Fermentation-Mediated Growth, Signaling, and Defense in Plants

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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 reprograming 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.

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

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

Introduction

Ethanolic fermentation and flood tolerance

In higher plants, molecular oxygen (O_2) deficiency can dramatically alter cellular metabolism resulting in reductions in productivity (Fukao & Bailey-Serres, 2004). Understanding and predicting plant metabolic responses to O_2 deficiency through changes in respiration and fermentation, and physiological processes like growth, has become an increasingly active area of research (Bailey-Serres & Freeling, 1990; Gibbs *et al.*, 2011). The capacity to ferment carbohydrates in higher plants derives from an ancient biochemical pathway that can be traced back to green algae that ferment starch under anaerobic conditions to numerous products including acetate, ethanol, formate, glycerol, lactate, H_2 and CO_2 (Gaffron & Rubin, 1942; Davies *et al.*, 1974; Catalanotti *et al.*, 2013; Xia *et al.*, 2016). The enhanced production of acetaldehyde and ethanol in higher plants by pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) during fermentation induced by hypoxia is well established (Kimmerer & MacDonald, 1987; Vartapetian *et al.*, 1997). During hypoxic conditions in roots associated with saturated soils and flooding, the suppression of ATP production by mitochondrial respiration may be partially compensated by ethanolic fermentation (Tadege *et al.*, 1998). Ethanolic fermentation capacity under hypoxia has been identified as a highly conserved characteristic of modern land

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

Numerous genetic studies have demonstrated that PDC and ADH play an important and often critical role in low-oxygen tolerance in plants (Ventura *et al.*, 2020). For example, in maize the *Adh1* null mutant, which fail to synthesize ADH1 isozymes, are very susceptible to hypoxia (Johnson *et al.*, 1994). In Arabidopsis, the *Adh1* null mutant also has reduced tolerance to hypoxia while in contrast, overexpressing *PDC1* or *PDC2* increases survival (Ismond *et al.*, 2003). Under hypoxia, pyruvate generated by glycolysis in roots cannot be effectively oxidized by aerobic respiration in mitochondria and instead is decarboxylated to acetaldehyde by PDC. To help regenerate NAD^+ required to maintain high rates of glycolysis, a fraction of the acetaldehyde is reduced to ethanol, followed by transport of acetaldehyde and ethanol from roots to the canopy via the transpiration stream (Atkinson *et al.*, 2008). ^{14}C-ethanol labeling of excised leaves and shoots revealed that ethanol in the xylem in poplar trees is rapidly assimilated into plant biomass, with 95% remaining in plant tissues after 24 hr (MacDonald & Kimmerer, 1993). Despite its volatile nature, < 5% of the label was lost to the atmosphere via leaf emissions of ^{14}C-ethanol and/or ^{14}C-acetaldehyde while <1% was lost as $^{14}CO_2$, suggesting the involvement of mitochondrial respiration. Likewise, while strong increases in leaf acetaldehyde emission have been reported from several species following root flooding, the majority of acetaldehyde is thought to be further oxidized to acetic acid which is much less volatile with a higher water solubility than ethanol or acetaldehyde (Kreuzwieser & Rennenberg, 2013).

In addition to roots, aerial tissues like stems and leaves of trees constitutively express ADH isozymes that can convert ethanol produced in hypoxic roots back to acetaldehyde (Kimmerer & Stringer, 1988; Harry & Kimmerer, 1991; Strommer, 2011). Ethanol oxidation and assimilation in aerobic stem and leaf tissues is therefore mediated by the reversible ADH reaction where ethanol is oxidized back to acetaldehyde (Kreuzwieser *et al.*, 1999) which is subsequently oxidized to acetic acid by an aldehyde dehydrogenase (ALDH: EC 1.2.1) (Wei *et al.*, 2009). Following acetate activation to acetyl-CoA by an acetyl-coenzyme A synthetase (ACS) (Lin & Oliver, 2008), this central C_2 metabolite is rapidly utilized in both anabolic (e.g. lipid biosynthesis) and catabolic (e.g. mitochondrial respiration) metabolism (**Figure 1**), thereby explaining the rapid incorporation of root derived ethanol into stem and leaf biomass (MacDonald & Kimmerer, 1993). Subsequent studies provided additional experimental evidence that enhanced leaf emissions of acetaldehyde and ethanol during root hypoxia involves root ethanol production, its transport to aerobic tissues via the transpiration stream, and its oxidation and assimilation in aerobic tissues (MacDonald & Kimmerer, 1993; Kreuzwieser *et al.*, 1999; Holzinger *et al.*, 2000; Kreuzwieser *et al.*, 2001; Kreuzwieser *et al.*, 2004; Rottenberger *et al.*, 2008; Kreuzwieser & Rennenberg, 2013; Kreuzwieser & Rennenberg, 2014). While these studies generally did not report acetic acid emissions, significant foliar emissions of acetic acid were observed from the tropical tree species that had the highest ethanol and acetaldehyde emission responses to flooding (Rottenberger *et al.*, 2008). Thus, these coupled below- and above-ground plant processes were proposed as a key physiological mechanism of flood tolerance (Kreuzwieser *et al.*, 2004).

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

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

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

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

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

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

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

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

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

ethanol, acetic acid, acetone, and methyl acetate (Jardine *et al.*, 2012; Dewhirst *et al.*, 2021; Jardine *et al.*, 2022) with the magnitude of these bursts greatly increasing under hypoxia (Jardine *et al.*, 2012). While the biochemical origin of acetone biosynthesis in plants remains uncertain, one possibility is the decarboxylation of acetoacetate in a similar mechanism as in bacteria (Fall, 2003). In bacteria, acetone is a well-known fermentation product generated from the decarboxylation of acetoacetate (Fall, 2003; Han *et al.*, 2011), and large scale production of acetone by solvent-producing strains of *Clostridium spp.* was among the first large-scale industrial fermentation processes to be developed (Jones & Woods, 1986). Evidence that acetone emissions in leaves is produced from acetoacetate decarboxylation and linked to fermentation was obtained from feeding detached leaves positionally-specific ^{13}C-pyruvate solutions via the transpiration stream. Direct ^{13}C-incorporation into large ^{13}C-acetone emissions was observed under pyruvate-2-^{13}C feeding, but not pyruvate-1-^{13}C (Jardine *et al.*, 2010). This is consistent with pyruvate entering the acetate fermentation pathway in plants which involves the decarboxylation of the C_1 of pyruvate followed by acetyl-CoA utilization by the mevalonate pathway leading to acetone production via acetoacetate decarboxylation (Suganuma *et al.*, 1993) (AAD, **Figure 1**).

