Bailey-Serres *et al.*, 2012). These two mechanisms involved in the activation of  
152 fermentation responses are integrated in *Arabidopsis* via an ATP-dependent shift in oleoyl-CoA  
153 (Schmidt *et al.*, 2018). A reduction in ATP/ADP ratio under hypoxia results in an increased  
154 C18:1/C18:O -CoA ratio. This triggers the release of ERFVII protein bound to the plasma  
155 membrane that is stable under hypoxia, enabling it to activate hypoxic responses in the nucleus.  
156 More recently, oxygen and energy sensing in plants was shown to be fine-tuned by rapamycin  
157 (TOR) kinase, a master energy sensor widely conserved in all eukaryotes. Reduced ATP  
158 production from carbohydrate metabolism under hypoxia reduces TOR activity, attenuating the  
159 activation of fermentation metabolism by ERF-VIIs. Thus, only under optimal energy available  
160 does TOR efficiently activate ERF-VIIs.

161

162 Part of the variability observed in root ethanolic fermentation and foliar ethanol and  
163 acetaldehyde emission rates of flood tolerant species may be related to the presence of a number  
164 of morphological, physiological, and anatomical adaptations to help increase oxygen supply to  
165 roots (Sauter, 2013; Jia *et al.*, 2021). This includes the development of air spaces in roots and



166 stems in structures like aerenchyma (Evans, 2004), as well as structures that enhance  
167 atmospheric uptake and diffusion of oxygen from aerial portions of the plant to roots including  
168 stem lenticels (Shimamura *et al.*, 2010), adventitious roots (Gonin *et al.*, 2019), prop roots  
169 (Zhang *et al.*, 2015), and pneumatophores (Kitaya *et al.*, 2002). In both flood tolerant and  
170 intolerant species, foliar emissions of acetaldehyde and ethanol do not remain constant following  
171 flooding. Emissions show a pattern of increasing typically 1-2 days after flooding followed by an  
172 emission rate drop after some days, reaching a steady emission level that is moderately higher  
173 than in non-flooded controls (Kreuzwieser & Rennenberg, 2013). The reason for the decrease in  
174 acetaldehyde and ethanol emissions despite the continuous root flooding is not known, but has  
175 also been observed in other studies that showed enhanced acetaldehyde and ethanol emissions as  
176 an intermittent response to flooding (Rottenberger *et al.*, 2008). Mechanisms behind this  
177 phenomenon may include improved oxygen transport to the roots via structural adjustments  
178 (aerenchyma, lenticels, adventitious roots, etc.), reduced TOR activity in roots which attenuates  
179 the activation of fermentation metabolism by ERF-VIIs, and an upregulation of acetaldehyde and  
180 ethanol oxidation in aerial tissues like leaves.

181

182 ***Light-dark transitions as evidence for fermentation metabolism capacity in leaves under***  
183 ***aerobic conditions***

184 The high activity of fermentation metabolism in leaves under aerobic conditions (Fall, 2003) was  
185 proposed to explain large emission bursts of acetaldehyde lasting several minutes following  
186 light-dark transitions after a period of photosynthesis (Karl *et al.*, 2002). Subsequent studies  
187 found large emission bursts of not only acetaldehyde following light/dark transitions, but also

188 ethanol, acetic acid, acetone, and methyl acetate (Jardine *et al.*, 2012; Dewhirst *et al.*, 2021;  
189 Jardine *et al.*, 2022) with the magnitude of these bursts greatly increasing under hypoxia (Jardine  
190 *et al.*, 2012). While the biochemical origin of acetone biosynthesis in plants remains uncertain,  
191 one possibility is the decarboxylation of acetoacetate in a similar mechanism as in bacteria (Fall,  
192 2003). In bacteria, acetone is a well-known fermentation product generated from the  
193 decarboxylation of acetoacetate (Fall, 2003; Han *et al.*, 2011), and large scale production of  
194 acetone by solvent-producing strains of *Clostridium spp.* was among the first large-scale  
195 industrial fermentation processes to be developed (Jones & Woods, 1986). Evidence that acetone  
196 emissions in leaves is produced from acetoacetate decarboxylation and linked to fermentation  
197 was obtained from feeding detached leaves positionally-specific <sup>13</sup>C-pyruvate solutions via the  
198 transpiration stream. Direct <sup>13</sup>C-incorporation into large <sup>13</sup>C-acetone emissions was observed  
199 under pyruvate-2-<sup>13</sup>C feeding, but not pyruvate-1-<sup>13</sup>C (Jardine *et al.*, 2010). This is consistent  
200 with pyruvate entering the acetate fermentation pathway in plants which involves the  
201 decarboxylation of the C<sub>1</sub> of pyruvate followed by acetyl-CoA utilization by the mevalonate  
202 pathway leading to acetone production via acetoacetate decarboxylation (Suganuma *et al.*, 1993)  
203 (AAD, **Figure 1**).

204

205 Recently, acetaldehyde emission bursts following light-dark transitions was suggested to not  
206 derive from the decarboxylation of pyruvate mediated by PDC, but instead from an intermediate  
207 of the photosynthesis-linked methylerythritol phosphate pathway (MEP) in chloroplasts (Jud *et*  
208 *al.*, 2016). Consistent with this idea, light/dark emission bursts of acetaldehyde, ethanol, acetic  
209 acid, and acetone were inhibited by drought (Jud *et al.*, 2016; Jardine *et al.*, 2022), increased

210 with cumulative photosynthesis (the total net amount of CO<sub>2</sub> assimilated during the light period,  
211 μmol CO<sub>2</sub> m<sup>-2</sup>), strongly suppressed by removing CO<sub>2</sub> from the atmosphere, and directly  
212 incorporated <sup>13</sup>CO<sub>2</sub> assimilated during the light period into <sup>13</sup>C<sub>2</sub>-acetyl-CoA (Jardine *et al.*, 2012).  
213 However, as photosynthetic production of triosephosphates (glyceraldehyde-3-phosphate, G3P)  
214 can be exported to the cytosol and converted to pyruvate via glycolysis, the role of the pyruvate  
215 overflow mechanism in the emission bursts of volatile fermentation products (acetaldehyde,  
216 ethanol, acetic acid, acetone and methyl acetate) following light-dark transitions, as originally  
217 proposed by Karl *et al.*, 2002, should still be considered as a potentially important mechanism  
218 (**Figure 1**: acetaldehyde production in the cytosol via the PDC catalyzed reaction). Chloroplasts  
219 may lack the ability to convert photosynthetically derived G3P to phosphoenolpyruvate (PEP)  
220 via glycolysis and consequently may require the export of G3P to the cytosol followed by re-  
221 importation as PEP (Sharkey & Monson, 2014) and/or pyruvate (Jardine *et al.*, 2010) in order to  
222 synthesize fatty acids and isoprenoids directly linked to recent photosynthate (**Figure 1**).  
223 Consistent with a pyruvate overflow mechanism involving PDC, light/dark emission bursts of  
224 acetaldehyde, ethanol, acetic acid, and acetone are greatly enhanced under hypoxia relative to  
225 aerobic conditions (Jardine *et al.*, 2012)

