

Effect of drought on soil microbial metabolisms driving carbon allocation and volatile organic compound cycling in the tropical rainforest at Biosphere 2

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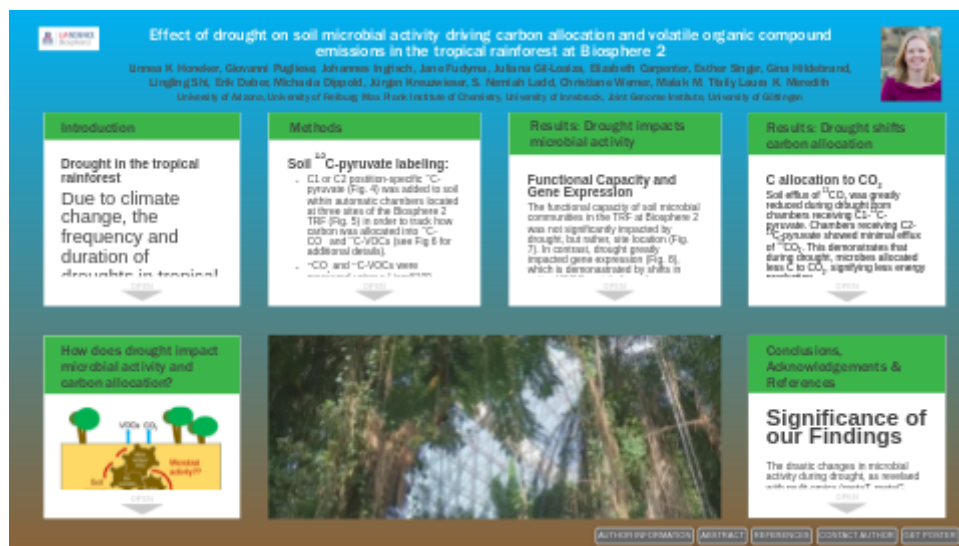
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Abstract

Droughts are occurring with increased frequency and duration in tropical rainforests due to climate change, having a significant impact on soil C dynamics. The role of microbes as drivers of changing C flow, particularly in relation to volatile organic compound (VOC) cycling, remains largely unknown. Here, we aimed to characterize microbial responses to drought using an integrative, multiple ‘omics approach, and hypothesized that microbial communities will adapt by altering their C allocation strategies. Specifically, during pre-drought, primary metabolic pathways will be more active with microbes using C towards growth, whereas during drought, microbes will divert C to secondary metabolite (including VOC) production in response to stress. To test this, we conducted an ecosystem-wide 66-day drought experiment in the tropical rainforest biome at Biosphere 2, a glass- and steel-enclosed facility near Tucson, AZ. To track carbon allocation by microbes, we injected C1 or C2 position-specific ¹³C-pyruvate solution into a 25 cm² region within a soil flux chamber collar (n=6 locations) and measured C isotope ratios of VOC and CO₂ emissions. Soil was collected at 0, 6, and 48 hours after pyruvate addition to examine responses in soil metatranscriptomics, metagenomics, and metabolomics (1H nuclear magnetic resonance [NMR] and Fourier-transform ion cyclotron resonance [FTICR]). Our results indicated that ¹³CO₂ (primarily emitted from C1-¹³C-pyruvate) fluxes decreased during drought, indicating diminished microbial activity. ¹³C-VOCs (primarily emitted from C2-¹³C-pyruvate) fluxes also differed between pre-drought and drought. Furthermore, drought-induced increases in activity of VOC-producing metabolic pathways, including acetate and acetone biosynthesis, were evident, as inferred from volatilome, metabolome, and metatranscriptome data. Overall, these results indicate that integration of multiple ‘omics datasets reveal specific impacts of drought on microbial activity affecting carbon flow in the tropical rainforest soil.

Effect of drought on soil microbial activity driving carbon allocation and volatile organic compound emissions in the tropical rainforest at Biosphere 2

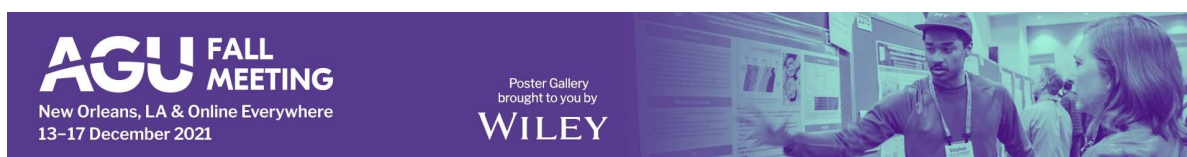


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PRESENTED AT:



INTRODUCTION

Drought in the tropical rainforest

Due to climate change, the frequency and duration of droughts in tropical rain forests (TRFs) are expected to increase, having a significant impact on soil carbon dynamics¹. The role of microbes as drivers of changing carbon flow, particularly in relation to metabolic pathways and volatile organic compounds (VOCs), remains largely unknown.

a.



b.



Fig 1. a) Biosphere 2, a glass- and steel-enclosed facility near Tucson, AZ, contains 5 biomes, including b) a tropical rainforest.

Biosphere 2 drought experiment

To examine how microbial activity, particularly in relation to carbon allocation, shifts during drought, we utilized the controllable conditions of the **glass and steel-enclosed TRF** at Biosphere 2 (Fig 1). Here we created a 66-day drought in order to study carbon cycling by plants and microbes before, during, and after drought (Water, Atmosphere, and Life Dynamics [WALD])². As part of this larger study, we performed multi-omics and traced carbon allocation by soil microbes using position-specific (C1 or C2) ¹³C-pyruvate labeling before and during drought. Our results will inform key processes in tropical soil carbon cycling.

METHODS

Soil ^{13}C -pyruvate labeling:

- C1 or C2 position-specific ^{13}C -pyruvate (Fig. 4) was added to soil within automatic chambers located at three sites of the Biosphere 2 TRF (Fig. 5) in order to track how carbon was allocated into $^{13}\text{C}\text{-CO}_2$ and $^{13}\text{C}\text{-VOCs}$ (see Fig 6 for additional details).
- $^{13}\text{CO}_2$ and $^{13}\text{C}\text{-VOCs}$ were measured using a Licor8100 coupled to Picarro G2201 and proton-transfer-reaction time-of-flight mass spectrometry (PTR-TOF-MS)



Fig 4. Diagram showing C1- and C2-position ^{13}C -labeling of pyruvate molecule.

