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**Supplementary Information**

## Extended Data Captions

**Extended Data Fig 1. Soil chamber locations and experimental setup**. A) Topological map of Biosphere 2 Tropical Rainforest, with locations of Sites 1 - 3. All circles represent collar locations where $CO_2$ and VOC data were collected and large circles represent a sub selection of sites collars additional samples for metatranscriptomics, metagenomics, and metabolomics were collected. B) Diagram showing soil pyruvate experimental setup with stencil placed inside a soil collar where $^{13}$C1- or $^{13}$C2-pyruvate was added within each 1 cm x 1 cm square. An automatic soil chamber was placed over the soil collar during the experiment and continuous measurements of $^{12}$C and $^{13}$C-$CO_2$ and VOCs were measured.

**Extended Data Fig 2. Continuous $CO_2$ emissions and proportion of C from pyruvate allocated to biosynthesis.** A) Total flux of $CO_2$ during pre-drought and drought conditions. Each point represents a single measurement per soil chamber (~ 64 measurements), of which there were 3 replicates each that were injected with either $^{13}$C1- or $^{13}$C2-pyruvate for each site (total n =18 each for pre-drought and drought), and lines show the data smoothed with the surrounding shaded area showing +/- SEM. B) Proportion of C from pyruvate allocated to biosynthesis. This was calculated as $^{13}C\text{-}CO_{2\text{-}C1}$/ ($^{13}C\text{-}CO_{2\text{-}C1}$ + $^{13}C\text{-}CO_{2\text{-}C2}$) on each set of C1/C2 chambers per site (n = 9 each for pre-drought and drought) with continuous emission data binned to 3 or 6 h intervals, from 0 to 48 h post pyruvate injection. (n= 9 each for pre-drought and drought). Boxes representing Q1 - Q3 with center line indication median, and bars extending to maximum and minimum values, excluding outliers.

**Extended Data Fig 3. GC-PTR-TOF data to identify $C_4H_6O_2$.** Two nearby locations A) and B) had two peaks with retention times 217 s and 275 s for $C_4H_6O_2$ corresponding to two different compounds. The retention time of 217 s matched the expected retention time for diacetyl, but the retention time of 275 s did not match with any known compounds. Therefore, $C_4H_6O_2$ may represent diacetyl and/or one additional unidentifiable compound, and is referred to as diacetyl$^+$ in the manuscript. The diacetyl peak (retention time of 217 s) is indicated with an arrow.

**Extended Data Fig 4. Metatranscriptomic and metagenomic functional and taxonomic diversity shows shifts in active microbial functional profiles and taxonomic diversity, but not in functional potential and total (active and inactive) taxonomic diversity**. PCA of (A) metatranscriptomic and (B) metagenomics data. P-values for differences between clusters in PCA was determined by PERMANOVA on Bray-Curtis distance matrices. Taxonomic profiles as inferred from metatranscriptomics data at the (C) kingdom-level for active archaea, bacteria, eukaryota, and viruses, D) phylum-level for bacteria and archaea, and E) phylum-level for fungi. Taxonomic profiles as inferred from metagenomics data at the F) kingdom-level for total archaea, bacteria, eukaryota, and viruses, G) phylum-level for bacteria and archaea, and H) phylum-level for fungi. For taxonomic profiles, arrows indicate direction of changes in relative abundance (up for increased, and down for decreased relative abundance in drought compared to pre-drought conditions). Arrows with no stars means the phylum was present in one condition (pre-drought or drought) and absent in the other condition. For panels A-B, P-values were determined using PERMANOVA on Bray-Curtis distance matrices. For panels C-H, P-values were determined using Linear Mixed Effect Models. *, $p < 0.05$; **, $p < 0.01$; (exact P-values: $P$=0.010 [Proteobacteria], $P$=0.016 [Gemmatimonadetes], $P$=0.0012 [Actinobacteria] (panel B), $P$=0.0050 [Ascomycota] (panel C)). metaT, metatranscriptomics; metaG, metagenomics; B, bacteria; A, archaea; F, fungi

**Extended Data Fig 5. Up- or down-regulation of genes encoding for enzymes that cycle $^{13}$C-enriched VOCs shift with pyruvate addition.** $Log_2$-fold change (FC) of genes involved in cycling of acetate, acetone, and diacetyl at 6 and 48 h in relation to 0 h to show effect of pyruvate addition on microbial activity during A) pre-drought and B) drought conditions. $Log_2$-FC values to indicate up-or down-regulation calculated with DESeq2. Production vs.

