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Beware of effects on isotopes of dissolved oxygen during storage of natural iron-rich water samples: A technical note

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Rationale: Investigations of the isotope ratios of dissolved oxygen ($\delta^{18}O_{DO}$) provide valuable information about the oxygen cycle in aquatic systems. However, oxidation of Fe(II) may change pristine $\delta^{18}O_{DO}$ values during storage and can lead to a misinterpretation. We sampled an Fe(II)-rich spring system and measured $\delta^{18}O_{DO}$ values at various time intervals in order to determine influences of Fe-oxidation.

Methods: Water samples were collected from an Fe-rich spring and related stream and the $\delta^{18}O_{DO}$ values were measured in fresh, 4- and 13-day-old samples with an isotope ratio mass spectrometer. Three replicates were measured for each sample with a 1σ of ± 0.2‰. On-site parameters and Fe(II) contents were also measured over the course of the spring system by multi-parameter probes and spectrophotometry.

Results: The $\delta^{18}O_{DO}$ values over the course of the spring system in fresh, 4- and 13-day-old samples revealed differences of up to 8‰. We explain this increase by the consumption of DO by Fe(II)-oxidation. After a flow length of 85 m the differences in $\delta^{18}O_{DO}$ values between fresh and older samples decreased because most of the Fe(II) was consumed.

Conclusions: False interpretations of $\delta^{18}O_{DO}$ values are possible if Fe-rich water samples are measured after too long storage, and we recommend measurement immediately after sampling.

1 | INTRODUCTION

Dissolved oxygen (DO) is one of the most important ecological parameters for the quality of water as it is essential for the survival of aerobic aquatic organisms. Additional measurements of its $^{18}O/^{16}O$ stable isotope ratio can offer valuable information about mechanisms of oxygen supply and consumption in aquatic systems. The ratios are expressed in a $\delta^{18}O_{DO}$ notation in permille [‰] versus the international Vienna Standard Mean Ocean Water (VSMOW). For instance, values around +24.6‰ indicate equilibration with atmospheric oxygen, while values above and below this threshold indicate the consumption of aqueous O_2 (DO) or the addition of DO by photosynthesis, respectively.[1,2]

One potential problem with $\delta^{18}O_{DO}$ measurements is that these reactions may continue after sampling. This in turn may lead to misinterpretations. A continuous supply of atmospheric O_2 can be minimized by collecting samples under near-exclusion to the atmosphere and by using butyl rubber-sealed sample containers.[3] Although slow diffusion of O_2 over longer periods between collection and measurement of samples cannot be completely ruled out, laboratory experiments have shown that samples remained stable, within the analytical uncertainty, for up to 3 months, when only considering influences of atmospheric O_2.

On the other hand, addition of DO due to ongoing photosynthesis after sampling or its removal due to consumption by respiration is efficiently prevented by preserving samples with

mercuric chloride ($HgCl_2$). This is often used as a standard technique to inhibit further biological activity.[4–6]

However, little attention has so far been paid to the possibility of changes to $\delta^{18}O_{DO}$ values by oxidation of reduced dissolved metal ions between sampling and measurement. One of the most common forms of such reduced mineral phases is Fe(II). In order to close this knowledge gap, our aims were to find out: (1) how quickly $\delta^{18}O_{DO}$ values may change after sampling when a critical mass of reduced metals is present in solution and (2) how strong such effects might be. For this purpose, we chose the Fe(II)-rich Espan spring and stream system in the city of Fürth, Germany (Figure 1). This is an ideal environment to test these questions, because it showed dissolved Fe(II) concentrations between 0 and 6.6 mg L^{-1} and DO concentrations between 2.3 and 11.0 mg L^{-1}. This study is also timely because to date only a few investigations exist about effects of metal oxidation on $\delta^{18}O_{DO}$ values, and most of them were carried out in either acidic or suboxic environments.[7–9] Also, their pH values were not specifically mentioned,[10] or they focused on experiments under controlled laboratory conditions.[11,12] In particular, the latter two studies showed that closed system iron oxidation leads to a consumption of dissolved oxygen and an associated increase in $\delta^{18}O_{DO}$ values over time. Oba and Poulson[11] provided a fractionation factor (α) of 0.9908 for similar conditions to those found in the Espan spring (i.e. neutral pH, temperature of 23°C and an iron concentration of 0.02 M). A similar value, of 0.980013, is available from experiments by Pati et al.[12]

