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Supplementary Information

for

Response of microbial community function to fluctuating
geochemical conditions within a legacy radioactive waste trench
environment

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20 **Results and Discussion**

21 **Reactive oxygen species detoxification**

22 The rapid trench water redox cycling involving the production of large quantities of
23 Fe(II), along with elevated concentrations of organic compounds, creates conditions
24 conducive to reactive oxygen species production (Page *et al.*, 2013; Minella *et al.*, 2015;
25 Klüpfel *et al.*, 2014; Page *et al.*, 2012; Tong *et al.*, 2016). Superoxide dismutase (SOD,
26 SUPEROX-DISMUT-RXN, EC:1.15.1.1) was found to be the predominant RXN related to
27 reactive oxygen species (ROS) detoxification at all time points, with a maximum at day 47
28 (Figure 5F). Catalase peaked at day 4 and superoxide reductases (SOR, 1.15.1.2-RXN) at day
29 47, both exhibiting similar relative abundances at the lowest values (days 0 and 4).

30 The general classic concept of strict anaerobes being unable to cope with O₂ and
31 reactive oxygen species has been long obsolete (Imlay, 2002). While SODs are well
32 distributed amongst aerobic and anaerobic organisms, catalases are more commonly found in
33 aerobes, and SORs in anaerobes (Sheng *et al.*, 2014). This is consistent with the results
34 presented above.

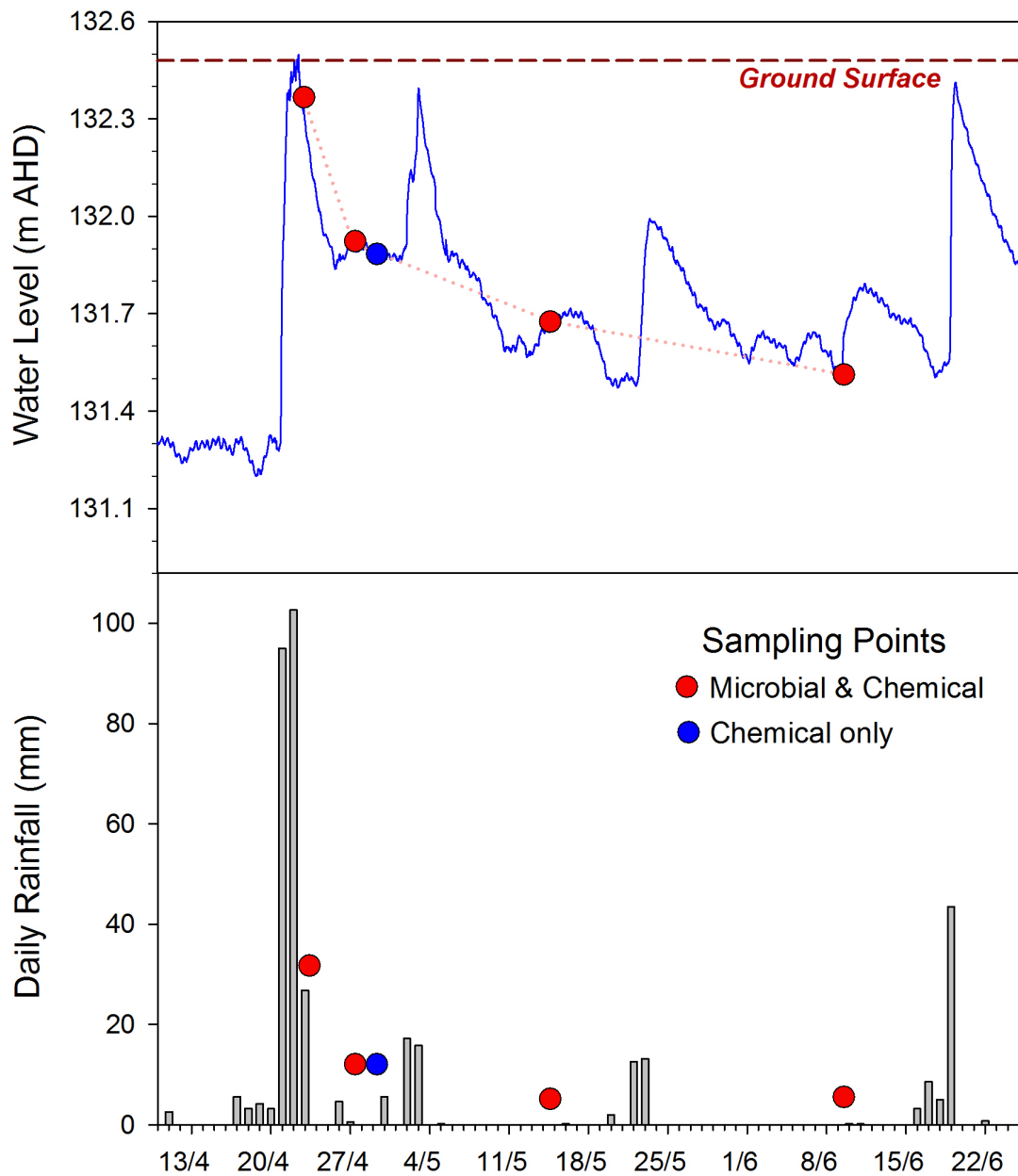
35 Levels of SOD have been correlated with the aerotolerance of anaerobes (Hassan, 1989;
36 Tally *et al.*, 1977). Gene copy numbers have been correlated with expression levels for
37 numerous proteins. The high relative abundance of SOD during the anaerobic phase could
38 relate to the physiological needs of the anaerobes and aerotolerant microbes thriving in the
39 trenches to deal with transient high oxygen concentrations (Brioukhanov *et al.*, 2002; Sheng
40 *et al.*, 2014).