226

227 The co-occurrence of fermentation volatile emission bursts of acetaldehyde, ethanol, acetic acid,  
228 and acetone following light-dark transitions provides additional evidence that leaves, known to  
229 constitutively express enzymes involved in fermentation (Kimmerer, 1987; Nguyen *et al.*, 2009),  
230 can have high activities under aerobic conditions. For example, in *Arabidopsis*, although  
231 *PDC1* and *PDC2* are strongly upregulated under hypoxia, their expression is already high under

232 aerobic conditions (Mithran *et al.*, 2014). The importance of ADH and PDC activity during  
233 fermentative metabolism to plant growth under aerobic conditions was recently highlighted in  
234 *Arabidopsis* lacking a functional *ADH1* or both *PDC1* and *PDC2* (Ventura *et al.*, 2020). Relative  
235 to the wild type, these mutants showed a higher growth penalty under aerobic conditions than  
236 under hypoxia. By demonstrating an important role in plant growth under aerobic conditions,  
237 these recent observations demonstrate that fermentation genes, known to be induced by hypoxia,  
238 go beyond their classical role during environmental hypoxia due to waterlogging or flooding  
239 (Ventura *et al.*, 2020).

240

#### 241 *Acetate fermentation and drought tolerance*

242 Although fermentation metabolism has traditionally been considered during root flooding  
243 (**Figure 2a**), its important roles in other biotic stresses like drought and high temperature have  
244 recently been revealed (**Figure 2b**). Drought, often occurring together with high temperature  
245 stress, can lead to large changes in cellular carbon and energy metabolism associated with  
246 enhanced osmotic (Fontes *et al.*, 2016) and oxidative (Jardine *et al.*, 2015) stress, including  
247 reduced stomatal conductance, net photosynthesis, and transpiration (Medrano *et al.*, 2002), and  
248 an acceleration of photorespiration (Voss *et al.*, 2013), potentially promoting the carbon  
249 starvation processes (McDowell *et al.*, 2022). Drought tolerant species have long been  
250 recognized to efficiently adjust energy metabolism during stress acclimation (Tari *et al.*, 2013).  
251 Recent studies demonstrated a central role of acetate fermentation in leaves during plant drought  
252 responses associated with local fermentation from carbohydrates (Kim *et al.*, 2017). Drought  
253 induced ethanol accumulation was observed in the aerial tissues of three conifer tree species

254 including Douglas-fir, lodgepole pine, and ponderosa pine (Manter & Kelsey, 2008). While  
255 acetate was not quantified, needles accumulated greater quantities of ethanol associated with  
256 lower leaf water potential than sapwood or phloem. Mediated by protein acetylation, drought  
257 tolerance is characterized by a large metabolic shift towards fermentation with acetate  
258 coordinating whole-plant reprogramming of transcription, cellular metabolism, hormone  
259 signaling, and chromatin modification (Kim et al., 2017).

260

261 Under aerobic conditions, the activation of acetate fermentation can lead to an accumulation of  
262 acetic acid in leaves and other tissues which promotes *de novo* jasmonic acid (JA) synthesis via  
263 an enrichment of histone H4 acetylation, thereby priming the JA signaling pathway for drought  
264 tolerance (Hu *et al.*, 2019). Exogenous acetic acid applied to *A. thaliana*, rapeseed (*Brassica*  
265 *napus*), maize (*Zea mays*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) confirmed that  
266 acetate-linked protein acetylation and signaling under drought is an evolutionarily conserved  
267 survival strategy in plants with the amount of acetate produced during drought directly  
268 correlating to survival (Kim et al., 2017). These conclusions were further supported by a study  
269 using transgenic Arabidopsis where *Pdc1* and *Aldh2b7* were overexpressed resulting in  
270 prolonged survival during drought (Rasheed et al., 2018). Moreover, soybean foliar acetic acid  
271 sprays (20 mM) promoted drought acclimation by reducing oxidative stress while enhancing root  
272 biomass, leaf area, net photosynthesis rates, and water use efficiency leading to improved growth  
273 performance (Rahman et al., 2021). Applications of acetic acid solutions also improved drought  
274 tolerance for rice (Ogawa *et al.*, 2021), cassava (Utsumi *et al.*, 2019), apple (Sun *et al.*, 2022),  
275 and cotton (Li *et al.*, 2021). Roots treated pre-treated acetic acid showed root-to-shoot  
276 jasmonates signals that partially overlap with those induced by drought, conferring an acclimated

277 state to shoots prior to drought (Ogawa *et al.*, 2021). Similar improvements to drought tolerance  
278 were observed in *Arabidopsis*, wheat, and rice from exogenous ethanol applications (Bashir *et*  
279 *al.*, 2022). In contrast, drought tolerance was not enhanced when key ALDH genes were mutated  
280 or when abscisic acid signaling was impaired. Ethanol was concluded to mediate enhanced  
281 drought tolerance through acetate biosynthesis, ABA signaling, and gluconeogenesis (Bashir *et*  
282 *al.*, 2022). NMR analysis during <sup>13</sup>C-ethanol treatment of *Arabidopsis* roots revealed a rapid  
283 production of <sup>13</sup>C-acetate as well as <sup>13</sup>C-labeled sugars, amino acids, and TCA cycle  
284 intermediates.

285

286 In addition to the utilization of acetate fermentation to enhance protein acetylation, the potential  
287 for drought-induced acetate fermentation to enhance acetylation of other biopolymers, such as  
288 structural polysaccharides in cell walls, was recently suggested (Jardine *et al.*, 2022). In poplar  
289 trees, drought induced elevated foliar acetic acid emissions together with acetaldehyde, ethanol,  
290 and acetone emissions and led to an increase in leaf bulk cell wall *O*-acetylation. The regulation  
291 of *O*-acetylation of plant cell wall polysaccharides is vital for the determination of wall  
292 architecture, mechanical strength, and function (Qaseem & Wu, 2020). Severe decreases in cell  
293 wall *O*-acetylation are associated with dwarfism, reduced mechanical strength of the stem,  
294 collapsed xylem vessels, and stunted plant growth (Lee *et al.*, 2011; Manabe *et al.*, 2013; Xiong  
295 *et al.*, 2013). In contrast, increased *O*-acetylation is known to enhance the thermal tolerance,  
296 mechanical strength, and hydrophobicity of major cell wall polysaccharides like xylan, with the  
297 degree and pattern of *O*-acetylation regulating intra-molecular interactions within the cell wall  
298 including cellulose-xylan and lignin-xylan interactions (Qaseem & Wu, 2020).