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

with cumulative photosynthesis (the total net amount of CO_2 assimilated during the light period, μmol CO_2 m^{-2}), strongly suppressed by removing CO_2 from the atmosphere, and directly incorporated $^{13}CO_2$ assimilated during the light period into $^{13}C_2$-acetyl-CoA (Jardine *et al.*, 2012). However, as photosynthetic production of triosephosphates (glyceraldehyde-3-phosphate, G3P) can be exported to the cytosol and converted to pyruvate via glycolysis, the role of the pyruvate overflow mechanism in the emission bursts of volatile fermentation products (acetaldehyde, ethanol, acetic acid, acetone and methyl acetate) following light-dark transitions, as originally proposed by Karl *et al.*, 2002, should still be considered as a potentially important mechanism (**Figure 1:** acetaldehyde production in the cytosol via the PDC catalyzed reaction). Chloroplasts may lack the ability to convert photosynthetically derived G3P to phosphoenolpyruvate (PEP) via glycolysis and consequently may require the export of G3P to the cytosol followed by re-importation as PEP (Sharkey & Monson, 2014) and/or pyruvate (Jardine *et al.*, 2010) in order to synthesize fatty acids and isoprenoids directly linked to recent photosynthate (**Figure 1**). Consistent with a pyruvate overflow mechanism involving PDC, light/dark emission bursts of acetaldehyde, ethanol, acetic acid, and acetone are greatly enhanced under hypoxia relative to aerobic conditions (Jardine *et al.*, 2012)

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

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

Acetate fermentation and drought tolerance

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

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

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

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

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

Central to the enhancement of drought tolerance mediated by acetate fermentation is the detoxification of acetaldehyde mediated by ALDH. Numerous *ALDH* genes have long been recognized as a key component of plant response to abiotic stress (Kirch *et al.*, 2004) by acting as "aldehyde scavengers" generated from lipid peroxidation (Singh *et al.*, 2013) and funneling acetaldehyde generated in fermentation metabolism to acetic acid (Wei *et al.*, 2009). In Arabidopsis, the *ALDH3I1* gene is transcriptionally activated by abiotic stress, with over-expression providing enhanced stress tolerance (Singh *et al.*, 2013). Moreover, both constitutive and stress-induced expression of both the chloroplastic *ALDH3I1* and the cytoplasmic *ALDH7B4* conferred tolerance to osmotic and oxidative stress, thereby protecting plants against lipid peroxidation of sensitive membranes central for example in photosynthesis and mitochondrial respiration (Singh *et al.*, 2013).

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

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

aerobic conditions has high rates of both aerobic fermentation and respiration to meet its high energetic and biosynthetic demands (Bucher *et al.*, 1995). As aerobic respiratory metabolism is important during both growth and defense processes (Millar *et al.*, 2011), fermentation under aerobic conditions in leaves and other plant tissues may help funnel respiratory substrates into mitochondria to support energy production and amino acid biosynthesis as well as into chloroplasts and the cytoplasm for the biosynthesis of fatty acids and secondary metabolites like terpenoids. For example, acetate fermentation under aerobic conditions coupled to acetate-dependent acetyl-CoA production was concluded to be very important in lipid biosynthesis in germinating tobacco pollen (Mellema *et al.*, 2002). Recently, a $^{13}C_2$-acetate solution directly injected into the xylem of poplar trees was shown to be efficiently transported to the canopy via the transpiration stream and utilized as a respiratory substrate in leaves (Jardine *et al.*, 2022). These observations demonstrated the potential for acetate fermentation under aerobic conditions to contribute to whole plant metabolism and growth. However, light appeared to inhibit the allocation of acetate into leaf respiratory metabolism during the day, with increased daytime allocation to biosynthetic processes speculated (Jardine *et al.*, 2022). The importance of acetate as a growth promoter was further highlighted in a recent study where exogenously supplied acetate was incorporated into biomass through major metabolic pathways (Hann *et al.*, 2022). This opens the door to acetate delivery to plants being used to enhance growth yields, especially for food production under controlled environments (Hann *et al.*, 2022).

Acetate fermentation and 'Defense Respiration' during drought and high temperature stress

Although variable, drought generally reduces plant respiration rates that are considered critical 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.

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

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

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^{-2} min^{-1} 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 ^{13}C-
417 labeling of respiratory CO_2 using the dynamic xylem solution injection (DXSI) technique for
418 continuous delivery of $^{13}C_2$-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 ºC 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

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

Conclusions and perspectives

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

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

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

metabolism coupled to respiration in fast growing tropical pioneer tree species like *Vismia guianensis* (Rodrigues *et al.*, 2020), may also be important in the re-growth of tropical forests following a disturbance. In addition, high temperature and drought stress characteristic of desert ecosystems may also stimulate high rates of fermentative metabolism as a key survival trait. For example, creosotebush (*Larrea tridentata*), which grows in well drained sandy soils, is vastly distributed in North American deserts, and was reported to have large temperature-stimulated leaf emissions of the fermentation volatiles acetaldehyde, acetic acid, acetone, ethanol, and methyl acetate during the summer monsoon in the Sonoran desert (Jardine *et al.*, 2010). Thus, plant fermentation studies across diverse plant functional traits like photosynthetic types (C_3, C_4, and crassulacean acid metabolism), growth rates, wood density, specific leaf area, etc. may lead to improvements in our predictive understanding of the roles of plant fermentative metabolism in the establishment and resilience of ecosystem structure and function (**Figure 4**).

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Competing Interests

None declared.

