

# Gas Phase Measurements of Pyruvic Acid and Its Volatile Metabolites

KOLBY J. JARDINE,<sup>\*,†</sup> EVAN D. SOMMER,<sup>†</sup> SCOTT R. SALESKA,<sup>‡</sup> TRAVIS E. HUXMAN,<sup>‡</sup> PETER C. HARLEY,<sup>§</sup> AND LEIF ABRELL<sup>||</sup>

The University of Arizona-Biosphere 2, Tucson, Arizona 85738, Department of Ecology and Evolutionary Biology, Departments of Chemistry & Biochemistry, and Soil, Water & Environmental Science, and National Center for Atmospheric Research

Received November 22, 2009. Revised manuscript received February 12, 2010. Accepted February 18, 2010.

Pyruvic acid, central to leaf carbon metabolism, is a precursor of many volatile organic compounds (VOCs) that impact air quality and climate. Although the pathways involved in the production of isoprenoids are well-known, those of several oxygenated VOCs remain uncertain. We present concentration and flux measurements of pyruvic acid and other VOCs within the tropical rainforest (TRF) biome at Biosphere 2. Pyruvic acid concentrations varied diurnally with midday maxima up to 15 ppbv, perhaps due to enhanced production rates and suppression of mitochondrial respiration in the light. Branch fluxes and ambient concentrations of pyruvic acid correlated with those of acetone, acetaldehyde, ethanol, acetic acid, isoprene, monoterpenes, and sesquiterpenes. While pyruvic acid is a known substrate for isoprenoid synthesis, this correlation suggests that the oxygenated VOCs may also derive from pyruvic acid, an idea supported by leaf feeding experiments with sodium pyruvate which resulted in large enhancements in emissions of both isoprenoids and oxygenated VOCs. While feeding with sodium pyruvate-2-<sup>13</sup>C resulted in large emissions of both <sup>13</sup>C-labeled isoprenoids and oxygenated VOCs, feeding with sodium pyruvate-1-<sup>13</sup>C resulted in only <sup>13</sup>C-labeled isoprenoids. This suggests that acetaldehyde, ethanol, and acetic acid are produced from pyruvic acid via the pyruvate dehydrogenase (PDH) bypass system (in which the 1-C carbon of pyruvic acid is lost as CO<sub>2</sub>) and that acetone is also derived from the decarboxylation of pyruvic acid.

## Introduction

Pyruvic acid lies at the heart of primary carbon and energy metabolism in plants, acting at the intersection of several key metabolic pathways (Figure 1). It is the end product of glycolysis and is also produced from pathway intermediates such as glyceraldehyde-3-phosphate from the Calvin cycle. It is converted to acetyl CoA by the action of pyruvate dehydrogenase (PDH) during aerobic respiration in mitochondria and fatty acid biosynthesis in plastids. Alternatively, it can be converted in the cytosol to acetaldehyde by pyruvate decarboxylase (PDC) and subsequently reduced to ethanol

or converted to acetyl CoA via the PDH bypass. The PDH bypass involves oxidation of acetaldehyde by aldehyde dehydrogenase (ALDH), forming acetic acid which is then activated to acetyl CoA by acetyl CoA synthetase (ACS). ACS activity is considered to be mainly localized to plastids where it supplies acetyl CoA for fatty acid biosynthesis (1). However, a cytosolic ACS could supply acetyl CoA for fatty acid elongation reactions and the biosynthesis of isoprenoids via the mevalonic acid (MVA) pathway. Evidence for the activity of the PDH bypass in plants under aerobic conditions has been obtained from pollen in which radiolabeled ethanol was converted into a variety of compounds including amino acids and lipids (2). In leaves, the potential for dedicating pyruvic acid to the PDH bypass was demonstrated from the purification of a high affinity PDC (3). Recently, direct evidence for the presence of PDH bypass for acetyl-CoA biosynthesis was obtained by feeding radiolabeled ethanol to *Arabidopsis* seedlings. Relative to wild type seedlings, ALDH mutants had greatly reduced incorporation rates of radiolabeled ethanol into fatty acids (4). However, direct measurements of the production of PDH bypass intermediates in leaves under normal aerobic conditions is lacking. In this study, we present new gas phase concentration and flux measurements of volatile intermediates derived from the PDH bypass from plants at the leaf, branch, and whole ecosystem scale.

