

Direct P K-edge X-ray absorption spectroscopy evidence of phosphorus immobilization in anoxic peat soils

Supplementary material

Adrian F. Florea^{1}, Dominik H. Zak², Case van Genuchten³, Camille Rivard^{4,5}, Hans Christian B. Hansen¹*

¹*Department of Plant and Environmental Science, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, Dk-1871 Frederiksberg C, Denmark.*

²*Department of Ecoscience, Aarhus University, C. F. Møllers Allé 3, 8000 Aarhus C.*

³ *Geochemistry Department, Geological Survey of Denmark and Greenland, Oester Voldgade 10, DK-1350, Copenhagen K, Denmark*

⁴ *LUCIA beamline, Synchrotron SOLEIL, 91190, Saint Aubin, France*

⁵ *TRANSFORM, INRAE, 44316, Nantes, France*

**Corresponding author. Tel.: +4525131343. E-mail address: aff@plen.ku.dk. Thorvaldsensvej 40, Dk-1871 Frederiksberg C, Denmark*

Section S1. Material and methods

S1.1 Soil characterization and solution analysis

A representative sample of approximately 30 g was collected and added into Chrom-Tech 50mL PVDF Maxi-Spin 0.45 μm filter tubes (Chrom-Tech tubes), for soil solution extraction and analysis. The Chrom-Tech tube was then sealed with Teflon tape and a screw cap and briefly allowed to leave the glovebox for centrifugation. Centrifugation was performed for 10 minutes at 21°C a Sigma 6-16K centrifuge using a 12169-H rotor at 5000 rpm, providing a 2795 g force. After centrifugation, the Chrom-Tech tubes containing the centrifuged soil were transferred back to the glove box, and the soil solution collected in the bottom of the Chrom-Tech tube was filtered through a Chromafil 0.45 μm syringe filter (RC-45/25) into a new 15 mL Falcon tube. The filtered soil solution was acidified using 20 μL of 2M H_2SO_4 mL⁻¹ solution to prevent sample oxidation, and the samples were then removed from the glovebox.

Inorganic phosphate concentrations in soil solution were determined by extracting pore water from moist soil samples via centrifugation at 2,795 g using Chrom-Tech 50 mL PVDF Maxi-Spin centrifuge tubes equipped with 0.45 μm filters. Phosphorus in solution was quantified spectrophotometrically using the molybdate-blue method (IOSWQ, 2004).

Dissolved Fe(II) concentrations in the soil solution samples were measured using the 1,10-phenanthroline method described by Fadrus and Maly (1975), following appropriate dilution. To assess potential oxidation of Fe(II) during solution extraction, Fe(II) concentrations were measured in selected samples before and after centrifugation. No significant Fe(II) oxidation was observed during centrifugation conducted outside the glovebox.

Soil solution pH and electrical conductivity were measured immediately after extraction inside the anoxic chamber using a Metrohm 914 portable pH/conductometer, while alkalinity was determined outside the glovebox by Gran titration (Gran, 1952).

The extent of Fe(III) reduction ($R_{\text{Fe(III)}}$) in field soil samples was monitored by determining the content of Fe(II) in the samples and was calculated as the ratio between $\text{Fe(II)}_{\text{HCl}} : \text{Fe}_{\text{ox}}$ (Eq. 1). Specifically, from the centrifuged soil (see above), 1 g was collected and exposed to 10 ml of 0.1 M HCl for 10 min on the shaking table at 120 rpm (Heiberg et al., 2012). Afterwards, the soil suspensions were filtered through 0.22 μm Macherey - Nagel Chromafil syringe filter and Fe(II) in the supernatant determined by the 1,10 - phenanthroline method of Fadrus and Maly (1975). Phosphorus concentrations in the same HCl extracts were quantified using the molybdate-blue method described above.

Oxalate-extractable aluminum, iron, and phosphorus (Al_{ox} , Fe_{ox} , and P_{ox}) were determined according to the method of Schoumans (2000), and elemental concentrations in the extracts were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES).

The oxalate extraction method is commonly used to assess the amorphous and poorly crystalline fraction of Fe(III)(oxyhydr)oxides in soils, with the P content in this extract serving as an indicator of P sorbed or occluded within these oxides. Oxalate has the capability to extract all amorphous iron (oxyhydr)oxides, iron(II) from various sources, including acid volatile sulfides, iron-containing carbonates, iron(II) absorbed onto sediment particles (Schwertmann 1964), and iron(II) associated with magnetite (Lovley et al., 1987). All results are reported on a soil dry mass (DM) basis.