41 **References**

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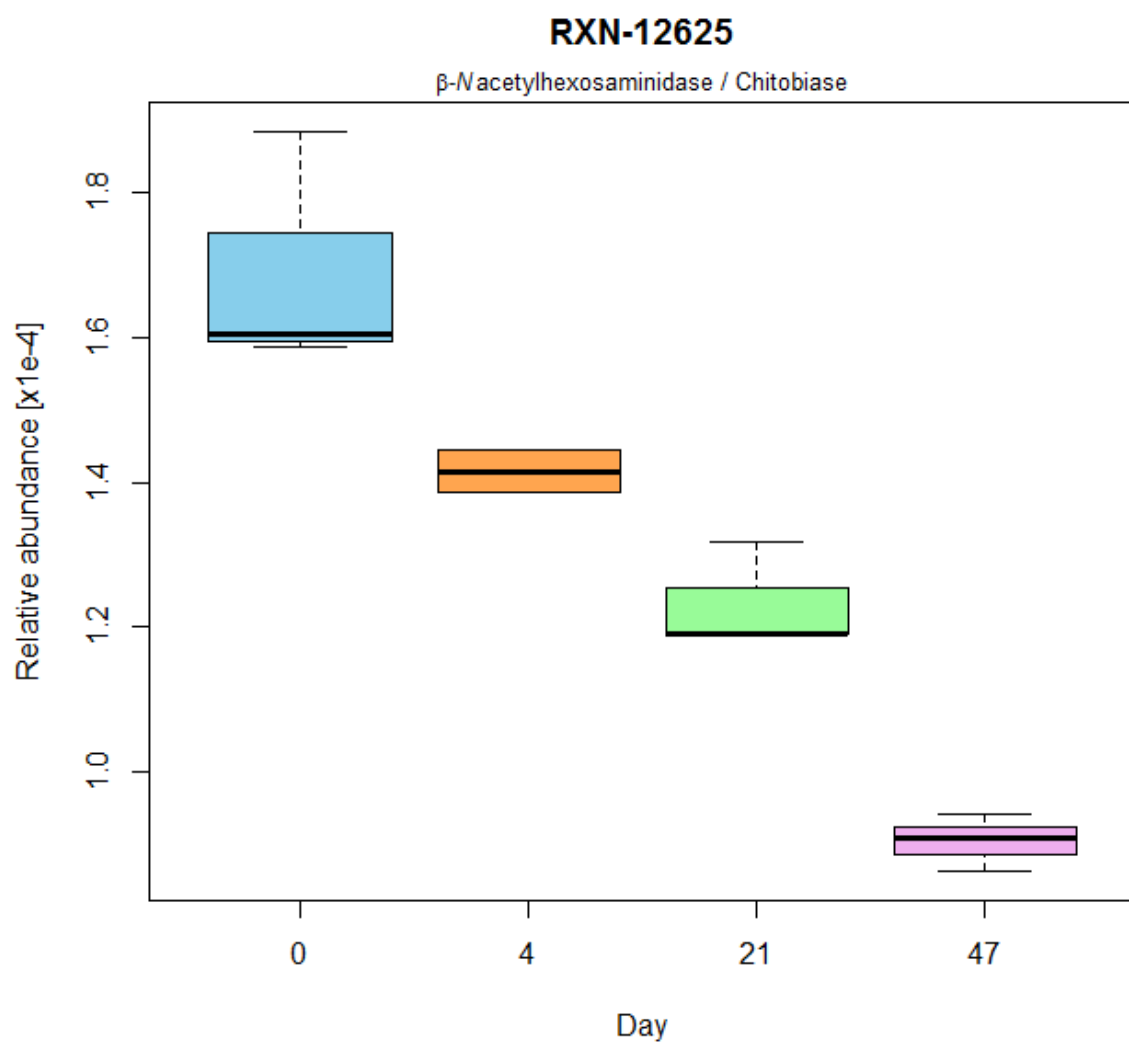
66 **Supplementary Figures**



