Triple oxygen isotope insight into terrestrial pyrite oxidation

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Edited by Mark H. Thiemens, University of California San Diego, La Jolla, CA, and approved February 28, 2020 (received for review October 9, 2019)

The mass-independent minor oxygen isotope compositions (\(\Delta ^{17}O\)) of atmospheric \(O_2\) and \(CO_2\) are primarily regulated by their relative partial pressures, \(p_{O_2}/p_{CO_2}\). Pyrite oxidation during chemical weathering on land consumes \(O_2\) and generates sulfate that is carried to the ocean by rivers. The \(\Delta ^{17}O\) values of marine sulfate deposits have thus been proposed to quantitatively track ancient atmospheric conditions. This proxy assumes direct \(O_2\) incorporation into terrestrial pyrite oxidation-derived sulfate, but a mechanistic understanding of pyrite oxidation—including oxygen sources—in weathering environments remains elusive. To address this issue, we present sulfate source estimates and \(\Delta ^{17}O\) measurements from modern rivers transecting the Annapurna Himalaya, Nepal. Sulfate in high-elevation headwaters is quantitatively sourced by pyrite oxidation, but resulting \(\Delta ^{17}O\) values imply no direct trophospheric \(O_2\) incorporation. Rather, our results necessitate incorporation of oxygen atoms from alternative, \(^{17}O\)-enriched sources such as reactive oxygen species. Sulfate \(\Delta ^{17}O\) decreases significantly when moving into warm, low-elevation tributaries draining the same bedrock lithology. We interpret this to reflect overprinting of the pyrite oxidation-derived \(\Delta ^{17}O\) anomaly by microbial sulfate reduction and reoxidation, consistent with previously described major sulfur and oxygen isotope relationships. The geologic application of sulfate \(\Delta ^{17}O\) as a proxy for past \(p_{O_2}/p_{CO_2}\) should consider both 1) alternative oxygen sources during pyrite oxidation and 2) secondary overprinting by microbial recycling.

atmospheric \(O_2\) | chemical weathering | \(\Delta ^{17}O\) Hima | Oasis sulfur cycle

A tmospheric molecular oxygen (\(O_2\)) governs biogeochemical cycles, Earth’s surface redox state, and the evolution of life; \(O_2\) partial pressure (\(p_{O_2}\)) has increased drastically in the geologic past in response to biologic and geologic drivers (1, 2). Despite this importance, quantitatively constraining \(p_{O_2}\) throughout Earth’s history remains challenging due to a lack of direct proxies (2). Recently, the minor oxygen isotope composition (\(^{17}O/^{16}O\), reported as \(\Delta ^{17}O\); Materials and Methods) of geologically preserved minerals has been proposed as one such proxy (1, 3–8). This approach utilizes the fact that photochemical reactions between \(O_2\), ozone (\(O_3\)), and carbon dioxide (\(CO_2\)) in the stratosphere generate anomalous, mass-independent \(^{17}O\) signatures that are mixed into the troposphere (9). In particular, tropospheric \(O_2\) carries a large negative \(\Delta ^{17}O\) anomaly, the magnitude of which reflects 1) the ratio of \(O_2\) to \(CO_2\) partial pressures (\(p_{O_2}/p_{CO_2}\)), which determines the strength of the signal acquired during stratospheric photochemistry, and 2) the amount of \(O_2\) generated by oxygenic photosynthesis in the biosphere, which dilutes stratospheric inputs (1, 6). Thus, if \(p_{CO_2}\) and biogenic productivity can be independently constrained, then tropospheric \(O_2\) \(\Delta ^{17}O\) is potentially a direct and quantitative \(p_{O_2}\) tracer.