Because these studies show that the oxidation of Fe(II) leads to noticeable increases in $\delta^{18}O_{DO}$ values and because Fe and oxygen turnover are critically important in many aqueous systems, it is essential that the effect of iron oxidation on $\delta^{18}O_{DO}$ values is closely examined for good quality analyses.[13–15] So far, little attention has been paid to this effect because Fe(II) is usually scarce in freshwaters that also freely exchange with the atmosphere. However, increased inputs of dissolved Fe(II) into rivers, lakes and oceans has been observed in recent years.[16–18] This so-called brownification is becoming a growing issue in aquatic systems. Therefore, the effect of iron oxidation on $\delta^{18}O_{DO}$ values demands further developments of sampling procedures and analytical techniques for optimal $\delta^{18}O_{DO}$

FIGURE 1 A, Location of the Espan spring in the metropolitan region Nuremberg Fürth. B, Overview map and C, satellite image of the Espan spring with sampling points

data acquisition and interpretation. Similar concerns exist for sampling of sedimentary waters that often have elevated Fe(II) concentrations.

Our study shows that iron oxidation in a natural and circumneutral aqueous system can exert noticeable effects on $\delta^{18}O_{DO}$ values. The extent of these effects was similar to those determined by Oba and Poulson[11] and Pati et al.[12] This effect could be traced in the first 45 m of the natural system. However, it became even more apparent with increasing time between sampling and analysis. Fresh and delayed analyses of samples that were stored for 4 and 13 days showed differences in their $\delta^{18}O_{DO}$ values of up to 8‰. Such discrepancies may lead to largely different interpretations of natural and Fe-rich water systems. Therefore, DO and $\delta^{18}O_{DO}$ values should be measured soon after sampling Fe-rich aqueous systems in order to avoid oxidation artefacts.

2 | METHODS

2.1 | Study site

The artesian Espan spring, located in the city of Fürth, Germany (49°28′15.8″N 11°00′53.0″E) (Figures 1A and 1B), was tapped by a drilling project in 1935 to a depth of 448.5 m below the ground surface. Its water stems from the lower Buntsandstein formation, which is rich in sulphates and dissolved Fe(II). The spring water flows into a 300 m long stream, the "Wetzendorfer Landgraben" (WL), that finally drains into the Pegnitz River (Figure 1C). A distinct red colouring of the stream bed indicates dominance of Fe. Close to the spring, the stream was initially undersaturated with respect to DO with values of 2.3 mg L^{-1} and saturated in Fe(II) with a maximum concentration of 6.6 mg L^{-1}.

2.2 | Sampling procedures

In a field campaign in February 2020 water was collected at 14 sampling points along the stream and divided into 3 sets of samples. One set was measured within less than 3 h, one was stored in the dark at 4°C for 4 days before measurement, and one was stored under the same conditions for 13 days before measurement. Samples that had experienced any longer time periods between sampling and measurement were not tested because we assumed that most of the geochemical alterations would occur in the first 2 weeks after sampling. We were also not able to test any shorter time periods and, due to field and transport logistics, isotope analyses within 3 h were the best possible option.

Samples for $\delta^{18}O_{DO}$ measurements were collected in 12-mL Exetainers® (Labco Ltd, Ceredigion, UK) that were prepared with 10 µL of a saturated HgCl$_2$ solution to prevent biological activity after sampling. The Exetainers were filled with water that was syringe-filtered using 0.45 µm pore size nylon filters until they were entirely full and free of air bubbles. They were then carefully closed with screw caps with a butyl septum in order to avoid atmospheric

contamination. Test series in the field and in the laboratory showed that the degree of atmospheric contamination during this filling procedure was negligible.[19]

Onsite parameters (pH, temperature, and DO) were measured with a HACH HQ40D multimeter i (Hach Lange GmbH, Düsseldorf, Germany).

The iron content was measured with an iron(II/III) cuvette test set (HACH) in combination with a portable HACH spectrophotometer (model DR 2800). These samples were also filtered with 0.45 µm pore size nylon filters to minimize iron precipitation and turbidity.