consumption indicated by black and gray bars, respectively. Significantly up- or down-regulated genes are outlined in color (blue = upregulated, red = downregulated), with exact P-values from DESeq2 analysis shown. ); *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

**Extended Data Fig 6. Acetone and acetate cycling gene transcripts.** Variance stabilization transformation (VST)-normalized transcript copies of A) acetate- and B) acetone-cycling genes at 0, 6, and 48 h post pyruvate injection. Bars represent Q1 - Q3 with center line indicating median, and bars extending to maximum and minimum values, excluding outliers, across all chambers and sites (n = 9 each for pre-drought and drought).

**Extended Data Fig 7. Drought induced a shift in both primary and secondary metabolic composition.** A) PCA biplot of samples collected during pre-drought (green; 0 h [n = 5]) or drought (orange; 0 h [n = 5] and 6 h [n = 1]) showing relationships between metabolic profiles identified with NMR which were mostly identified as primary metabolites with relatively low molecular weight. Arrows represent the loadings of individual metabolites. B) PCA biplot of samples collected during pre-drought (green) or drought (orange) showing relationships between profiles of metabolic classes identified with FTICR-MS, capturing mostly high molecular weight metabolites including secondary metabolites. Arrows represent the loadings of metabolic classes driving these patterns. P-values indicate significance of clustering between pre-drought and drought conditions using PERMANOVA on bray-curtis dissimilarity matrix. C) Each stacked bar represents the composition of compound classes identified with FTICR-MS for all samples (n = 18 [6 chambers sampled at 0, 6, and 48 h post pyruvate addition] averaged across pre-drought and drought conditions). Exact P-values are shown as determined by Linear Mixed Effects Models; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

**Extended Data Fig 8. Modules of co-expressed genes that are associated with pre-drought or drought conditions and $^{13}$C-enrichment of VOCs.** A) WGCNA cluster dendrogram reveals 9 clusters of co-expressed genes, or modules. The four modules with significant correlations to drought or pre-drought (pink, green, magenta, and brown) are indicated. B) Correlations of the pink, green, magenta, and brown module eigengenes with environmental conditions (pre-drought = 0, drought = 1) and efflux of $^{13}$C-enriched (VOC $^{13}C/(^{12}C + ^{13}C)$) acetate and acetone from chambers receiving $^{13}$C2-pyruvate (acetate-C2 and acetone-C2, respectively) across three time points (0, 6, and 48 h post pyruvate injection; total n = 27 [3 measurements each for each soil collar receiving $^{13}$C2-pyruvate]). Pearson correlation coefficients are shown with FDR-corrected p-values below in parentheses. White boxes indicate no correlation ($P<0.1$). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

**Extended Data Table 9. Metabolic concentrations (µM) detected using NMR.** Samples displayed are a subset of total samples based on the quality of NMR data. Most samples are from 0 h, except for P24 during drought which is from 6 h post pyruvate injection.

