Supplementary Information for

A fungal-mediated cryptic selenium cycle linked to manganese biogeochemistry

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**Supplementary Information Text**

**Materials and Methods**

*Synchrotron-based X-ray Absorption Spectroscopy.* Samples were shipped frozen to the APS and allowed to thaw just before analysis. Manganese and Se K-edge XAS spectra were collected on the same samples using a Si(111) monochromator. The incident beam energy was calibrated using Mn and Se foils, with the maximum of the first derivative of the K-edges set to 6539 and 12658 eV for Mn and Se, respectively. Fluorescence spectra were collected with a 13-element Ge detector positioned 90° to the incident beam. Successive scans were collected and monitored in the near-edge region (i.e. a shift in white line energy) to ensure that no radiation-induced oxidation or reduction of Se or Mn in the sample occurred during data collection. Three to four scans were collected per sample at room temperature from about -200 to +800 eV around the Mn (6539 eV) and Se (12658 eV) K-edges. Data reduction, normalization, and analysis of the bulk XAS data was performed using the Athena program in Demeter (version 0.9.26)\(^1\). The structure of the mycogenic Mn oxides as well as the speciation of solid-phase Se was determined using the EXAFS (extended X-ray absorption fine structure) region. X-ray absorption near edge structure (XANES) analysis was used primarily to determine the Se oxidation state of the samples. Spectra were background subtracted, \(k^3\)-weighted, and analyzed from 3-12 Å\(^{-1}\) (EXAFS) or across the region -30 to +40 eV (XANES) relative to the K-edge. Principal component analysis (PCA) and target transformation analysis (TTA) of the EXAFS spectra were performed to establish the number of components representing the entire dataset (PCA) and to evaluate the fitness of our model compounds to the overall data set (TTA), as described previously\(^2, 3\). A spectral reference library of model compounds was used to
identify and quantify the structural components using linear combination fitting (LCF). In our LCF approach, binding energies were fixed, negative component contributions were prohibited, and components were summed to 1.0. Goodness of fit was established by minimization of the $R$ factor (normalized sum of squares;NSS) parameter$^4$. Residuals were also checked to ensure that there was no systematic beat pattern suggesting missing components. For Mn, the library of model compounds was described previously$^5$, and included $\delta$-MnO$_2$, triclinic Na-birnessite, hexagonal acid birnessite, groutite ($\alpha$-MnOOH), feitknechtite ($\beta$-MnOOH), hausmannite (Mn$_3$O$_4$), Na-buserite, synthetic todorokite (((Na,Ca,K)(Mg,Mn)Mn$_6$O$_{14}$$\cdot$5H$_2$O), pyrolusite ($\beta$-MnO$_2$), rhodochrosite (MnCO$_3$), aqueous MnSO$_4$, and MnCl$_2$. For Se, the library included sodium selenate (NaSeO$_4$), sodium selenite (NaSeO$_3$), selenous acid (H$_2$SeO$_3$), grey crystalline Se(0), black amorphous Se(0), red amorphous Se(0) synthesized and stabilized with glucose$^6$, seleno-DL-cystine, Se-methyl-L- selenocysteine, seleno-DL-methionine, iron selenide (FeSe), and zinc selenide (ZnSe).
Fig S1: Comparison of total aqueous Se removal when supplied as 100 µM Se(IV) (A) or 100 µM Se(VI) (B), in the presence of no biomass or Mn oxides, synthetic acid birnessite, synthetic δ-MnO2, *P. sporulosum* + biogenic Mn oxides (bio-MnO2), *Stagonospora* sp. + biogenic Mn oxides. See Fig 3, Fig S3, and Table S1 for confirmation of formation and structure of mycogenic Mn oxides produced by *P. sporulosum* and *Stagonospora* sp. Data points represent calculated means of duplicates and error bars represent actual percentages removed for each duplicate. Symbols without error bars have a range of percent removed equal to or smaller than the size of the symbol.
**Fig S2:** Biotic control experiment results. Aqueous selenite (Se(IV); blue) and selenate (Se(VI); red) measured during growth with *P. sporulosum* (top panels) and *Stagonospora* sp. (bottom panels) in either 100 µM Se(IV) (left panels) or 100 µM Se(VI) (right panels) without Mn. Data points represent calculated means of duplicates and error bars represent actual percentages removed for each duplicate. Symbols without error bars have a range of percent removed equal to or smaller than the size of the symbol.
Fig S3: Mn content over time in solution (open squares) and solid (filled circles) for P. sporulosum (A), and Stagonospora sp. (B). Data points represent calculated means of duplicates and error bars represent actual values measured for each duplicate. Symbols without error bars have a range of measured values equal to or smaller than the size of the symbol.
**Fig S4:** Se K-edge EXAFS spectra (solid black lines) and calculated LCFs (dotted blue and red lines) of solid-associated Se for *P. sporulosum* (left panel) and *Stagonospora* sp. (right panel) grown in the presence of 100 µM Mn and 100 µM Se(IV) (top) or 100 µM Mn and 100 µM Se(VI) (bottom).
**Fig S5:** TEM images of *P. sporulosum* (top) and *Stagonospora* sp. (bottom) grown in Mn + Se(IV) (left) and Mn + Se(VI) right. Black (electron dense) round spots in images A, B, C, and E are EDS-confirmed Se nanoparticles. Panels B, F, and H are HR-TEM images that show rumpled sheet-like morphology of Mn oxides produced by both species. Panel C, showing Se(0) produced by *P. sporulosum* grown in Mn + Se(VI) was the only Se(0) observed via TEM for this condition.
**Fig S6:** Mn K-edge EXAFS spectra (solid black lines) and calculated LCFs (dotted grey lines) for *P. sporulosum* (left panel) and *Stagonospora* sp. (right panel) solid-associated Se, when the fungi were grown in the presence of 100 µM Mn (top), 100 µM Mn and 100 µM Se(IV) (middle) or 100 µM Mn and 100 µM Se(VI) (bottom).
**Fig S7**: Stacked X-ray diffraction (XRD) patterns collected after 31 days of growth of *P. sporulosum* (left) and *Stagonospora* sp. (right) in 100 μM Mn only (black, top), 100 μM Mn + 100 μM Se(IV) (blue, middle), and 100 μM Mn + 100 μM Se(VI) (red, bottom). XRD of fungal biomass grown without Mn or Se is shown on the bottom of each plot in grey. Vertical grey lines at 5.5, 16.7, and 28.9 °2θ emphasize the (001) basal peak, (200)/(110), and (310)/(020) reflections, respectively, for biogenic hexagonal birnessite. The peak at ~9 °2θ likely represents chitin in the fungal cell walls.
Table S1: Linear combination fit (LCF) results for Mn K-edge EXAFS and Se K-edge EXAFS shown in Figures S4 and S5 for samples collected after 14 days (grey shading) and 31 days (white) of growth in 100 µM Mn only, 100 µM Mn and 100 µM Se(IV), or 100 µM Mn and 100 µM Se(VI). Data provided as relative percent of each phase in the total Mn or Se signal.

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Notes:
NA – not analyzed
SI References