Figures

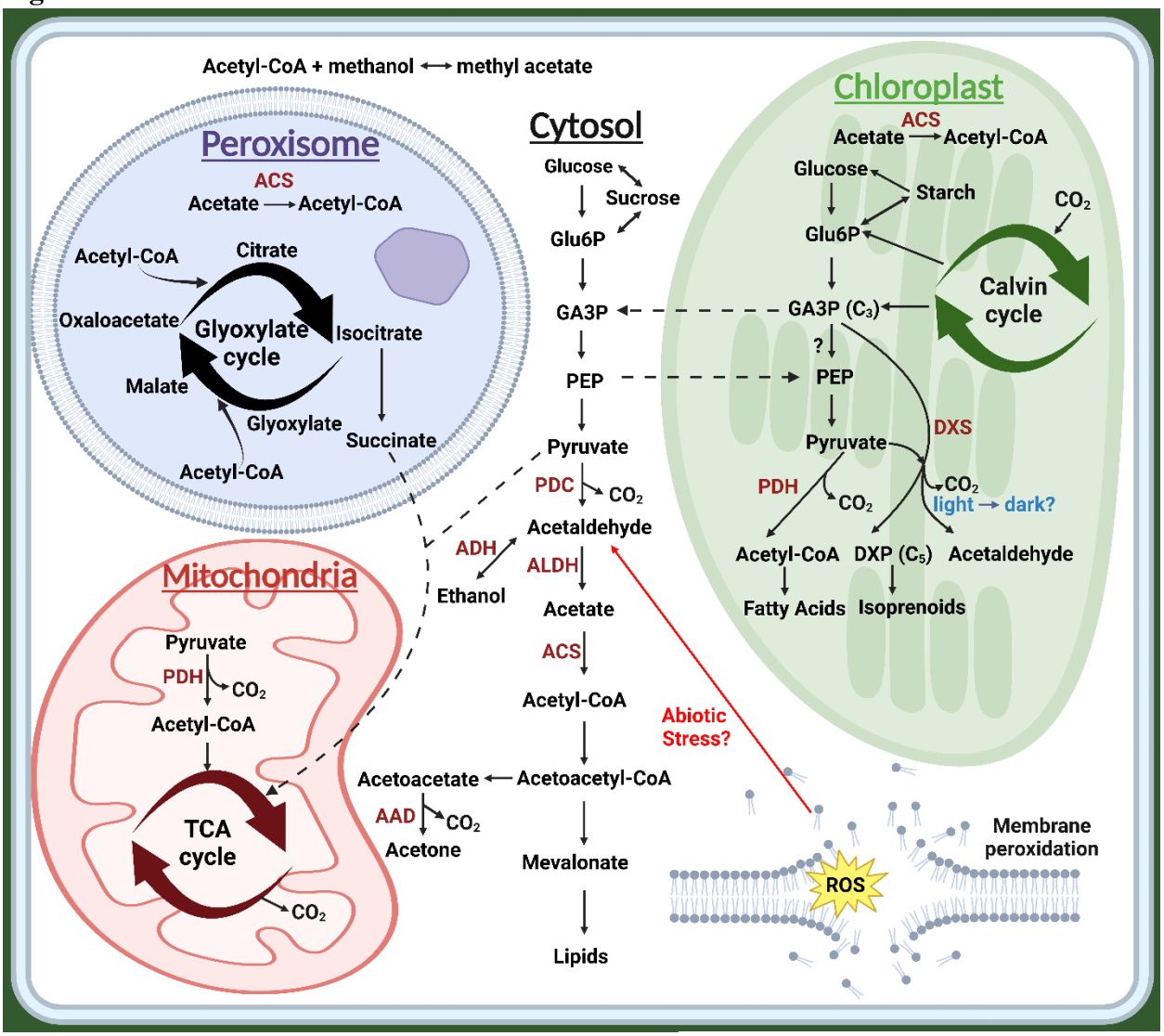

Figure 1: Graphic summary of acetate metabolism of photosynthetic plant cells including the volatile fermentation intermediates acetaldehyde, ethanol, acetic acid, acetone, and methyl acetate. Following the activation of acetate to acetyl-CoA in the cytosol, peroxisome and chloroplast, acetate is efficiently utilized by the mevalonate pathway for the biosynthesis of lipids and secondary metabolites, oxidized to CO_2 via the coupled activities of the glyoxylate cycle and mitochondrial respiration, and utilized to support fatty acid biosynthesis in chloroplasts. Two putative alternative sources of acetaldehyde are also highlighted including the potential production of acetaldehyde from photosynthesis-linked intermediates of the isoprenoid pathway following light-dark transitions, and the production of acetaldehyde from membrane peroxidation by reactive oxygen species (ROS) during abiotic stress. Key enzymes (and their Enzyme Commission number, EC) mediating these transformations are acetoacetate decarboxylase (AAD, EC 4.1.1.4), acetyl-CoA synthetase (ACS, EC 6.2.1.1), pyruvate dehydrogenase (PDH, EC 1.2.4.1), pyruvate decarboxylase (PDC, EC 4.1.1.1), alcohol dehydrogenase (ADH, EC 1.1.1.1), aldehyde dehydrogenase (ALDH, EC 1.2.1.3), and 1-deoxy-D-xylulose 5-phosphate synthase (DXS, EC 2.2.1.7). Metabolite abbreviations include

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

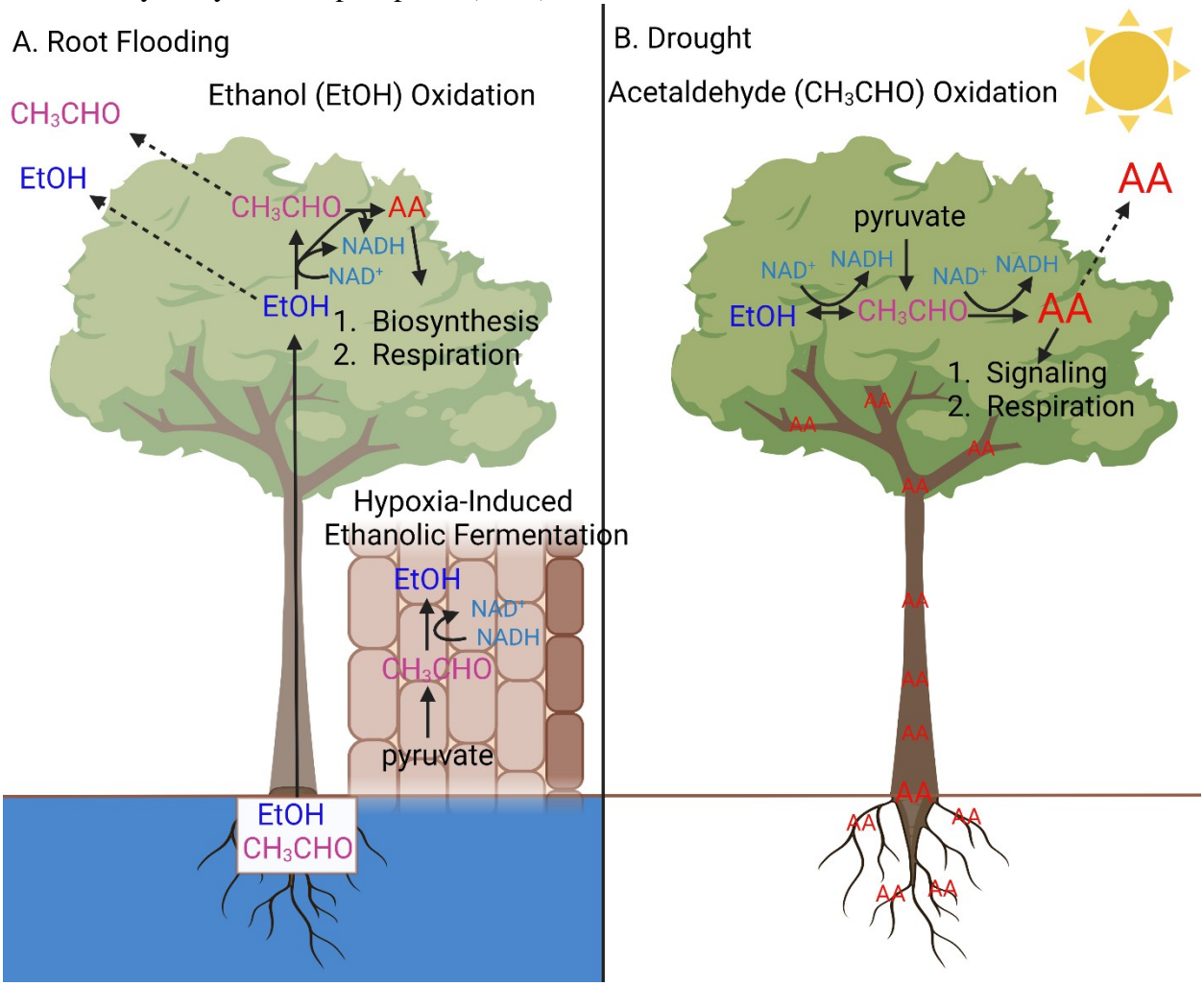

Figure 2: Graphical representation of tree fermentation processes during root hypoxia stress induced by flooding and drought stress associated with decreased precipitation and cloud-free conditions. In scenario **A** (Root Flooding), Ethanol (EtOH) production during ethanolic fermentation in roots under hypoxia helps regenerate Nicotinamide Adenine Dinucleotide (NAD^+) consumed during the oxidation of sugars by glycolysis. Transport of EtOH and its precursor acetaldehyde (CH_3CHO) to the canopy via the transpiration stream results in enhanced emissions of these volatiles to the atmosphere. However, coupled to Nicotinamide Adenine Dinucleotide Hydrogen (NADH) formation in leaves and other aerobic tissues, the majority of EtOH is oxidized back to acetaldehyde which is further oxidized to acetic acid (AA). Finally, AA is then utilized by both catabolic (aerobic respiration) and biosynthetic (e.g. lipid synthesis) processes associated with growth. In scenario **B** (Drought), transpiration is greatly reduced and AA is directly produced in roots, stems and leaves via acetate fermentation under aerobic conditions where it coordinates whole plant signaling and other defense processes via acetylation and respiration.