Sulfate (\(SO_4^{2-}\))-bearing minerals are particularly attractive reservoirs for this method of reconstructing \(p_{O_2}\) in the geologic past because oxidative pyrite (\(FeS_2\)) weathering consumes \(O_2\) (10, 11). If oxidation of pyrite contained in exhumed sedimentary rocks on land follows the reaction

\[FeS_2 + \frac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ , \tag{1}\]

then contemporaneous tropospheric \(O_2\)—including its anomalous \(^{17}O\) composition—could be directly incorporated into resulting sulfate (12). Isotopically labeled oxidation experiments support this idea; \(\sim 8 \text{ to } 15\% \) of sulfate oxygen atoms have been shown to be sourced directly from dissolved \(O_2\) under well-oxygenated experimental conditions (12, 13). In light of these results and the fact that sulfate oxygen isotopes do not equilibrate with water on geologically relevant timescales, preserved barite (\(BaSO_4\)), gypsum (\(CaSO_4\)), and carbonate-associated sulfate \(\Delta ^{17}O\) values have been used to reconstruct \(p_{O_2}/p_{CO_2}\) throughout Earth’s history (3–8, 14, 15). Such interpretations require two criteria to be met: 1) This direct \(O_2\) consumption mechanism (Eq. 1) contributes a significant proportion of pyrite oxidation-derived sulfate, and 2) primary \(\Delta ^{17}O\) signals are not overprinted or diluted in the environment prior to being preserved in the rock record. Tropospheric \(O_2\) \(\Delta ^{17}O\) compositions in the geologic past are thus typically reconstructed using the most negative sulfate \(\Delta ^{17}O\) value from a given geologic unit (i.e., least overprinted) and scaling by a \(\sim 8\% \) to \(15\% \) \(O_2\) incorporation factor (7, 15).

However, anoxic laboratory experiments and modern field observations complicate this picture. Specifically, both biotic and

Significance

Pyrite is oxidized during weathering to form dissolved sulfate that is carried to the ocean by rivers. This process is thought to incorporate atmospheric \(O_2\)-derived oxygen; geologically preserved sulfate has thus been proposed to directly trace \(O_2\) isotope compositions. However, this mechanism has not been thoroughly tested in modern weathering environments. We show that dissolved sulfate in Himalayan rivers is predominantly derived from pyrite, yet its oxygen isotope compositions preclude direct \(O_2\) incorporation. Rather, alternative oxygen sources (e.g., reactive oxygen species) may be incorporated during oxidation, prompting reconsideration of the pyrite oxidation mechanism and the interpretation of geologically preserved sulfate as a direct \(O_2\) tracer.

Author contributions: J.D.H. and D.T.J. designed research; J.D.H. and H.O. performed research; A.V.T., E.T.T., and M.J.B. contributed new reagents/analytic tools; J.D.H., H.O., and D.T.J. analyzed data; J.D.H., H.O., A.V.T., E.T.T., M.J.B., and D.T.J. wrote the paper. The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi/10.1073/pnas.1917518117/-/DCSupplemental.

abiotic experiments (10, 11, 13, 16, 17) imply that sulfate oxygen can be quantitatively sourced from meteoric water,

\[
\text{Fe}_2\text{S}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+. \tag{2}
\]

O\text{2} is then consumed indirectly during subsequent oxidation of Fe\text{3+} to Fe\text{2+}. If pyrite oxidation in the environment exclusively follows Eq. 2, then product sulfate \Delta^{17}O is decoupled from atmospheric pO\text{2}/pCO\text{2}. Recent studies using major oxygen and sulfur isotope compositions (\delta^{18}O, \delta^{34}S; Materials and Methods) suggest that pyrite oxidation in some modern river systems occurs in suboxic groundwater aquifers and quantitatively incorporates meteoric water-derived oxygen (15–20). Consistent with this interpretation, detailed studies of shale bedrock drill cores indicate that pyrite oxidation occurs in low-O\text{2} pores and microfractures within a deep, sharp reaction front independent of erosion rate (21). Such results raise the question as to why direct O\text{2} incorporation into sulfate is evident in the geologic past but is apparently absent from modern pyrite weathering environments. Furthermore, pyrite oxidation must proceed via one-electron transfer steps regardless of the exact mechanism involved (11, 22, 23), raising the possibility that reactive oxygen species (ROS; e.g., H\text{2}O\text{2}, O\text{2}·, and ONOO·) may provide additional, isotopically unique oxygen sources to sulfate (24, 25). ROS incorporation could help reconcile modern and ancient observations, but its environmental importance remains unknown.

To provide insight, we constrain the sulfate budget in a suite of highly erosive mountainous rivers. Building upon previous work reporting \delta^{13}O and \delta^{18}S evolution (20), we document sulfate source and \Delta^{17}O values for samples collected throughout the Marsyandi River basin, Annapurna Himalaya, Nepal. These results update our understanding of fluvial sulfate oxygen isotope systematics, with implications for modern weathering budgets and geologic pO\text{2}/pCO\text{2} reconstructions.

