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Fungal-Mineral Interactions Modulating Intrinsic Peroxidase-like Activity of Iron Nanoparticles: Implications for the Biogeochemical Cycles of Nutrient Elements and Attenuation of Contaminants

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ABSTRACT

Fungal mediated extracellular reactive oxygen species (ROS) are essential for biogeochemical cycles of carbon, nitrogen, and contaminants in terrestrial environments. These ROS levels may be modulated by iron nanoparticles that possess intrinsic peroxidase (POD)-like activity (nanozymes). However, it remains largely undescribed how fungi modulate the POD-like activity of the iron nanoparticles with various crystallinities and crystal facets. Using well-controlled fungal-mineral cultivation experiments, here we showed that fungi possessed a robust defect engineering strategy to modulate POD-like activity of the attached iron minerals by decreasing the catalytic activity of poorly ordered ferrihydrite but enhancing that of well crystallized hematite. The dynamics of POD-like activity were found to reside in molecular trade-offs between lattice oxygen and oxygen vacancies in the iron nanoparticles, which may be located in a cytoprotective fungal exoskeleton. Together, our findings unveil coupled POD-like activity and oxygen redox dynamics during fungal-mineral interactions which increase understanding of the catalytic mechanisms of POD-like nanozymes and microbial mediated biogeochemical cycles of nutrient elements as well as the attenuation of contaminants in terrestrial environments.

Keywords: Cytoprotective exoskeleton, fungal biomineralization, Fenton reaction, iron (oxyhydr)oxide, molecular trade-offs, nanozyme, oxygen vacancy, reactive oxygen species

Synopsis

Fungal-mineral interactions modulate both mineral nanozyme activity and extracellular reactive oxygen species (ROS) levels that are essential to the biogeochemical cycles of nutrient elements and the attenuation of contaminants in terrestrial environments.

■ INTRODUCTION

In natural environments, fungi are often interconnected to naturally-occurring iron nanomaterials.[1–3] Fungal mediated transformations of iron (Fe) minerals, i.e., fungal-mineral interactions or biomineralization, drives many biogeochemical processes in ecosystems including the transport and fate of contaminants,[4] turnover of carbon (C) and nitrogen (N),[5] and inhibition of pathogens.[6] Fungal-mineral interactions also promote organismal evolution and protect microorganisms from external stressors by the formation of an ultrathin cytoprotective exoskeleton.[7,8] As by-products of aerobic metabolism, fungi are known to generate reactive oxygen species (ROS), i.e., extracellular superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$), which play a central role in these biogeochemical cycles of nutrient elements (e.g., C, N, and Fe) and contaminants.[9,10] Although ROS play a key role in cellular growth and defence systems,[11] elevated ROS levels have detrimental impacts on cells via oxidative stress and cytotoxicity,[11,12] raising the question why and how ROS concentrations can be maintained at subtoxic levels which is essential for fungal growth and biogeochemical cycling.

A common strategy of fungal ROS regulation is the production of various enzymes, e.g., peroxidase, catalase or other oxidases.[12,13] However, this strategy may not only consume considerable energy but is also ineffective in “extreme” conditions, e.g., acid mine drainage systems, sites contaminated with high levels of pollutants, and at extremes of pH, alkalinity, and temperature.[14] An alternative strategy of fungal ROS regulation is to utilize attached Fe nanomaterials that are abundant in nature[1,15] and can

act as biomimetic catalysts (so-called "nanozymes").[16,17] Nanozymes, i.e., nanomaterials with enzyme-like activities, are superior to natural enzymes in several aspects, such as their high stability, large surface areas available for bioconjugation, and multifunctionality.[18] These biological-nanoparticle interactions may reflect perfectly the unique ecological, biological, and morphological plasticity of fungi in response to extreme environments.[19] Based on their distinct properties of particle size, shape, specific surface area, and crystallinity, biocompatible Fe minerals, ranging from poorly ordered ferrihydrite to well crystalline hematite,[20,21] are predicted to possess a distinct catalytic activity and thus mitigate differential oxidative stress on fungi. However, the mechanisms by which fungi modulate the catalytic activity of attached iron nanomaterials, of varied crystallinity and crystal facets, for the purpose of maintaining subtoxic ROS levels and nutrient acquisition, remain largely undescribed. This lack of knowledge limits our understanding of fungal- and mineral-mediated biogeochemical processes in terrestrial ecosystems.