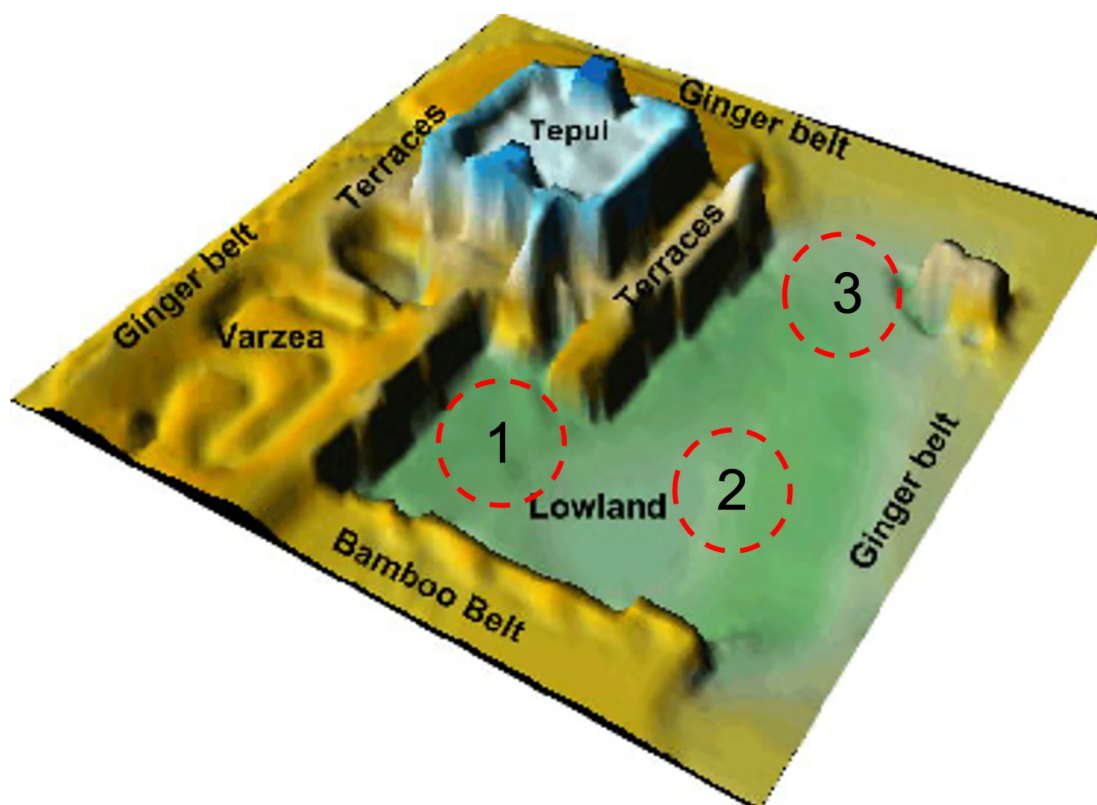


Fig 5. Topographical map of the Biosphere 2 TRF showing three sites where soil pyruvate labeling was performed.

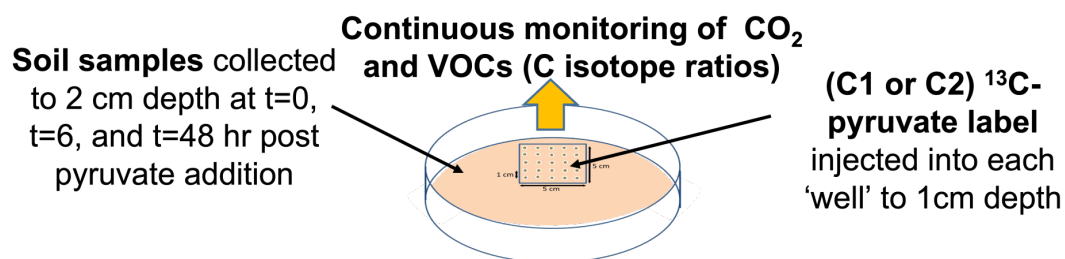


Fig. 6. Position specific ¹³C-pyruvate labeling (see Fig 4) experiment was performed in automatic soil chamber.

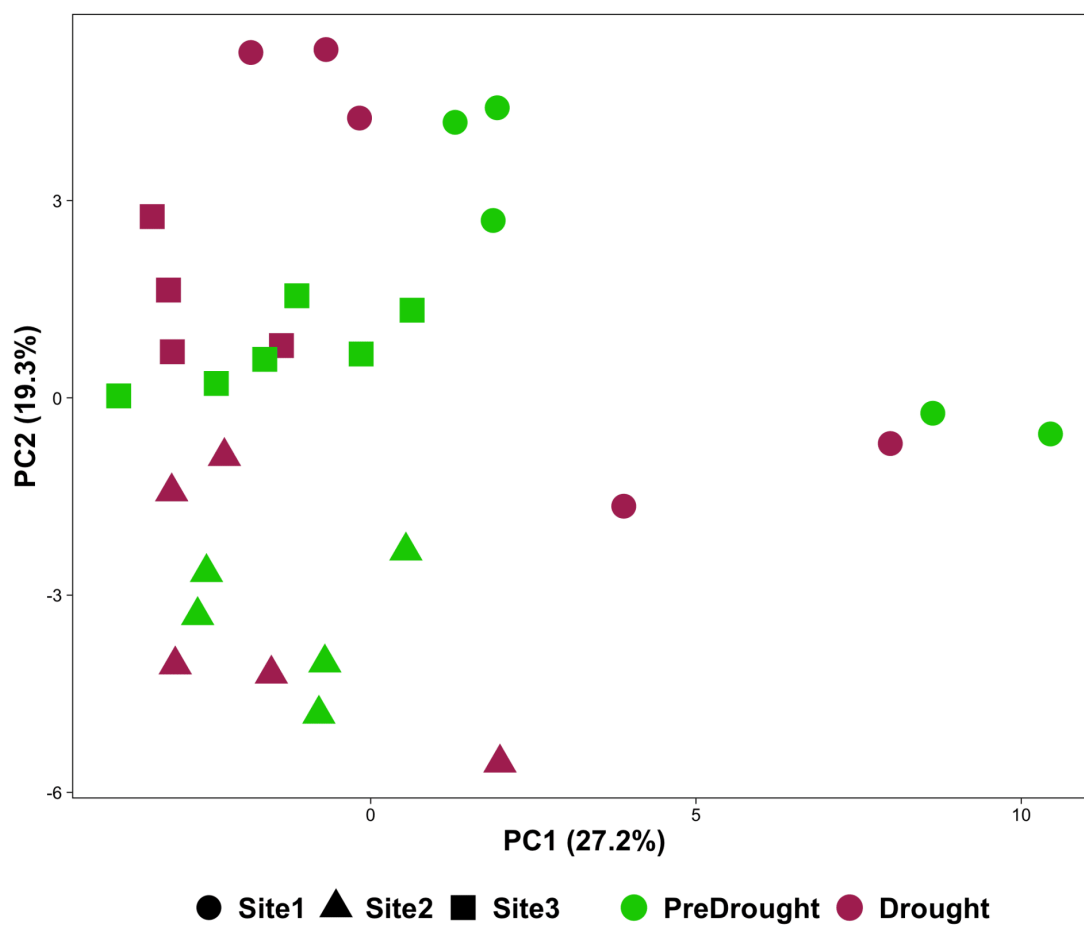
Soil sample analyses:

- **Metabolomics (metaB):** Water extractions were performed on soil and sent to PNNL (Pacific Northwest National Laboratory) for metabolomic analysis using Fourier-transform ion-coupled resonance mass spectrometry (FTICR-MS).
- **Metagenomics (metaG) and metatranscriptomics (metaT):** DNA and RNA were extracted and sent to Joint Genome Institute (JGI) for sequencing, assembly, annotation, and submission to Integrated Microbial Genomics (IMG).

RESULTS: DROUGHT IMPACTS MICROBIAL ACTIVITY

Functional Capacity and Gene Expression

The functional capacity of soil microbial communities in the TRF at Biosphere 2 was not significantly impacted by drought, but rather, site location (Fig. 7). In contrast, drought greatly impacted gene expression (Fig. 8), which is demonstrated by shifts in active KEGG metabolic pathways, particularly towards biosynthesis of secondary metabolites (Fig. 9).



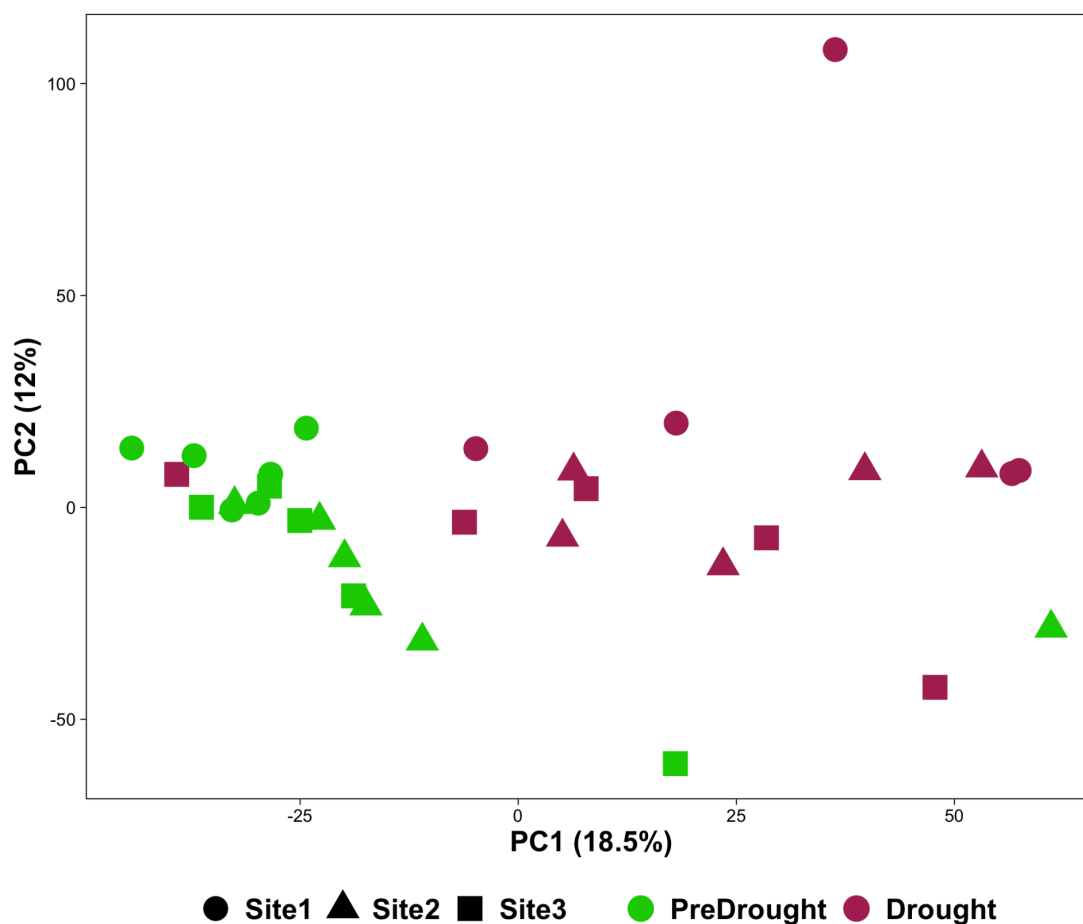


Fig. 8. PCA plot of metaT data showing that drought is the largest driver of gene expression.