**Study Setting**

The Marsyandi River is located in the southern flank of the Annapurna Himalaya, central Nepal (Fig. L1). Headwaters drain the Tethyan Sedimentary Series (TSS); although some Manaslu granite exposures exist within our study region, bedrock is primarily described as a continental margin sequence containing variably metamorphosed limestone and silticlastics with interbedded pyrite-rich black shales (26–28). The TSS lies entirely within the Himalayan orogenic rain shadow; vegetation is sparse (29), and mean annual precipitation (MAP) never exceeds 1,000 mm y\text{−1} (average \sim 500 mm y\text{−1}) (30). Mean annual temperature (MAT) within the TSS ranges from –10 \textdegree C in the highest elevations to near 15 \textdegree C in the lowest elevations (Fig. 1B). Downstream of the TSS, the Higher Himalayan Crystalline Series (HHCS) contains two main bedrock units: Formation I (FI), dominated by silicate gneiss, and Formation II (FII), dominated by calc-silicate metamorphic rocks (26, 31). MAP increases markedly due to the influence of the Indian Summer Monsoon, reaching 2,500 mm y\text{−1} (30). Wheres MAT exhibits only modest increases (Fig. 1B). Further downstream, the Lesser Himalayan Series (LHS) is described as undifferentiated lower to medium-grade metasedimentary and metavolcanic rocks (27) and by MAP and MAT values near those within the HHCS. Importantly, evaporitic successions have never been reported in central Nepal (29).

*Schildrich-ri hydrothermal hot springs along fault zones do suggest the presence of basinal brines or buried halite (32, 33). However, hot springs are deficient in both SO\text{2}− and Ca\text{2+} relative to Cl− and Na\text{+} (33), indicating minimal contribution of gypsum dissolution to hydrothermal solutes and thus precluding evaporite weathering as a major driver of observed sulfate trends.

**Results**

Samples were collected in May (premonsoon) and September (monsoon season) 2002 across a \sim 120-km transect starting at the Marsyandi River headwaters in the TSS (5,000-m elevation) and ending in the LHS near the base of the Himalaya (750-m elevation; Fig. 1B) (29, 32, 34, 35). Most samples were collected from small tributaries draining single lithologies (median catchment area = 20 km\text{2}; Dataset S2) and are independent of one another. Tributaries span a range of catchment slopes (24.0\textdegree to 41.1\textdegree), MAP (407 mm y\text{−1} to 1,330 mm y\text{−1}), MAT (5 \textdegree C to 15 \textdegree C), and glacial extent (0 to 46% by area; Dataset S2). This approach allows us to isolate the effect of geomorphic environmental changes on riverine chemistry while holding lithology and catchment area roughly constant (36). To investigate main-stream evolution, we additionally report results from the Marsyandi River at four locations throughout the basin.

**Sulfate Sources.** Sulfate source contributions—including atmospheric inputs and carbonate, evaporite, granite, and shale weathering—were estimated using a conservative tracer mixing model and previously published major ion concentrations (Materials and Methods) (29). Results indicate that the fraction of riverine sulfate derived from pyrite oxidation in shale, termed \chi_{py}, ranges from 40 to 100% (Fig. 1B). \chi_{py} is highest in sulfate-rich TSS tributaries, ranging from 60 to 100% and averaging 91 \pm 11% (\mu \pm 1\sigma, n = 25; Dataset S1). Slightly lower \chi_{py} values in the most downstream TSS samples could result from partial HHCS or Manaslu granite bedrock exposure in these tributaries (29). Across the entire sample set, \chi_{py} decreases moving downstream (slope = –0.003% km\text{−1}, p = 6.5 \times 10^{-5}), largely due to the sharp drop within tributaries draining HHCS FII calc-silicate metamorphic rocks. This drop coincides with the Main Central Thrust (35) and is likely driven by contributions from limestone weathering in this region, as suggested by our weathering model results (Dataset S1). In contrast, when considering high TSS samples that are sufficiently upstream of potential HHCS and Manaslu granite influence (≤40 km from the source) (29), \chi_{py} remains near 100% and does not correlate with downstream distance (p > 0.05). Importantly, results account for precipitation-derived sulfate, including anthropogenic sources (i.e., acid rain). These inputs never exceed 11% of total sulfate in any sample, averaging only 2.0 \pm 2.5% (n = 38) across the entire sample set and 1.0 \pm 1.1% (n = 25) within the TSS, consistent with low measured sulfate concentrations in Himalayan rain water (37, 38). We thus primarily limit our sulfate \Delta^{17}O isotope measurements to upstream TSS catchments.