Because of the broad applications of Fe (oxyhydr)oxide mineral nanozymes (mainly as peroxidase (POD)-like activity), ranging from biosensors, biomedical diagnosis to environmental remediation, their catalytic mechanisms have gained extensive attention.[22–24] A growing body of evidence has revealed that nanoparticle surfaces[25–27] rather than the classical Fenton catalysis[23] are responsible for the catalytic activity of Fe (oxyhydr)oxide mineral nanozymes. It is known that the catalytic activity occurring on nanoparticle surfaces is ~50-fold more effective in driving $HO^{\bullet}$ production than dissolved ferric iron ions,[28] probably attributable to a higher catalytic reactivity of

ferrous ions bound on mineral facets than that of ferric iron ions.[29] These mineral nanoparticles are usually adsorbed to negatively-charged, bioactive molecules in the cell wall and extracellular materials exuded by fungi,[30] creating a suitable environment to form a cytoprotective exoskeleton.[17,31] To date, the activity descriptors and the location of POD-like nanozymes remain unclear.

In this research, we examined the dynamics of iron mineral nanozymes, with various crystallinities and crystal facets, when interacting with a fungus. We investigated the molecular trade-offs between lattice oxygen (O_L) and oxygen vacancy sites (O_V) that may drive the POD-like activity of iron (oxyhydr)oxide mineral nanozymes under physiological conditions. To this end, we performed a series of well-controlled fungal-mineral cultivation experiments by selecting *Trichoderma guizhouense* NJAU4742 as the model fungus and a diverse set of Fe(III) (oxyhydr)oxides as model iron minerals due to their ubiquity in the terrestrial environment and their strong catalytic activities.[1,32] The Fe minerals examined included poorly ordered ferrihydrite, as well as crystalline goethite and hematite, but also hematite nanoplates with the (001) plane as the dominant facet (named as hematite (001) thereafter)[29] and hematite nanocubes equally enclosed by (012), (102) and (-112) planes (named as hematite (012) thereafter).[33] We hypothesized that fungi can drive Fe mineral nanozyme activity by modulating the Fe mineral nanoparticles with surface deficiency (also called defect engineering[34]). Because of missing or dislocated atoms (e.g., O in this study), these defect sites locally break the regular periodic arrangement of atoms in the crystalline structures.[35] During this defect engineering, O_L may be removed by

fungal activity from anion sites leaving behind surface defects (e.g., O_V). Thus, the molecular trade-offs between O_L and O_V on minerals result in the introduction of oxygen defects that may serve as reactive centers to drive intrinsic nanozyme activity, owing to their lower adsorption energies of H_2O_2 and desorption energy of $HO^{\bullet}$.[34] During these engineered defects, fungi regulate POD-like activity of iron (oxyhydr)oxide mineral nanozymes that catalyze H_2O_2 into $HO^{\bullet}$ and form the oxidized substrates,[23] thus changing ROS levels around the cell.

■ MATERIALS AND METHODS

Iron Mineral Preparation. Iron (oxyhydr)oxide minerals with various crystallinity and crystal facets, including ferrihydrite, goethite, hematite, hematite (001) and hematite (012), were selected in this study. All chemicals were analytical reagents and purchased from J&K Scientific, Beijing, China. In brief, ferrihydrite was prepared by adding 330 mL of 1M KOH to 500 mL of 0.05 M $Fe(NO_3)_3 \cdot 9H_2O$.[37] Goethite was prepared by mixing100 mL of 1M $Fe(NO_3)_3 \cdot 9H_2O$ with 180 mL of 5 M KOH, and then aging (60 h, 70°C). Hematite was synthesized by mixing 2 L of 0.04 M $Fe(NO_3)_3 \cdot 9H_2O$ with 0.002 M HNO_3 (98°C) and then aging (7 d, 98°C). Synthesis of hematite (001) was done as follows:[29] 1.35 g of CH_3COONa and 0.5 g of $FeCl_3 \cdot 6H_2O$ were first dissolved into the 14.5 mL polyethylene glycol (PEG), stirred vigorously (30 min), transferred into a Teflon-lined autoclave (20.0 mL), and then heated at 200°C for 12 h in an electronic oven. Preparation of hematite (012) was done as follows:[29,33] 1.62 g

$FeCl_3$•$6H_2O$ was first dissolved in 50 mL 0.04 mol/L cetyltrimethylammonium bromide (CTAB) aqueous solution. Then, the whole mixture was stirred for 30 min and transferred into a 20 mL Teflon-lined stainless-steel autoclave, sealed, and maintained at 120°C for 32 h.

The ionic impurities in the five suspensions were removed by dialyzing with deionized water for 3 d. The pellets were then vacuum freeze-dried, stored in a desiccator, and used for cultivation experiments within one week. The Brunauer-Emmett-Teller (BET) surface areas of five Fe minerals were determined as follows (Units: $m^2\ g^{-1}$): 280 for ferrihydrite, 25 for goethite, 38 for hematite, 31 for hematite (001), and 0.4 for hematite (012).