2.3 | Laboratory methods

The stable isotope ratios of DO were measured on a Delta Advantage isotope ratio mass spectrometer that was linked to a Gasbench II autosampler and an extraction unit (all from Thermo Fisher Scientific). The procedure was modified from a method by Barth et al[3] with isolation of DO into a headspace that employs a helium extraction technique developed by Kampbell et al[20] and Wassenaar and Koehler.[4] Prior to analyses, the headspace was automatically generated in each vial on the Gasbench II with an autosampler that was equipped with a double-hole needle. This needle extruded water via the lower hole by injecting a continuous helium flow at its upper hole after penetration for about 2.5 cm underneath the septum. Immediately after headspace generation samples were placed for 30 minutes on a horizontal shaker that moved at a rate of ~250 strokes per minute. This mobilized all present DO as free O$_2$ into the headspace. The samples were then placed back on the Gasbench II autosampler after its switchover to connection to the isotope ratio mass spectrometer. The headspace was extracted in a helium stream via another dry double-hole needle on the autosampler. The O$_2$ was then separated by a CP-Molsieve 5 Å capillary column (25 m length × 0.53 mm OD × 0.05 mm ID; Agilent, Santa Clara, CA, USA). Finally, the purified O$_2$ was transferred in continuous flow to the mass spectrometer that was tuned for mass separation of m/z 34 and 32 for direct measurement of the $^{18}O/^{16}O$ ratios.

The obtained data sets were corrected for linearity and instrumental drift effects during each run. The $\delta^{18}O$-value of laboratory air was used as an internal standard with a known value of +23.88‰.[21]

The isotope ratios are reported in permille (‰) as δ-values relative to the Vienna Standard Mean Ocean Water (VSMOW) as:

$$\delta = \left(R_{sample}/R_{SMOW} - 1 \right)$$

All samples were measured in triplicate and the 1σ standard deviations were less than ±0.2‰.

The cation contents (including uranium) were determined with an iCAP Qs inductively coupled plasma mass spectrometer (Thermo Fisher Scientific) with a SC-2DXS autosampler (Elemental Scientific, Omaha, NE, USA). These samples were preserved with two drops of 65% HNO$_3$ Suprapur and measured three times. The anions were

measured by ion chromatography (ICS 2000; Thermo Dionex, Sunnyvale, CA, USA). The -σ standard deviation of all triplicate metal and major ion measurements was alway better than 1%.
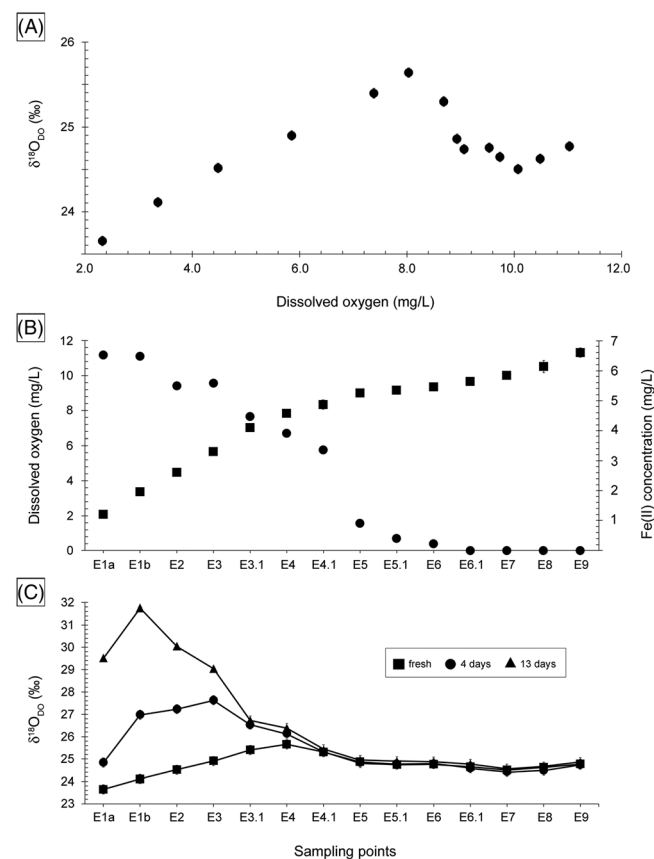
FIGURE 2 A, Correlation between $\delta^{18}O_{DO}$ and DO of fresh samples. B, Oxygen and Fe(II) concentrations over the course of the stream (squares: DO, circles: Fe(II)). C, $\delta^{18}O_{DO}$ values over the course of the stream for fresh, 4- and 13-day-old samples (squares: fresh, circles: 4 days, triangles: 13 days)

2.4 | Calculations of saturation states

The saturation states were calculated as a function of pH, pE, ion concentrations as well as the alkalinity and temperature with the programme PhreeqC (version 3).[22]

3 | RESULTS AND DISCUSSION

Our data showed that:

1. $\delta^{18}O_{DO}$ values increased by up to 3‰ within 4 days and by up to 8‰ within 13 days after sampling, and
2. these differences between fresh and stored samples became increasingly smaller because of decreasing Fe(II) contents over the downstream evolution of the stream (Figures 2B and 2C).