299

300 Central to the enhancement of drought tolerance mediated by acetate fermentation is the  
301 detoxification of acetaldehyde mediated by ALDH. Numerous *ALDH* genes have long been  
302 recognized as a key component of plant response to abiotic stress (Kirch *et al.*, 2004) by acting  
303 as “aldehyde scavengers” generated from lipid peroxidation (Singh *et al.*, 2013) and funneling  
304 acetaldehyde generated in fermentation metabolism to acetic acid (Wei *et al.*, 2009). In  
305 Arabidopsis, the *ALDH3II* gene is transcriptionally activated by abiotic stress, with over-  
306 expression providing enhanced stress tolerance (Singh *et al.*, 2013). Moreover, both constitutive  
307 and stress-induced expression of both the chloroplastic *ALDH3II* and the  
308 cytoplasmic *ALDH7B4* conferred tolerance to osmotic and oxidative stress, thereby protecting  
309 plants against lipid peroxidation of sensitive membranes central for example in photosynthesis  
310 and mitochondrial respiration (Singh *et al.*, 2013).

311

312 ***Acetate fermentation under aerobic conditions is linked to respiration, lipid biosynthesis, and***  
313 ***growth***

314 In contrast to hypoxic conditions which inhibit mitochondrial respiration while stimulating  
315 fermentation, aerobic conditions can support high rates of respiratory metabolism. Although  
316 fermentation was traditionally considered only in hypoxic tissues, the emerging view is that this  
317 process also occurs under aerobic conditions in plants regulated by the cell energy status that can  
318 decline due to both decreases in usable energy production during abiotic stress like flooding and  
319 drought, but also during high energy demand associated with accelerated growth rates. Similar to  
320 the yeast pathway termed the “pyruvate dehydrogenase bypass” as an alternate route for acetyl-  
321 CoA production, acetate generated during aerobic fermentation in plants can be activated to  
322 acetyl CoA by an acetyl CoA synthetase (Wei *et al.*, 2009). Germinating tobacco pollen under

323 aerobic conditions has high rates of both aerobic fermentation and respiration to meet its high  
324 energetic and biosynthetic demands (Bucher *et al.*, 1995). As aerobic respiratory metabolism is  
325 important during both growth and defense processes (Millar *et al.*, 2011), fermentation under  
326 aerobic conditions in leaves and other plant tissues may help funnel respiratory substrates into  
327 mitochondria to support energy production and amino acid biosynthesis as well as into  
328 chloroplasts and the cytoplasm for the biosynthesis of fatty acids and secondary metabolites like  
329 terpenoids. For example, acetate fermentation under aerobic conditions coupled to acetate-  
330 dependent acetyl-CoA production was concluded to be very important in lipid biosynthesis in  
331 germinating tobacco pollen (Mellema *et al.*, 2002). Recently, a  $^{13}\text{C}_2$ -acetate solution directly  
332 injected into the xylem of poplar trees was shown to be efficiently transported to the canopy via  
333 the transpiration stream and utilized as a respiratory substrate in leaves (Jardine *et al.*, 2022).  
334 These observations demonstrated the potential for acetate fermentation under aerobic conditions  
335 to contribute to whole plant metabolism and growth. However, light appeared to inhibit the  
336 allocation of acetate into leaf respiratory metabolism during the day, with increased daytime  
337 allocation to biosynthetic processes speculated (Jardine *et al.*, 2022). The importance of acetate  
338 as a growth promoter was further highlighted in a recent study where exogenously supplied  
339 acetate was incorporated into biomass through major metabolic pathways (Hann *et al.*, 2022).  
340 This opens the door to acetate delivery to plants being used to enhance growth yields, especially  
341 for food production under controlled environments (Hann *et al.*, 2022).

342

### 343 ***Acetate fermentation and ‘Defense Respiration’ during drought and high temperature stress***

344 Although variable, drought generally reduces plant respiration rates that are considered critical  
345 for not only survival during the drought, but also rapid recovery of productivity following the



346 release of water stress (Atkin & Macherel, 2009). Decreases in photosynthesis are routinely  
347 observed to be more extreme than those of respiration in response to drought (Schwalm *et al.*,  
348 2010). Together with the increased temperature associated with many droughts, a conversion to a  
349 negative whole plant carbon balance with increased risk of carbon starvation has been observed  
350 under drought (Zhao *et al.*, 2013). How drought causes shifts in respiratory substrates in plant  
351 tissues is poorly understood. In stems, a paradox was presented that together with decreasing  
352 respiratory rates, an increase in respiratory substrates was observed including sugars and sugar  
353 alcohols and to a lesser extent, amino acids and organic acids (Rodríguez-Calcerrada *et al.*,  
354 2021). Although acetate was not reported, one hypothesis is the glycolysis of accumulated sugars  
355 and a metabolic shift away from a direct entry of pyruvate into the TCA cycle and towards  
356 acetate biosynthesis (Kim *et al.*, 2017) leads to a large increase in acetate utilized as a respiratory  
357 substrate during drought.

358

359 While maintenance and growth respiration are often treated separately in most terrestrial models,  
360 the lack of an underlying biochemical basis to separate these processes has been noted (O'Leary  
361 *et al.*, 2019). Although lacking experimental evidence of its existence, we propose the new  
362 concept of 'Defense Respiration' fueled by acetate fermentation. This process would not only  
363 lead to enhanced acetylation of proteins and structural polysaccharides involved in defense  
364 signaling and modified cell wall chemical and physical properties under reduced tissue water  
365 potential, but also provide acetate substrate for both biosynthesis and alternative energy  
366 production via aerobic respiration. To test this hypothesis, future studies aiming to characterize  
367 shifts in respiratory rates and substrate composition during drought should aim to specifically  
368 include tissue acetate concentrations in analytical methods.