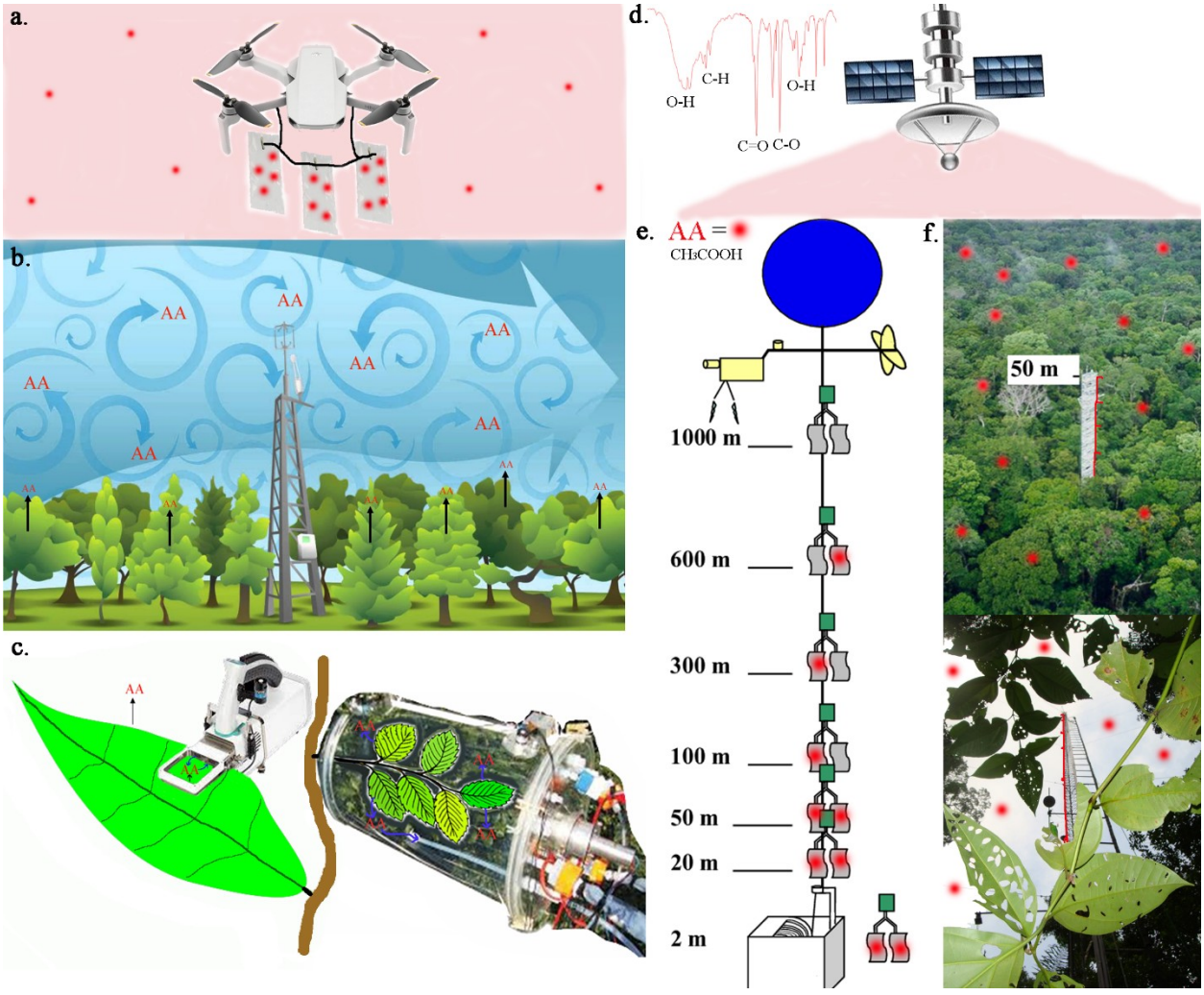

Figure 3: Graphical summary of emerging methods to quantify acetic acid (CH_3COOH, AA) emissions from the terrestrial biosphere. **a.** Drone based air sample collections to characterize above canopy AA flux horizontal heterogeneity at the landscape scale, **b.** Ecosystem-scale vertical AA flux measurements using eddy covariance, **c.** Individual leaf and branch AA flux measurements using environmentally controlled dynamic chambers, **d.** Satellite-based remote sensing of atmospheric AA using IR-absorbance spectroscopy, **e.** Vertical tropospheric gradients of AA to characterize regional scale AA sources and sinks, and **f.** fine-scale within canopy sources and sinks determined by high resolution vertical concentration gradient system on a flux tower within and above a forest canopy.