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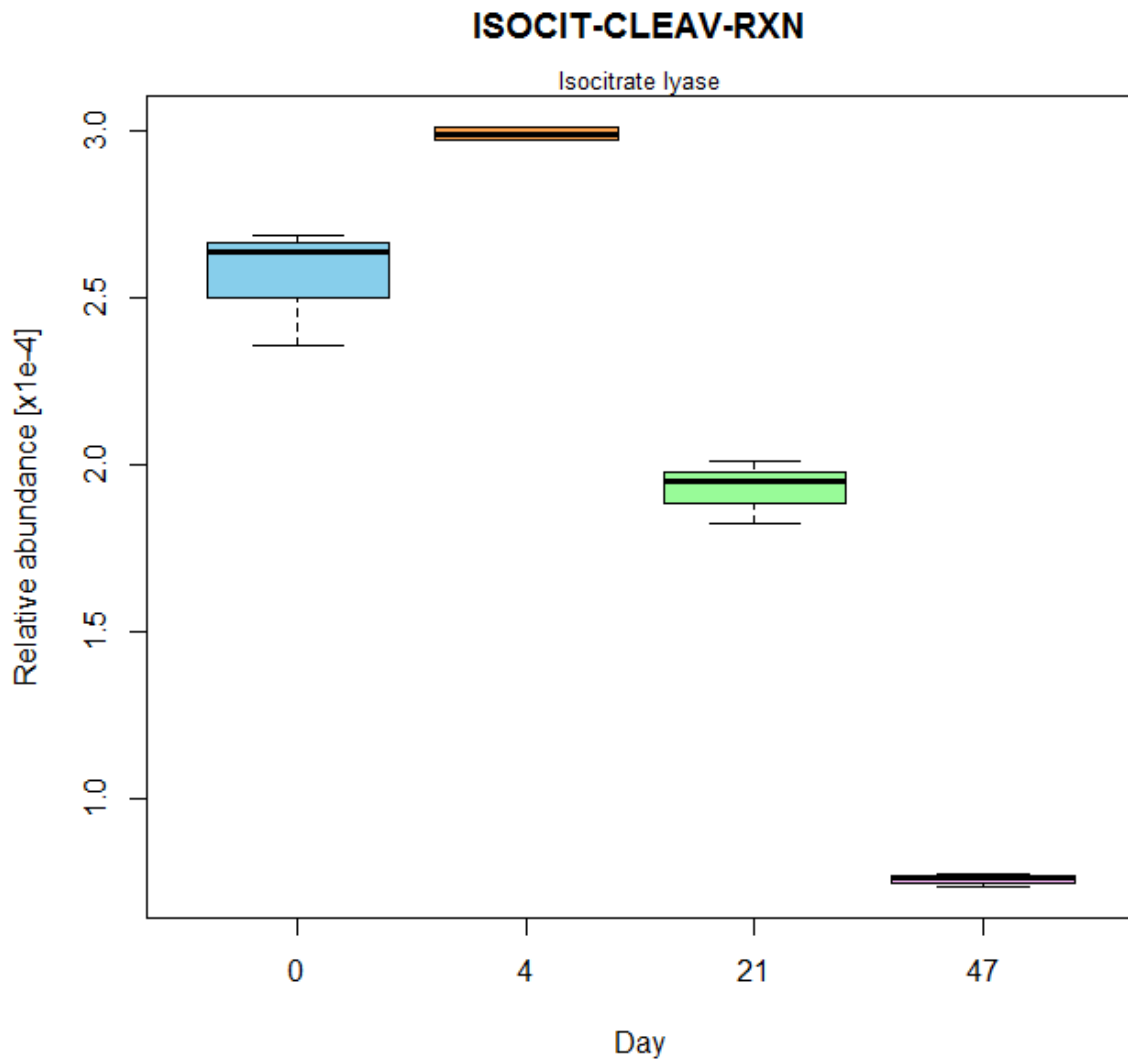
68 **Figure S1. Daily rainfall at Lucas Heights (ANSTO) Meteorological Station from April to July**
 69 **2015 (bottom). Corresponding trench water levels across the sampling period, along with the**
 70 **ground surface elevation shown by the dashed line (top). Circles depict dates of chemical and**
 71 **microbial sampling. Rainfall data is courtesy of the Australian Government, Bureau of Meteorology.**