Although acetone is emitted from all plant species studied to date and is one of the dominant VOCs released by many ecosystems (5), the metabolic origin of acetone in plants remains unclear (except in cyanogenic plants, which release both acetone and hydrogen cyanide in response to herbivory (6)). Attempts to demonstrate its production from acetoacetic acid, as has been shown in animals and bacteria, have generally failed (7). However, during <sup>13</sup>CO<sub>2</sub> labeling experiments on needles of Gray pine (*Pinus sabiniana*), acetone was released with all three carbons showing up to 50% labeling (8), demonstrating that acetone is produced, in part, from recently photoassimilated carbon. In this study, we investigated the possibility that volatile isoprenoids and oxygenated VOCs including acetone are produced as a result of pyruvic acid metabolism. Because 1-C from pyruvate is typically lost during decarboxylation, while the other 2 C's are used for production of downstream metabolites, we used pyruvate-1-<sup>13</sup>C and pyruvate-2-<sup>13</sup>C labeling experiments to identify and examine the cellular fate of volatile pyruvate products.

We also report the development of an online technique for the quantification of pyruvic acid concentrations in air based on proton transfer reaction mass spectrometry (PTR-MS) and thermal desorption-gas chromatography–mass spectrometry (TD-GC-MS). We demonstrate the utility of these techniques by measuring pyruvic acid concentrations in the atmosphere of the tropical rainforest (TRF) biome at Biosphere 2.

## Experimental Section

VOC concentration and flux measurements were made with a commercial high sensitivity PTR-MS instrument (IONICON Analytik, Innsbruck, Austria). The parent ions for each VOC studied were monitored with a 1 s dwell time and include *m/z* 45 (acetaldehyde), *m/z* 47 (ethanol), *m/z* 59 (acetone), *m/z* 61 (acetic acid), *m/z* 69 (isoprene), *m/z* 83 (C<sub>6</sub> aldehydes and alcohols), *m/z* 89 (pyruvic acid), *m/z* 137 (monoterpenes), and *m/z* 205 (sesquiterpenes). For natural abundance and <sup>13</sup>C-labeling studies on individual leaves, the ions corre-

\* Corresponding author phone: 520-838-6177; e-mail: jardine@email.arizona.edu.

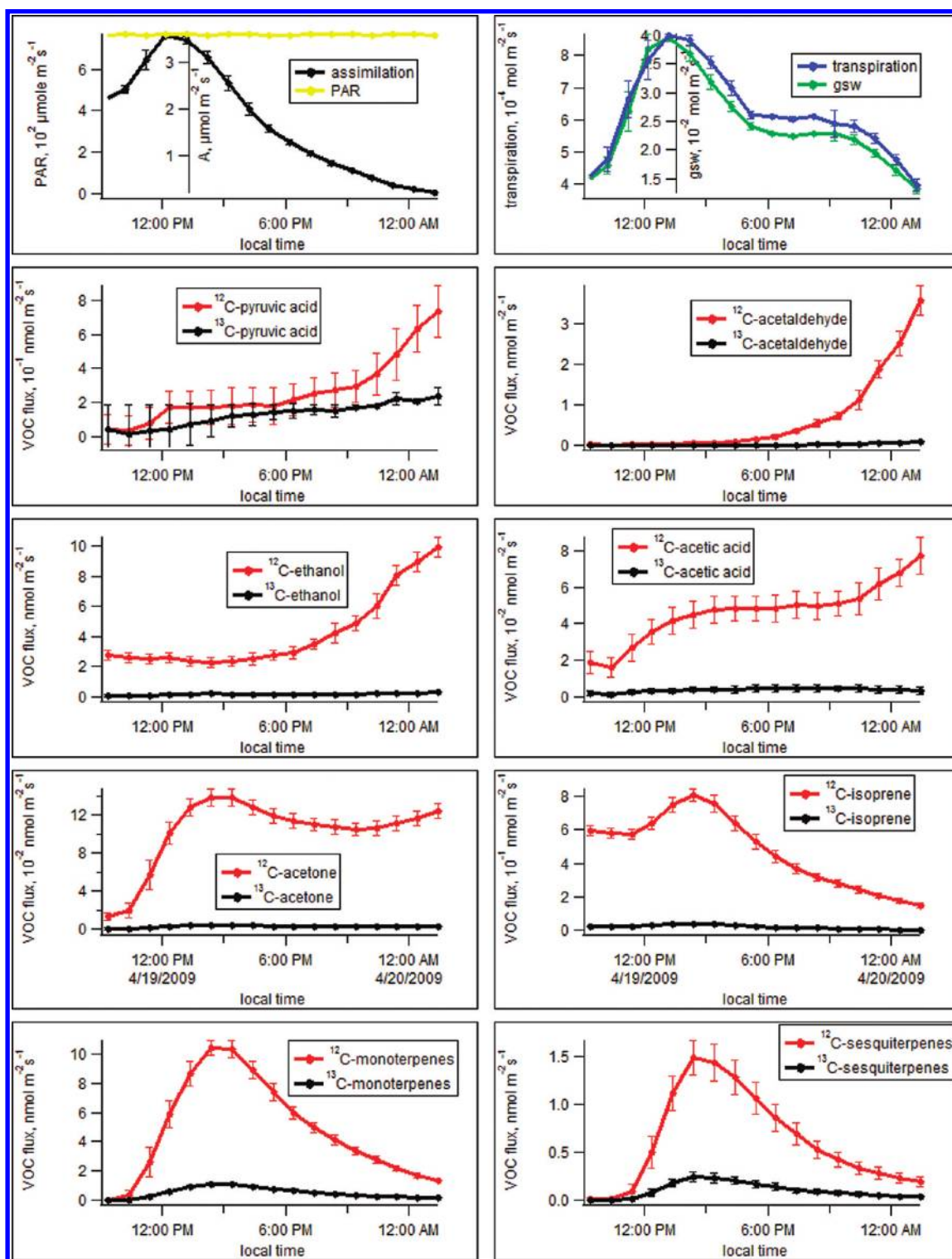
<sup>†</sup> The University of Arizona-Biosphere 2.