369

370 *Remote chemical sensing of acetate fermentation in terrestrial ecosystems through emerging*  
371 *methods in land-atmosphere fluxes and atmospheric concentrations of acetic acid*

372 Despite the increased recognition of ethanolic and acetate fermentation under both hypoxic and  
373 aerobic conditions, relatively few measurements of plant-atmosphere gas-exchange of  
374 fermentation volatiles exist in Earth's major biomes including both managed and natural  
375 ecosystems (Kesselmeier & Staudt, 1999; Kesselmeier, 2001; Jardine *et al.*, 2012). This is in  
376 part, because fermentation products acetaldehyde, ethanol, acetone, and acetic acid deriving from  
377 pyruvate decarboxylation by PDC are present in low concentrations in plant tissues where they  
378 are rapidly metabolized and transported between tissues in the aqueous and gaseous phases, and  
379 are highly volatile allowing them to escape the plant via stomatal emissions (Jardine *et al.*,  
380 2010). However, it should be noted that acetic acid establishes chemical equilibria between the  
381 protonated volatile acid form (acetic acid) which can escape the plant as a gas, and the non-  
382 volatile acetate anion, which is restricted to the aqueous phase. Thus, intracellular and  
383 extracellular pH affects the relative fraction of acetate and acetic acid. For example, the slightly  
384 alkaline pH of a typical plant cytoplasm (pH of 7.5-6.5) is expected to favor the dissociation of  
385 acetic acid to acetate. In contrast, pH of the apoplast is more acidic with typical values between  
386 4-6 (Yu *et al.*, 2000), favoring a larger fraction in the volatile acetic acid form with 50% acetic  
387 acid and 50% acetate at pH of 4.8, the pKa of acetic acid. How intra- and extra-cellular  
388 variations in pH control the volatility and solubility of acetate/acetic acid in plants, and therefore  
389 determine its function and fate from the allocation to anabolic and catabolic metabolism to the  
390 formation of gas-phase acetic acid, which can escape the plant, is an area of active research.

391

392 Real-time observations of fermentation volatile gas-exchange fluxes between leaves, stems, and  
393 roots, individual plant canopies, whole ecosystems and the atmosphere offers new opportunities  
394 for non-invasive studies of fermentation metabolism in plants and their biological and  
395 environmental controls. Gas-exchange methods can be adapted to both process-based laboratory  
396 studies under controlled environmental conditions, and field studies aimed at developing a  
397 predictive understanding of the response of ecosystems to environmental change, where  
398 environmental conditions are monitored, but not controlled. Limited studies on plant acetic acid  
399 emissions have found emissions to be positively influenced by environmental parameters  
400 affecting stomatal opening and/or biosynthesis such as photosynthetically active radiation and  
401 leaf temperature (Kesselmeier *et al.*, 1998; Gabriel *et al.*, 1999; Kesselmeier, 2001). Using  
402 dynamic plant chambers on potted trees grown outdoors continuously flushed with purified air,  
403 acetic acid emissions were observed between 0.7 and 8.1 nmol m<sup>-2</sup> min<sup>-1</sup> from numerous tree  
404 species (Kesselmeier *et al.*, 1998).

405

406 Current methods to quantify acetate fermentation responses to drought stress require destructive  
407 tissue sampling, greatly limiting the temporal and spatial scales that can be studied. Moreover,  
408 metabolomics techniques based on tissue extractions often miss acetate while most trace volatile  
409 components like acetaldehyde, ethanol, acetic acid, and acetone are lost during tissue extraction  
410 and sample preparations (Qualley & Dudareva, 2009). In contrast, chemical sensing techniques  
411 are under development using techniques to quantify plant-atmosphere emissions of volatile  
412 fermentation products like acetic acid as well as vertical atmospheric concentration profiles from  
413 a variety of spatial and temporal scales ranging from individual leaves, branches, ecosystems,  
414 landscapes, regions and even global scales (**Figure 3**). Towards the goal of developing a

415 comprehensive understanding of whole plant acetate transport and metabolism (**Figures 1-2**),  
416 future studies could leverage recent advances in continuous non-destructive whole plant <sup>13</sup>C-  
417 labeling of respiratory CO<sub>2</sub> using the dynamic xylem solution injection (DXSI) technique for  
418 continuous delivery of <sup>13</sup>C<sub>2</sub>-acetate to plant canopies via the transpiration stream (Jardine, K *et*  
419 *al.*, 2022).

420

421 In contrast to real-time measurement techniques like PTR-MS and SIFT-MS, offline techniques  
422 have been reported for gaseous acetic acid measurements based on thermal desorption pre-  
423 concentration (Dewhirst *et al.*, 2020) as well as aqueous collections of gaseous acetic acid  
424 followed by analysis of acetate in the laboratory by gas chromatography (Kesselmeier *et al.*,  
425 1998). While modern gas chromatography-mass spectrometer systems show high selectivity and  
426 sensitivity for low ppb acetic acid quantification in low air values (e.g. 100 ml), great care must  
427 be taken to minimize and take into account any artifacts caused by the creation of acetic acid  
428 during the thermal desorption process (Dewhirst *et al.*, 2020). However, due to relatively strong  
429 interactions with surfaces including the stationary phase of the analytical column leading to  
430 severe peak tailing and other chromatographic issues, organic acids like acetic acid typically do  
431 not perform well in GC analysis without derivatization. Alternatively, owing to the high-water  
432 solubility of acetic acid, it can be quantitatively collected by bubbling air samples through a  
433 sterile, pH buffered aqueous solution followed by later quantification of aqueous acetate and/or  
434 acetic acid in the laboratory (Kesselmeier *et al.*, 1998). To avoid the chromatography issues with  
435 non-derivatized acetic acid, one possibility is to quantify the aqueous acetic acid concentration

436 indirectly using colorimetric acetate assay kits commonly used to quantify acetate concentrations  
437 in tissue extracts (Dewhirst et al., 2020).

438

439 Using online mass spectrometry, temperature-dependent emissions of acetaldehyde, ethanol,  
440 acetic acid, and methyl acetate were quantified in real-time from individual leaves and branches  
441 during drought response in poplar trees (Dewhirst *et al.*, 2021; Jardine *et al.*, 2022). Acetic acid  
442 emission has also been evaluated at the ecosystem scale using continuous vertical profiles of  
443 acetic acid ambient concentrations within and above a tropical forest canopy in South America  
444 (Jardine et al., 2011) and in North America using the technique of eddy covariance where acetic  
445 acid emission fluxes are calculated every 30 min from the covariance between above canopy  
446 vertical wind speed and ambient acetic acid concentrations (Kim et al., 2010; Park et al., 2013).  
447 However, due to the low concentrations (low ppb), high water solubility, and “stickiness” of  
448 acetic acid, gas phase emission measurements from plants and ecosystems remains highly  
449 technical. It can be completely lost if the gas sample encounters condensed water along the  
450 sample path and often shows memory effects in unheated tubing due to surface adsorption and  
451 desorption from many materials at ambient temperate. Thus, operating online trace gas sensors  
452 for volatile organic compounds like PTR-MS and SIFT-MS at the highest sensitivity possible,  
453 regular calibration with a certified primary standard for acetic acid, using high purity water  
454 sources for regulating humidity and preventing condensation in plant enclosures, and heating all  
455 gas sample tubing to 50-60 °C to prevent condensation and wall losses during gas sample  
456 transport to the analyzer is critical (Jardine *et al.*, 2022).