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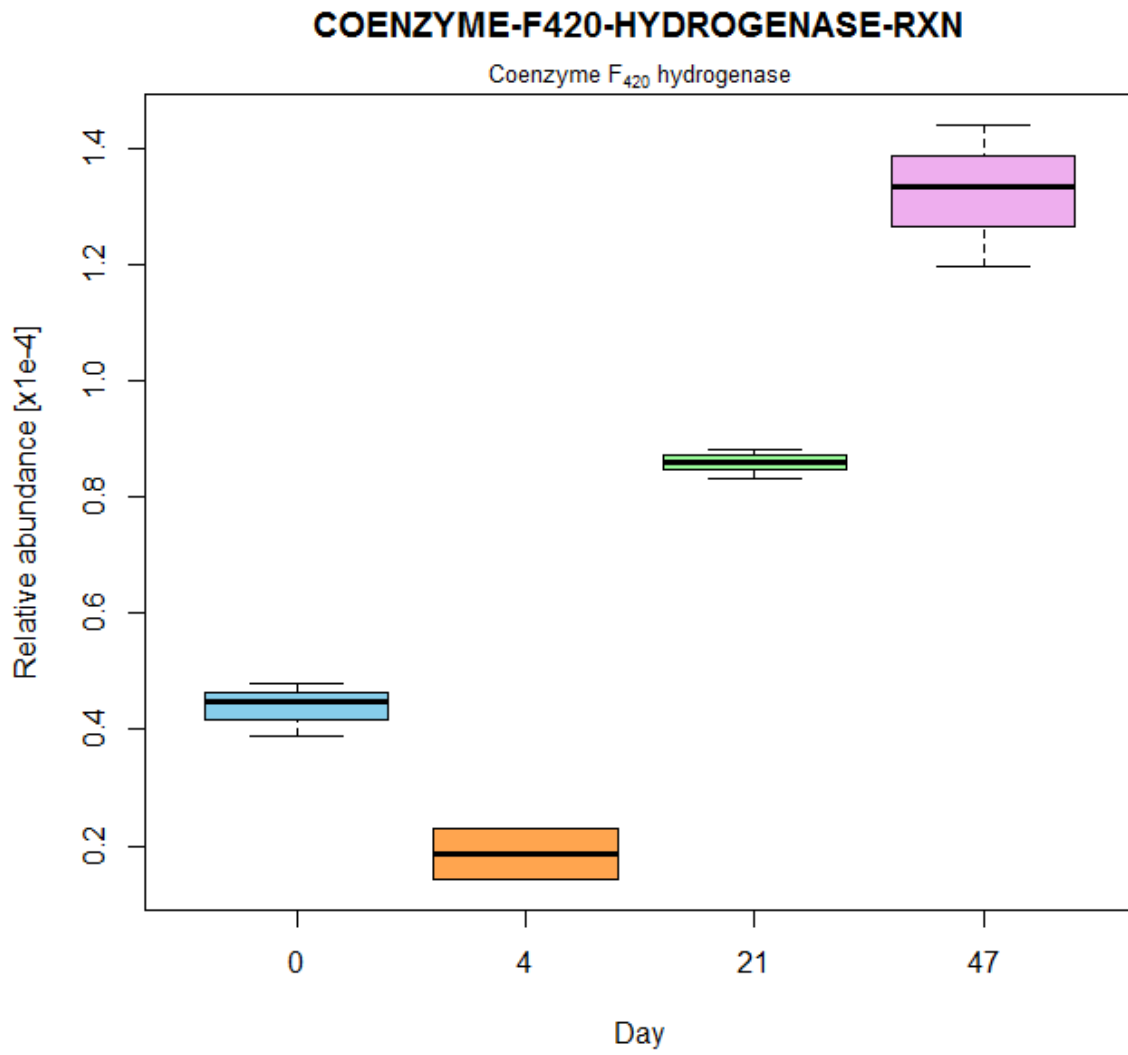
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74 **Figure S2. Chitobiase.** Marker for the degradation of chitobiose and/or chitin.



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76 **Figure S3. ISOCIT-CLEAV-RXN.** Marker for the glyoxylate pathway.