Fungal-Mineral Cultivation Experiments. *Trichoderma guizhouense* NJAU 4742[6] was used as the experimental fungus. All cultivation experiments were performed at 28°C in the dark. For fungal growth, the following medium was used (per liter): 20 g glucose, 100 mg urea, 100 mg yeast extract, 1500 mg KH_2PO_4, 1400 mg $(NH_4)_2SO_4$, 400 mg $MgSO_4$•$7H_2O$, 100 mg $CaCl_2$•$2H_2O$, 5 mg $FeSO_4$•$7H_2O$, 2.5 mg $ZnSO_4$•$7H_2O$, 1.8 mg $MnSO_4$•H_2O, 5 mg $CoCl_2$, and 600 mg NaCl. The cultivation experiments were initiated by adding 0.1% (w/v) iron mineral to the liquid medium that was inoculated with$10^4\ mL^{-1}$ of *T. guizhouense* conidia and then incubated in a shaking incubator (170 rpm).

POD-like Nanozyme Activity Assay. POD-like nanozyme activity assays were carried out using 3,3′,5,5′-tetramethylbenzidine (TMB) solution (10 μL of 5 mg mL^{-1})

in dimethyl sulfoxide (DMSO) as the substrate in 2-mL tubes. Each tube contained 1 mg Fe mineral nanoparticles in the test TMB solution (1 mL) in 0.2 M NaAc-HAc buffer (pH 3.6). After H_2O_2 input (to a final concentration of 50 mM), a blue colour was observed at 28°C, and measured at 652 nm every 15 s for up to 10 min using a SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The control was set without addition of H_2O_2 or TMB solution. The POD-like activity (units) of Fe (oxyhydr)oxide mineral nanozymes was calculated as follows:[36]

$$b_{\text{nanozyme}} = V/(\varepsilon \times l) \times (\Delta A/\Delta t) \quad (1)$$

where b_{nanozyme} is the nanozyme activity expressed in catalytic units; V is the total reaction volume (μL); ε is the molar absorption coefficient of the substrate; and l is the path length of light (cm). The $\Delta A/\Delta t$ is the initial rate of change in absorbance at 652 nm in min^{-1}.

High Resolution X-ray photoelectron spectroscopy (XPS) Analyses. XPS analyses were conducted using a PHI 5000 Versa Probe (UlVAC-PHI, Japan) spectrometer that equipped with a monochromatic Al X-ray source (1486.6 eV). For achieving the absolute binding energy, the C 1s signal (284.8 eV) was used as an internal reference. The surface charge was balanced using a flood gun at 6 eV. The base pressure of spectrometer was 6.7×10^{-10} Torr. For wide-scan spectra, an energy range of 0–1100 eV was used with a step size of 1 eV and a pass energy of 80 eV. High-resolution XPS spectra were collected with a step size of 0.06 eV and pass energy of 40 eV. The energy precision was 0.06 eV. The high-resolution O 1s and C 1s XPS spectra were

fitted using the CasaXPS software.[38]

Nano-Scale Secondary Ion Mass Spectrometry (NanoSIMS) Analyses. Fungal-mineral cultivated samples were fixed, dehydrated, embedded, and cut into 1 μm-thick sections using a Diatome diamond knife (Leica UCT ultramicrotome). The samples were then gold-coated and examined using scanning electron microscopy (SEM) (Zeiss EVO18 scanning electron microscope) with a 20-kV accelerating potential. The samples were next analyzed using a NanoSIMS 50L (Cameca, Gennevilliers, France) instrument at the School of Earth System Science, Tianjin University, China. Prior to NanoSIMS analysis, a gold coating layer (~15 nm) was first pre-sputtered using a high primary beam current (pre-sputtering). Secondary ions of $^{12}C^{14}N^-$ and $^{16}O^-$ were collected by electron multipliers.[26] The NanoSIMS image sizes were 25 × 25 μm^2. The $^{12}C^{14}N^-$ ion was interpreted as representing fungal biomass or bioactive molecules, while $^{16}O^-$ ion was interpreted as mineral O, respectively.[26] The charging effect was compensated for using an electron flood gun. Thickness measurements of the cytoprotective exoskeleton, based on the line profile analysis from NanoSIMS images, were conducted using ImageJ (version1.45) combined with the OpenMIMS plugin (http://www.nrims.hms.harvard.edu/NRIMS_ImageJ.php).

Transmission Electron Microscopy (TEM) and Physicochemical Analyses. To observe the morphology of mineral particles, samples were dispersed in ethanol and then dropped onto C-coated copper grids. After being air-dried, the samples were observed by TEM (JEM-1400 PLUS) operating at an acceleration voltage of 120 keV.