In the following, we first discuss how the $\delta^{18}O_{DO}$ values changed in the fresh samples over the course of the stream and how they were impacted by dissolved oxygen and Fe(II). We then discuss the $\delta^{18}O_{DO}$ values measured after 4 and 13 days and how they differ from those of the fresh samples.

Figure 2A displays the correlation between DO and $\delta^{18}O_{DO}$ in fresh samples (Tables 1 and 2). Initially, the DO and its associated $\delta^{18}O_{DO}$ value rose to 8.0 mg L^{-1} and +25.7‰ at sampling point E4. After this point, a rise in DO correlated to a decrease in $\delta^{18}O_{DO}$ with values of 10.1 mg L^{-1} and +24.5‰, respectively, at sampling point E7. Towards the end of the stream, the DO and the $\delta^{18}O_{DO}$ values increased again to a final value of 11.0 mg L^{-1} and +24.8‰ at sampling point E9. Figure 2B shows how the directly measured DO and Fe(II) concentrations changed over the course of the stream. While the amount of DO increased continuously from 2.3 mg L^{-1} at sampling point E1a to 11.0 mg L^{-1} at sampling point E9, the Fe(II) content decreased from 6.6 mg L^{-1} at E1a to 0.0 mg L^{-1} at E9.

TABLE 1 $\delta^{18}O_{DO}$ values of fresh, 4- and 13-day-old samples

| Sampling location	Distance to the well (m)	$\delta^{18}O_{DO}$ (‰) fresh	$\delta^{18}O_{DO}$ (‰) 4 days	$\delta^{18}O_{DO}$ (‰) 13 days
E1a	0	23.7	24.8	29.5
E1b	0	24.1	27.0	31.8
E2	15	24.5	27.2	30.0
E3	45	24.9	27.6	29.0
E3.1	65	25.4	26.5	26.7
E4	85	25.7	26.1	26.4
E4.1	115	25.3	25.3	25.4
E5	145	24.9	24.8	24.9
E5.1	175	24.7	24.8	24.9
E6	205	24.8	24.8	24.9
E6.1	235	24.7	24.6	24.8
E7	265	24.5	24.4	24.6
E8	295	24.6	24.5	24.7
E9	300	24.8	24.7	24.9

TABLE 2 pH, Fe(II) and DO concentrations over the course of the spring determined in the field

| Sampling location	Distance to the well (m)	pH	Fe(II) (mg/L)	DO (mg/L)
E1a	0	6.1	6.6	2.3
E1b	0	6.5	6.6	3.4
E2	15	6.5	5.6	4.5
E3	45	6.7	5.7	5.9
E3.1	65	6.5	4.5	7.4
E4	85	7.1	3.9	8.0
E4.1	115	7.5	3.4	8.7
E5	145	7.9	0.9	8.9
E5.1	175	7.6	0.4	9.1
E6	205	7.9	0.2	9.5
E6.1	235	7.9	0.0	9.7
E7	265	8.0	0.0	10.1
E8	295	8.0	0.0	10.5
E9	300	8.6	0.0	11.0

The continuous rise in DO (Figures 2A and 2B; Table 2) can best be explained by the dissolution of atmospheric O_2 over the course of the first 85 m of the stream. Further downstream additional photosynthetic impacts were found as evidenced by green mats of cyanobacteria and algae.

The $\delta^{18}O_{DO}$ values of below +24.6‰ measured at the spring at sampling point E1a seemed to result from a photosynthetic influence. However, the water only came into contact with light after sampling point E1a thus excluding photosynthesis. Therefore, one possible mechanism for these unexpected low values could have been radiolysis. This is plausible because the area is known for high radiation originating from the bedrock and Buntsandstein.[23] Furthermore, the spring also showed elevated uranium contents of up to 170 μg L^{-1}.