KEGG pathway	PreDrought	Drought	
map01100 Metabolic pathways	83	140	↑ Pre-drought ↑ Drought
map01220 Degradation of aromatic compounds	8	0	
map00622 Xylene degradation	5	0	
map00362 Benzoate degradation	7	0	
map00330 Arginine and proline metabolism	6	0	
map00910 Nitrogen metabolism	4	0	
map00650 Butanoate metabolism	11	5	
map00270 Cysteine and methionine metabolism	9	4	
map00680 Methane metabolism	8	4	
map05111 Biofilm formation - <i>Vibrio cholerae</i>	8	0	
map02025 Biofilm formation - <i>Pseudomonas aeruginosa</i>	10	3	
map02040 Flagellar assembly	6	0	
map02020 Two-component system	24	7	
map02024 Quorum sensing	11	4	
map03070 Bacterial secretion system	4	0	
map00230 Purine metabolism	5	11	
map00860 Porphyrin and chlorophyll metabolism	0	23	
map00760 Nicotinate and nicotinamide metabolism	0	6	
map00564 Glycerophospholipid metabolism	0	4	
map00520 Amino sugar and nucleotide sugar metabolism	0	7	
map00500 Starch and sucrose metabolism	0	10	
map00450 Selenocompound metabolism	0	5	
map00340 Histidine metabolism	0	7	
map00240 Pyrimidine metabolism	0	5	
map00051 Fructose and mannose metabolism	0	7	
map01230 Biosynthesis of amino acids	5	19	
map00540 Lipopolysaccharide biosynthesis	0	6	
map00400 Phenylalanine, tyrosine and tryptophan biosynthesis	0	7	
map00970 Aminoacyl-tRNA biosynthesis	0	4	
map00541 O-Antigen nucleotide sugar biosynthesis	0	6	
map01110 Biosynthesis of secondary metabolites	22	78	
map01240 Biosynthesis of cofactors	8	27	
map00900 Terpenoid backbone biosynthesis	0	4	
map00906 Carotenoid biosynthesis	0	5	
map00790 Folate biosynthesis	0	4	

Substrate/nutrient metabolism

Biosynthesis: biomass

Others: Environment sensory and communication

Substrate/nutrient metabolism

Biosynthesis: biomass

Biosynthesis: products

Fig. 9. Kegg orthology (KO) functional groups were mapped to KEGG pathways. Results show a distinct impact of drought on active microbial metabolic pathways.

Metabolomics - secondary metabolites

MetaB data, as measured using FTICR, also revealed drought to be a major driver of soil metabolic composition, supporting a drought-induced shift in microbial activity. During drought, compound classes shifted compared to pre-drought; lipids and proteins decreased while lignins, tannins, and condensed hydrocarbons increased (Fig. 10). After mapping metabolites to KEGG pathways, it was evident that biosynthesis of phenylpropanoid was upregulated during drought, a pathway which includes lignin production. Increased lignins during drought could also be due to microbial depletion of more easily degradable compounds, leaving lignin, a more recalcitrant compound, behind. Decreased lipids during drought could signify less microbial biomass.

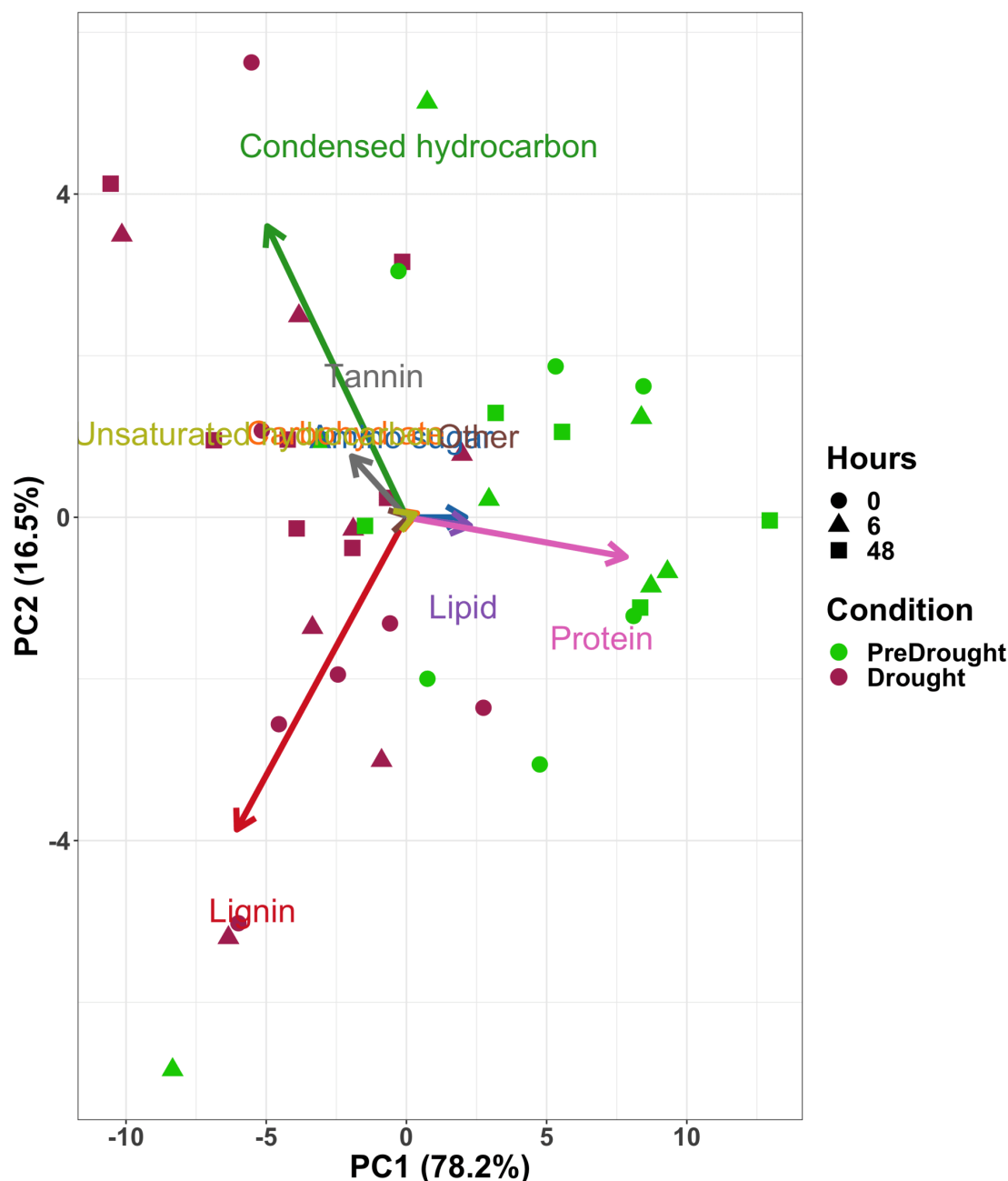


Fig. 10. PCA plot of metaB data showing that drought is a major driver of metabolomic profiles, an indicator of microbial activity.