**Sulfate Isotopes.** Riverine sulfate \Delta^{17}O ranges from 0.041 to 0.180%; Vienna Standard Mean Ocean Water (VSMOW) (average = 0.117 \pm 0.038%; VSMOW; n = 29; Dataset S1) and exhibits a significant decrease moving downstream (slope = –0.001% km\text{−1}; p = 3.8 \times 10^{-3}; R^2 = 0.35; SI Appendix, Fig. S1). Sulfate \Delta^{17}O is negatively correlated with both \delta^{18}O (slope = –0.005% \cdot 10^{-4}; p = 3.8 \times 10^{-3}; R^2 = 0.44; Fig. 24) and \delta^{34}S (slope = –0.002% \cdot 10^{-4}; p = 0.032% \cdot 10^{-4}; R^2 = 0.14; Dataset S1). Because sulfate oxygen can be sourced directly from water (Eq. 2) (10, 11, 16, 17, 39), we additionally report offsets between sulfate and concomitant river water \delta^{18}O and \Delta^{17}O, termed \Delta\delta^{18}O and \Delta\Delta^{17}O (Fig. 2B and Materials and Methods). Water \delta^{34}S was measured directly (20), whereas \Delta^{17}O was calculated using the global meteoric water line (40). \Delta\delta^{18}O ranges from –0.033 to 0.103% (average = 0.048 \pm 0.034% ; n = 26; Dataset S1) and displays a statistically significant decrease with increasing \Delta^{17}O (slope = –0.008% \cdot 10^{-4}; p = 8.3 \times 10^{-4}; R^2 = 0.39; Fig. 2B). In contrast, both \Delta\delta^{18}O and \Delta\Delta^{17}O values do not correlate with \chi_{py} estimates nor
with estimated fractional dolomite, gneiss/granite, limestone, or evaporite end-member weathering contributions ($p > 0.05$). Unlike $\chi_{py}$, which is largely stable near 100% within the TSS, sulfate $\Delta^{17}\text{O}$ values in tributaries draining this region display large and systematic decreases moving downstream (SI Appendix, Fig. S1).

**Discussion**

**Fluvial Sulfate Oxygen Source.** We observe spatially coherent sulfate oxygen isotope signals throughout the Marsyandi River basin. Headwater sulfate exhibits large, positive $\Delta^{17}\text{O}$ values ($\Delta\delta^{17}\text{O} \approx +0.10\%o$) as well as $\delta^{18}\text{O}$ near that of local meteoric water ($\delta^{18}\text{O}_{\text{water}} \approx 5\%o$). Consistent with previous observations based on sulfate $\delta^{18}\text{O}$ from a suite of global rivers (18–20), these results imply that pyrite weathering in mountainous headwaters occurs primarily within suboxic groundwater aquifers. Sulfate $^{17}\text{O}$ compositions indicate no appreciable $O_2$ incorporation during pyrite oxidation (Eq. 1). Any $O_2$ contribution would produce sulfate with $\Delta^{17}\text{O}$ below the meteoric water line (i.e., $\Delta\delta^{17}\text{O} < 0$), since $\delta^{17}\text{O} = -0.5\%o$ VSMOW in modern tropospheric $O_2$ (6, 9). However, the opposite is observed (Fig. 2B). Furthermore, mass-dependent isotope fractionation associated with anoxic pyrite weathering (Eq. 2) likely cannot explain observed $^{17}\text{O}$ enrichment, since this would require a mass law of $\theta \approx 0.54$ to 0.55, considerably higher than any known low-temperature microbial or abiotic processes (1, 41). Rather, positive $\Delta\delta^{17}\text{O}$ values require either 1) overprinting by atmospherically derived (precipitation or aerosol) sulfate inputs (1, 41) or 2) an additional, $^{17}\text{O}$-enriched oxygen source that is incorporated into sulfate during pyrite oxidation.

