European Journal of Soil Science

WILEY

European Journal of Soil Science

RESEARCH ARTICLE OPEN ACCESS

Liming Enhances Soil Phosphorus Cycling in Long-Term Agricultural Fields

Ye Wang[1] | Sara L. Bauke[1] | Martina I. Gocke[1] | Christian von Sperber[2] | Julien Guigue[3] | Kathlin Schweitzer[4] | Sabine J. Seidel[5,6] | Federica Tamburini[7] | Wulf Amelung[1,8]

[1]Institute of Crop Science and Resource Conservation (INRES)—Soil Science and Soil Ecology, University of Bonn, Bonn, Germany | [2]Department of Geography, McGill University, Montreal, Quebec, Canada | [3]Chair of Soil Science, Technical University of Munich, Freising, Germany | [4]Department of Crop and Animal Sciences, Humboldt-Universität Zu Berlin, Berlin, Germany | [5]Crop Science, Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany | [6]Department of Agricultural Sciences, Institute of Organic Farming, BOKU University, Vienna, Austria | [7]Institute of Agricultural Sciences, ETH Zürich, Lindau, Switzerland | [8]Institute for Bio- and Geosciences—IBG-3: Agrosphere, Forschungszentrum Jülich GmbH, Jülich, Germany

Correspondence: Sara L. Bauke (sarabauke@uni-bonn.de)

Received: 26 July 2024 | **Revised:** 3 November 2025 | **Accepted:** 4 November 2025

Funding: This study was funded by the German Federal Ministry of Education and Research (BMBF) in the framework of the funding measure "Soil as a Sustainable Resource for the Bioeconomy—BonaRes," project BonaRes (Module A): BonaRes Center for Soil Research, subproject "Sustainable Subsoil Management—Soil3" (Grants 031B0026A, 031B0151A, and 031B1006A) and by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2070-390732324 (PhenoRob). Ye Wang would like to thank the China Scholarship Council for financial support (No. 201606040185).

Keywords: Ca addition | long-term field experiment | P fertilization | phosphate oxygen isotopes ($\delta^{18}O_P$) | sequential P fractionation | subsoil

ABSTRACT

Liming enhances both organic phosphorus (P) mineralization and the precipitation of inorganic phosphates with calcium (Ca) cations. To better understand how P storage and cycling in soil profiles are regulated by the interaction of long-term P fertilization and liming, we collected soil samples from three German arable long-term field experiments in Berlin-Dahlem (Albic Luvisol; sandy topsoil [0–30 cm], and loamy subsoil [> 30 cm]), Dikopshof (Haplic Luvisol; silty-loamy topsoil, and clayey-loamy subsoil), and Thyrow (Albic Luvisol; sandy soil). Treatments within each of these experiments had received mineral fertilization with NKPCa (N: nitrogen; K: potassium; P: phosphorus; Ca: calcium, referring to liming), NKCa, NKP, and NK or no fertilizer application (none) for at least 60 years. Soil P stocks down to 100 cm depth were assessed by Hedley sequential P fractionation and the oxygen isotopic composition of 1 M HCl-extractable phosphate ($\delta^{18}O_P$) was analyzed as an indicator of the degree of microbial P cycling over the decades of experimental duration. We found that mineral P fertilization increased soil total P stocks in all P fractions regardless of differences in soil clay content among the different experiments. Liming significantly decreased $NaHCO_3$-Pi (Pi: inorganic P) and NaOH-Pi stocks by up to 50% across the three experiments and soil depths, but tended to increase Po (organic P) stocks in these fractions by up to 40%, reflecting enhanced P uptake into plant and microbial biomass when acidic soil conditions were improved by lime application. Soil HCl-Pi stocks in treatments with long-term P fertilization and liming were larger by a factor of up to 1.8 compared to the unfertilized control plots, while especially the plots without P fertilization showed smaller $\delta^{18}O_P$ values of 11‰ in the subsoil. These results indicate that, on the one hand, biological P cycling was enhanced in fertilized treatments, but on the other hand, soluble Pi was precipitated

---

Ye Wang and Sara L. Bauke contributed equally to this study.

as secondary Ca–P minerals into stable P fractions. These changes occurred both in the topsoil and upper subsoil (30–50 cm). We conclude that the combined application of long-term P fertilization and liming to the surface soil also increased the utilization of subsoil P.

1 | Introduction

Phosphorus (P) determines food production as a primary limiting nutrient in many agricultural systems (Gao and Deluca 2018). To ensure crop yields for a growing population, P fertilization is used to increase or maintain soil P contents. However, the availability of P in soils to plants is controlled by pH-dependent sorption–desorption processes on reactive surfaces (Curtin and Syers 2001), and further varies among soil orders and with clay content (He et al. 1999; Gocke et al. 2021). Several management practices such as the application of lime or other fertilizers rich in calcium (Ca) are used to adjust soil pH (Sumner and Yamadu 2002; Huang et al. 2021; Tiecher et al. 2023). Therefore, both P and Ca fertilizer applications are critical for P availability and solubility, and further influence plant P uptake from the soil (Penn and Camberato 2019; Pfahler et al. 2020).