457

458 Extending beyond the ecosystem scale, horizontal heterogeneity at the landscape scale of surface  
459 emissions of volatile organic compounds has recently been demonstrated for volatile isoprenoids  
460 using a drone fitted with a volatile collection system (Batista *et al.*, 2019). While acetic acid has  
461 not yet been quantified across landscapes using drones, this is expected to change in the near  
462 future given the rapid pace of development in both drone and atmospheric sensor technologies  
463 (Galle *et al.*, 2021). Development of real-time infrared spectroscopy methods, such as cavity  
464 ring-down spectroscopy (CRDS) and tunable laser direct absorption spectroscopy (TILDAS)  
465 methods for trace analysis of gaseous ethanol, acetic acid, acetaldehyde, acetone, and other  
466 fermentation volatile fluxes is on the horizon (Crunaire *et al.*, 2006; Zhou *et al.*, 2016) and  
467 promises to bridge the gap between larger real-time mass-spectrometer systems, and more field  
468 portable systems that require reduced footprint and energy requirements. While a commercial  
469 TILDAS system is available for high sensitivity real-time observations of formaldehyde  
470 (Aerodyne Research, Inc., <100 ppt detection limit with 1 second averaging), in general further  
471 developments in CRDS and TILDAS selectivity and sensitivity are needed to reach the necessary  
472 limits of detection needed to quantify concentrations and fluxes of fermentation volatiles; Gas-  
473 phase concentrations are typically observed in plant enclosures and forested atmospheres in the  
474 low ppb range. Furthermore, vertical gradient measurements of volatile constituents of the lower  
475 troposphere are routinely collected during atmospheric measurement campaigns using tethered  
476 balloon systems. For example, a tethered balloon system was developed to collect volatile  
477 organic compounds at different heights up to 1 km in altitude into Teflon bags using a timer-  
478 controlled pump system placed at different lengths on the tether as the balloon ascends into the  
479 lower troposphere (Tseng *et al.*, 2009). Finally, a global view of regional surface source of acetic  
480 acid has recently been demonstrated based on infrared atmospheric sounding interferometer

481 (Franco et al., 2020). Consistent with a terrestrial vegetation source, acetic acid abundance was  
482 found to be highly correlated to model-derived emissions of isoprene and monoterpenes,  
483 biomarkers of terrestrial photosynthesis. While careful validation at each spatial scale is required,  
484 these developments offer the exciting possibility of monitoring both managed and natural  
485 ecosystem fermentation response to abiotic stress across spatial and temporal scales by  
486 evaluating changes in atmospheric concentrations of acetic acid and other volatile fermentation  
487 products from individual leaves (minutes-hours) to regions (days-year).

488

### 489 *Conclusions and perspectives*

490 Plant fermentation is an ancient metabolic pathway that may be critical in surviving hypoxic  
491 conditions experienced by roots during flooding. However, emerging evidence suggests that  
492 rather than a strict dependence on oxygen availability in tissues, fermentation can also be active  
493 under aerobic conditions linked to a drop in cellular energy status. Key adaptations to root  
494 hypoxia in flood tolerant species, including enhanced uptake of atmospheric oxygen in aerial  
495 tissues and delivery to roots, is lacking in flood intolerant species. This likely explains why much  
496 higher foliar emissions of ethanol and acetaldehyde have been observed from some flood  
497 intolerant species during flooding compared with flood tolerant species, in contrast to early  
498 predictions. Moreover, high tissue concentrations and atmospheric emissions of fermentation  
499 volatiles have been observed under aerobic conditions in well drained soils associated with  
500 temperature-linked growth processes as well as drought stress. Further, genomic and  
501 transcriptomic studies have revealed that a metabolic shift towards acetate fermentation occurs in  
502 roots and leaves which coordinates drought tolerance in plants via protein acetylation and the  
503 activation of the jasmonate signaling pathway. Additional studies revealed that acetate

504 fermentation under aerobic conditions improves plant growth and that co-occurrence of acetate  
505 fermentation, aerobic respiration, and the utilization of acetate in biosynthetic pathways helps  
506 plants meet the high energetic and carbon demands of fast growth rates. This recent research  
507 appears to resolve the paradox from earlier work of why fermentation enzymes are so abundant  
508 in leaves when they are the least likely tissue to experience hypoxia. While destructive methods  
509 are mainly used to study fermentation patterns in plants, the emerging frontier of quantifying  
510 biosphere-atmosphere fluxes of fermentation volatiles and atmospheric vertical concentration  
511 gradients may provide a means to study dynamic fermentation responses in plants during growth  
512 and environmental stress from leaves, branches, ecosystems, landscapes and whole regions. This  
513 may enable studies aimed at improving the quantitative understanding of the plant physiological  
514 and ecological roles of fermentation under hypoxic and aerobic conditions. This includes  
515 potential critical roles in supporting productivity during favorable conditions for net carbon  
516 assimilation and growth, as well as defense processes linked to survival during abiotic stress in a  
517 changing climate.

518

519 Although field observations to quantify plant fermentative metabolism patterns across diverse  
520 plant functional types are in general lacking, ecosystems regularly exposed to root flooding like  
521 mangroves (McKee & Mendelsohn, 1987) and low-lying tropical forests may be considered to  
522 have high rates of fermentative metabolism (Kreuzwieser *et al.*, 2004). For example, in  
523 Amazonian floodplain forests, more than 1000 tree species are exposed to extended annual  
524 submergence lasting up to 9 months each year, with full submergence of young trees (Parolin,  
525 2009). Despite hypoxia, restricted photosynthesis rates, and extremely low light levels during the  
526 submergence, leafed seedlings survival rates are high (Parolin, 2009). Aerobic fermentation



527 metabolism coupled to respiration in fast growing tropical pioneer tree species like *Vismia*  
528 *guianensis* (Rodrigues *et al.*, 2020), may also be important in the re-growth of tropical forests  
529 following a disturbance. In addition, high temperature and drought stress characteristic of desert  
530 ecosystems may also stimulate high rates of fermentative metabolism as a key survival trait. For  
531 example, creosotebush (*Larrea tridentata*), which grows in well drained sandy soils, is vastly  
532 distributed in North American deserts, and was reported to have large temperature-stimulated  
533 leaf emissions of the fermentation volatiles acetaldehyde, acetic acid, acetone, ethanol, and  
534 methyl acetate during the summer monsoon in the Sonoran desert (Jardine *et al.*, 2010). Thus,  
535 plant fermentation studies across diverse plant functional traits like photosynthetic types (C<sub>3</sub>, C<sub>4</sub>,  
536 and crassulacean acid metabolism), growth rates, wood density, specific leaf area, etc. may lead  
537 to improvements in our predictive understanding of the roles of plant fermentative metabolism in  
538 the establishment and resilience of ecosystem structure and function (**Figure 4**).