We first consider atmospheric inputs. Many oxygen-bearing gases carry positive $^{17}\text{O}$ anomalies that can be transferred to sulfate in the atmosphere. For instance, aqueous-phase $SO_2$ oxidation by $^{17}\text{O}$-enriched $H_2O_2$ or $O_3$ in the atmosphere generates sulfate in rainwater and aerosols with positive $\Delta^{17}\text{O}$ values (41, 42). It is therefore possible that aerosol and/or rainwater inputs contribute to observed riverine sulfate signals. However, although correct in the required $\Delta^{17}\text{O}$ directionality, atmospheric deposition alone fails to explain our results for three reasons:

1) According to our conservative tracer mixing model, precipitation accounts for $2.0 \pm 2.5\%$ of fluvial sulfate in the entire sample set and only $1.0 \pm 1.1\%$ within the TSS, where $\Delta^{17}\text{O}$ values are highest. If we assume all pyrite oxidation–derived sulfate follows Eq. 2 with no mass-independent isotope fractionation, then mass balance considerations require

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**Fig. 1.** (A) Marsyandi basin map. Black lines delineate catchment areas upstream of each sample location. Background color represents elevation. Inset shows study location (red box) within Nepal. (B) Downstream evolution of elevation (blue line) and local MAT (red line) moving along the main-stem Marsyandi. Also shown is the estimated fraction of sulfate derived from pyrite oxidation ($\chi_{py}$) for each sample; tributaries are plotted at their confluence with the main stem. Background shading indicates bedrock lithology. TSS tributaries, white squares; HHCS FII tributaries, gray circles; HHCS FI tributaries, light gray triangles; LHS tributaries, dark gray diamonds; main-stem Marsyandi, black triangles.

**Fig. 2.** Oxygen isotope compositions. (A) Colored points, $\Delta^{17}\text{O}$ vs. $\delta^{18}\text{O}$ scatter plot for all sulfate samples; gray line, Marsyandi basin river water isotope array. Water $\delta^{18}O$ was measured directly (20), and $\Delta^{17}\text{O}$ was calculated using the global meteoric water line (40). (B) Scatter plot of $\Delta\delta^{18}\text{O}$ vs. $\delta^{16}\text{O}$, the offsets between sulfate and concomitant river water $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ (Materials and Methods). For A and B, symbol colors refer to the estimated fraction of sulfate derived from pyrite oxidation ($\chi_{py}$) and shapes represent TSS tributaries (squares), LHS tributaries (diamonds), and the main-stem Marsyandi (triangles). $\Delta^{17}\text{O}$ and $\Delta\delta^{17}\text{O}$ uncertainty ($\mu \pm 1\sigma$) is the long-term instrument reproducibility (Materials and Methods); $\delta^{16}\text{O}$ and $\Delta\delta^{18}\text{O}$ uncertainty is smaller than marker points (20). A and B show that sulfate is generally enriched in $^{17}\text{O}$ relative to meteoric water, opposite of what would be expected from direct atmospheric $O_2$ incorporation.
precipitation-derived sulfate with $\Delta^{17}O$ values up to $\sim 9\%$ VSMOW (higher if any contribution by Eq. 1 is invoked). This is nearly an order of magnitude higher than measured rainwater sulfate $\Delta^{17}O$ (41).

Still, it has previously been shown that $\sim 25\%$ of sulfate in Rocky Mountain headwater streams draining sulfate-poor gneiss and granite lithologies can be sourced from atmospheric deposition in snowpack (43). However, Marsyandi headwater stream sulfate concentrations are $\sim 20\times$ higher than those of ref. 43. Thus, if we assume snowpack sulfate $\Delta^{17}O$ values are similar in the Himalaya and Rocky Mountains (i.e., $0.8$ to $1.5\%$), then mass balance considerations would require snowpack sulfate concentrations of $\sim 150 \mu M$. This is an order of magnitude higher than reported Himalayan precipitation and Rocky Mountain snowpack concentrations (37, 38, 43).