Total phosphorus content was determined following hot acid digestion of 0.5 g of combusted soil (520 °C) using 5 mL of 6 M HCl, with P quantified by the molybdate-blue colorimetric method. Moreover, the total inorganic P (P_{inorg}) was determined following hot acid digestion

of 0.5 g of dry soil using 5 mL of 6 M HCl, with P quantified by the molybdate-blue colorimetric method. The difference between P_T and P_{inorg} is represented by the organic P (P_{org}) fraction. Total carbon and nitrogen contents were measured on dry soil samples using an Elementar Vario Macro Cube elemental analyzer.

Total elemental concentrations of Fe, Al, Ca, and Mg were determined by XRF on finely ground, pelletized soil samples using an Olympus Delta Premium DP-6000 analyzer, following Florea et al. (2026). Soil solutions obtained by centrifugation were analyzed for dissolved cations (ICP-OES), anions (ion chromatography, 930 Compact IC Flex), and DOC (Shimadzu TOC-L CPH after acidification), as previously described in Florea et al. (2026). Alkalinity and electrical conductivity were measured for all soil solution samples.

S1.2. Sample preparation for XAS analysis

Approximately 50 g of soil from each collected soil sample were dried inside an anoxic glovebox under a controlled inert atmosphere (N_2/H_2) to prevent oxidation during handling. Following drying, the samples were manually milled using an agate mortar and pestle to obtain a homogeneous fine powder. All preparation steps, including drying and milling, were performed inside the glovebox under anoxic conditions. The maintenance of the anoxic state throughout sample preparation was verified by Fe(II) extraction with HCl, conducted immediately after drying and milling (Figure S1). The presence and stability of Fe(II) served as a redox indicator confirming that no significant oxidation occurred during soil processing. From each dried and milled soil sample, resin-embedded pellets (diameter 1.75 cm; thickness 0.2 cm; volume 0.481 cm³) were prepared to ensure mechanical stability and reproducible geometry for XRF and XAS measurements. Each pellet contained approximately 0.2 g of soil, corresponding to an average bulk density of $\sim 0.42 \text{ g cm}^{-3}$ ($\approx 420 \text{ kg m}^{-3}$), consistent with highly decomposed peat material (Qui et al 2025). The powdered soil was embedded in Epo-Tek 301-

2 resin and cast into uniform pellets using silicone molds (17.5 mm × 2 mm) inside the anoxic glovebox. The resin was allowed to cure under anoxic conditions to prevent oxidation of redox-sensitive phases. The prepared pellets were subsequently stored and sealed in 200 ml airtight Cole-Parmer glass vials within the glovebox and maintained under anoxic conditions, until XAS analysis. During transportation, anoxic conditions were preserved by maintaining the pellets in the sealed vials. Upon arrival at the synchrotron facility, the vials were opened inside an anoxic glovebox. For transfer to the beamline instrument, the samples were placed in gas-tight anoxic bags sealed within the glovebox to prevent any exposure to atmospheric oxygen prior to analysis.

To enable direct comparison of redox-dependent mineralogical and chemical changes, oxic reference samples were prepared from the same sampling batch. These samples were dried and milled under ambient laboratory atmosphere and embedded in resin following the same protocol used for the anoxic samples, but outside the glovebox. Oxic pellets were stored in sealed glass vials under atmospheric conditions prior to XAS analysis.

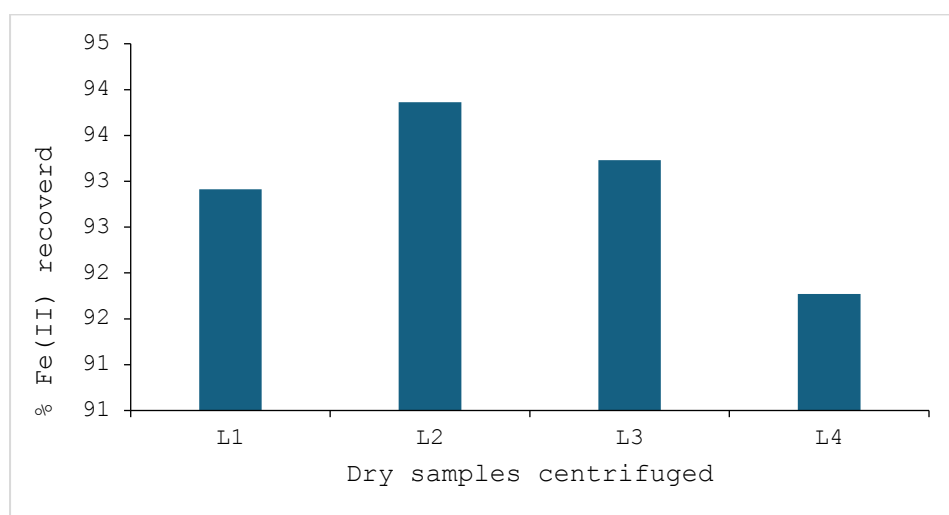


Figure S1: Fe(II)_{HCl} measured in the centrifugation method testing the anoxic soil samples.