<sup>‡</sup> Department of Ecology and Evolutionary Biology.

<sup>§</sup> National Center for Atmospheric Research.

<sup>||</sup> Departments of Chemistry & Biochemistry, and Soil, Water, & Environmental Science.



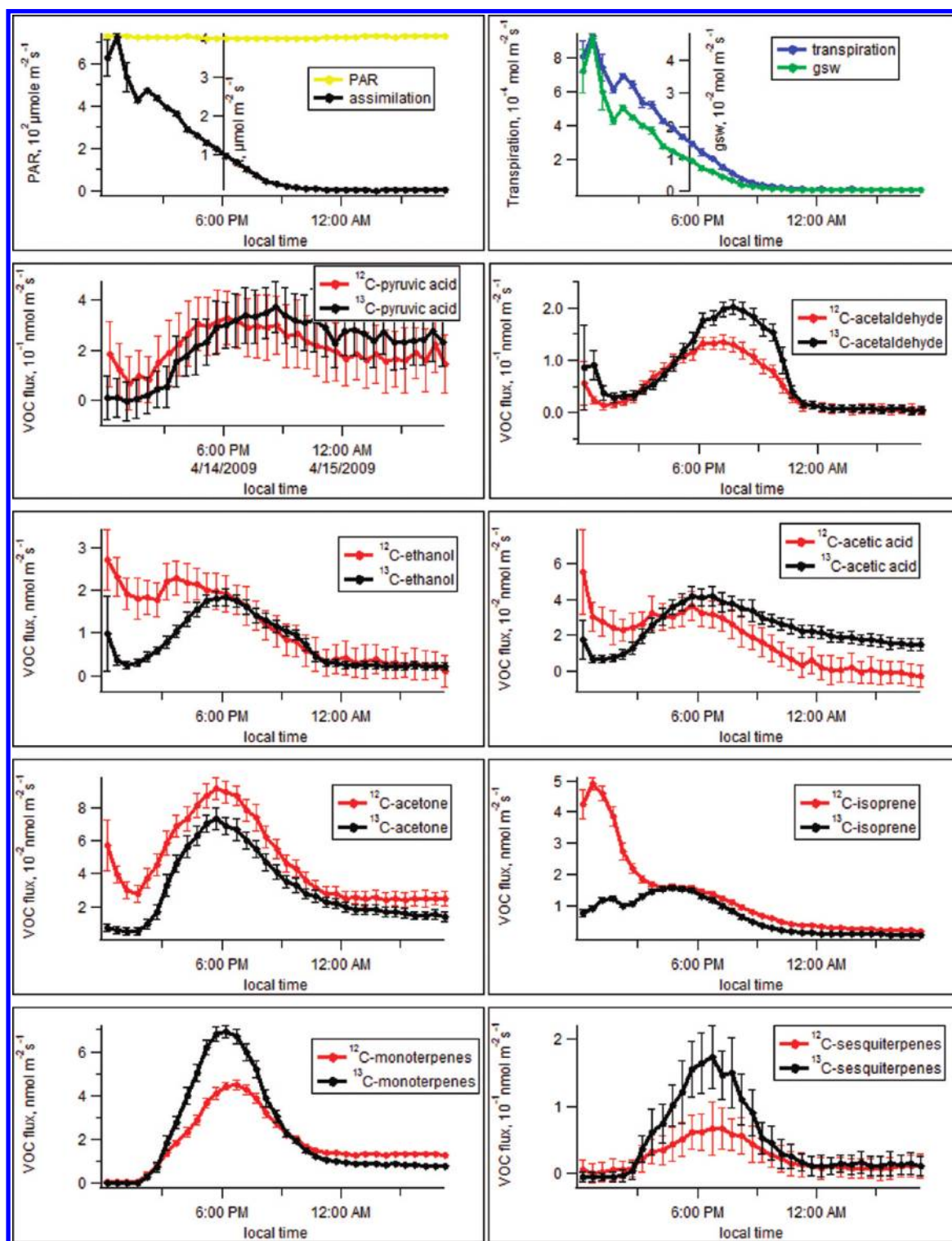


**FIGURE 2.** Emissions of VOCs and  $^{13}\text{C}$ -labeled VOCs from a detached *Mangifera indica* leaf placed in 44.7 mM sodium pyruvate. Photosynthetically active radiation (PAR) and rates of net carbon assimilation, transpiration, and stomatal conductance to water (gsw) are also shown.

the maximum acetaldehyde, ethanol, acetic acid, acetone, monoterpene, and sesquiterpene emission rates were 3.6, 10.0, 0.1, 0.1, 10.4, and 1.5  $\text{nmol m}^{-2} \text{s}^{-1}$ , respectively. These represent emission rates 2–120 times greater than those of leaves in distilled water (Figure 2 and Supporting Information, Figure S5). In repeated experiments with different leaves, considerable variability in the VOC emission rates was observed, but sodium pyruvate feeding always resulted in the enhancement of VOC emissions relative to distilled water controls. When data from the first hour were omitted (due

to the initial emission burst), pyruvic acid emissions significantly correlated with acetaldehyde, acetic acid, and ethanol emissions, with linear correlation coefficients of 0.98, 0.95, and 0.97, respectively (Supporting Information, Figure S6). Correlations between pyruvic acid and acetone were weaker (correlation coefficient of 0.61) suggesting that there may be other important controls on acetone emissions.