2) Riverine sulfate $\delta^{18}O$, $\delta^{34}S$, and $\Delta^{17}O$ trends are identical during premonsoon and monsoon seasons despite differences in air mass trajectories, precipitation amounts, and the importance of wet vs. dry deposition. In particular, we would expect significantly lower $\Delta^{17}O$ during the dry premonsoon season, since atmospheric sulfate produced by gas-phase OH$^+$ does not carry mass-independent $\Delta^{17}O$ signal (42). Similarly, if aerosol deposition were driving observed trends, then we would expect a much larger positive signal during the monsoon season when air masses cross the (heavily polluted) Indian subcontinent (41). This is not observed. Rather, for sites in which premonsoon and monsoon sampling can be compared directly, there exists no statistically significant seasonal difference in either sulfate $\delta^{18}O$ (May to September: $-0.1 \pm 2.8\% ; \mu \pm 1\sigma ; n = 4$) or sulfate $\Delta^{17}O$ (May to September: $-0.002 \pm 0.030\% ; \mu \pm 1\sigma ; n = 3$).

3) Fluvial sulfate concentrations and $\Delta^{17}O$ values both decrease as MAP increases moving downstream, opposite of the expected atmospheric deposition trend. Highly erosive tributaries draining pyrite-rich TSS lithologies exhibit particularly positive $\Delta^{17}O$ values, up to $1.80\%$ VSMOW, despite receiving only $\sim 400 \text{ mm y}^{-1}$ MAP (Dataset S2). Similar to snowpack, it is possible that glacial meltwater contributes atmospheric precipitation- or aerosol-derived sulfate to headwater streams (44). However, we observe no statistical correlation between sulfate $\Delta^{17}O$ and glacial extent across our sample set ($p > 0.05$; Datasets S1 and S2), suggesting that glacier meltwater contributions alone cannot explain observed trends. Thus, while atmospheric deposition can contribute to fluvial sulfate with positive $\Delta^{17}O$ values (43), such contributions are likely negligible in the Marsyandi River basin.

Marsi River headwater sulfate is thus quantitatively rock derived. The question then arises: What is the source of $^{17}O$-enriched oxygen to pyrite oxidation-derived sulfate? Pyrite oxidation proceeds via one-electron transfer steps occurring at anode sites on the mineral surface and thus involves oxygen-bearing sulfur intermediates species ($S_2O_3^{2-}$, $SO_4^{2-}$, and $SO_2^{2-}$) (11). Sulfite ($SO_3^{2-}$), the most likely terminal precursor to sulfate, rapidly reaches isotopic equilibrium with water (45), raising the possibility that pyrite oxidation-derived sulfate $^{17}O$ is buffered to water regardless of the original oxygen source. However, the measured equilibrium sulfate–water $^{18}O$ effect (45) is significantly larger than headwater $\Delta^{18}O$ values observed here ($\sim 5\%$; Fig. 2B), suggesting sulfate–water–isotope equilibrium cannot explain observed $\delta^{18}O$ and $\Delta^{17}O$ trends.

Rather, we hypothesize that ROS isotope signatures are incorporated into pyrite oxidation-derived sulfate, consistent with laboratory experimental results (10, 11, 16, 17, 22, 39). Electrochemical studies treat pyrite as a semiconductor with a sulfur anode and an iron cathode; this model states that sulfate oxygen is quantitatively derived from $H_2O$ or other $O(-II)$-bearing species, whereas $O_2$ is reduced via ROS intermediates on iron cathode sites (22, 23). Furthermore, pyrite surfaces are almost always covered in Fe(III) hydroxide patches (11, 46); these patches have been shown to disproportionate $H_2O_2$, generating $O_2$ and $H_2O$ with unique isotope signatures relative to those of bulk fluid (23). If $H_2O_2$-derived $H_2O$ is adsorbed onto pyrite surfaces, then these molecules could be preferentially incorporated into neighboring anode sulfur sites despite their low molarity relative to bulk water. In addition to explaining the origin of $^{17}O$-enriched sulfate, this mechanism could potentially reconcile the apparent nonstoichiometric $O_2$ incorporation observed in isotope labeling pyrite oxidation experiments (i.e., values other than $25\%$, $50\%$, $75\%$, or $100\%$ $O_2$-derived) (12, 13). That is, if isotopically labeled $O_2$ is reduced to $H_2O$ via ROS intermediates on pyrite surfaces, then the apparent incorporation of this signature into sulfate would depend on the relative amount of adsorbed surface sites occupied by these molecules and would not be constrained by sulfate oxygen stoichiometry.