The positive correlation between DO and $\delta^{18}O_{DO}$ can be explained by equilibration with atmospheric oxygen to a value of +24.6‰. However, the $\delta^{18}O_{DO}$ values also increased above this value and therefore it cannot be explained by equilibration alone. A plausible explanation is that Fe-oxidation occurred (Equation (1)). This process consumed DO, while more O_2 was replenished from the atmosphere. This effect seems to have exerted a stronger influence on the $\delta^{18}O_{DO}$ values than the oxygen supply from the atmosphere.

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \qquad (1)$$
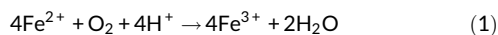
The decrease in $\delta^{18}O_{DO}$ values with an increase in DO after sampling point E4 can be explained by increasing photosynthesis. This process was probably suppressed under the influence of Fe(II)-oxidation. It is also possible that downstream of E4 less Fe(II) was oxidized because it precipitated as siderite (Equation (2)). Alternatively, the Fe(II) could have been adsorbed onto already formed iron oxides.

$$Fe^{2+} + CO_3^{2-} \rightarrow FeCO_3 \qquad (2)$$
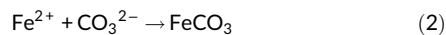
The positive correlation between DO and $\delta^{18}O_{DO}$ downstream of sampling point E7 was probably associated with decreases in photosynthesis. This was also obvious by the disappearance of green algae mats.

Figure 2C displays the $\delta^{18}O_{DO}$ values of fresh samples compared with those after 4 and 13 days. It shows that the $\delta^{18}O_{DO}$ values after 4 days of storage and subsequent measurement between points E1 and E4.1 differ from those of the fresh samples. Between sampling points E1a and E1b the $\delta^{18}O_{DO}$ value increased from +24.8‰ to +27.0‰ and then at a lesser rate towards sampling point E3. After this point the value decreased to +25.3‰ in sampling location E4.1. Downstream of this sampling point no differences between fresh and stored samples could be found (Figure 2C).

The curve after 13 days between sampling locations E1a and E4.1 differed from those for the fresh and 4-day-old samples. First the value of +29.5‰ at E1 increased to +31.8‰ and then decreased to +25.4‰ at sampling location E4.1. In addition to these differences, the values between E1a and E4.1 after 4 and 13 days were higher than in the fresh samples. These differences amounted to +1.2‰ after 4 days at sampling location E1a, +2.9‰ at E1b, +2.7‰ at E2 and E3, +1.1‰ at E3.1 and +0.5‰ at sampling location E4. Between 4 and 13 days the differences in $\delta^{18}O_{DO}$ values amounted to +4.7‰ at sampling location E1a, +4.8‰ at E1b, +2.8‰ at E2, +1.4‰ at E3, +0.3‰ at E3.1 and +0.1‰ at sampling point E4.

The increase in $\delta^{18}O_{DO}$ values after 4 and 13 days compared with fresh samples between sampling points E1 and E4 shows that DO was consumed. Moreover, downstream of sampling point E4 only small rates of DO consumption seemed obvious.

The abundant Fe(II) quickly reacts with DO under circumneutral conditions (Equation (1)). This oxidation of Fe was also apparent as red to orange precipitates in the stream bed. Similar precipitates were found in the sample vials after 4 and 13 days (Figures 3A and 3B).

It is known that iron oxidation can have an impact on $\delta^{18}O_{DO}$ values through the consumption of oxygen as demonstrated in laboratory experiments by Oba and Poulson[11] and Pati et al.[12] Here we were able to trace a similar effect in a natural spring system with

FIGURE 3 Red to orange precipitates in A, the spring and B, in vials after 4 days

increasing $\delta^{18}O_{DO}$ values over the first 45 m of the stream. This process took place despite continuous additions of atmospheric O_2. With the obvious Fe-oxide precipitations the oxygen consumption must have resulted to a large part from this process. Calculations with PhreeqC also showed that Fe-oxides can form directly in the stream and also in the vials after sampling (see Tables S1 and S2, supporting information).