Prior to the arrival of exogenous pyruvate- $2\text{-}^{13}\text{C}$ ,  $^{12}\text{C}$ -isoprene emissions from leaves in sodium pyruvate- $2\text{-}^{13}\text{C}$  generally tracked assimilation rates. During the decline in



**FIGURE 3.** Emissions of VOCs and  $^{13}\text{C}$ -labeled VOCs from a detached *Mangifera indica* leaf placed in 44.7 mM sodium pyruvate-2- $^{13}\text{C}$ . Photosynthetically active radiation (PAR) and rates of net carbon assimilation, transpiration, and stomatal conductance to water (gs<sub>w</sub>) are also shown.

assimilation rate and  $^{12}\text{C}$ -isoprene emissions, a pulse in  $^{13}\text{C}$ -isoprene emissions occurred as the exogenous pyruvate-2- $^{13}\text{C}$  arrived in the leaf. At this time, emission rates of all  $^{13}\text{C}$ -labeled VOCs including  $^{13}\text{C}$ -pyruvic acid were strongly enhanced, exceeding in most cases the  $^{12}\text{C}$ -isotopologue emission rates (Figure 3). The measured stable carbon isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) were strongly enriched in  $^{13}\text{C}$  derived from the sodium pyruvate-2- $^{13}\text{C}$ . At the peak in  $^{13}\text{C}$ -VOC emission rates, isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) for sesquiterpenes, monoterpenes, isoprene, acetone, acetaldehyde, acetic acid, and

ethanol were 266%, 157%, 100%, 78%, 154%, 124%, and 100%, respectively.