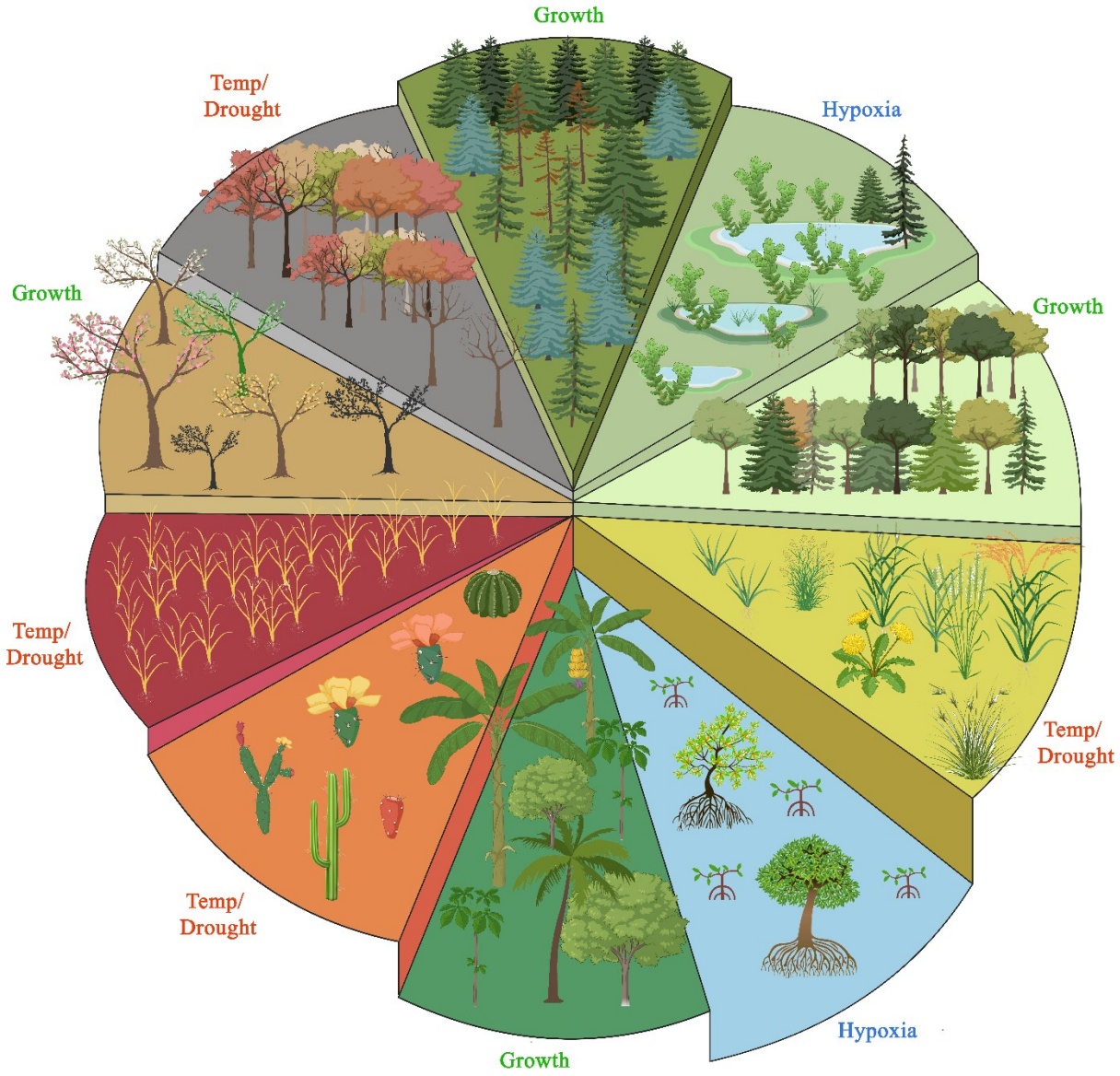

Figure 4: Exploring physiological and ecological roles of plant fermentation metabolism in diverse ecosystems globally under a changing climate. The dominant process anticipated to be linked with plant fermentation activity in each ecosystem is listed as root response to hypoxia (blue), growth and development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

References

Atkin OK, Macherel D. 2009. The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals of Botany* **103**(4): 581-597.

Atkinson CJ, Harrison-Murray R, Taylor J. 2008. Rapid flood-induced stomatal closure accompanies xylem sap transportation of root-derived acetaldehyde and ethanol in Forsythia. *Environmental and Experimental Botany* **64**(2): 196-205.

Awala SK, Yamane K, Izumi Y, Fujioka Y, Watanabe Y, Wada KC, Kawato Y, Mwandemele OD, Iijima M. 2016. Field evaluation of mixed-seedlings with rice to alleviate flood stress for semi-arid cereals. *European Journal of Agronomy* **80**: 105-112.

Bailey-Serres J, Freeling M. 1990. Hypoxic stress-induced changes in ribosomes of maize seedling roots. *Plant Physiology* **94**(3): 1237-1243.

Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voesenek LA, van Dongen JT. 2012. Making sense of low oxygen sensing. *Trends in plant science* **17**(3): 129-138.

Bashir K, Todaka D, Rasheed S, Matsui A, Ahmad Z, Sako K, Utsumi Y, Vu AT, Tanaka M, Takahashi S. 2022. Ethanol-mediated novel survival strategy against drought stress in plants. *Plant and Cell Physiology* **63**(9): 1181-1192.

Batista CE, Ye J, Ribeiro IO, Guimarães PC, Medeiros AS, Barbosa RG, Oliveira RL, Duvoisin Jr S, Jardine KJ, Gu D. 2019. Intermediate-scale horizontal isoprene concentrations in the near-canopy forest atmosphere and implications for emission heterogeneity. *Proceedings of the National Academy of Sciences* **116**(39): 19318-19323.

Bucher M, Brander KA, Sbicego S, Mandel T, Kuhlemeier C. 1995. Aerobic Fermentation in Tobacco Pollen. *Plant Molecular Biology* **28**(4): 739-750.

Bui LT, Novi G, Lombardi L, Iannuzzi C, Rossi J, Santaniello A, Mensuali A, Corbineau F, Giuntoli B, Perata P. 2019. Conservation of ethanol fermentation and its regulation in land plants. *Journal of Experimental Botany* **70**(6): 1815-1827.

Catalanotti C, Yang W, Posewitz MC, Grossman AR. 2013. Fermentation metabolism and its evolution in algae. *Frontiers in plant science* **4**: 150.

Copolovici L, Niinemets Ü. 2010. Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. *Plant, cell & environment* **33**(9): 1582-1594.

Crunaire S, Tarmoul J, Fittschen C, Tomas A, Lemoine B, Coddeville P. 2006. Use of cw-CRDS for studying the atmospheric oxidation of acetic acid in a simulation chamber. *Applied Physics B* **85**(2): 467-476.

Davies DD, Grego S, Kenworthy P. 1974. The control of the production of lactate and ethanol by higher plants. *Planta* **118**(4): 297-310.

Dewhirst RA, Afseth CA, Castanha C, Mortimer JC, Jardine KJ. 2020. Cell wall O-acetyl and methyl esterification patterns of leaves reflected in atmospheric emission signatures of acetic acid and methanol. *PloS one* **15**(5): e0227591.

Dewhirst RA, Lei J, Afseth CA, Castanha C, Wistrom CM, Mortimer JC, Jardine KJ. 2021. Are Methanol-Derived Foliar Methyl Acetate Emissions a Tracer of Acetate-Mediated Drought Survival in Plants? *Plants* **10**(2): 411.

Evans DE. 2004. Aerenchyma formation. *New phytologist* **161**(1): 35-49.

Fall R. 2003. Abundant oxygenates in the atmosphere: A biochemical perspective. *Chemical reviews* **103**(12): 4941-4952.

Fontes C, Jardine K, Dawson TE, Gimenez B, Higuchi N, Chambers JQ 2016. The Effect of the 2015/16 El Niño on Hydraulic Characteristics of Central Amazonian Trees. *AGU Fall Meeting Abstracts*. B31G-0544.

Franco B, Clarisse L, Stavrakou T, Müller JF, Taraborrelli D, Hadji-Lazaro J, Hannigan JW, Hase F, Hurtmans D, Jones N. 2020. Spaceborne measurements of formic and acetic acids: a global view of the regional sources. *Geophysical Research Letters* **47**(4): e2019GL086239.

Fukao T, Bailey-Serres J. 2004. Plant responses to hypoxia–is survival a balancing act? *Trends in plant science* **9**(9): 449-456.

Gabriel R, Schäfer L, Gerlach C, Rausch T, Kesselmeier J. 1999. Factors controlling the emissions of volatile organic acids from leaves of Quercus ilex L.(Holm oak). *Atmospheric Environment* **33**(9): 1347-1355.

Gaffron H, Rubin J. 1942. Fermentative and photochemical production of hydrogen in algae. *The Journal of General Physiology* **26**(2): 219-240.

Galle B, Arellano S, Bobrowski N, Conde V, Fischer TP, Gerdes G, Gutmann A, Hoffmann T, Itikarai I, Krejci T. 2021. A multi-purpose, multi-rotor drone system for long-range and high-altitude volcanic gas plume measurements. *Atmospheric Measurement Techniques* **14**(6): 4255-4277.