| Compound | | Pre-drought | | | | | Drought | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name | Formula | P15-0hr | P24-0hr | P26-0hr | P34-0hr | P35-0hr | P12-Ctrl | P15-0hr | P24-6hr | P26-0hr | P34-0hr | P34-0hr |
| 2-Oxoisocaproate**P | C6H10O3 | 6 | 5 | D | 5 | D | ND | ND | ND | ND | ND | D |
| 3-Hydroxybutyrate | C4H8O3 | ND | ND | ND | ND | D | ND | ND | ND | ND | ND | ND |
| Acetate | C2H4O2 | 22 | 38 | 30 | 55 | 26 | 19 | 35 | 31 | 31 | 24 | 11 |
| Acetone | C3H6O | ND | D | D | ND | ND | D | ND | ND | ND | ND | D |
| Alanine**P | C3H7NO2 | 24 | 21 | 15 | 20 | 17 | 18 | 9 | 13 | 9 | 7 | 9 |
| Benzoate | C7H6O2 | ND | ND | ND | D | ND | ND | ND | ND | ND | ND | ND |
| Betaine | C5H11NO2 | ND | ND | ND | ND | ND | D | D | ND | ND | ND | ND |
| Ethanol | C2H6O | 12 | 17 | 21 | 19 | 18 | 28 | 24 | 12 | 22 | 22 | 12 |
| Formate***P | CH2O2 | 17 | 38 | 40 | 23 | 31 | ND | D | 6 | 16 | D | 11 |
| Glutamate | C5H9NO4 | 12 | 12 | 8 | 6 | 10 | 11 | 7 | 10 | 10 | 10 | 6 |
| Glycerol | C3H8O3 | 6 | 8 | 8 | 6 | 7 | 5 | 6 | 7 | 5 | 8 | 6 |
| Glycine*P | C2H5NO2 | 12 | 9 | 6 | 8 | 7 | 5 | D | 7 | 6 | D | D |
| Isoleucine | C6H13NO2 | 7 | 7 | D | D | D | D | D | D | D | D | D |
| Lactate | C3H6O3 | 6 | 7 | D | 12 | 7 | D | D | 11 | D | D | 7 |
| Leucine*P | C6H13NO2 | 12 | 11 | 7 | 7 | 6 | 7 | 6 | 6 | 6 | D | D |
| Methanol | CH4O | 150 | 375 | 367 | 222 | 326 | 508 | 329 | 203 | 370 | 372 | 322 |
| Phenylalanine*P | C9H11NO2 | D | D | ND | D | ND | ND | ND | ND | ND | ND | ND |
| Pyroglutamate*P | C5H7NO3 | 22 | 19 | 10 | 16 | 9 | 8 | 5 | 12 | 8 | D | D |
| Pyruvate**P | C3H4O3 | 11 | 13 | 10 | 11 | 10 | D | 5 | 9 | 5 | D | 7 |
| Succinate | C4H6O4 | ND | ND | ND | ND | ND | ND | D | D | ND | ND | ND |
| Threonine | C4H9NO3 | 6 | 7 | D | D | D | D | D | D | D | D | D |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trehalose*[D] | C12H22O11 | ND | ND | ND | ND | ND | 18 | D | 7 | 6 | 13 | D |
| Uracil*[P] | C4H4N2O2 | D | 5 | D | D | D | ND | ND | D | ND | ND | D |
| Valine*[P] | C5H11NO2 | 15 | 13 | 8 | 9 | 8 | 8 | 6 | 8 | 6 | 5 | 5 |

ND, not detected (below the level of detection where concentration < 1.9 μM); D, detected (below level of quantification, where concentration 2 - 5 μM)

[P], Compound higher during pre-drought (Linear Fixed Effect Model (DF = 7), where ND and D were given values of 1 and 4 μM respectively, Oxoisocaproate $P$=0.0014, Alanine $P$=0.0074, Formate $P$=4.2E-4, Glycine $P$=0.017, Leucine $P$=0.016, Phenylalanine $P$=0.030, Pyroglutamate $P$=0.018, Pyruvate $P$=0.0011, Uracil $P$=0.022, Valine $P$=0.012)

[D], Compound higher during drought (Trehalose $P$=0.020)

*, $P$<0.05; **, $P$<0.01; ***, $P$<0.001

**Extended Data Table 10. Sequence coverage for metagenomics and metatranscriptomics.** Read counts are post-filtering (as described in the methods section).