In the single experiment with 44.7 mM sodium pyruvate-1- $^{13}\text{C}$ , small emissions of  $^{13}\text{C}$ -labeled pyruvic acid from the leaf were observed after several hours (Figure 4). Significant emissions of  $^{13}\text{C}$ -labeled isoprenoids occurred, resulting in increased stable carbon isotope ratios relative to natural abundance sources: 28.8%, 59.5%, and 32.9% compared to 5.5%, 11.1%, 16.6% for isoprene, monoterpenes, and sesquiterpenes, respectively. However, significant emissions of

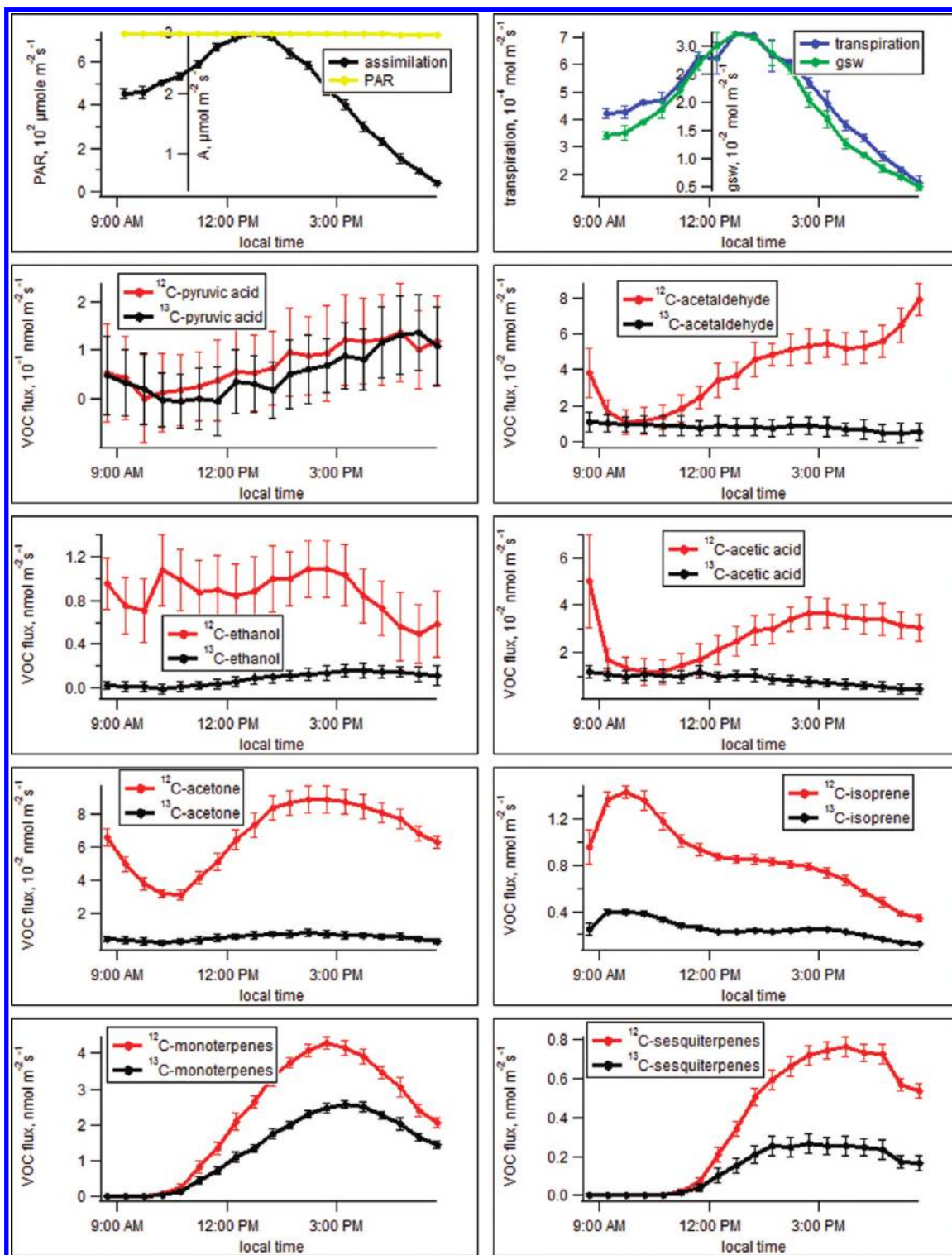


FIGURE 4. Emissions of VOCs and  $^{13}\text{C}$ -labeled VOCs from a detached *Mangifera indica* leaf placed in 44.7 mM sodium pyruvate- $1\text{-}^{13}\text{C}$ . Photosynthetically active radiation (PAR) and rates of net carbon assimilation, transpiration, and stomatal conductance to water (gsw) are also shown.

$^{13}\text{C}$ -labeled acetaldehyde, ethanol, acetic acid, and acetone emissions were not observed.