Theoretically, abiotic and biotic sulphide oxidation could change the $\delta^{18}O_{DO}$ values in experiments and water courses similar to the Espan spring.[7,10,24,25] However, in our case no sulphide could be detected over the entire course of the stream nor in the sediment waters. Post-sampling biological sulphide oxidation or consumption of oxygen by respiration could also be ruled out as mechanisms for increases in $\delta^{18}O_{DO}$ because the samples were preserved by $HgCl_2$ and then stored at $4°C$ in the dark.

In the following we discuss specific differences between the sampling points over time in more detail.

3.1 | Differences between fresh and 4-day-old samples

Compared with sampling points E1b to E3, E1a showed a relatively small increase in $\delta^{18}O_{DO}$ values between fresh and 4-day-old samples. This indicates that oxygen was consumed through Fe-oxidation at sampling points E1b to E3. One possible reason for this observation could be a higher pH value of ~6.5 (Table 2) at these sampling points. Under neutral to circumneutral conditions, Fe-oxidation occurs more rapidly, while acidic pH values can slow down Fe(II)-oxidation.

Specifically, the initial pH of 6.1 in sample E1a can be considered as more acidic and may have hampered Fe-oxidation. Downstream of sampling point E3.1, the $\delta^{18}O_{DO}$ values of 4-day-old samples successively approached the values of samples that were analyzed immediately after sampling. Starting at point E4.1, they were almost identical. This indicates that, downstream of this location, hardly any Fe becomes oxidized and thus less DO is consumed. Another indication for this changeover is that the amount of orange precipitate that was found in all sampling vials between points E1a and E4 was missing in all samples downstream of sampling point E4 even after longer storage times.

3.2 | Differences between 4- and 13-day-old samples

Increases in the $\delta^{18}O_{DO}$ values between 4- and 13-days storage time were obvious in sampling points E1a to E3. This indicates ongoing oxygen consumption and thus Fe(II)-oxidation. This process was confirmed by an even more pronounced orange precipitate. The stronger increase in comparison with the 4-day-old samples can mostly be explained by the longer reaction time.

At sampling point E1b the effect on $\delta^{18}O_{DO}$ values between 4 and 13 days was less pronounced than between fresh and 4-day-old samples. Theoretically, more Fe(II) should have been oxidized. This would create a stronger impact on the isotope ratios because of the longer reaction time. Two explanations are possible for this observation: either no oxygen was left to be consumed by the Fe(II) or no Fe(II) was left to consume the oxygen. When applying the stoichiometry of Equation (1) to calculate how much oxygen can be consumed by a specific amount of Fe(II) via oxidation, it becomes evident that not enough Fe(II) was present to entirely consume the available oxygen in the vials.

We also used a Rayleigh model to calculate how much oxygen would still be remaining in the vials after 4 and 13 days. This approach outlines the change in isotope ratios in a diminishing reservoir with known fractionation factors.[25] It implemented the fractionation factors determined by Oba and Poulson[11] and Pati et al.[12] This calculation confirmed that even after these longer reaction times DO was still available in all vials. Thus, only a decrease in Fe(II) concentrations can be responsible for the low impact on $\delta^{18}O_{DO}$ between 4 to 13 days. Some preliminary tests showed that about 0.1 μM Fe(II) per hour was removed from the water. Note, however, that part of this rate can also be due to formation of Fe(II) minerals such as siderite and/or the adsorption of Fe(II) onto already existing iron oxides.

Based on calculation of the saturation indices (SI values, cf. Tables S1 and S2, supporting information) a mixture of ferrihydrite, hematite, goethite and siderite is expected (Equations (2)–(4)).

$$4Fe^{2+} + 3O_2 + 6H_2O \rightarrow 4Fe(OH)_3 \qquad (3)$$

$$2Fe(OH)_3 \rightarrow Fe_2O_3 + 3H_2O \qquad (4)$$
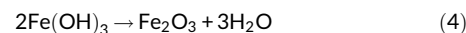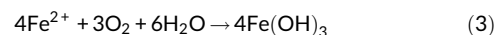
At sampling point E2 the value after 13 days only increased by +2.8‰. This shows that the amount of DO consumed between 4 and

13 days decreased in comparison with sampling point E1b. This implies that at sampling point E2 less iron was oxidized between 4 and 13 days than at sampling point E1b. Again, this could be due to a shortage in DO or Fe(II). Reasons for a lower Fe(II) content could have been either that the Fe has already been used up by oxidation or precipitated as siderite and/or it had been adsorbed on already existing minerals. Sampling locations E3, E3.1 and E4 repeated the same observations as that at location E2, albeit at slightly reduced differences.