RESULTS: DROUGHT SHIFTS CARBON ALLOCATION

C allocation to CO₂

Soil efflux of ¹³CO₂ was greatly reduced during drought from chambers receiving C1-¹³C-pyruvate. Chambers receiving C2-¹³C-pyruvate showed minimal efflux of ¹³CO₂. This demonstrates that during drought, microbes allocated less C to CO₂, signifying less energy production.

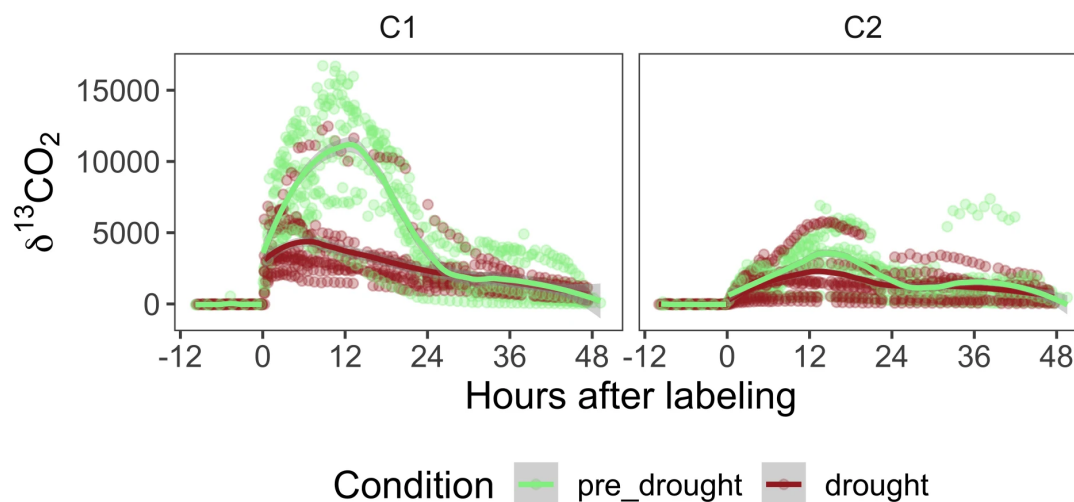


Fig. 11. Soil efflux of ¹³CO₂ over time post C1-¹³C-pyruvate or C2-¹³C-pyruvate labeling during pre-drought and drought conditions. Drought induced a decrease in C allocation to CO₂.

C allocation to VOCs

¹³C-VOC fluxes for all compounds with detectable ¹³C-enrichment (acetone, acetate, and C₄H₆O₂ [Diacyetyl], and ethylene glycol) were greater during drought from chambers receiving C2-¹³C-pyruvate (Fig. 12). This signifies that microbes allocated a greater amount of C to VOC production during drought. Furthermore, acetate and acetone are fermentation products, indicating that fermentation processes may be stimulated during drought, perhaps in anaerobic micro-sites. Here we will focus on acetate and acetone.