## Discussion

In this study, the fact that pyruvic acid is a precursor in the biosynthesis of volatile isoprenoids and several oxygenated VOCs is demonstrated by the increase in emissions upon pyruvate feeding and, more importantly, the appearance of  $^{13}\text{C}$ -label in these VOCs from the 2-C of pyruvate (Figure 3).

Oxygenated VOCs may be produced from pyruvic acid by the PDH bypass in the cytosol, while pyruvic acid and isopentenyl pyrophosphate (IPP) can be transported into the chloroplast and feed directly into the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway for isoprene and monoterpene production (Figure 1). The bias in  $^{13}\text{C}$ -monoterpene production over  $^{13}\text{C}$ -isoprene may reflect the fact that terpene synthases have a greater affinity for dimethylallyl pyrophosphate (DMAPP) than does isoprene synthase (13, 14).

Acetyl CoA for sesquiterpene biosynthesis in the cytosol may derive from the exchange of intermediates such as IPP between the cytosol and chloroplasts or possibly from acetate produced by the PDH bypass.

The lack of  $^{13}\text{C}$  label in oxygenated VOCs following sodium pyruvate- $1\text{-}^{13}\text{C}$  leaf feeding suggests the involvement of the PDH bypass; the carbon lost as  $\text{CO}_2$  in the PDC reaction is that in the 1-C position of pyruvate. The absence of label in acetone suggests that acetone production in plants, like that of acetaldehyde, acetic acid, and ethanol, is associated with the decarboxylation of pyruvate. Although the mechanism for acetone production from pyruvate in leaves needs to be explored in more detail, one possible mechanism is the decarboxylation of the 1-C of pyruvate accompanied by a methyl transferase reaction. Surprisingly, emissions of  $^{13}\text{C}$ -labeled isoprene, monoterpenes, and sesquiterpenes occurred at a much higher rate than expected for natural abundance carbon sources (Figure 4). One possible explanation for this is that a portion of the  $^{13}\text{C}$ -label released from pyruvate- $1^{13}\text{C}$  as  $^{13}\text{CO}_2$  is reassimilated by the Calvin cycle and subsequently incorporated into isoprene and monoterpenes via the plastidic MEP pathway. Because sesquiterpenes derive from the MVA pathway in the cytosol, incorporation of label into sesquiterpenes may involve exchange of labeled IPP between cellular compartments. Therefore, our observations support previous studies demonstrating that volatile isoprenoids are derived from two pathways in plants, the MVA pathway in the cytosol and the plastidic MEP pathway, and linked by the exchange of common intermediates such as IPP and pyruvic acid (15).

Although the affinity of PDH for pyruvic acid greatly exceeds that of PDC (16), should pyruvic acid concentrations become elevated and/or mitochondrial PDH become less active in leaves, the PDC reaction may be able to compete with the PDH reaction. In the light, carbon fixation in chloroplasts provides triose-phosphate to the cytosol, including glyceraldehyde-3-phosphate which can then be converted to pyruvic acid by glycolysis. It is generally accepted that day respiration (mitochondrial nonphotorespiratory  $\text{CO}_2$  production) in C3 leaves is suppressed relative to respiration in the dark (17). This is partially attributed to the light induced inhibition of mitochondrial PDH (18). Although the plastidial PDH may be more active in the light than in the dark (leading to increased fatty acid biosynthesis), photosynthesis and reduced day respiration may lead to the accumulation of primary photosynthetic products like pyruvic acid (19). As pyruvic acid accumulates in the light, one consequence could be increased production rates of volatile isoprenoids and oxygenated VOCs. This agrees with many observations that the emissions of these compounds from plants are light and temperature dependent, a fact that has enabled the development of global biogenic VOC emission models (20). While light dependent emissions of both isoprene and monoterpene have been clearly demonstrated (21), the light dependence of sesquiterpene (22) and oxygenated VOC (23) emissions remains uncertain. Although other important factors, including possible diurnal patterns in enzyme activities involved in their production (24), control the production of isoprenoids, our results suggest that the biosynthesis of volatile isoprenoids and some oxygenated VOCs (acetaldehyde, acetone, acetic acid, and ethanol) may demonstrate a light dependency at least in part due to the accumulation of pyruvic acid during photosynthesis. However, because there are other sources of pyruvic acid, including glycolysis, that are not light dependent, a complete dependence of the emission rates on light is not expected. This model is supported by previous studies that demonstrated a diurnal variation of pyruvic acid concentrations in leaves. For example, pyruvic acid concentrations in poplar leaves peak

during midday (25). Also, with extended illumination of leaves, the concentration of pyruvic acid continues to increase (26).