As a measure of soil P characterization, the sequential extraction procedure suggested by Hedley et al. (1982) is widely used to assign soil P into different chemical fractions: resin-P, $NaHCO_3$-P, NaOH-P, HCl-P, and residual-P. These fractions comprise conceptual pools of soil P and are assumed to represent decreasing bioavailability with increasing extractant strength, although several studies have also pointed out that the fractions obtained by Hedley extraction cannot be assigned to any specific chemical P forms (Klotzbücher et al. 2019; Gu and Margenot 2021). In general, P fertilization increases soil P content in all sequential fractions, whereas continuous cultivation with insufficient P fertilization strongly depletes labile P fractions (resin-P and $NaHCO_3$-P), and also moderately depletes stable ones (HCl-P and residual-P) (Tiessen et al. 1983; Campbell et al. 1986; Bauke et al. 2018).

Unlike for the effects of P fertilization on soil P fractions, the effects of Ca fertilizer application and increased soil pH on P concentration in soil solution are more ambiguous. The addition of Ca and an increase in soil pH toward less acidic or neutral conditions alter soil conditions by stabilizing soil structure, by promoting mineralization of soil organic matter (Filipek 2011; Wang, Yao, et al. 2021), and by increasing the proportions of multivalent P species (HPO_4^{2-} and PO_4^{3-}) in soil solution compared to monovalent $H_2PO_4^-$ (Naidu et al. 1990; Murphy 2007). With these increased proportions of multivalent P species, soil P availability may either decrease due to the enhanced P sorption and precipitation (Beck and Sanchez 1994; Boschetti et al. 2009), or increase when adsorbing surfaces become more negatively charged as pH increases (Barrow 1984; Curtin and Syers 2001). In consequence, a number of studies have reported increased P fertilizer use efficiency by crops, but no or inconsistent changes in concentrations of plant-available soil P (e.g., Azeez et al. 2020; Qaswar et al. 2020; Huang et al. 2021; Tiecher et al. 2023). It thus remains unclear how long-term P and Ca fertilizer application affect soil P availability both in the topsoil, and in deeper soil horizons of the subsoil (e.g., up to 100 cm depth).

In addition to the assessment of soil P fractions, the oxygen isotopic composition of phosphate ($\delta^{18}O_P$) can be a tracer for biological P cycling (Tamburini et al. 2010, 2014; Bauke 2021; von Sperber et al. 2023). The P–O bond in phosphate is stable and no oxygen isotope exchange with ambient water occurs in abiotic conditions (Liang and Blake 2007), while oxygen exchange occurs rapidly under enzymatic activity (Blake et al. 2005). A complete exchange of oxygen atoms between phosphate and water is catalyzed by the intracellular enzyme pyrophosphatase when inorganic phosphate (Pi) is recycled in microbial or plant cells, resulting in isotopic equilibrium (Blake et al. 2005; Chang and Blake 2015). Thus, the similarity of $\delta^{18}O_P$ values to the isotopic equilibrium value indicates the extent of biological P cycling in the soils (Bauke 2021; von Sperber et al. 2023).

Compared to other bioavailable P fractions, which may be subject to diurnal or seasonal variability, $\delta^{18}O_P$ values of the HCl fraction of the sequential extraction procedure retain long-term information of biological P cycling (Helfenstein et al. 2018) due to the continuous precipitation of biologically cycled P into secondary Ca–P minerals (Amelung et al. 2015; Joshi et al. 2016; Bauke et al. 2018). $\delta^{18}O_P$ values within the soil profile can thus be used to determine to which depth soil P is affected by biological activity in different soil textures and fertilization regimes (Amelung et al. 2015; Bauke et al. 2018; Wang, Bauke, et al. 2021). It was reported that full fertilization of NPK (nitrogen, phosphorus, and potassium) increased biological cycling of soil P, leading to $\delta^{18}O_P$ values closer to equilibrium values in comparison to treatments without P fertilization (Bauke et al. 2018), but that enhanced biological cycling of subsoil P was also already mediated by increased supply of only N (Wang, Bauke, et al. 2021). A first analysis of the combined effects of P and Ca addition has been provided for topsoils of a long-term grassland experiment (Pfahler et al. 2020), indicating a close link between $\delta^{18}O_P$ values and soil pH. However, we still lack knowledge on the $\delta^{18}O_P$ values in agricultural soil profiles extending into the subsoil in response to the interaction of long-term P and Ca fertilizer application.