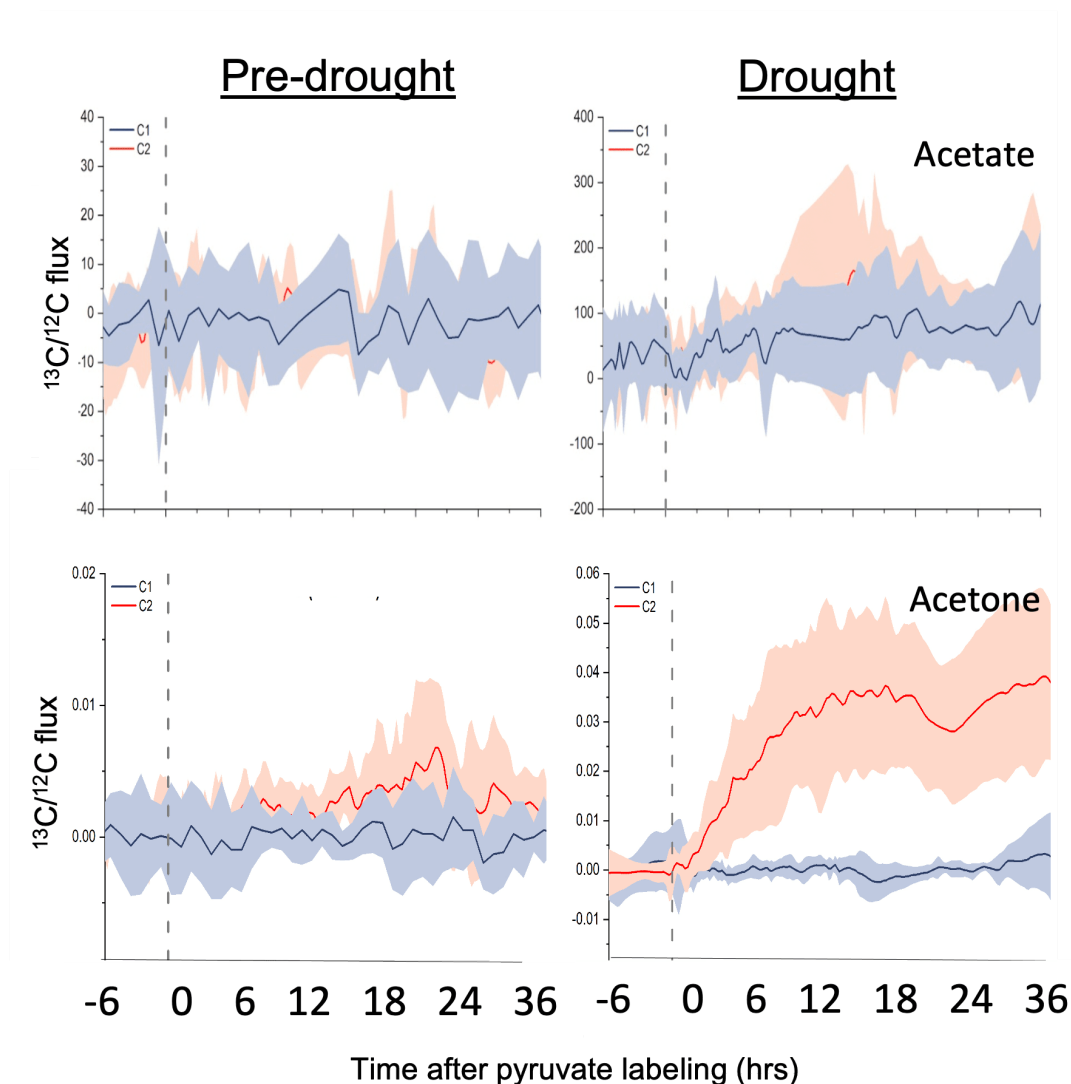


Fig. 12. ^{13}C -VOC fluxes for VOCs with detectable ^{13}C -enrichment from 6 hours before to 36 hours after C1- or C2- ^{13}C -pyruvate addition.

Active pyruvate metabolic pathways

To determine which metabolic pathways were active in the production of acetate and acetone, gene expression (metaT) data was analyzed (Fig. 13).

For acetate production, both pyruvate dehydrogenase (PDH) - quinone and pyruvate oxidase (PO) genes were upregulated during drought (Fig. 13). While acetate can be a fermentation by-product, PDH-quinone and PO-mediated reactions do not directly produce energy and can occur aerobically. During stationary growth phase, *E. coli* upregulate PDH-quinone³. In *Cornebacterium*, PDH-quinone is upregulated in conditions where readily available carbon sources, such as glucose, are low⁴. These examples show that PDH-quinone is upregulated during times of low growth and resource limitations, similar to conditions found during drought, however, the exact purpose for PDH-quinone remains elusive. A third pathway to acetate is also possible, via acetyl-CoA and acetaldehyde (fermentation), however, there were no upregulation

of genes during drought along this pathway. In conclusion, drought induced non-fermentative pathways of acetate biosynthesis may serve some protective services to increase microbial community survival during stressed conditions.

For acetone production, there were no clear patterns in upregulation of genes along the pathway that was identified leading from pyruvate to acetone (Fig. 13). This indicates that either acetone was being rapidly metabolized to downstream products, or there is an uncharacterized pathway from pyruvate to acetone. We are currently doing further analysis to determine the microbial pathway for acetone production.

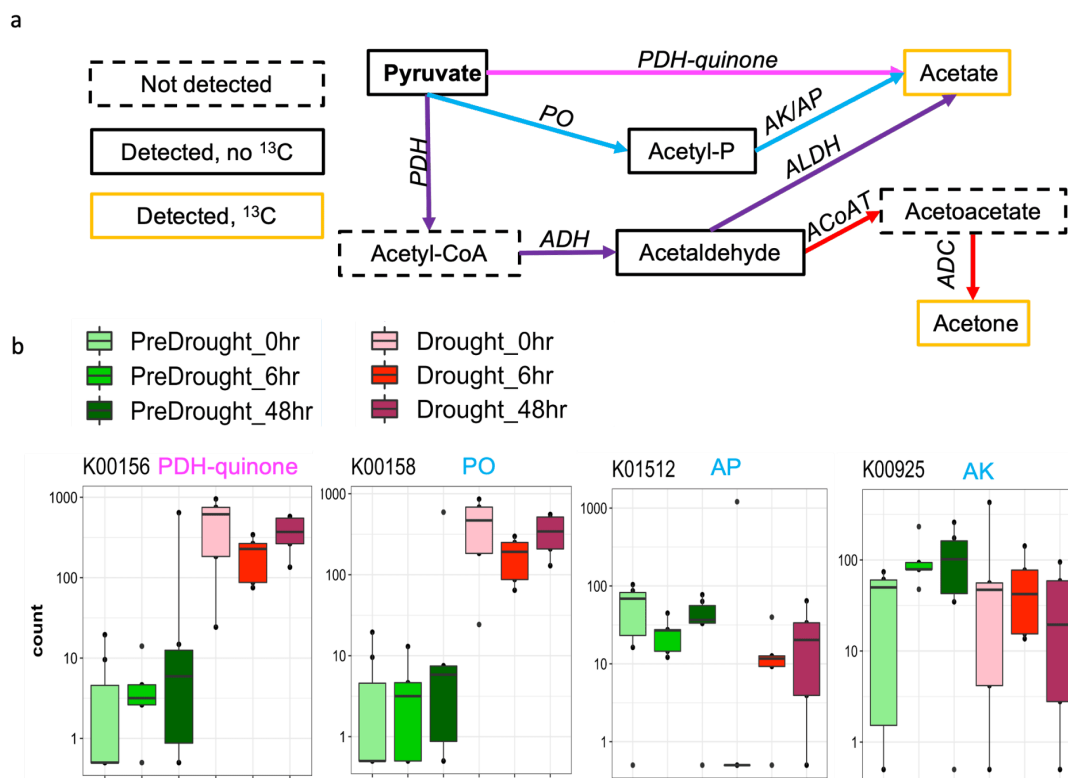


Fig. 13. a) Potential metabolic pathways where C from pyruvate can be transferred to acetate and acetone. Different pathways to acetate production are highlighted in pink, blue, and purple, and to acetone production in red and purple (PDH and ADH only). b) Gene expression along pathways to acetate production. Both pyruvate dehydrogenase (PDH) - quinone and pyruvate oxidase (PO) are upregulated during drought. AK, acetate kinase; AP, acetylphosphatase; ADH, acetylaldehyde dehydrogenase; ALDH, aldehyde dehydrogenase; ACoAH, acetyl-CoA hydrolase; ADC, acetoacetate decarboxylase

HOW DOES DROUGHT IMPACT MICROBIAL ACTIVITY AND CARBON ALLOCATION?