539

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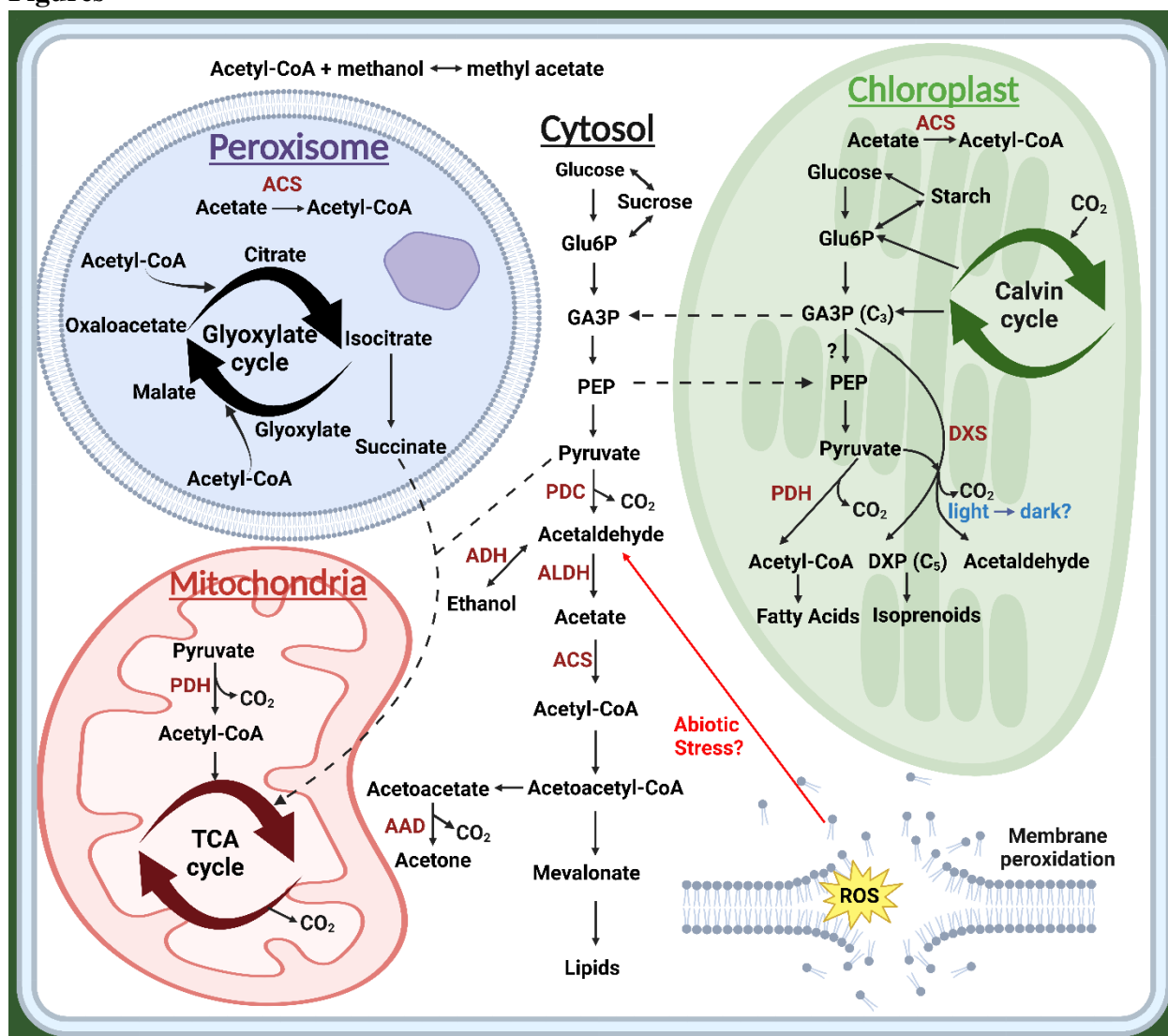
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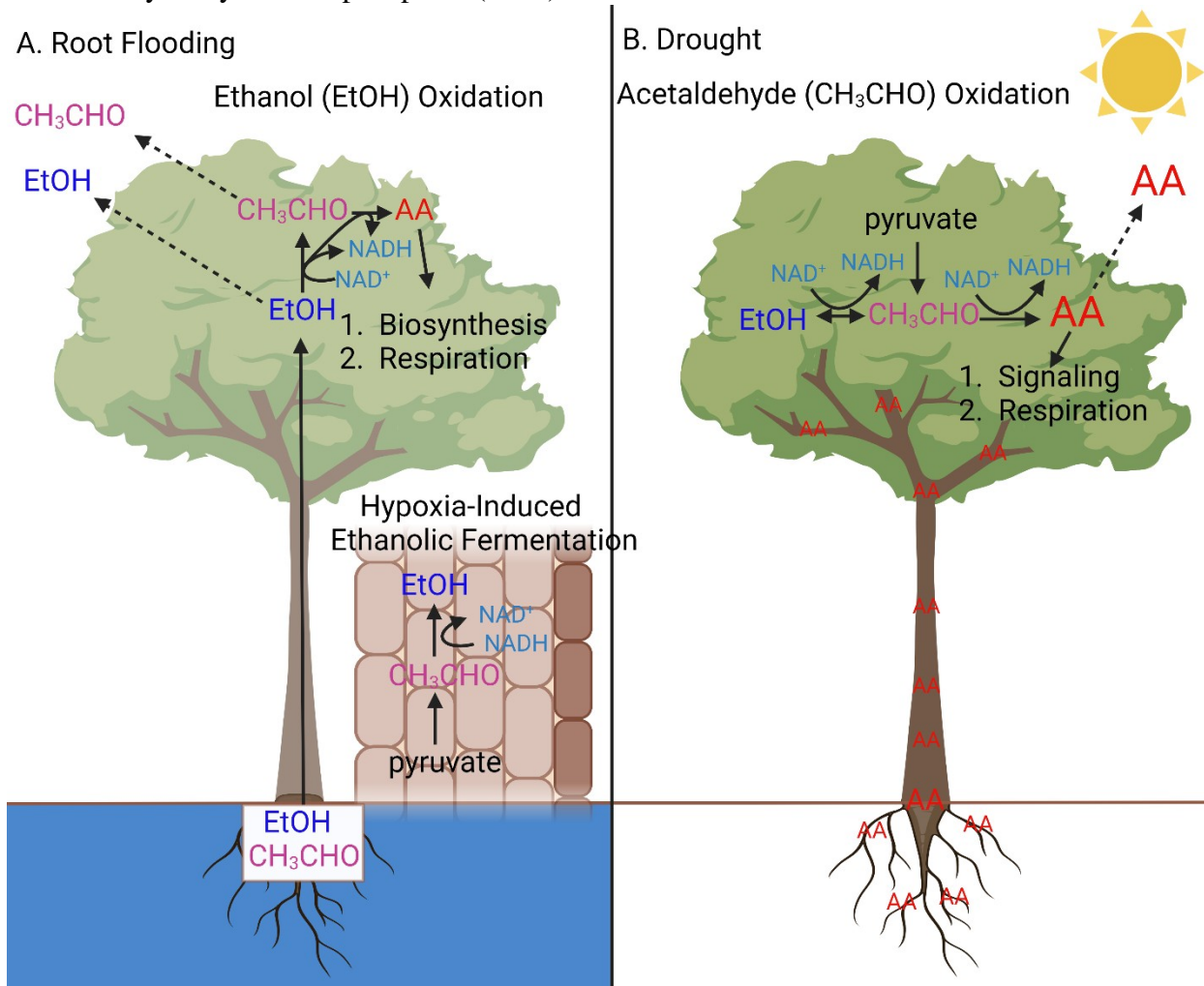
551 *Competing Interests*