The BET surface areas of Fe minerals were obtained from N adsorption isotherms obtained using a Micromeritics Tristar3000 instrument.[29] Extracellular $O_2^{\bullet-}$ and H_2O_2 were detected using specific stains.[10] *T. guizhouense* colonies on potato dextrose agar (PDA) plates were flooded with nitroblue tetrazolium (NBT) chloride and 3,3′-diaminobenzidine (DAB) for $O_2^{\bullet-}$ and H_2O_2, respectively. The NBT assay was composed of NBT chloride (2.5 mM, Sigma, St Louis, MO, USA) and 3-(N morpholino) propanesulfonate NaOH (5 mM, pH 7.6). The plates were first incubated with the stains in the dark and then imaged using a stereomicroscope (Leica DM 5000B, Leica Microsystems, Germany). In this assay, blue and brown precipitates indicate the presence of $O_2^{\bullet-}$ and H_2O_2, respectively.

Statistical Analyses. Significant differences were determined by Duncan's multiple range test at $p < 0.05$. One-way analysis of variance (ANOVA) was used to assess the data (means ± SD, $n = 3$) using IBM SPSS statistics 20.0.

■ RESULTS AND DISCUSSION

Contrasting POD-like Activity of Fe (Oxyhydr)oxide Mineral Nanozyme Responses to Fungal Growth. Predictably, the morphology (Figure 1A) and crystalline structures (Figure S1, Supporting Information (SI)) of five selected Fe (oxyhydr)oxide mineral nanozymes were distinct. TEM and X-ray diffraction (XRD) revealed that ferrihydrite was two-line ferrihydrite, poorly crystalline and aggregated as nano-sized particles, while goethite and hematite were well crystallized and

possessed a larger particle size than ferrihydrite. Although all the hematites possessed the same X-ray diffraction pattern (Figure S1A), their morphologies were clearly different, i.e., hematite of hexagonal shape, hematite (001) with a dominant (001) facet, and hematite (012) composed of (012), (102) and (-112) planes (Figure 1A). After 120 h cultivation, the crystalline structure of goethite, hematite, hematite (001) and hematite (012) remained stable, which may be attributable to surface-sorbed organic carbon that prevents these minerals from undergoing phase transformation[39] (Table S1). However, the poorly crystalline structure of ferrihydrite changed to a large extent (Figure S1B), i.e., with the presence of several prominent peaks at 9.0, 12.2, 19.2, 32.1, and 35.3 Å, suggesting an unstable structure of the poorly ordered ferrihydrite compared to well crystallized goethite and hematite during fungal cultivation experiments.

NBT and DAB staining[10] confirmed hotspots with concentrated $O_2^{\bullet-}$ on hyphae as well as a more diffuse distribution of H_2O_2 along the hyphae after 120 h cultivation of the fungus in the presence of ferrihydrite (Figure 1B), which was in agreement with our previous observations for fungal + hematite cultivation.[26,40] Fungal activity (Figure S2) created an acidic environment (Figure S2A), initiating the decay of H_2O_2 in the presence of Fe mineral nanoparticles through POD-like nanozyme activity[22] (Figure 1C). During fungal-mineral cultivation, fungal biomass increased gradually with cultivation time, in line with an increase in biological activity[41] (Figure S3), implying that the fungus was bioactive over the entire cultivation period. Intriguingly, the POD-like activity of Fe mineral nanozymes was affected differentially by growth of the fungus (Figure 1D,E). Specifically, the colorimetric signal of poorly ordered

ferrihydrite gradually became more intense with cultivation time; conversely, the colorimetric signal of well crystallized hematite, hematite (001), and hematite (012) decreased in intensity with cultivation time (Figure 1D). The colorimetric signal in the presence of goethite changed only slightly. These observations suggested that over 120 h cultivation, POD-like activity decreased gradually for poorly ordered ferrihydrite but markedly increased for well crystallized hematite.

By calculating the POD-like activity of Fe mineral nanozymes, it was evident that at the initial time point (0 h), ferrihydrite had the highest POD-like activity (0.075), which was approximately 3 times and 37 times higher than that of goethite (0.024) and hematite (0.002). Furthermore, the activity of the hematite (001) facet corresponded to ~80% of the POD-like activity from hematite. Compared to ferrhydrite and hematite, the POD-like activity of goethite only slightly decreased during 120 h cultivation. Therefore, during fungal-mineral interactions, the POD-like activity of ferrihydrite and goethite dropped over 3 and 1 times, respectively. In contrast, the POD-like activity of hematite increased aprroximately 17 times. Together, these results provide the first evidence that fungal growth has a contrasting effect on POD-like activity of the different Fe (oxyhydr)oxide mineral nanozymes, i.e., reducing the catalytic activity of poorly crystalline ferrihydrite but enhancing the catalytic activity of well crystallized hematite.