Gibbs DJ, Lee SC, Md Isa N, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J. 2011. Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* **479**(7373): 415-418.

Gonin M, Bergougnoux V, Nguyen TD, Gantet P, Champion A. 2019. What makes adventitious roots? *Plants* **8**(7): 240.

Han B, Gopalan V, Ezeji TC. 2011. Acetone production in solventogenic Clostridium species: new insights from non-enzymatic decarboxylation of acetoacetate. *Applied microbiology and biotechnology* **91**(3): 565-576.

Hann EC, Overa S, Harland-Dunaway M, Narvaez AF, Le DN, Orozco-Cárdenas ML, Jiao F, Jinkerson RE. 2022. A hybrid inorganic–biological artificial photosynthesis system for energy-efficient food production. *Nature Food* **3**(6): 461-471.

Harry DE, Kimmerer TW. 1991. Molecular-Genetics and Physiology of Alcohol-Dehydrogenase in Woody-Plants. *Forest Ecology and Management* **43**(3-4): 251-272.

Holzinger R, Sandoval-Soto L, Rottenberger S, Crutzen P, Kesselmeier J. 2000. Emissions of volatile organic compounds from Quercus ilex L. measured by Proton Transfer Reaction Mass Spectrometry under different environmental conditions. *Journal of Geophysical Research: Atmospheres* **105**(D16): 20573-20579.

Hu Y, Lu Y, Zhao Y, Zhou D-X. 2019. Histone acetylation dynamics integrates metabolic activity to regulate plant response to stress. *Frontiers in plant science* **10**: 1236.

Ismond KP, Dolferus R, De Pauw M, Dennis ES, Good AG. 2003. Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. *Plant Physiology* **132**(3): 1292-1302.

Jardine K, Abrell L, Kurc S, Huxman T, Ortega J, Guenther A. 2010. Volatile organic compound emissions from Larrea tridentata (creosotebush). *Atmospheric Chemistry and Physics* **10**(24): 12191-12206.

Jardine K, Barron-Gafford G, Norman J, Abrell L, Monson R, Meyers K, Pavao-Zuckerman M, Dontsova K, Kleist E, Werner C. 2012. Green leaf volatiles and oxygenated metabolite emission bursts from mesquite branches following light–dark transitions. *Photosynthesis research* **113**(1): 321-333.

Jardine K, Lei J, Som S, Souza D, Clendinen CS, Mehta H, Handakumbura P, Bill M, Young RP. 2022. Light-Dependence of Formate (C1) and Acetate (C2) Transport and Oxidation in Poplar Trees. *Plants* **11**(16): 2080.

Jardine K, Wegener F, Abrell L, Van Haren J, Werner C. 2014. Phytogenic biosynthesis and emission of methyl acetate. *Plant, cell & environment* **37**(2): 414-424.

Jardine K, Yañez Serrano A, Arneth A, Abrell L, Jardine A, Artaxo P, Alves E, Kesselmeier J, Taylor T, Saleska S. 2011. Ecosystem-scale compensation points of formic and acetic acid in the central Amazon. *Biogeosciences* **8**(12): 3709-3720.

Jardine KJ, Chambers JQ, Holm J, Jardine AB, Fontes CG, Zorzanelli RF, Meyers KT, De Souza VF, Garcia S, Gimenez BO. 2015. Green leaf volatile emissions during high temperature and drought stress in a Central Amazon rainforest. *Plants* **4**(3): 678-690.

Jardine KJ, Dewhirst RA, Som S, Lei J, Tucker E, Young RP, Portillo-Estrada M, Gao Y, Su L, Fares S. 2022. Cell wall ester modifications and volatile emission signatures of plant response to abiotic stress. *Plant, cell & environment*.

Jardine KJ, Sommer ED, Saleska SR, Huxman TE, Harley PC, Abrell L. 2010. Gas phase measurements of pyruvic acid and its volatile metabolites. *Environmental science & technology* **44**(7): 2454-2460.

Jia W, Ma M, Chen J, Wu S. 2021. Plant morphological, physiological and anatomical adaption to flooding stress and the underlying molecular mechanisms. *International journal of molecular sciences* **22**(3): 1088.

Johnson JR, Cobb BG, Drew MC. 1994. Hypoxic induction of anoxia tolerance in roots of Adh1 null Zea mays L. *Plant Physiology* **105**(1): 61-67.

Jones DT, Woods D. 1986. Acetone-butanol fermentation revisited. *Microbiological reviews* **50**(4): 484-524.

Jud W, Vanzo E, Li Z, Ghirardo A, Zimmer I, Sharkey TD, Hansel A, Schnitzler JP. 2016. Effects of heat and drought stress on post-illumination bursts of volatile organic compounds in isoprene-emitting and non-emitting poplar. *Plant, cell & environment* **39**(6): 1204-1215.

Karl T, Curtis A, Rosenstiel T, Monson R, Fall R. 2002. Transient releases of acetaldehyde from tree leaves− products of a pyruvate overflow mechanism? *Plant, cell & environment* **25**(9): 1121-1131.

Keiluweit M, Wanzek T, Kleber M, Nico P, Fendorf S. 2017. Anaerobic microsites have an unaccounted role in soil carbon stabilization. *Nature communications* **8**(1): 1-10.

Kesselmeier J. 2001. Exchange of short-chain oxygenated volatile organic compounds (VOCs) between plants and the atmosphere: A compilation of field and laboratory studies. *Journal of atmospheric chemistry* **39**(3): 219-233.

Kesselmeier J, Bode K, Gerlach C, Jork E-M. 1998. Exchange of atmospheric formic and acetic acids with trees and crop plants under controlled chamber and purified air conditions. *Atmospheric Environment* **32**(10): 1765-1775.

Kesselmeier J, Staudt M. 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *Journal of atmospheric chemistry* **33**(1): 23-88.

Kim J-M, To TK, Matsui A, Tanoi K, Kobayashi NI, Matsuda F, Habu Y, Ogawa D, Sakamoto T, Matsunaga S. 2017. Acetate-mediated novel survival strategy against drought in plants. *Nature Plants* **3**(7): 1-7.

Kim S, Karl T, Guenther A, Tyndall G, Orlando J, Harley P, Rasmussen R, Apel E. 2010. Emissions and ambient distributions of Biogenic Volatile Organic Compounds (BVOC) in a ponderosa pine ecosystem: interpretation of PTR-MS mass spectra. *Atmospheric Chemistry and Physics* **10**(4): 1759-1771.

Kimmerer TW. 1987. Alcohol dehydrogenase and pyruvate decarboxylase activity in leaves and roots of eastern cottonwood (Populus deltoides Bartr.) and soybean (Glycine max L.). *Plant Physiology* **84**(4): 1210-1213.

Kimmerer TW, MacDonald RC. 1987. Acetaldehyde and ethanol biosynthesis in leaves of plants. *Plant Physiology* **84**(4): 1204-1209.

Kimmerer TW, Stringer MA. 1988. Alcohol dehydrogenase and ethanol in the stems of trees: evidence for anaerobic metabolism in the vascular cambium. *Plant Physiology* **87**(3): 693-697.

Kirch H-H, Bartels D, Wei Y, Schnable PS, Wood AJ. 2004. The ALDH gene superfamily of Arabidopsis. *Trends in plant science* **9**(8): 371-377.