Section S2: Results

S2.1. Iron reduction and phosphorus dynamics

Residual sorption capacity (Eq 2 in main text) for the sampling points in November 2024.

$$L1 = 74.6 \text{ mmol kg}^{-1}$$

$$L2 = 136 \text{ mmol kg}^{-1}$$

$$L3 = 170 \text{ mmol kg}^{-1}$$

$$L4 = 203 \text{ mmol kg}^{-1}$$

S2.2. Macro XAS analysis

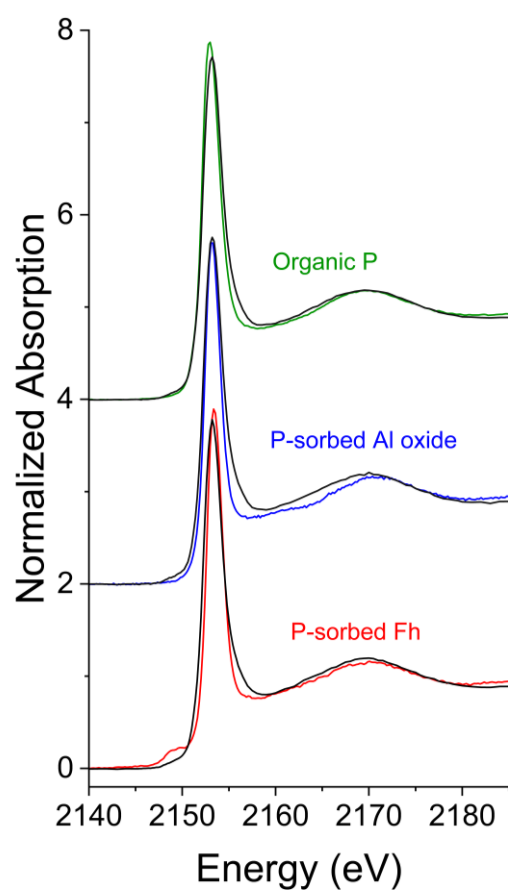


Figure S2: P K-edge XANES spectra of organic P (green), P sorbed to Al oxide (blue), and P sorbed to ferrihydrite (red) reference compounds and experimental spectra. Experimental

spectra are shown as black lines and corresponding fitted/reference spectra as colored lines.

Spectra are vertically offset for clarity

S2.3. Micro-XRF and μ -XAS analysis

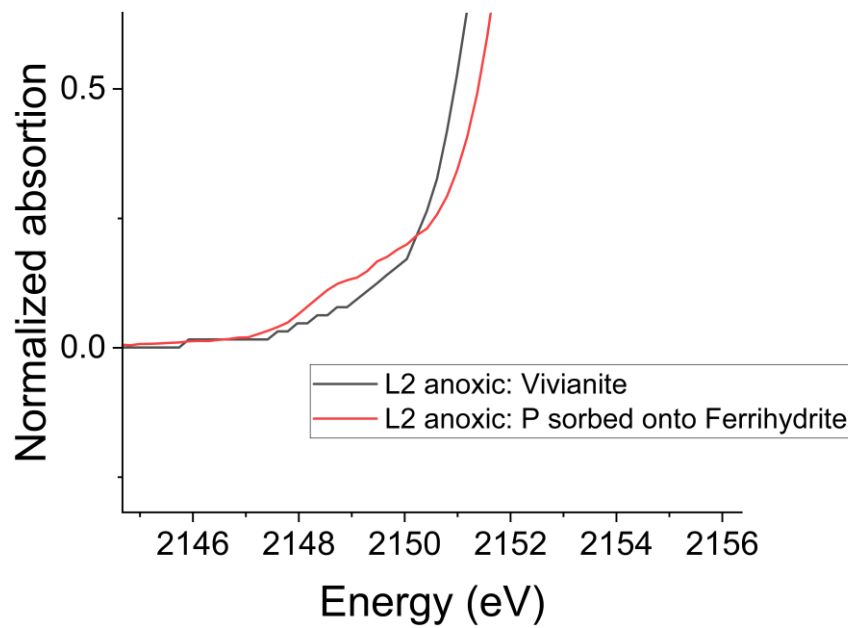


Figure S3: Pre-edge zoom in for vivianite-like solids and Fe(III)-bound P in L2 anoxic samples.