At sampling point E4.1 and further downstream no additional oxygen seems to have been consumed even after storage times of 13 days. This was confirmed by negligible changes in $\delta^{18}O_{DO}$ between fresh samples and those analyzed after 13 days. This also suggests that only negligible amounts of Fe(II) were available for DO consumption.

The observed changes seem to depend on available reactive Fe(II) in the samples. In terms of $\delta^{18}O_{DO}$ analyses, such presence of reduced metals can severely compromise the reliability of results. This also means that higher $\delta^{18}O_{DO}$ values after 4 and 13 days of storage could lead to a misinterpretation of how aqueous systems operate in terms of oxygen cycles.

The value of +23.7‰ at sampling point E1a in a fresh sample suggests a slight influence of photosynthesis because it lies below the atmospheric value of +24.6‰. However, increased values of the same samples that were stored for 4 days (+24.8‰) and for 13 days (+29.5‰) would lead to the false interpretation that the water was mostly influenced by atmospheric equilibration and/or consumption of oxygen by respiration. This interpretation stands in contrast to the one established from samples that were analyzed shortly after collection.

The strength of the Fe-oxidizing effect on the $\delta^{18}O_{DO}$ values depends on how much time has passed between sampling and measurement, and on how much Fe(II) or other reduced metals are present. While we cannot exclude these effects within less than 3 h after sampling, this is the shortest time period that could be achieved. We therefore assume that these values are close to the real situation in the system.

If samples have to be stored for longer time periods before measurement one can also consider stabilizing them by acidification as Fe(II)-oxidation is known to considerably slow down under acidic conditions.[26] We have undertaken first attempts in this direction. However, while acidification slowed down the effects of Fe(II)-oxidation it did not completely exclude effects on the $\delta^{18}O_{DO}$ values. This is an issue that needs to be further researched. These considerations may also become important for laboratory studies of $\delta^{18}O_{DO}$.[27,28]

4 | CONCLUSIONS

This work is among the first to test natural abundance in the $^{18}O/^{16}O$ ratios of dissolved oxygen ($\delta^{18}O_{DO}$ values) in a natural field study with circumneutral iron-rich waters. DO concentrations over the course of the spring system showed a continuous increase due to influences by the atmosphere and by photosynthesis. In contrast, the Fe(II) contents decreased continuously due to oxidation, precipitation as siderite and/or adsorption onto already existing minerals.

Our results also confirmed that freely available Fe(II) can have considerable influences on $\delta^{18}O_{DO}$ values through its oxidation. This trend can continue after sampling even if samples are tightly sealed against atmospheric influences and poisoned with $HgCl_2$ to avoid biological activity after sampling. This implies the risk that Fe(II)-oxidation after sampling may lead to wrong data interpretation. Moreover, the strength of this effect on the $\delta^{18}O_{DO}$ values depends on the storage time.

In order to ensure that iron oxidation has negligible influences on the $\delta^{18}O_{DO}$ values, we propose the following measures:

1. first, test for Fe(II) and O_2 contents in waters considered for sampling;
2. minimize the amount of time between sampling and measurement; and
3. investigate Fe(II) contents in vials after $\delta^{18}O_{DO}$ measurements in order to determine possible effects of iron oxidation on the $\delta^{18}O_{DO}$ value for corrections.

Overall, a good understanding of metal species that can become oxidized by available DO is required when analyzing and interpreting water samples for their $\delta^{18}O_{DO}$ values. We were able to show this in an iron-rich system that offers considerable amounts of Fe(II). However, other metal oxidations such as those of manganese or aluminium may also have to be taken into account. So far, first attempts to preserve water against metal oxidation after sampling by acidification were not successful. We therefore recommend rapid analyses after sampling as the best approach for iron-rich solutions. As an alternative we recommend extraction of the DO into a headspace and its transfer into helium-flushed vials directly on site. The latter would offer rapid isolation of the extracted O_2 and would thus minimize further influences by oxidizable metals or other factors such as photosynthesis or respiration as long as the vials are kept tightly closed.

Quantification of dissolved oxygen (DO) in combination with $\delta^{18}O_{DO}$ values provides valuable information about sources and sinks and remain a useful tool in the investigation of oxygen cycling in aquatic systems. However, possible interactions with metals need to be considered even after sampling.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.