There are at least two pathways by which ROS incorporation could explain our observed positive sulfate $\Delta^{17}O$ values: 1) in situ production of $^{17}O$-enriched $H_2O_2$, either by microbes (47) or abiotically during $O_2$ reduction on pyrite surfaces (23), or 2) delivery of atmospheric $H_2O_2$ to the site of pyrite oxidation, for example, by rainwater (24, 25, 42). Although the mass-dependent fractionation relationships are currently not known for any step of the pyrite oxidation mechanism, the analogous $H_2O_2$-producing Mehler reaction has been shown to follow $\theta \approx 0.50$ (48). If $O_2$ reduction on pyrite surfaces follows a similar $\theta$ value as that of the Mehler reaction, then this process would generate $^{17}O$-enriched $H_2O_2$ that could be disproportionated and incorporated into sulfate. Future research is clearly needed to constrain these fractionation factors.

Alternatively, it has been shown that rainwater contains up to $\sim 30 \mu M$ $H_2O_2$ with $\Delta^{17}O$ values near $1.0\%$ VSMOW (25). A substantial fraction of this $H_2O_2$ is transferred to river water; measured concentrations reach $\sim 150 \text{ nM}$ (49, 50). If we assume $1)^{17}O$-enriched sulfate oxygen is sourced from a mixture of river water and $H_2O_2$, 2) rainwater $H_2O_2$ $\Delta^{17}O$ values reported in the literature (25) are generally representative, and 3) disproportionation and incorporation into sulfate molecules does not impart a mass-independent anomaly (12), then atmospherically derived $H_2O_2$ could contribute 7 to 15% of sulfate oxygen in Marsyandi headwaters (Fig. 3). This result satisfies both $\delta^{18}O$ and $\Delta^{17}O$ observations. Still, it remains unclear how and to what extent rainwater $H_2O_2$ would contribute to the site of pyrite oxidation; while this estimate based on the limited existing literature ROS $\Delta^{17}O$ data (24) appears reasonable, we emphasize that we do not quantitatively trust these results.

Regardless of the exact delivery mechanism invoked, pyrite oxidation-derived sulfate oxygen in mountainous rivers must be sourced primarily from meteoric water with supplemental contributions from a $^{17}O$-enriched source. Electrochemical models and limited $\Delta^{17}O$ measurements suggest this source is likely $H_2O_2$, but other ROS (e.g., $O_2^-$ or $OH^+$) or oxidants (e.g., $NO_3^-$) containing positive $^{17}O$ anomalies (51) could, instead, be driving observed signals. Future work is needed to provide detailed constraints on the importance of each of these species and pathways.

**Downstream Evolution and Implications for Global Sulfur Budgets.** Moving downstream, riverine sulfate becomes enriched in $^{34}S$ and $^{18}O$ and loses its positive $^{17}O$ anomaly. This transition occurs within tributaries draining the TSS and is therefore
Fig. 3. Sulfate oxygen source mixing diagram. White circles are measured riverine sulfate oxygen isotope compositions (Fig. 2A); blue circle is the high-elevation headwater meteoric water composition. Gray region is a mixing array between high-elevation meteoric water and the measured range of precipitation-derived H$_2$O$_2$ compositions (24); shading represents fraction of H$_2$O$_2$ contribution. Red region is a hypothesized MSR fractionation array starting from a “primary” headwater sulfate composition; shading represents the range of possible MSR $\theta$ values (58). The black dotted line is a mixing line between the same starting composition and tropospheric O$_2$. The primary composition used to define this mixing space was chosen such that the majority of data fall within the bounds defined by primary sulfate, MSR fractionation, and O$_2$ incorporation. Measured sulfate compositions cannot be explained as a binary mixing between meteoric water and tropospheric O$_2$, especially in $^{18}$O-enriched headwater TSS tributaries.

Fig. 4. Sulfate major isotope compositions. Colored points: $\delta^{34}$S vs. $\delta^{18}$O for all samples in ref. 20. Symbol colors represent $\chi_{py}$ values, and shapes refer to TSS tributaries (squares), HHCS Fil tributaries (circles), HHCS FII tributaries (right-pointing triangles), LHS tributaries (diamonds), and the main-stem Marsyandi (down-pointing triangles). Histograms: literature compilations of all reported $\delta^{34}$S and $\delta^{18}$O values from global rivers (Dataset S4 and Materials and Methods). Marsyandi River basin $\delta^{18}$O and $\delta^{34}$S compositions span nearly the entire global range and approach global median values moving downstream.
downstream tributary data fall below the MSR fractionation line and imply secondary incorporation of $^{17}$O-depleted oxygen into sulfate, most likely from tropospheric $O_2$.