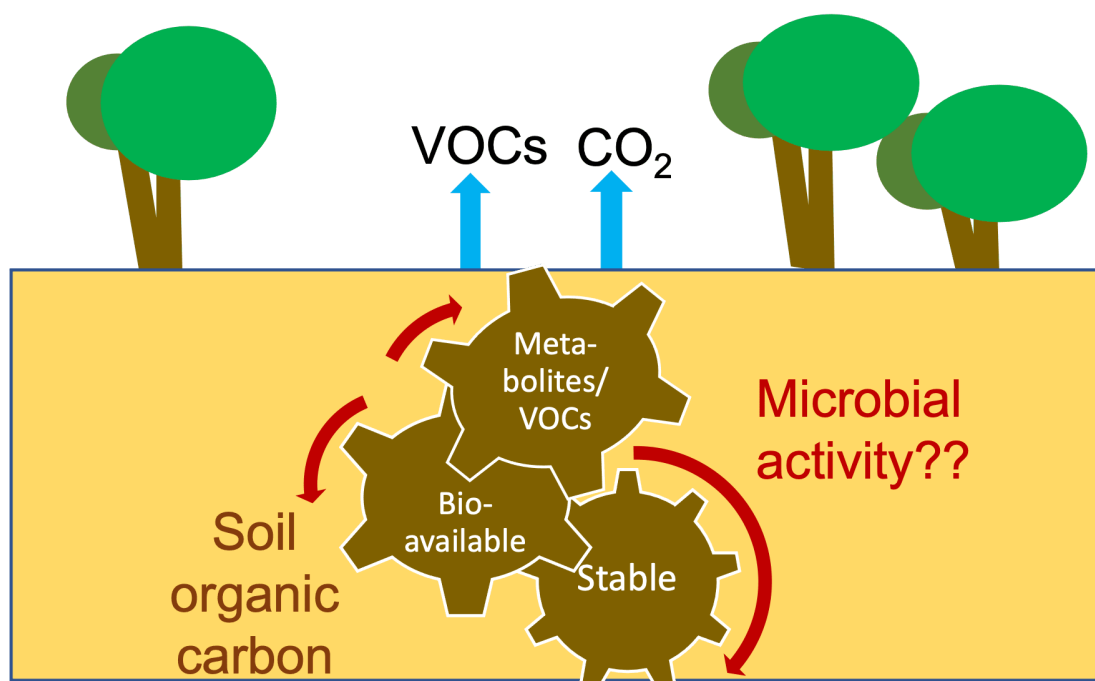


Fig. 2. Conceptual model depicting microbial activity as driver of carbon cycling and VOC/CO₂ emissions to the atmosphere.

Research questions:

1. How does drought affect soil microbial activity and carbon allocation towards energy vs. biosynthesis?
2. What impact does this have on microbial contribution to atmospheric gas exchange (CO₂ and VOCs) (Fig. 2)?

We address these research questions from two perspectives:

1. Overall microbial activity through multi-omics - metagenomics (metaG), metatranscriptomics (metaT), and metabolomics (metaB).
2. Stable isotope - track how carbon from position-specific (C1 or C2) ¹³C-pyruvate is allocated into CO₂, primary metabolites, and volatile organic compounds (VOCs).

Hypotheses

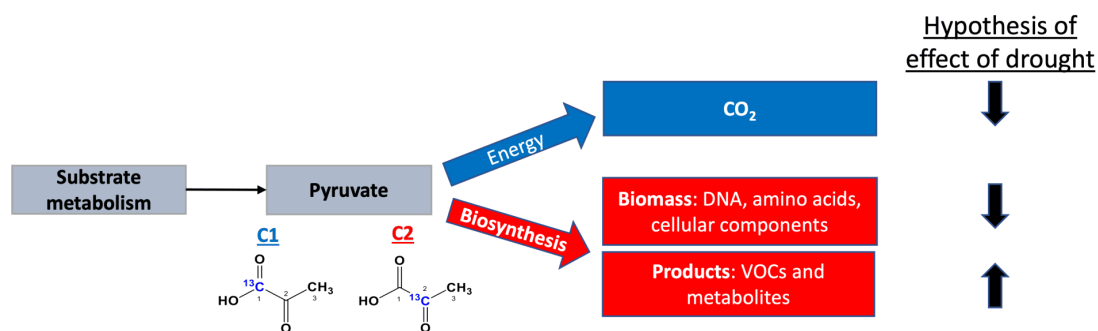
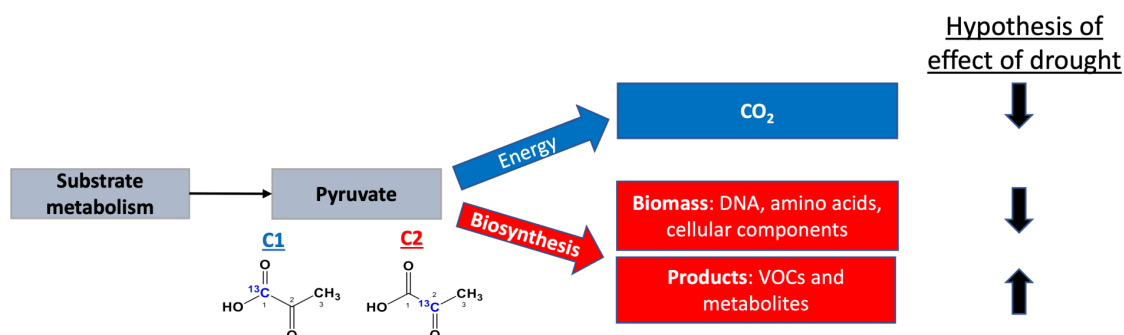
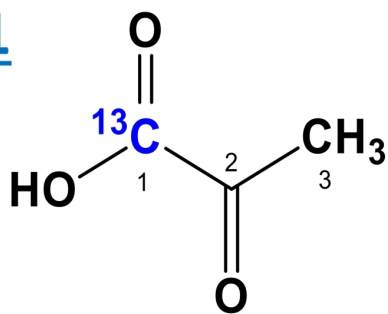
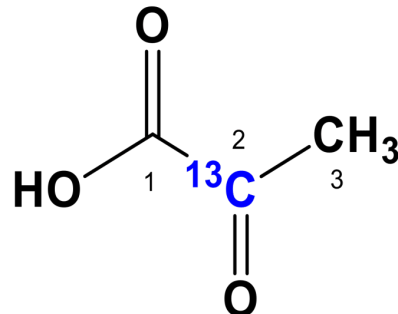


Fig. 3. Theoretical framework showing possible routes of carbon allocation, and our hypotheses of how drought will impact allocation. Furthermore, we expect that C1 carbon from C1-¹³C-pyruvate (blue) will go towards CO₂, and C2 from C2-¹³C-pyruvate (red) will go towards biosynthesis.