Natural emissions of VOCs from the terrestrial biosphere are estimated to be an order of magnitude larger than anthropogenic emissions globally with volatile isoprenoids and oxygenated VOCs as the dominant species (20). The results from this study may aid in the development of mechanistic predictive models for the emissions of VOCs from the biosphere to the atmosphere for use in air quality and climate models.

## Acknowledgments

This research was supported by a joint postdoctoral fellowship program between Biosphere 2 Earth Science division and the National Center for Atmospheric Research. We would like to kindly thank John Adams and Joey Martinez at Biosphere 2 for providing much of the required infrastructure needed in this study. Instrumentation support is acknowledged from NSF (CHE 0216226) and from Edward P. Bass.

## Supporting Information Available

More information on the technical details of the PTR-MS and TD-GC-MS configuration, the setup for ambient air concentration and branch flux measurements in the TRF, leaf feeding experiments, and additional figures including Figure S1: diurnal concentration patterns of VOCs near the top of the TRF biome (26 m), Figure S2: bar plot of the dominant ions produced during electron-impact ionization mass spectra of pyruvic acid, Figure S3: pyruvic acid sample peaks measured with TD-GC-MS, Figure S4: diurnal patterns of branch level emission rates of VOCs from a *Mangifera indica* tree in the TRF biome, Figure S5: emissions of VOCs and  $^{13}\text{C}$ -labeled VOCs from a detached *Mangifera indica* leaf placed in distilled water, and Figure S6: linear correlation between VOC and pyruvic acid emissions during sodium pyruvate leaf feeding. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## Literature Cited

- Oliver, D. J.; Nikolau, B. J.; Wurtele, E. S. Acetyl-CoA-Life at the metabolic nexus. *Plant Sci.* **2009**, *176* (5), 597–601.
- Mellema, S.; Eichenberger, W.; Rawlyer, A.; Suter, M.; Tadege, M.; Kuhlmeier, C. The ethanolic fermentation pathway supports respiration and lipid biosynthesis in tobacco pollen. *Plant J.* **2002**, *30* (3), 329–336.
- Nguyen, T.; Drotar, A. M.; Monson, R. K.; Fall, R. A high affinity pyruvate decarboxylase is present in cottonwood leaf veins and petioles: A second source of leaf acetaldehyde emission. *Phytochemistry* **2009**, *70* (10), 1217–1221.
- Wei, Y.; Lin, M.; Oliver, D. J.; Schnable, P. S. The roles of aldehyde dehydrogenases (ALDHs) in the PDH bypass of Arabidopsis. *BMC Biochem.* **2009**, *10*, 7.
- Karl, T.; Potosnak, M.; Guenther, A.; Clark, D.; Walker, J.; Herrick, J. D.; Geron, C. Exchange processes of volatile organic compounds above a tropical rain forest: Implications for modeling tropospheric chemistry above dense vegetation. *J. Geophys. Res., [Atmos.]* **2004**, *109*, D18306. 10.1029/2004JD004738.
- Fall, R.; Custer, T. G.; Kato, S.; Bierbaum, V. M. New Directions: The biogenic acetone-HCN connection. *Atmos. Environ.* **2001**, *35* (9), 1713–1714.
- Fall, R. Abundant oxygenates in the atmosphere: A biochemical perspective. *Chem. Rev.* **2003**, *103* (12), 4941–4951.
- Curtis, A. J. Ph.D. Dissertation, University of Colorado, 2002.
- Kim, S.; Karl, T.; Helmig, D.; Daly, R.; Rasmussen, R.; Guenther, A. Measurement of atmospheric sesquiterpenes by proton transfer reaction-mass spectrometry (PTR-MS). *Atmos. Meas. Tech.* **2009**, *2*, 99–112.
- Talbot, R. W.; Andreae, M. O.; Berresheim, H.; Jacob, D. J.; Beecher, K. M. Sources and sinks of formic, acetic, and pyruvic acids over central Amazonia. 2. Wet season. *J. Geophys. Res., [Atmos.]* **1990**, *95* (D10), 16799–16811.
- Talbot, R. W.; Mosher, B. W.; Heikes, B. G.; Jacob, D. J.; Munger, J. W.; Daube, B. C.; Keene, W. C.; Maben, J. R.; Artz, R. S.