552 None declared.



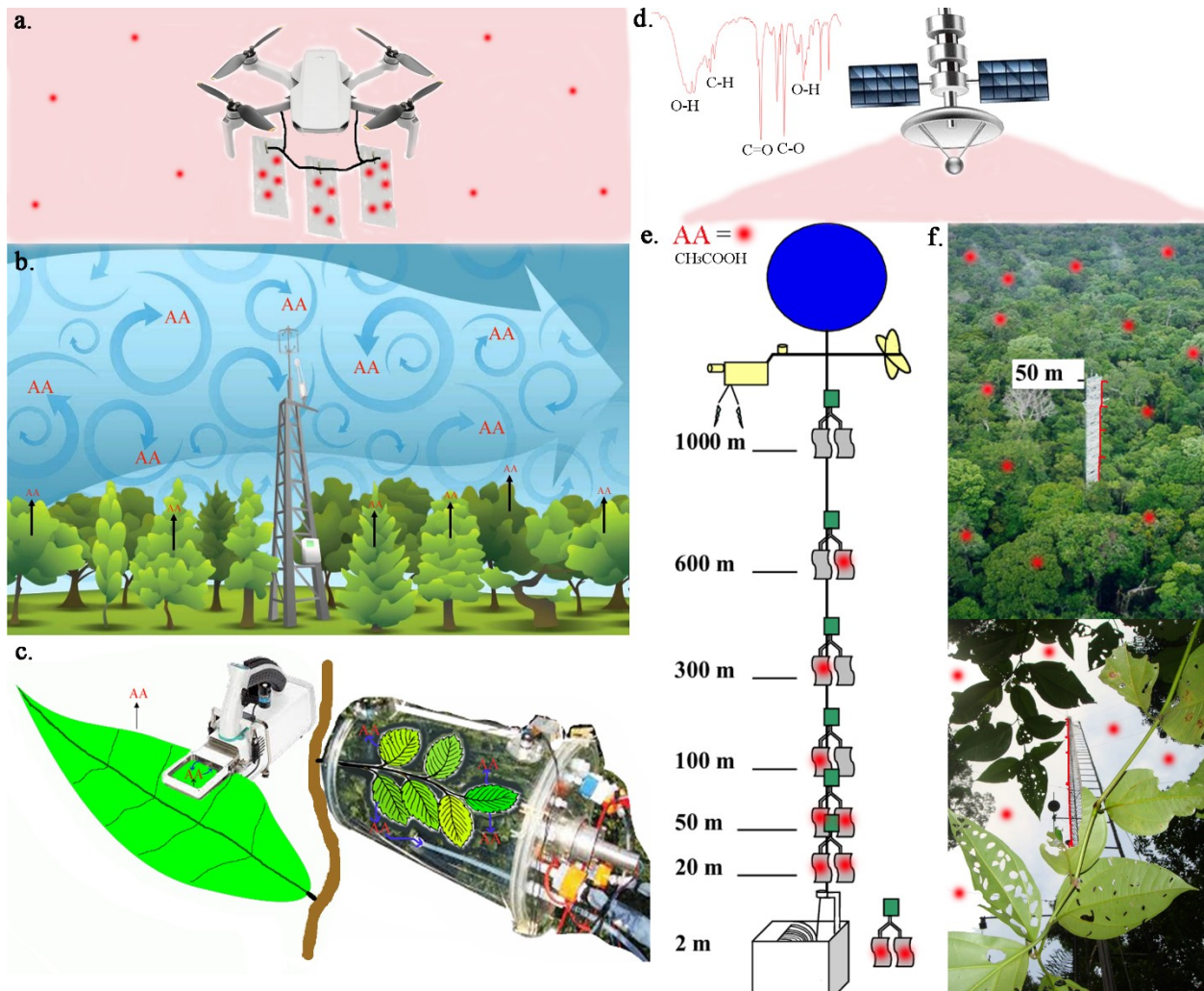
554  
 555 **Figure 1:** Graphic summary of acetate metabolism of photosynthetic plant cells including the  
 556 volatile fermentation intermediates acetaldehyde, ethanol, acetic acid, acetone, and methyl  
 557 acetate. Following the activation of acetate to acetyl-CoA in the cytosol, peroxisome and  
 558 chloroplast, acetate is efficiently utilized by the mevalonate pathway for the biosynthesis of lipids  
 559 and secondary metabolites, oxidized to CO<sub>2</sub> via the coupled activities of the glyoxylate cycle and  
 560 mitochondrial respiration, and utilized to support fatty acid biosynthesis in chloroplasts. Two  
 561 putative alternative sources of acetaldehyde are also highlighted including the potential  
 562 production of acetaldehyde from photosynthesis-linked intermediates of the isoprenoid pathway  
 563 following light-dark transitions, and the production of acetaldehyde from membrane  
 564 peroxidation by reactive oxygen species (ROS) during abiotic stress. Key enzymes (and their  
 565 Enzyme Commission number, EC) mediating these transformations are acetoacetate  
 566 decarboxylase (AAD, EC 4.1.1.4), acetyl-CoA synthetase (ACS, EC 6.2.1.1), pyruvate  
 567 dehydrogenase (PDH, EC 1.2.4.1), pyruvate decarboxylase (PDC, EC 4.1.1.1), alcohol  
 568 dehydrogenase (ADH, EC 1.1.1.1), aldehyde dehydrogenase (ALDH, EC 1.2.1.3), and 1-deoxy-  
 569 D-xylulose 5-phosphate synthase (DXS, EC 2.2.1.7). Metabolite abbreviations include

570 glyceraldehyde-3-phosphate (GA3P), glucose-6-phosphate (Glu6P), phosphoenolpyruvate (PEP),  
 571 and 1-deoxy-D-xylulose 5-phosphate (DXP).