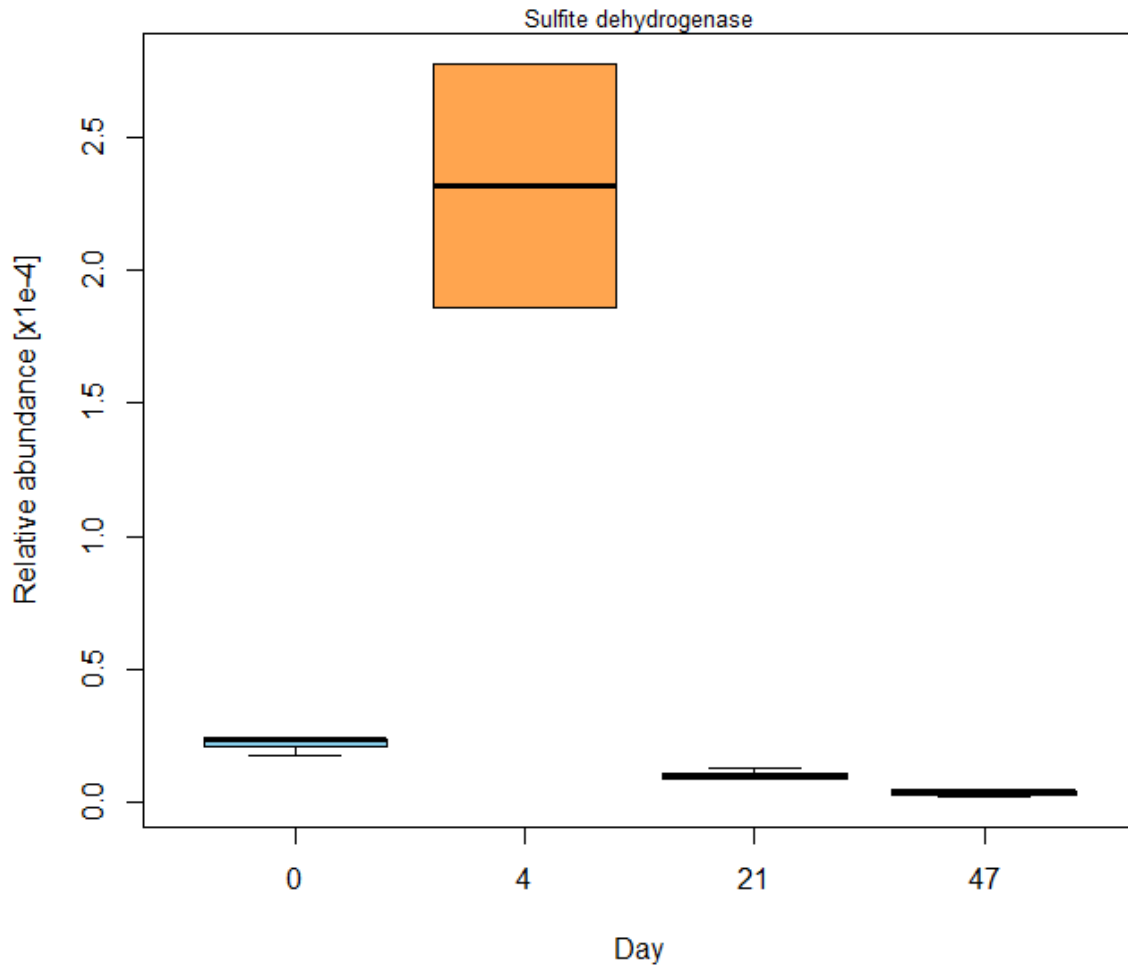
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79 **Figure S4. COENZYME-F420-HYDROGENASE-RXN.** Indicator of methanogenesis from H₂ and CO₂.