Kitaya Y, Yabuki K, Kiyota M, Tani A, Hirano T, Aiga I. 2002. Gas exchange and oxygen concentration in pneumatophores and prop roots of four mangrove species. *Trees* **16**(2): 155-158.

Kreuzwieser J, Harren FJ, Laarhoven LJ, Boamfa I, Te Lintel-Hekkert S, Scheerer U, Hüglin C, Rennenberg H. 2001. Acetaldehyde emission by the leaves of trees–correlation with physiological and environmental parameters. *Physiologia Plantarum* **113**(1): 41-49.

Kreuzwieser J, Papadopoulou E, Rennenberg H. 2004. Interaction of flooding with carbon metabolism of forest trees. *Plant Biology* **6**(03): 299-306.

Kreuzwieser J, Rennenberg H. 2013. Flooding-driven emissions from trees. *Biology, controls and models of tree volatile organic compound emissions*: 237-252.

Kreuzwieser J, Rennenberg H. 2014. Molecular and physiological responses of trees to waterlogging stress. *Plant, cell & environment* **37**(10): 2245-2259.

Kreuzwieser J, Scheerer U, Rennenberg H. 1999. Metabolic origin of acetaldehyde emitted by poplar (Populus tremula× P. alba) trees. *Journal of Experimental Botany* **50**(335): 757-765.

Lee C, Teng Q, Zhong R, Ye Z-H. 2011. The four Arabidopsis reduced wall acetylation genes are expressed in secondary wall-containing cells and required for the acetylation of xylan. *Plant and Cell Physiology* **52**(8): 1289-1301.

Li C, Kong X, Luo Z, Li W, Tang W, Zhang D, Ma C, Dong H. 2021. Exogenous application of acetic acid improves the survival rate of cotton by increasing abscisic acid and jasmonic acid contents under drought stress. *Acta Physiologiae Plantarum* **43**(2): 1-10.

Lin M, Oliver DJ. 2008. The role of acetyl-coenzyme a synthetase in Arabidopsis. *Plant Physiology* **147**(4): 1822-1829.

MacDonald RC, Kimmerer TW. 1993. Metabolism of transpired ethanol by eastern cottonwood (Populus deltoides Bartr.). *Plant Physiology* **102**(1): 173-179.

Manabe Y, Verhertbruggen Y, Gille S, Harholt J, Chong S-L, Pawar PM-A, Mellerowicz EJ, Tenkanen M, Cheng K, Pauly M. 2013. Reduced wall acetylation proteins play vital and distinct roles in cell wall O-acetylation in Arabidopsis. *Plant Physiology* **163**(3): 1107-1117.

Manter DK, Kelsey RG. 2008. Ethanol accumulation in drought-stressed conifer seedlings. *International journal of plant sciences* **169**(3): 361-369.

McDowell NG, Sapes G, Pivovaroff A, Adams HD, Allen CD, Anderegg WR, Arend M, Breshears DD, Brodribb T, Choat B. 2022. Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. *Nature Reviews Earth & Environment* **3**(5): 294-308.

McKee KL, Mendelssohn IA. 1987. Root metabolism in the black mangrove (Avicennia germinans (L.) L): response to hypoxia. *Environmental and Experimental Botany* **27**(2): 147-156.

Medrano H, Escalona JM, Bota J, Gulías J, Flexas J. 2002. Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of Botany* **89**(7): 895-905.

Mellema S, Eichenberger W, Rawyler A, Suter M, Tadege M, Kuhlemeier C. 2002. The ethanolic fermentation pathway supports respiration and lipid biosynthesis in tobacco pollen. *Plant Journal* **30**(3): 329-336.

Millar AH, Whelan J, Soole KL, Day DA. 2011. Organization and regulation of mitochondrial respiration in plants. *Annual review of plant biology* **62**(1): 79-104.

Mithran M, Paparelli E, Novi G, Perata P, Loreti E. 2014. Analysis of the role of the pyruvate decarboxylase gene family in A rabidopsis thaliana under low-oxygen conditions. *Plant Biology* **16**(1): 28-34.

Nguyen T, Drotar A-M, Monson R, Fall R. 2009. A high affinity pyruvate decarboxylase is present in cottonwood leaf veins and petioles: A second source of leaf acetaldehyde emission? *Phytochemistry* **70**(10): 1217-1221.

O'Leary BM, Asao S, Millar AH, Atkin OK. 2019. Core principles which explain variation in respiration across biological scales. *New phytologist* **222**(2): 670-686.

Ogawa D, Suzuki Y, Yokoo T, Katoh E, Teruya M, Muramatsu M, Ma JF, Yoshida Y, Isaji S, Ogo Y. 2021. Acetic-acid-induced jasmonate signaling in root enhances drought avoidance in rice. *Scientific reports* **11**(1): 1-13.

Park J-H, Goldstein A, Timkovsky J, Fares S, Weber R, Karlik J, Holzinger R. 2013. Eddy covariance emission and deposition flux measurements using proton transfer reaction–time of flight–mass spectrometry (PTR-TOF-MS): comparison with PTR-MS measured vertical gradients and fluxes. *Atmospheric Chemistry and Physics* **13**(3): 1439-1456.

Parolin P. 2009. Submerged in darkness: adaptations to prolonged submergence by woody species of the Amazonian floodplains. *Annals of Botany* **103**(2): 359-376.

Qaseem MF, Wu A-M. 2020. Balanced xylan acetylation is the key regulator of plant growth and development, and cell wall structure and for industrial utilization. *International journal of molecular sciences* **21**(21): 7875.

Qualley AV, Dudareva N. 2009. Metabolomics of plant volatiles. *Plant Systems Biology*: 329-343.

Quimio CA, Torrizo LB, Setter TL, Ellis M, Grover A, Abrigo EM, Oliva NP, Ella ES, Carpena AL, Ito O. 2000. Enhancement of submergence tolerance

in transgenic rice overproducing pyruvate decarboxylase. *Journal of Plant Physiology* **156**(4): 516-521.

Rahman M, Mostofa MG, Keya SS, Rahman A, Das AK, Islam R, Abdelrahman M, Bhuiyan SU, Naznin T, Ansary MU. 2021. Acetic acid improves drought acclimation in soybean: an integrative response of photosynthesis, osmoregulation, mineral uptake and antioxidant defense. *Physiologia Plantarum* **172**(2): 334-350.

Rasheed S, Bashir K, Kim J-M, Ando M, Tanaka M, Seki M. 2018. The modulation of acetic acid pathway genes in Arabidopsis improves survival under drought stress. *Scientific reports* **8**(1): 1-15.

Raymond P, Al-Ani A, Pradet A. 1985. ATP production by respiration and fermentation, and energy charge during aerobiosis and anaerobiosis in twelve fatty and starchy germinating seeds. *Plant Physiology* **79**(3): 879-884.

Rodrigues TB, Baker CR, Walker AP, McDowell N, Rogers A, Higuchi N, Chambers JQ, Jardine KJ. 2020. Stimulation of isoprene emissions and electron transport rates as key mechanisms of thermal tolerance in the tropical species Vismia guianensis. *Global Change Biology* **26**(10): 5928-5941.