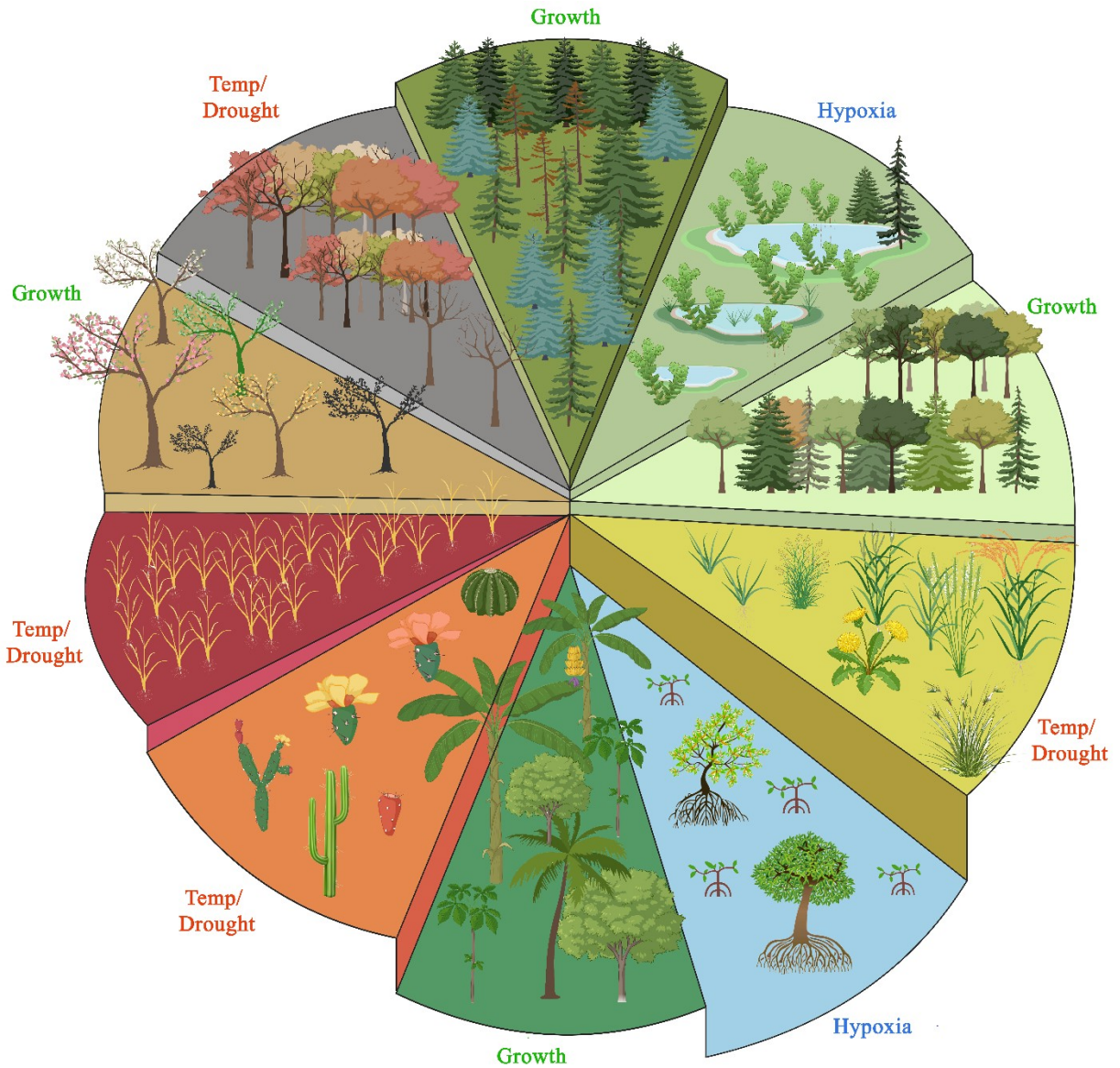


572

573 **Figure 2:** Graphical representation of tree fermentation processes during root hypoxia stress  
 574 induced by flooding and drought stress associated with decreased precipitation and cloud-free  
 575 conditions. In scenario **A** (Root Flooding), Ethanol (EtOH) production during ethanolic  
 576 fermentation in roots under hypoxia helps regenerate Nicotinamide Adenine Dinucleotide  
 577 (NAD<sup>+</sup>) consumed during the oxidation of sugars by glycolysis. Transport of EtOH and its  
 578 precursor acetaldehyde (CH<sub>3</sub>CHO) to the canopy via the transpiration stream results in enhanced  
 579 emissions of these volatiles to the atmosphere. However, coupled to Nicotinamide Adenine  
 580 Dinucleotide Hydrogen (NADH) formation in leaves and other aerobic tissues, the majority of  
 581 EtOH is oxidized back to acetaldehyde which is further oxidized to acetic acid (AA). Finally,  
 582 AA is then utilized by both catabolic (aerobic respiration) and biosynthetic (e.g. lipid synthesis)  
 583 processes associated with growth. In scenario **B** (Drought), transpiration is greatly reduced and  
 584 AA is directly produced in roots, stems and leaves via acetate fermentation under aerobic  
 585 conditions where it coordinates whole plant signaling and other defense processes via acetylation  
 586 and respiration.



587  
 588 **Figure 3:** Graphical summary of emerging methods to quantify acetic acid ( $\text{CH}_3\text{COOH}$ , AA)  
 589 emissions from the terrestrial biosphere. **a.** Drone based air sample collections to characterize  
 590 above canopy AA flux horizontal heterogeneity at the landscape scale, **b.** Ecosystem-scale  
 591 vertical AA flux measurements using eddy covariance, **c.** Individual leaf and branch AA flux  
 592 measurements using environmentally controlled dynamic chambers, **d.** Satellite-based remote  
 593 sensing of atmospheric AA using IR-absorbance spectroscopy, **e.** Vertical tropospheric gradients  
 594 of AA to characterize regional scale AA sources and sinks, and **f.** fine-scale within canopy  
 595 sources and sinks determined by high resolution vertical concentration gradient system on a flux  
 596 tower within and above a forest canopy.



597

598 **Figure 4:** Exploring physiological and ecological roles of plant fermentation metabolism in  
 599 diverse ecosystems globally under a changing climate. The dominant process anticipated to be  
 600 linked with plant fermentation activity in each ecosystem is listed as root response to hypoxia  
 601 (blue), growth and development (green), and high temperature/drought responses (red).

602

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