Linking Fe Mineral Nanozyme Activity to the Molecular Trade-offs Between Lattice Oxygen and Oxygen Vacancy Sites. Iron cations are known to catalyze

257 H_2O_2 to HO$^{\bullet}$ through Fenton catalysis.[15] However, the absence of dissolved Fe and
258 solid-phase Fe(II) during the initial 24 h cultivation and the disproportionate changes
259 in dissolved Fe and solid Fe(II) with POD-like activity (Figure S2B,C), implied that
260 Fe(II) ions in our systems were not responsible for the changed POD-like activity. This
261 observation was consistent with a previous report which found that catalytic reactions
262 on the mineral nanoparticle surface were multiple times more effective than dissolved
263 Fenton agents.[42] Furthermore, high-resolution XPS spectroscopy, a near-surface
264 sensitive technique,[43] was used to gain insight into changes in the molecular structures
265 of Fe and O in the iron(III) (oxyhydr)oxides. Wide scan XPS spectra of the Fe minerals
266 indicated the presence of Fe, O, and C (Figure S4A). Changes in the peak intensities of
267 surface Fe (Figure S5A,B) were obviously more pronounced than those for bulk Fe
268 (Figure S5C), and almost no shift of the Fe local coordination position during fungal-
269 mineral interactions was observed. Combined with Fe chemistry in solution (Figure
270 S5B,C), these spectroscopic features suggest that part of the Fe is transferred from the
271 minerals into solution, and that surface Fe structures (Figure S5) may not dominate the
272 changed POD-like activity of Fe mineral nanozymes (Figure 1D,E).

273 Surface O anions of mineral nanozymes are a significant redox partner for
274 transition metal (TM) cations (e.g., Fe in this study) due to the strong hybridization
275 between TM 3d and O 2*p* electronic states.[43] In stark contrast to the stable Fe spectra
276 over time (Figure S5), O 1*s* core-level XPS spectra showed that the chemical states of
277 O atoms from Fe mineral nanozymes had a dramatic change. One peak centered at
278 ~529.7 eV is assigned to the O_L bonded to Fe, whereas the others centered at ~531.6

and ~532.5 eV are attributed to adsorbed O species or non-lattice O (O_{NL}), mainly composed of O_V (i.e., occupied by hydroxyl species (OH^-) and its deprotonated form O^{2-}) and carbon-oxygen functional groups (O_C, i.e., C–O and C–O=C).[44] Based on the integrated area of Gaussian-resolved peaks, the O_{NL} percentage (i.e., $O_V + O_C$) for all of the examined Fe mineral nanozymes increased with cultivation time (Figure 2). Changes in O_{NL} and POD-like activity reveal that O_{NL}, the mixture of vacant oxygen (O_V) and carbon-oxygen functional groups (O_C), cannot be used as the determinant of catalytic activity in Fe mineral nanozymes.

The catalytic activity of mineral nanozymes is attributed to the ability of Fe (oxyhydr)oxides to undergo rapid redox cycles by releasing and storing O, where O_V sites chiefly contribute to the catalytic activity, owing to O_C being short of a prominent catalytic capacity.[38,44] To disentangle the relative contribution of O_V from the total O_{NL}, we re-evaluated the proportion of surface O_V (i.e., c(O_V)) by combining with C 1s core-level XPS spectra (Figure S4B, Tables S1–S2, SI), based on the following equation 2:[38]

$$c(O_V) = c_{oxygen} \times c(\text{adsorbed O}) - c_{carbon} \times [c(\text{C-O}) + 2 \times c(\text{O-C=O})] \quad (2)$$

Note that Eq. (2) is used to approximate the content of adsorbed hydroxyl species (OH^- and its deprotonated form O^{2-}) from the difference between total adsorbed O species (OH^-, O^{2-}, C-O, O-C=O) and carbon-oxygen groups (C-O, O-C=O). [38,44] Remarkably, the percentage of V_O exhibited a pronounced positive and linear relationship ($R > 0.6$, $p < 0.01$, n = 18) with POD-like activity for all the examined Fe mineral nanozymes