Rodríguez-Calcerrada J, Rodrigues AM, António C, Perdiguero P, Pita P, Collada C, Li M, Gil L. 2021. Stem metabolism under drought stress–a paradox of increasing respiratory substrates and decreasing respiratory rates. *Physiologia Plantarum* **172**(2): 391-404.

Rottenberger S, Kleiss B, Kuhn U, Wolf A, Piedade MTF, Junk W, Kesselmeier J. 2008. The effect of flooding on the exchange of the volatile C 2-compounds ethanol, acetaldehyde and acetic acid between leaves of Amazonian floodplain tree species and the atmosphere. *Biogeosciences* **5**(4): 1085-1100.

Sauter M. 2013. Root responses to flooding. *Current Opinion in Plant Biology* **16**(3): 282-286.

Schmidt RR, Fulda M, Paul MV, Anders M, Plum F, Weits DA, Kosmacz M, Larson TR, Graham IA, Beemster GT. 2018. Low-oxygen response is triggered by an ATP-dependent shift in oleoyl-CoA in Arabidopsis. *Proceedings of the National Academy of Sciences* **115**(51): E12101-E12110.

Schwalm CR, Williams CA, Schaefer K, Arneth A, Bonal D, Buchmann N, Chen J, Law BE, Lindroth A, Luyssaert S. 2010. Assimilation exceeds respiration sensitivity to drought: A FLUXNET synthesis. *Global Change Biology* **16**(2): 657-670.

Sharkey TD, Monson RK. 2014. The future of isoprene emission from leaves, canopies and landscapes. *Plant, cell & environment* **37**(8): 1727-1740.

Shimamura S, Yamamoto R, Nakamura T, Shimada S, Komatsu S. 2010. Stem hypertrophic lenticels and secondary aerenchyma enable oxygen transport to roots of soybean in flooded soil. *Annals of Botany* **106**(2): 277-284.

Shukla V, Lombardi L, Iacopino S, Pencik A, Novak O, Perata P, Giuntoli B, Licausi F. 2019. Endogenous hypoxia in lateral root primordia controls root architecture by antagonizing auxin signaling in Arabidopsis. *Molecular Plant* **12**(4): 538-551.

Singh S, Brocker C, Koppaka V, Chen Y, Jackson BC, Matsumoto A, Thompson DC, Vasiliou V. 2013. Aldehyde dehydrogenases in cellular

responses to oxidative/electrophilicstress. *Free radical biology and medicine* **56**: 89-101.
Spicer R, Holbrook NM. 2007. Effects of carbon dioxide and oxygen on sapwood respiration in five temperate tree species. *Journal of Experimental Botany* **58**(6): 1313-1320.
Strommer J. 2011. The plant ADH gene family. *The Plant Journal* **66**(1): 128-142.
Suganuma N, Shimokawa R, Katoh T, Nagai T. 1993. Presence of acetone and acetoacetate decarboxylase in soybean moot nodules. *Soil Science and Plant Nutrition* **39**(4): 653-660.
Sun T, Zhang J, Zhang Q, Li X, Li M, Yang Y, Zhou J, Wei Q, Zhou B. 2022. Exogenous application of acetic acid enhances drought tolerance by influencing the MAPK signaling pathway induced by ABA and JA in apple plants. *Tree Physiology*.
Tadege M, Brändle R, Kuhlemeier C. 1998. Anoxia tolerance in tobacco roots: effect of overexpression of pyruvate decarboxylase. *The Plant Journal* **14**(3): 327-335.
Tari I, Laskay G, Takács Z, Poór P. 2013. Response of sorghum to abiotic stresses: A review. *Journal of agronomy and crop science* **199**(4): 264-274.
Tseng K-H, Chen C-L, Lin M-D, Chang K-H, Tsuang B-J. 2009. Vertical profile of ozone and accompanying air pollutant concentrations observed at a downwind foothill site of industrial and urban areas. *Aerosol and Air Quality Research* **9**(4): 421-434.
Utsumi Y, Utsumi C, Tanaka M, Ha CV, Takahashi S, Matsui A, Matsunaga TM, Matsunaga S, Kanno Y, Seo M. 2019. Acetic acid treatment enhances drought avoidance in cassava (Manihot esculenta Crantz). *Frontiers in plant science* **10**: 521.
Vartapetian BB, Jackson MB. 1997. Plant adaptations to anaerobic stress. *Annals of Botany* **79**(suppl_1): 3-20.
Vartapetian BB, Pulli S, Fagerstedt K. 1997. Plant response and adaptation to anaerobiosis. *Annals of Botany* **79**: R1-R1.
Ventura I, Brunello L, Iacopino S, Valeri MC, Novi G, Dornbusch T, Perata P, Loreti E. 2020. Arabidopsis phenotyping reveals the importance of alcohol dehydrogenase and pyruvate decarboxylase for aerobic plant growth. *Scientific reports* **10**(1): 1-14.
Voss I, Sunil B, Scheibe R, Raghavendra A. 2013. Emerging concept for the role of photorespiration as an important part of abiotic stress response. *Plant Biology* **15**(4): 713-722.
Wei Y, Lin M, Oliver DJ, Schnable PS. 2009. The roles of aldehyde dehydrogenases (ALDHs) in the PDH bypass of Arabidopsis. *BMC biochemistry* **10**(1): 1-10.
Weits DA, Kunkowska AB, Kamps NC, Portz KM, Packbier NK, Nemec Venza Z, Gaillochet C, Lohmann JU, Pedersen O, van Dongen JT. 2019. An apical hypoxic niche sets the pace of shoot meristem activity. *Nature* **569**(7758): 714-717.
Weits DA, van Dongen JT, Licausi F. 2021. Molecular oxygen as a signaling component in plant development. *New phytologist* **229**(1): 24-35.
Xia A, Jacob A, Tabassum MR, Herrmann C, Murphy JD. 2016. Production of hydrogen, ethanol and volatile fatty acids through co-fermentation of macro- and micro-algae. *Bioresource technology* **205**: 118-125.

Xiong G, Cheng K, Pauly M. 2013. Xylan O-acetylation impacts xylem development and enzymatic recalcitrance as indicated by the Arabidopsis mutant tbl29. *Molecular Plant* **6**(4): 1373-1375.

Yu Q, Tang C, Kuo J. 2000. A critical review on methods to measure apoplastic pH in plants. *Plant and soil* **219**(1): 29-40.

Zabalza A, Van Dongen JT, Froehlich A, Oliver SN, Faix B, Gupta KJ, Schmalzlin E, Igal M, Orcaray L, Royuela M. 2009. Regulation of respiration and fermentation to control the plant internal oxygen concentration. *Plant Physiology* **149**(2): 1087-1098.

Zhang X, Chua VP, Cheong H-F. 2015. Hydrodynamics in mangrove prop roots and their physical properties. *Journal of hydro-environment research* **9**(2): 281-294.

Zhao J, Hartmann H, Trumbore S, Ziegler W, Zhang Y. 2013. High temperature causes negative whole-plant carbon balance under mild drought. *New phytologist* **200**(2): 330-339.

Zhou S, Han Y, Li B. 2016. Simultaneous detection of ethanol, ether and acetone by mid-infrared cavity ring-down spectroscopy at 3.8 μm. *Applied Physics B* **122**(7): 1-8.