- Carboxylic-Acids in the Rural Continental Atmosphere over the Eastern United-States during the Shenandoah Cloud and Photochemistry Experiment. *J. Geophys. Res., [Atmos.]* **1995**, *100* (D5), 9335–9343.
- (12) Fall, R.; Karl, T.; Hansel, A.; Jordan, A.; Lindinger, W. Volatile organic compounds emitted after leaf wounding: On-line analysis by proton-transfer-reaction mass spectrometry. *J. Geophys. Res., [Atmos.]* **1999**, *104* (D13), 15963–15974.
- (13) Kampranis, S. C.; Ioannidis, D.; Purvis, A.; Mahrez, W.; Ninga, E.; Katerelos, N. A.; Anssour, S.; Dunwell, J. M.; Degenhardt, J.; Makris, A. M.; Goodenough, P. W.; Johnson, C. B. Rational conversion of substrate and product specificity in a *Salvia* monoterpene synthase: Structural insights into the evolution of terpene synthase function. *Plant Cell* **2007**, *19* (6), 1994–2005.
- (14) Datukishvili, N. T.; Tarkhnishvili, G. M.; Mikeladze, D. G.; Beridze, T. G.; Sanadze, G. A. Isolation and purification of protein responsible for the conversion of dimethylallylpyrophosphate from poplar leaves into isoprene. *Russ. J. Plant Physiol.* **2001**, *48* (2), 222–225.
- (15) Rohmer, M. From molecular fossils of bacterial hopanoids to the formation of isoprene units: discovery and elucidation of the methylerythritol phosphate pathway. *Lipids* **2008**, *43* (12), 1095–107.
- (16) Tadege, M.; Dupuis, I.; Kuhlemeier, C. Ethanol fermentation: new functions for an old pathway. *Trends Plant Sci.* **1999**, *4* (8), 320–325.
- (17) Atkin, O. K.; Evans, J. R.; Siebke, K. Relationship between the inhibition of leaf respiration by light and enhancement of leaf dark respiration following light treatment. *Aust. J. Plant Physiol.* **1998**, *25* (4), 437–443.
- (18) Tovar-Mendez, A.; Miernyk, J. A.; Randall, D. D. Regulation of pyruvate dehydrogenase complex activity in plant cells. *Eur. J. Biochem.* **2003**, *270* (6), 1043–1049.
- (19) Raghavendra, A. S.; Padmasree, K.; Saradadevi, K. Interdependence of Photosynthesis and Respiration in Plant-Cells - Interactions between Chloroplasts and Mitochondria. *Plant Sci.* **1994**, *97* (1), 1–14.
- (20) Guenther, A.; Hewitt, C. N.; Erickson, D.; Fall, R.; Geron, C.; Graedel, T.; Harley, P.; Klinger, L.; Lerdau, M.; Mckay, W. A.; Pierce, T.; Scholes, B.; Steinbrecher, R.; Tallamraju, R.; Taylor, J.; Zimmerman, P. A Global-Model of Natural Volatile Organic-Compound Emissions. *J. Geophys. Res., [Atmos.]* **1995**, *100* (D5), 8873–8892.
- (21) Lerdau, M.; Gray, D. Ecology and evolution of light-dependent and light-independent phytogetic volatile organic carbon. *New Phytol.* **2003**, *157* (2), 199–211.
- (22) Duhl, T. R.; Helmig, D.; Guenther, A. Sesquiterpene emissions from vegetation: a review. *Biogeosciences* **2008**, *5* (3), 761–777.
- (23) Jardine, K.; Karl, T.; Harley, P.; Lerdau, M.; Mak, J.; Guenther, A. Plant physiological and environmental controls over the exchange of acetaldehyde between plants and the atmosphere. *Biogeosciences Discuss.* **2008**, *5*, 1–33.
- (24) Loivamaki, M.; Louis, S.; Cinege, G.; Zimmer, I.; Fischbach, R. J.; Schnitzler, J. P. Circadian rhythms of isoprene biosynthesis in grey poplar leaves. *Plant Physiol.* **2007**, *143* (1), 540–51.
- (25) Magel, E.; Mayrhofer, S.; Muller, A.; Zimmer, I.; Hampp, R.; Schnitzler, J. P. Photosynthesis and substrate supply for isoprene biosynthesis in poplar leaves. *Atmos. Environ.* **2006**, *40*, S138–S151.
- (26) Roeske, C. A.; Chollet, R. Role of metabolites in the reversible light activation of pyruvate, ortho-phosphate dikinase in *Zea mays* Mesophyll Cells. *In-Vivo. Plant Physiol.* **1989**, *90* (1), 330–337.

ES903544P