(Figure 3), strongly revealing that surface OH^- and O^{2-} are the key regulators of POD-like activity in Fe mineral nanozymes. The presence of V_O was further confirmed by Fe K-edge extended X-ray absorption fine structure (EXAFS) spectra (Figure S5C, SI) which showed a pronounced intensity decrease for ferrihydrite and goethite cultivations but an increase for hematite, hematite (001) and hematite (012) was observed at 1.7 $Å^{-1}$ with time which matched with the changes in Fe–O bonds and thus the existence of V_O in the bulk minerals.[45] Our spectral evidence suggested that fungal activity causes Fe migration from mineral nanoparticles (Figure S5, SI) to solution (Figure S2B,C, SI), leaving spaces with O-deficient interfaces, and thus O atoms on the Fe mineral lattice (Fe–O) easily escape to create O_V (Fe–O + Fe–O → 2Fe–O_V + O_2).[46] The O-deficient interfaces and the formed O_V sites were evidenced by an increase in surface hydroxyl species (OH^-) at around 532.5 eV (Figure 2) and a decrease in Fe–O bonds at 1.7 $Å^{-1}$ (Figure S5C). The O_V sites are shown to be mainly occupied by adsorbed hydroxyl species.[44,46] In summary, we show unexpected and exciting findings that fungi modulate the defect engineering, i.e., the molecular trade-offs between O_L and O_V in mineral nanoparticles during fungal-mineral interactions, probably with benefits in regulating Fe mineral nanozyme activity and maintaining ROS concentrations at subtoxic levels.

High-Resolution Detection of Fungal Nanozyme Catalytic Sites. Fungus-mediated catalytic reactions must mainly occur at fungal surfaces because firstly, fungal exudated $O_2^{\bullet-}$ does not diffuse far from its formation site[47] and secondly, $HO^\bullet$ has a very short half-life (~several nanoseconds).[48] To localize the catalytic sites of the Fe mineral nanozymes on fungal hyphae, NanoSIMS imaging was used to provide high spatial

resolution (down to 50 nm[49]) characterization of both mineral O anions and organic substances. After 120 h cultivation, fungal-mineral cultivated samples were fixed, embedded in epoxy resin, and cut into transverse sections (1 μm-thick) (Figure 4A). SEM images clearly showed the position of fungal hyphae, while NanoSIMS images further indicated that the hyphae were surrounded by a layer that contained mineral O ($^{16}O^-$) and organic substances ($^{12}C^{14}N^-$) (Figure 4A), consistent with observations of the whole hyphae which exclude possible interference from the epoxy resin used for embedding (Figure S6, SI). Furthermore, synchrotron radiation-based scanning transmission X-ray microscopy (STXM) images also supported the presence of a heterogenous mineral O layer on the examined hyphae (Figure S7, SI). These observations are compatible with previous reports suggesting that the cytoprotective exoskeleton can act as a "cell-in-shell" structure.[50–52] In nature, hard cytoprotective exoskeletons are commonly used to protect microbial soft tissues from environmental stress.[8] To determine the thickness of the cytoprotective exoskeleton on the hyphae, a region of interest (ROI) analysis[53] was conducted. The results from ROI analysis revealed that the thickness of the cytoprotective exoskeleton was approximately 0.1–0.6 μm (Figure 4B). These observations demonstrate that the POD-like activity of Fe mineral nanozymes may reside mainly in this thin cytoprotective exoskeleton, which provides a similar protective barrier analogous to fungal spores.

Proposed catalytic reactions in the fungal cytoprotective exoskeleton. Although cytoprotective exoskeleton coatings have been demonstrated to form on taxonomically and ecologically diverse bacteria,[52] yeast cells,[50,54] and filamentous fungi,[55] this study

provides the first evidence that links POD-like activity in the cytoprotective exoskeleton to the molecular trade-offs between O_L and O_V in the Fe mineral nanoparticles. Fungal biomineralization can alter the surface properties (Figures 2, S4, and S5) of Fe minerals through Fe(III) reduction (Figure S2B), cation migration (Figure S2C), and co-precipitation (Figures 4,5) processes.[2,26,40] These processes break the regular periodic arrangement of atoms (e.g., Fe and O) in the crystalline structures (Figures 2 and S5) and leave behind surface defect sites (Figure S5).[35] The surface defect sites are reported to serve as reactive centers to drive intrinsic nanozyme activity.[34] We accordingly propose that during fungal-mineral interactions, the fungal cell wall and exudated extracellular polymeric substances (EPS, Figure S3)[30] create abundant nucleation sites which retain Fe ions and mineral nanoparticles (Figure 5), and that this intimate relationship between metals and biopolymers further promotes the rapid formation of a cytoprotective exoskeleton (Figure 4).[17,31] The cytoprotective exoskeleton is capable of complex, artificial biochemical cascades (including biocatalysis) on the cells.[16,17]

Furthermore, the catalytic reactions occurring in the fungal cytoprotective exoskeleton may include multiple steps (Figure 5). First, H_2O_2 excreted by the fungus (Figure 1B) may react with Fe minerals and produces surface Fe(II) (Figure S3B) and peroxide radicals through Fenton-like reactions.[56] On the mineral surface, Fe minerals are hydrated and in a first step the adsorbed H_2O can be exchanged with exuded H_2O_2 which is thermodynamically favourable ($\Delta G° < 0$).[57] Secondly, the dissociation of H_2O_2 leads to Fe(II) oxidation with hydroxyl species OH^- and $HO^•$ ligands. Hydroxyl species