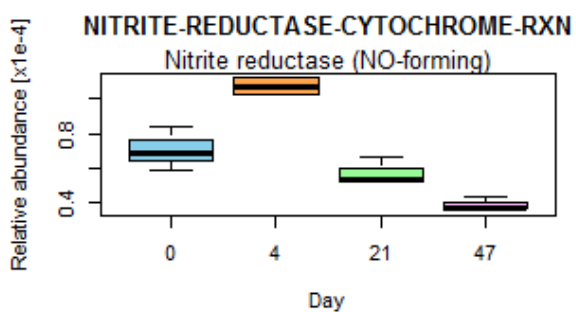
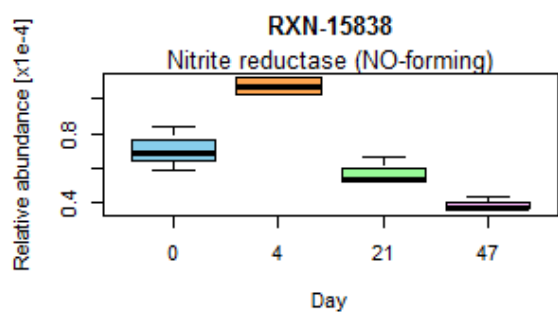
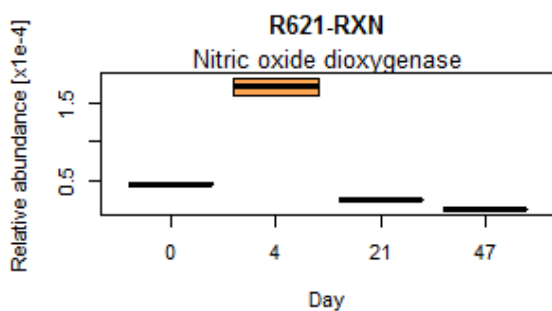
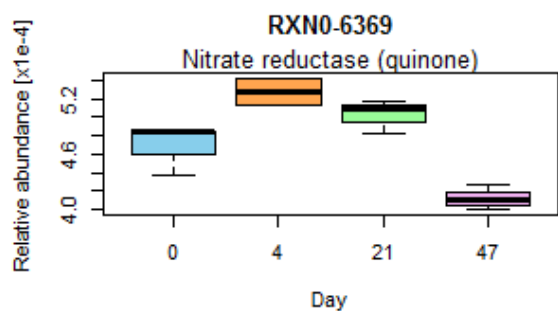
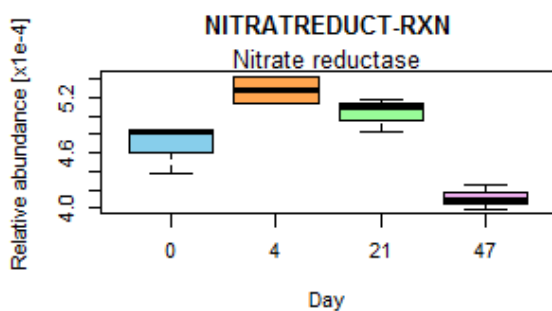
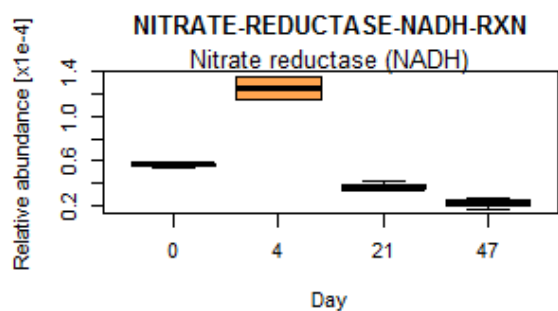
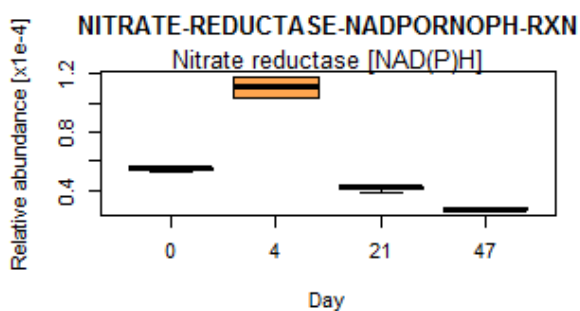
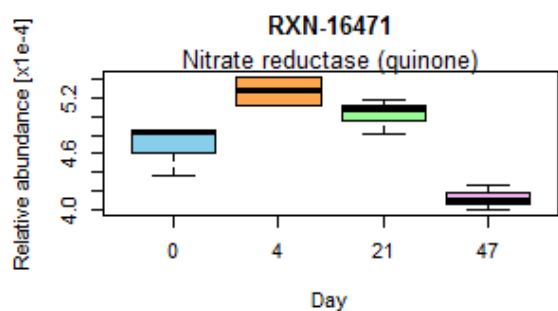
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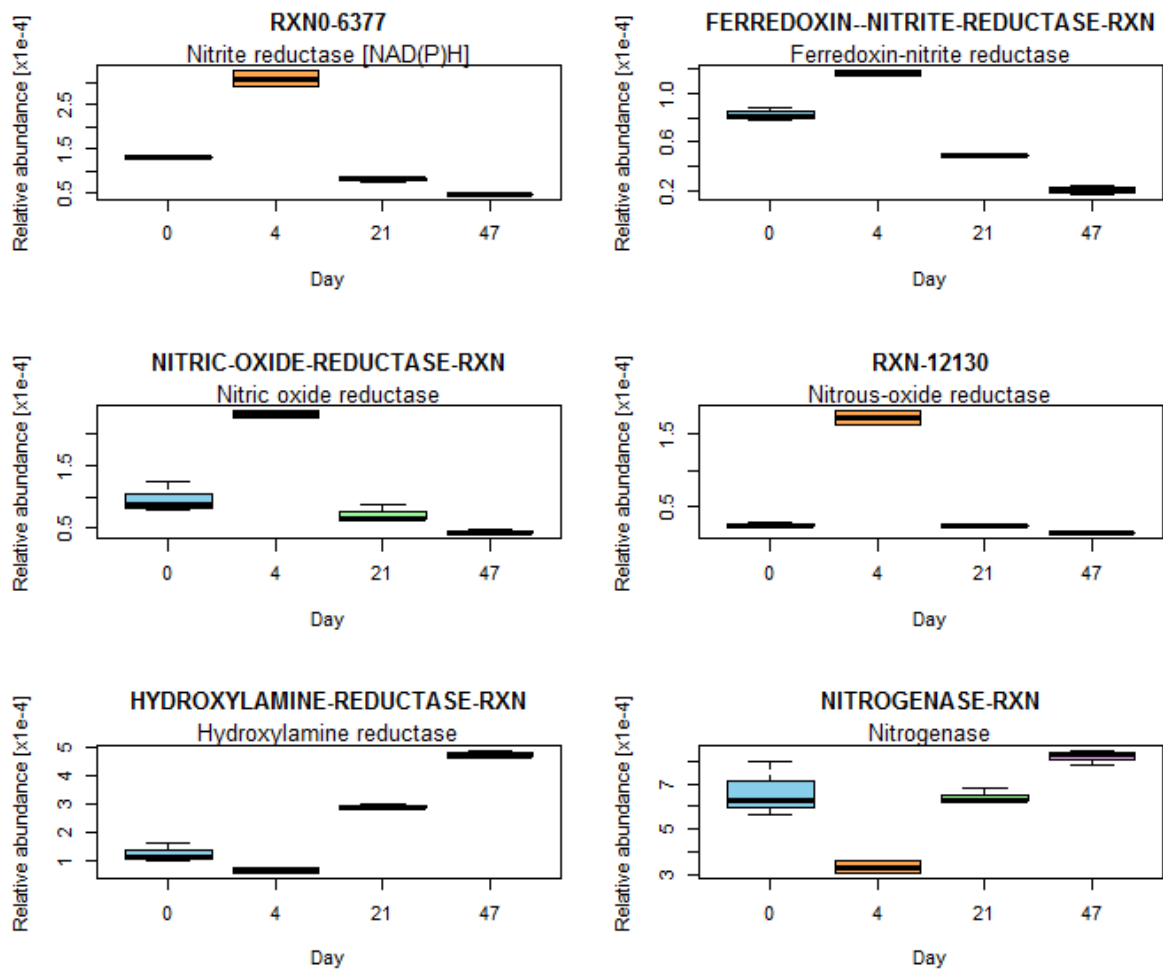
SULFITE-DEHYDROGENASE-RXN