| | Metagenomics | | | Metatranscriptomics | | |
|---|---|---|---|---|---|---|
| Sample | Reads | Bases (Gb) | Mapped to assembly (%) | Reads | Bases (Gb) | Mapped to assembly (%) |
| P11SSC1_190916_c | 142713986 | 21.4 | 49.1 | 124166928 | 18.0 | 77.9 |
| P11SSC1_190916_f | 224647504 | 33.6 | 60.0 | 134601358 | 19.5 | 81.2 |
| P11SSC1_190916_i | 155786656 | 23.3 | 53.4 | 101082054 | 14.5 | 77.6 |
| P11SSC1_191110_c | 227806600 | 34.1 | 64.9 | 82931902 | 12.0 | 81.1 |
| P11SSC1_191119_f | 132381506 | 19.8 | 55.9 | 107176982 | 15.4 | 82.3 |
| P11SSC1_191119_i | 257335184 | 38.5 | 64.8 | 76710842 | 11.0 | 81.1 |
| P12SS_CTRL_191116_c | 135568486 | 20.3 | 52.9 | 92715120 | 13.2 | 77.0 |
| P12SS_CTRL_191116_f | 103105310 | 15.4 | 48.0 | 146171918 | 20.8 | 73.4 |
| P12SS_CTRL_191116_i | 89432070 | 13.4 | 45.8 | 85117324 | 12.1 | 81.9 |
| P15SSC1_190912_c | 141457632 | 21.2 | 44.5 | 95565802 | 13.6 | 77.1 |
| P15SSC1_190913_f | 136093864 | 20.4 | 41.0 | 102381422 | 14.5 | 73.1 |
| P15SSC1_190915_i | 116566008 | 17.5 | 39.7 | 105544744 | 15.0 | 67.5 |
| P15SSC1_191107_f | 126857110 | 19.0 | 43.0 | 22699542 | 3.2 | 68.0 |
| P15SSC1_191107_i | 141678932 | 21.2 | 43.5 | 104936440 | 14.9 | 75.1 |
| P15SSCI_191107_c | 131734810 | 19.7 | 45.6 | 106270712 | 15.0 | 78.9 |
| P24SSC2_190915_c | 131640432 | 19.7 | 54.6 | 93137922 | 13.3 | 80.2 |
| P24SSC2_190915_f | 108754462 | 16.3 | 50.0 | 145088652 | 20.6 | 77.5 |
| P24SSC2_190915_i | 143103050 | 21.4 | 55.0 | 120662996 | 17.1 | 73.7 |
| P24SSC2_191109_c | 122604752 | 18.4 | 49.7 | 124732280 | 17.6 | 74.2 |
| P24SSC2_191109_f | 145461884 | 21.8 | 59.2 | 79879116 | 11.5 | 74.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| P24SSC2_191109_i | 101194304 | 15.2 | 44.3 | 72649070 | 10.2 | 77.6 |
| P26SSC2_190918_c | 138246448 | 20.7 | 58.3 | 76395558 | 10.8 | 63.8 |
| P26SSC2_190918_f | 110179686 | 16.5 | 54.9 | 94868962 | 13.4 | 65.5 |
| P26SSC2_190918_i | 112067984 | 16.8 | 49.2 | 116842828 | 16.7 | 76.7 |
| P26SSC2_191112_c | 98449944 | 14.7 | 50.9 | 85137010 | 12.0 | 72.2 |
| P26SSC2_191112_f | 119958450 | 18.0 | 58.9 | 78330638 | 10.9 | 68.4 |
| P26SSC2_191112_i | 175881098 | 26.3 | 62.4 | 97683038 | 13.7 | 68.9 |
| P34SSC2_190917_c | 132462676 | 19.8 | 55.5 | 71554676 | 10.2 | 77.8 |
| P34SSC2_190917_f | 101846050 | 15.3 | 48.6 | 183218676 | 25.8 | 78.6 |
| P34SSC2_190917_i | 127521864 | 19.1 | 51.5 | 85630146 | 12.0 | 69.7 |
| P34SSC2_191111_c | 154773318 | 23.2 | 57.8 | 83204978 | 12.0 | 84.2 |
| P34SSC2_191111_f | 134298460 | 20.1 | 56.9 | 78791904 | 10.8 | 72.9 |
| P34SSC2_191111_i | 112046872 | 16.8 | 53.5 | 68499682 | 9.9 | 80.1 |
| P35SSC1_190914_c | 112270418 | 16.8 | 56.0 | 16633060 | 2.3 | 65.2 |
| P35SSC1_190914_f | 112035746 | 16.8 | 55.5 | na | na | na |
| P35SSC1_190914_i | 128627610 | 19.3 | 60.0 | 14394504 | 2.0 | 37.3 |
| P35SSC1_191108_c | 195950226 | 29.3 | 64.4 | 13623088 | 1.9 | 59.7 |
| P35SSC1_191108_f | 128984880 | 19.3 | 56.8 | 97460190 | 13.5 | 72.1 |
| P35SSC1_191108_i | 109718370 | 16.4 | 55.1 | 86907854 | 12.1 | 74.3 |

a, 0 h; f, 6 h; i, 48 h
na, Metatranscriptomics of sample P35SSC1_191108_c were not completed due to sequencing issues