366 on the mineral surfaces (Table S1) can enhance V_O production (Figures 2 and S5C) and
367 further produce adsorption sites for OH^- and H_2O (Figure 2).[44] As a result, surface
368 hydroxylated minerals yield more HO•. Finally, a release of HO• radicals from these
369 species into solution occurs but this may be undetectable owing to its short half-life.[57]
370 The HO• radicals usually react with substrates on sites (Figure 1C-E), whereas the
371 estimated diffusion distance of H_2O_2 is about 1.5 mm.[48] Thus, we surmise that fungal
372 exuded H_2O_2 can diffuse in the fungal cytoprotective exoskeleton (Figure 4) and then
373 react with Fe mineral nanozymes (Figure 1C-E). As a sink of HO• radicals, the EPS
374 matrix (Figures 4 and S3)[30] around the hyphae will protect the fungusfrom being
375 exposed to its own ROS. Because the catalytic behaviour of Fe mineral nanozymes
376 (Figure 1D,E) strongly depends on their surface area, crystal morphology, and
377 crystalline structure,[24,58] poorly ordered ferrihydrite had a higher (3–30 times) catalytic
378 activity than well crystallized goethite and hematite (Figure 1D,E).

379 **Environmental Implications.** Using a combination of classic POD colorimetric
380 reactions, high-resolution XPS spectra and NanoSIMS images, our findings
381 unambiguously reveal key traits that determine the POD-like activity of Fe mineral
382 nanoparticles during fungal-mineral interactions. Specifically, fungi drive POD-like
383 activity by modulating the molecular trade-offs between O_L and O_V in Fe(III)
384 (oxyhydr)oxide mineral nanoparticles, which might occur mainly at a thin (hundreds of
385 nm-thickness) cytoprotective fungal exoskeleton. In particular, our findings indicate
386 varying effects for different mineral facets of the same (hematite) mineral on POD-like
387 activity, pointing to the importance of further exploration of structure-activity

388 relationships[59,60] that influence the POD-like activity of mineral nanozymes.

389 Although large amounts of nutrients can be closely associated with minerals, i.e.,
390 coexisting as mineral-organo associations in terrrestrial environments,[21,61] fungi have
391 been demonstrated to possess the ability to access carbon and other nutrient elements
392 (e.g., nitrogen, phosphorus, and iron) from mineral-associated organic matters through
393 regulation of ROS levels. [5,26,40,61-64] In this study, our findings suggest that fungal
394 regulation of the ROS level may be mainly located at their cytoprotective exoskeleton
395 layers, and therefore play a more important role in the biogeochemical cycling of C, N,
396 P, and Fe than has been previously appreciated (e.g., extracellular enzymes and carbon
397 based metabolism[65]). This emphasises that the fungal cytoprotective exoskeleton acts
398 as an "artificial cell coating" [17] to enable new or augmented biological functions, e.g.,
399 preserving enzymatic activity[66] and cell viability,[67] as well as enhancing extracellular
400 electron transfer.[68,69] In summary, the consequences of our findings regarding the
401 molecular trade-offs between oxygen anions improve our understanding of the role of
402 mineral nanomaterials in fungal mediated biogeochemical cycles of carbon and other
403 elements in terrestrial environments.

404 Fungi have acquired many of their biofunctionalities over ~1.0-billion-years of
405 evolution,[70] but fungal evolution in degrading organic pollutants lags behind the rapid
406 increase of synthetic organic pollutants in the new human-influenced geological epoch,
407 the Anthropocene.[71] Our findings suggest that fungi may evolve in successfully coping
408 with organic pollutants by interacting with surrounding redox-active Fe minerals and

forming cytoprotective exoskeleton layers. These cytoprotective exoskeleton layers not only prevent the intimate contact between cells and pollutants but also improve the degradation efficiency of organic pollutants by producing non-selective strong oxidant HO• radicals.[8,17,50] More broadly, given that teragram (Tg)-level abundance of mineral nanoparticles[1] and ~12 Gt (gigaton ton, 10^9 t) C of fungal biomass[72,73] are present in terrestrial ecosystems, the current research has prompted us to further explore fungal-mineral interactions that produce HO• radicals which have multiple environmental and ecological implications, including nutrient acquisition[62] and the potential *in situ* degradation of organic contaminants.[75] Since fungi constitute a vast kingdom of approximately 2–6 million or more species,[19] future studies are warranted to explore whether modulation of molecular trade-offs between oxygen anions in Fe minerals is a widespread fungal property.

■ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org.

Associated content in support of the main manuscript includes additional methods, twelve figures, and three data tables (PDF).

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The authors declare no competing financial interest.

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Figures Caption

Figure 1. Morphology and POD-like activity of Fe mineral nanozymes during fungal-mineral interactions. (a) TEM images of original Fe mineral nanozymes. Scale bars represent 100 nm (white) and 1 μm (blue), respectively. (b) Location of $O_2^{\bullet-}$ (blue) and H_2O_2 (pink) in fungal hyphae shown by staining. Scale bar, 10 μm. (c) Catalytic reaction of POD mimics (i.e., nanozymes) in the presence of H_2O_2 (electron acceptor) with colourless 3,3′,5,5′-tetramethylbenzidine (TMB) to form blue oxidized TMB (oxTMB). NP, iron mineral nanoparticles. (d) Photograph of colorimetric reaction after 10 min incubation of Fe mineral nanozymes (1 mg) with H_2O_2 (50 mM) + TMB (10 μL, 5 mg mL^{-1}) in pH 3.6 buffer. Tubes 1–6 represent Fe mineral nanozymes at the cultivation times of 0, 24, 48, 72, 96, and 120 h, respectively. (e) Changes in the POD-like activity of Fe mineral nanozymes with cultivation time. Data are means ± SD (n = 3). *T.*+Fh, *T. guizhouense* plus ferrihydrite. *T.*+Goe, *T. guizhouense* plus goethite. *T.*+Hem, *T. guizhouense* plus hematite. *T.*+Hem (001), *T. guizhouense* plus hematite (001). *T.*+Hem (012), *T. guizhouense* plus hematite (012). POD, peroxidase.

Figure 2. Molecular trade-offs between O_L and O_V during fungal-mineral interactions. (a) O 1*s* core-level XPS spectra of Fe mineral nanozymes. (b) The relative abundance of O_L, O_V and O_C. In the O1*s* spectrum: the cyan peak located at ~529.7 eV is assigned to the lattice oxygen (O_L) bonded to cations, while the blue and orange peaks centered at around 531.6 and 532.5 eV are referred to the non-lattice O (O_{NL}) or adsorbed O species, mainly composed of O vacancy (O_V) and carbon-oxygen groups (O_C). Specifically, O_V, occupied by hydroxyl species (OH^-) and its deprotonated form O^{2-},

exhibit a strong catalytic capacity, while O_C, occupied by carbon-oxygen groups (C–O and C–O=C), is suggested to have a reduced catalytic capacity.[38,44] *T.*+Fh, *T. guizhouense* plus ferrihydrite experiment. *T.*+Goe, *T. guizhouense* plus goethite. *T.*+Hem, *T. guizhouense* plus hematite. *T.*+Hem (001), *T. guizhouense* plus hematite (001). *T.*+Hem (012), *T. guizhouense* plus hematite (012).

Figure 3. Relationship between O_V and POD-like activity. *T.*+Fh, *T. guizhouense* plus ferrihydrite. *T.*+Goe, *T. guizhouense* plus goethite. *T.*+Hem, *T. guizhouense* plus hematite. *T.*+Hem (001), *T. guizhouense* plus hematite (001). *T.*+Hem (012), *T. guizhouense* plus hematite (012). O_V, oxygen vacancy. POD, peroxidase. Data are means ± SD (n = 3). $^{**}p < 0.01$.

Figure 4. Cross-sectional (1 μm-thickness) observation of the fungal cytoprotective exoskeleton on a single hypha after 120 h cultivation. (a) Correlative SEM and NanoSIMS images. (b) Thickness of cytoprotective exoskeleton based on the line profile analysis from NanoSIMS images. Secondary ions for $^{16}O^-$ and $^{12}C^{14}N^-$ indicate the presence of mineral O and fungal hyphae, respectively. Blue and white arrows indicate the presence of fungal hyphae and cytoprotective exoskeleton, respectively. “n” in (b) is the number of hyphae. Scale bar = 2 μm. *T.*+Fh, *T. guizhouense* plus ferrihydrite. *T.*+Goe, *T. guizhouense* plus goethite. *T.*+Hem, *T. guizhouense* plus hematite. *T.*+Hem (001), *T. guizhouense* plus hematite (001). *T.*+Hem (012), *T. guizhouense* plus hematite (012). SEM, scanning electron microscopy. NanoSIMS, nanoscale secondary ion mass spectrometry.

706 **Figure 5**. Schematic of fungal-mediated molecular trade-offs between lattice oxygen
707 and oxygen vacancy sites controlling intrinsic peroxidase-like activity of iron
708 (oxyhydr)oxide mineral nanoparticles.