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82 **Figure S5. SULFITE-DEHYDROGENASE-RXN (EC:1.8.2.1).** Assimilatory sulfate reduction.

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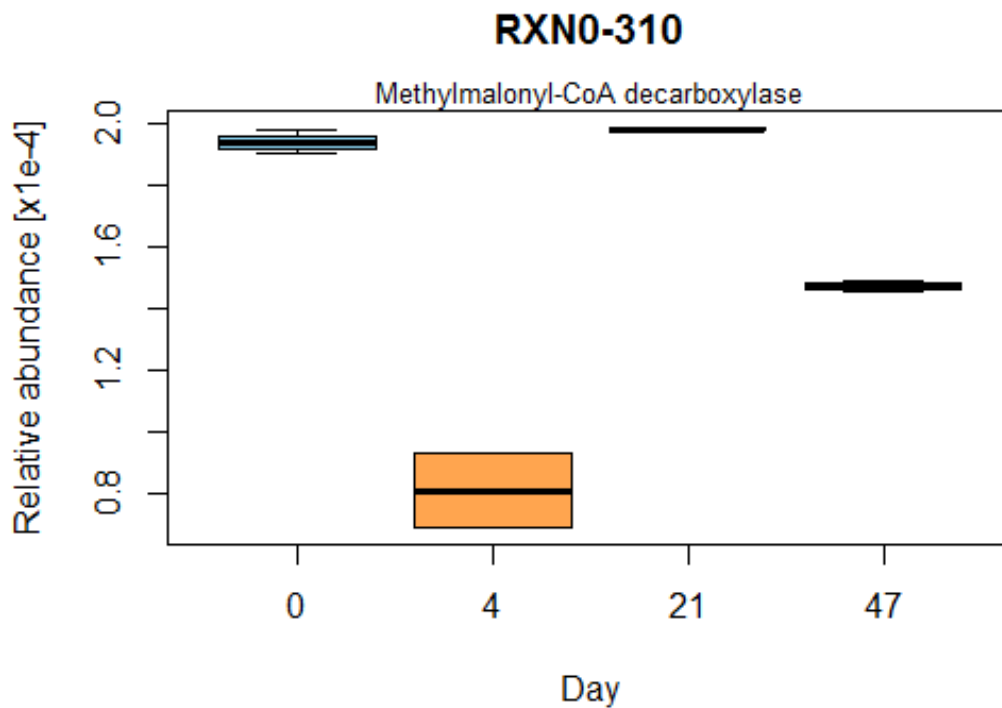
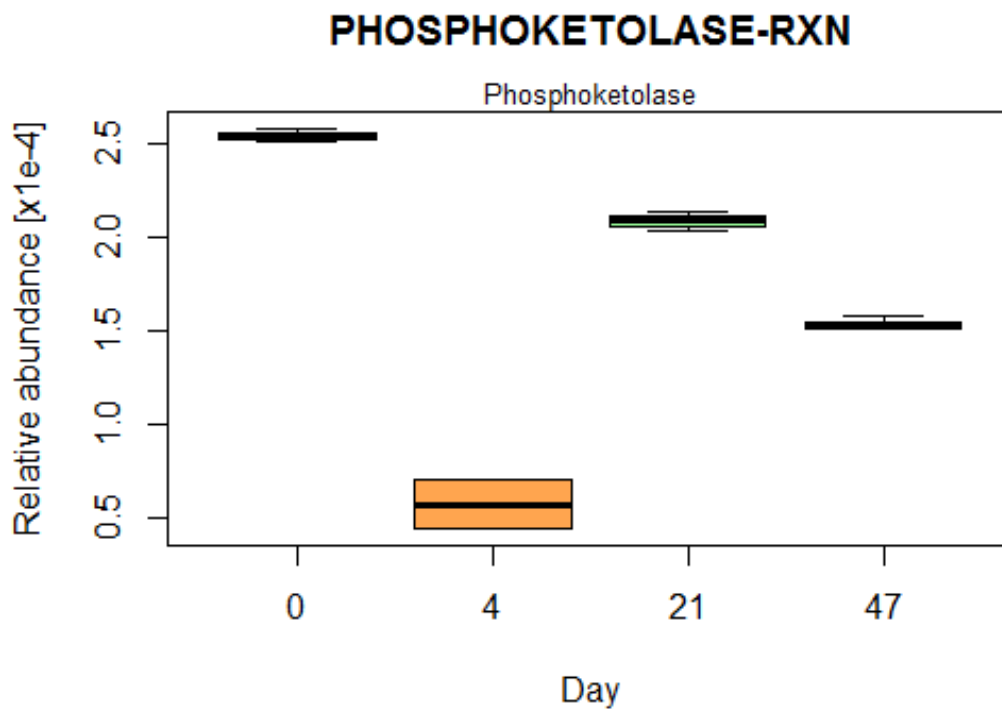