We hypothesize that during drought (Fig. 3):

- 1) Microbial gene expression will shift, representing a change in microbial activity.
- 2) Carbon allocation towards CO₂, producing energy, will decrease.
- 2) Carbon allocation towards biosynthesis of biomass will decrease, as microbes enter stationary growth phase, and biosynthesis of products will increase, with the production of compounds that will improve microbial survival during drought.



C1C2



CONCLUSIONS, ACKNOWLEDGEMENTS & REFERENCES

Significance of our Findings

The drastic changes in microbial activity during drought, as revealed with multi-omics (metaT, metaG, and metaB) and stable isotope labeling, demonstrates a very specific microbial response to decreased water availability. Therefore, microbes are not passive during drought, but rather, actively shift carbon from energy production to biosynthesis of products, resulting in decreased CO₂ emissions and increased carbon storage in the soil, with the exception of VOC production, which could impact global atmospheric carbon levels. Regarding the later, further research is needed to determine how total carbon released to the atmosphere as VOCs compares to that released as CO₂.

Furthermore, the high time resolution of soil gas flux (CO₂ and VOCs) helped us to identify clear trends of microbial activity over time in relation to ¹³C-pyruvate addition. These online measurement techniques (Licor/Picarro/PTR-TOF-MS) can be used with other isotopic labels to characterize specific microbial metabolisms.

Summary of Findings

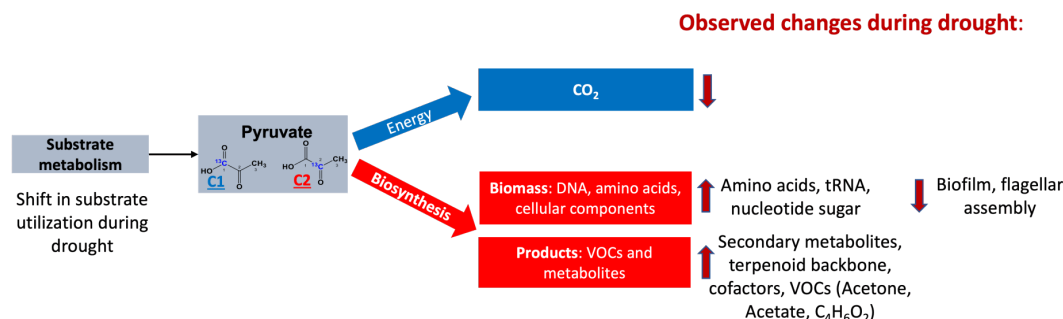


Fig. 15. Summary of drought induced changes to microbial allocation of resources. Drought decreased C allocation toward energy production (CO₂) and increased resource allocation biosynthesis of products, including secondary metabolites and VOCs.

- Multi-omics (metaT, metaG, and metaB) revealed a drought induced shift in microbial activity; substrate utilization and biosynthesis of biomass changed and secondary metabolite biosynthesis increased (Fig. 15).
- Stable isotope (C1- or C2-¹³C-pyruvate) analysis revealed a drought induced shift from energy production to biosynthesis of VOCs (Fig 15).
- An increase in acetate and acetone production during drought could indicate increased fermentation reactions, which would be unexpected in the drier soil, however, gene expression data supports non-fermentative pathways to acetate production.
- Some biomass production actually increases during drought, such as amino acids (AA), however, AAs could be used in osmoregulation

and tRNA to meet increased demand for secondary metabolite production.

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ABSTRACT

Droughts are occurring with increased frequency and duration in tropical rainforests due to climate change, having a significant impact on soil C dynamics. The role of microbes as drivers of changing C flow, particularly in relation to volatile organic compound (VOC) cycling, remains largely unknown. Here, we aimed to characterize microbial responses to drought using an integrative, multiple 'omics approach, and hypothesized that microbial communities will adapt by altering their C allocation strategies. Specifically, during pre-drought, primary metabolic pathways will be more active with microbes using C towards growth and energy, whereas during drought, microbes will divert C to secondary metabolite (including VOC) production in response to stress. To test this, we conducted an ecosystem-wide 66-day drought experiment in the tropical rainforest biome at Biosphere 2 a glass- and steel-enclosed facility near Tucson, AZ. To track carbon allocation by microbes, we injected C1 or C2 position-specific ^{13}C -pyruvate solution into a 25 cm² region within a soil flux chamber collar (n=6 locations) and measured C isotope ratios of VOC and CO₂ emissions. Soil was collected at 0, 6, and 48 hours after pyruvate addition to examine responses in soil metatranscriptomics, metagenomics, and metabolomics (Fourier-transform ion cyclotron resonance [FTICR]). Our results indicated that $^{13}\text{CO}_2$ (primarily emitted from C1- ^{13}C -pyruvate) fluxes decreased during drought, indicating diminished microbial activity. ^{13}C -VOCs (primarily emitted from C2- ^{13}C -pyruvate) fluxes also differed between pre-drought and drought, with acetone, acetate, diacetyl, and ethylene glycol increasing during drought. Using metatranscriptomic expression data, we examined the potential metabolic pathways that shifted leading to acetate and acetone production. Overall, these results indicate that integration of multiple 'omics datasets reveal specific impacts of drought on microbial activity affecting carbon flow in the tropical rainforest soil.

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