Sulfate generated by MSR can be reoxidized either biotically or abiotically. Aerobic, chemolithoautotrophic sulfide-oxidizing bacteria (SOB) are known to rapidly oxidize sulfide to sulfate in the presence of $O_2$ (59). However, aerobic SOB likely utilize an electron transport chain rather than an oxygenase enzyme (60); resulting sulfate oxygen atoms are thus exclusively derived from $H_2O$ and not from $O_2$ directly (61). Alternatively, abiotic $H_2S$ oxidation, while kinetically slower than oxidation by SOB (59), would directly incorporate $O_2$ and could explain observed downstream trends. This mechanism—MSR followed by secondary $H_2$ oxidation—has been previously interpreted to reflect tropospheric $O_2$ contributions during pyrite oxidation (62). We, instead, interpret this result as reflecting continued secondary sulfate recycling, although the potential for alternative sulfate sources and anthropogenic inputs in the Mississippi River cannot be discounted (62).

If the mechanism proposed here is true more generally, then the loss of $O_2$ incorporation into sulfate occurs not in erosive, pyrite-rich headwaters but rather during secondary sulfate recycling in lowland floodplains. This difference could carry implications for sulfate $^{17}$O compositions through geologic time.

**Conclusion and Implications for Earth History.** The utility of sulfate $^{17}$O as a paleo-$pO_2$ tracer is predicated on tropospheric $O_2$ incorporation via oxidative pyrite weathering. In this study, we targeted highly erosive rivers draining pyrite-rich shale lithologies to isolate the $^{17}$O signature of pyrite oxidation-derived sulfate; results under modern conditions suggest a complex reaction network that does not directly incorporate $O_2$. However, $O_2$ is the only major atmospheric species that carries a negative $^{17}$O anomaly; observed $^{17}$O values in Precambrian sulfate-bearing rocks therefore require atmospheric $O_2$ incorporation into sulfate precursors (3–5, 14, 15). Here we hypothesize that such incorporation may occur during secondary sulfate recycling in floodplains; this mechanism predicts that floodplain area could act as an additional, previously unrecognized control on sulfate $^{17}$O composition. Still, open questions remain regarding the interpretation of geologic sulfate $^{17}$O records, including the dependence of ROS $^{17}$O compositions on $pO_2/pCO_2$ and the implications for paleoatmospheric compositions. Answering these questions will require mechanistic studies in modern settings in addition to new, high-resolution $^{17}$O measurements of geologic sulfate throughout the Phanerzoic Eon.

**Materials and Methods**

The materials and methods are summarized here; further details are provided in SI Appendix. All data used in this study are reported in Datasets S1–S4.

**Isotope Measurements and Data Reporting.** Site location and sample collection details, as well as major ion, $^{16}$O/$^{18}$O, and $^{34}$S/$^{32}$S analytical procedures, have been described previously (20, 29, 32, 34, 35). Sulfate $^{16}$O/$^{18}$O ratios were measured by laser fluorination by $\delta$O coupled with isotope ratio mass spectrometry following ref. 63. Uncertainty (±1σ) was taken as the long-term reproducibility of a suite of primary sulfate standards ($σ = 0.016$). Isotope ratios are reported in conventional delta notation, $\delta^18O = \left( \frac{18O_{\text{sample}}}{18O_{\text{standard}}} - 1 \right) \times 10^3 \%$.

**ACKNOWLEDGMENTS.** We thank Julien Foriel for laboratory assistance, Kevin Sutherland for helpful discussions on early versions of the manuscript, and the editor and two anonymous reviewers whose comments greatly improved the overall strength and clarity of this paper. This research was supported by NSF Early-Concept Grants for Exploratory Research Grant EAR1833941, American Chemical Society Petroleum Research Fund Grant 59455-ND2 (to both J.D.H. and D.T.J.), and NSF Marine Geology and Geophysics Grant OCE-1821958 (to D.T.J.).

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