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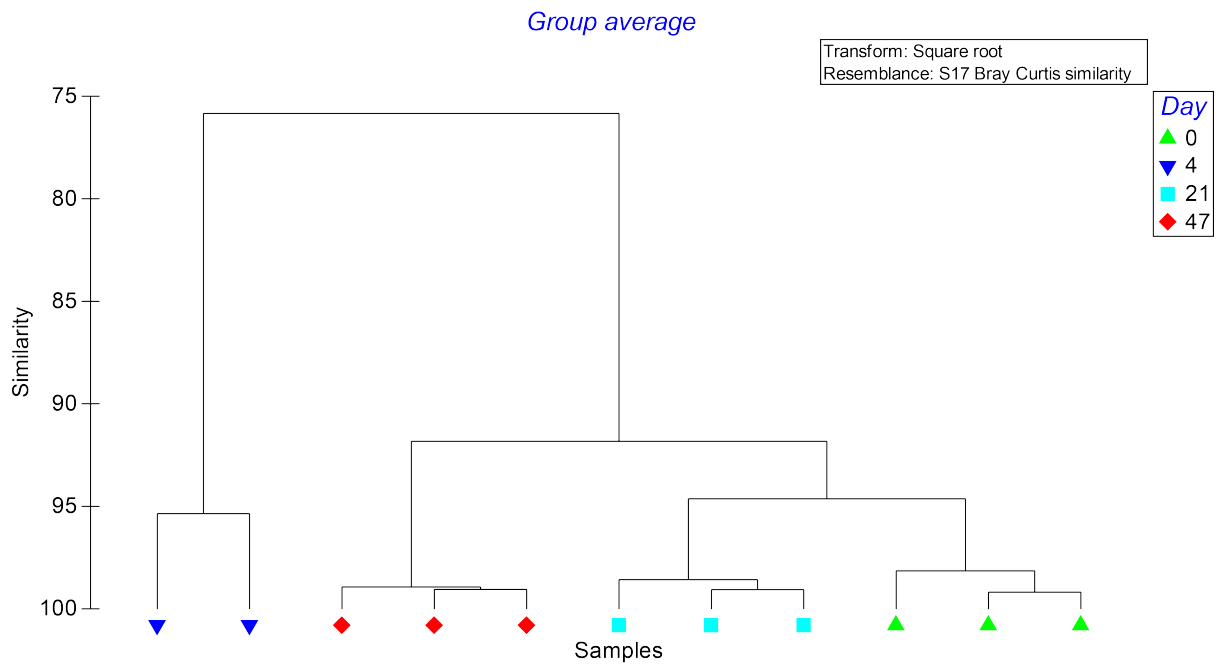
86 **Figure S6. Relevant RXNs related to the nitrogen cycle.**

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89 **Figure S7. PHOSPHOKETOLASE-RXN and RXN0-310.** Markers for heterolactic and propionate
 90 fermentation respectively.



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92 **Figure S8. Bray-Curtis similarity tree of the functional profiles for the individual sampling**
 93 **replicates.**

94 **Supplementary Files**

95 Supplementary file 1. Spreadsheet with YSI data.

96 Supplementary file 2. Krona HTML file with the taxonomy.

97 Supplementary file 3. Spreadsheet with the relative abundances of all the RXNs.

98 Supplementary file 4. Table with the raw HUMAnN2 output.

99 All supplementary files can be viewed at: <https://dx.doi.org/10.6084/m9.figshare.3817356>