Section S3: Discussion

S3.1. Decoupling of Fe(III) reduction and dissolved P release in alkaline peat soils

$$P_{\text{mob}} = \text{Fe(II)}_{\text{HCl}} * \frac{P_{\text{ox}}}{P_{\text{SC}}} \quad \text{Eq.3 main text}$$

P mobilization (P_{mob}) calculation in L1, L2 L3 and L4 samples according to equation 6:

L1 calculation: $P_{\text{mob}} = 188.7 \times 79\% \times 16.5/224.4 \times 0.4 \text{ m depth} \times 10000\text{m}^2 \times 305 \text{ kg m}^{-1} \times 31/1000 = 415 \text{ kg P mobilized}$

L2 calculation: $P_{\text{mob}} = 227.6 \times 60\% \times 17.8/272.9 \times 0.4 \text{ m depth} \times 10000\text{m}^2 \times 416 \text{ kg m}^{-1} \times 31/1000 = 460 \text{ kg P mobilized}$

L3 calculation: $P_{\text{mob}} = 216.3 \times 42\% \times 12.7/260.8 \times 0.4 \text{ m depth} \times 10000\text{m}^2 \times 509 \text{ kg m}^{-1} \times 31/1000 = 278 \text{ kg P mobilized}$

L4 calculation: $P_{\text{mob}} = 203.6 \times 23\% \times 18.5/250 \times 0.4 \text{ m depth} \times 10000\text{m}^2 \times 564 \text{ kg m}^{-1} \times 31/1000 = 242 \text{ kg P mobilized}$

S3.2. Mechanistic interpretation and implications for P immobilization in neutral anoxic peat soils

Saturation indices (SI) were calculated for key Fe- and Ca-bearing phosphates including vivianite ($\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) ($\text{pK}_{\text{sp}} = -37.7$) (Allison et al. (1991), and hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$) ($\text{pK}_{\text{sp}} = -44.3$).

Geochemical equilibrium modeling was conducted using Visual MINTEQ version 3.1 to simulate aqueous speciation and evaluate mineral saturation states relevant to phosphorus dynamics (Gustafsson 2014). The calculations were performed at a fixed temperature of 8°C, reflecting typical subsurface conditions at the study sites, and the input parameters included measured concentrations of Fe^{2+} , PO_4^{3-} , pH, and dissolved organic carbon (DOC), as well as major cations (e.g., Ca^{2+} , Mg^{2+}) and anions (e.g., SO_4^{2-}), based on field and laboratory analyses. Positive SI values mean supersaturation, while negative values indicate undersaturation.

References

- Allison, J. D., Brown, D. S., & Novo-Gradac, K. J. (1991). MINTEQA2/PRODEFA2: A geochemical assessment model for environmental systems—Version 3.0 user's manual (EPA/600/3-91/021). U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.
- Fadrus, H., & Malý, J. (1975). Suppression of iron(III) interference in the determination of iron(II) in water by the 1,10-phenanthroline method. *Analyst*, 100(1193), 549–554.
- Florea, A. F., Zak, D. H., Petersen, R. J., Dreher, C. L., Kappler, A., Hansen, H.C.B. (2026). Phosphorus dynamics in acidic and neutral fen soils: does vivianite limit phosphorus release?, *Geoderma*, Volume 466, 117685, ISSN 0016-7061, <https://doi.org/10.1016/j.geoderma.2026.117685>.
- Gran, G. (1952) Determination of the Equivalence Point in Potentiometric Titrations. Part II *Analyst*, 77, 661-671.
- Gustafsson, J. P. (2014). Visual MINTEQ, version 3.1. KTH, Royal Institute of Technology, Stockholm, Sweden. (Software and database documentation)
- Heiberg, L., Koch, C.B., Kjaergaard, C., Jensen, H.S., Hansen, H.C.B., 2012. Vivianite precipitation and phosphate sorption following iron reduction in anoxic soils. *J. Environ.Qual.* 41 (3), 938–949. <https://doi-org.ep.fjernadgang.kb.dk/10.2134/jeq2011.0067>.
- IOSWQ. International organization for Standardization Water quality — Determination of phosphorus — Ammonium molybdate spectrometric method 2004, ISO 6878.

- Lovley, D., Stolz, J., Nord, G. et al. Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. *Nature* 330, 252–254 (1987). <https://doi.org/10.1038/330252a0>
- Schoumans, O.F., 2000. Determination of the degree of phosphate saturation in non-calcareous soils. In *Methods of phosphorus analysis for soils, sediments, residuals and waters.*; Pierzynski, G.M., Ed.; Raleigh NC, USA. Coop. Ser. Bull. 396, Publ. SERA-IEG-17.: North Carolina State University, South., pp. 31–34.
- Schwertmann, U., 1964. Differenzierung der Eisenoxide des Bodens durch photochemische Extraktion mit saurer Ammoniumoxalatlösung. *Z. Pflanzenern/ihr., Diing., Bodenkd.*, 105: 194--202. Schwertmann, U., 1964. Differenzierung der Eisenoxide des Bodens durch photochemische Extraktion mit saurer Ammoniumoxalatlösung. *Z. Pflanzenern/ihr., Diing., Bodenkd.*, 105: 194--202.