Here, we assumed that P and Ca additions and the related pH increase do not only improve P supply in the surface soil but also promote the cycling of subsoil P due to more and deeper growth of crop roots in fertilized conditions. We sampled three long-term field experiments with and without P fertilization and liming. Sequential P fractionation and analysis of the oxygen isotope composition of phosphate in the HCl–P fraction ($\delta^{18}O_P$) were used to assess how P fertilization and liming affect P stocks in different fractions and soil depths as well as the biological cycling of P. In particular, we hypothesized that (1) continued P fertilization over several decades (> 60 years) increases soil P stocks in all fractions, while liming decreases labile Pi (sum of resin-Pi and $NaHCO_3$-Pi) stocks due to Ca-P precipitation and (2) long-term P fertilization and liming increase biological soil P cycling at all soil depths, indicated by $\delta^{18}O_P$ values in the HCl–P fraction close to the expected equilibrium value.

Summary

- Interactive effects of P fertilizer and lime application are often assessed only for topsoil horizons.
- Decadal application of liming decreased stocks of inorganic phosphorus throughout the soil profile.
- Combined application of P and Ca fertilizer enhanced subsoil P cycling compared to nonfertilized treatments.
- Long-term P fertilization and liming can increase the utilization of subsoil P reserves.

2 | Materials and Methods

2.1 | Study Site and Samples

The three long-term fertilization experiments were located in Berlin-Dahlem (52°28′ N, 13°17′ E), Dikopshof (50°48′ N700 7°00′ E), and Thyrow (52°15′ N, 13°14′ E), Germany. The experiment in Dahlem was established in 1923 with a nonrandomized block design (i.e., experimental replications were arranged in blocks, but treatments within the blocks always occurred in the same order) with three replicate blocks of plots of 20 m^2 each. The soil is characterized as Albic Luvisol (IUSS Working Group WRB 2015) and developed from sand deposited above a glacial till with soil texture ranging from sandy in the topsoil (0–30 cm) to loamy sand in the subsoil (30–100 cm). The mean annual temperature in Dahlem is 9.9°C with a mean annual precipitation of 562 mm. The soil pH ranges from 4.2 to 6.8 and soil bulk density is 1.4–1.8 $g\,cm^{-3}$. The 4-year crop rotation is fodder beet (*Beta vulgaris* var. *crassa*), winter wheat (*Triticum aestivum* L.), potato (*Solanum tuberosum* L.), and again winter wheat. Four treatments for mineral fertilizer application were considered here: NKPCa, NKCa, NKP, and NK. The average amounts per crop rotation of N (ammonium sulfate nitrate), K (magnesium potassium), and, in the designated treatments, P (superphosphate), and Ca (calcium carbonate) fertilizer were 105, 125, 34, and 200 $kg\,ha^{-1}\,year^{-1}$, respectively (Table 1).

The experiment at the University of Bonn research station Dikopshof started in 1904. The soil was classified as a Haplic Luvisol (IUSS Working Group WRB 2015) with a mean annual temperature of 10.1°C and a mean annual precipitation of 630 mm. The soil texture is a silty loam in the topsoil and clay loam in the subsoil. The soil pH is 5.8–7.0 and soil bulk density ranges from 1.4 to 1.6 $g\,cm^{-3}$ across different soil depths. The field experiment is a nonrandomized block design without replicates and each plot covers an area of 277.5 m^2. The 5-year crop rotation comprises sugar beet (*B. vulgaris*), winter wheat (*T. aestivum* L.), winter rye (*Secale cereal* L.), persian clover (*Trifolium resupinatum* L.), and oat/potato (*Avena* L./*S. tuberosum* L., potato replaced oat in 1953). The three treatments considered in this study were: NKPCa, NKCa, NKP, and no fertilization as a control (none). Within each 5-year rotation, average fertilizer amounts of 46 $kg\,ha^{-1}\,year^{-1}$ N, 116 $kg\,ha^{-1}\,year^{-1}$ K, 31 $kg\,ha^{-1}\,year^{-1}$ P, and 229 $kg\,ha^{-1}\,year^{-1}$ Ca were applied as ammonium nitrate, potash, superphosphate, and burnt lime (CaO), respectively, according to the experimental design (Table 1). Although field management varied in the first 50 years of the field experiment, it has not changed since 1953. More details on the field management were reported by Rueda-Ayala et al. (2018) and Seidel et al. (2021).

The experiment at Thyrow was established in 1937 with a randomized block design with four replicate plots, each having a plot area of 72 m^2. The mean annual temperature is 9.2°C and mean annual precipitation is 510 mm. The soil is classified as Cutanic Albic Luvisol (IUSS Working Group WRB 2015) and developed from periglacial sand over a loamy substrate of calcareous glacial till (Bauke et al. 2018). The soil texture is sandy with soil pH between 4.1 and 6.2. The soil bulk density varies from 1.66 to 1.85 $g\,cm^{-3}$ across different soil depths. The experiment was conducted under a rotation of spring barley (*Hordeum vulgare* L.), potato (*S. tuberosum* L.), spring barley (*H. vulgare* L.), and maize (*Zea mays* L.). It has 4 treatments of mineral fertilizer application: NKPCa, NKCa, NKP, and NK. N (ammonium nitrate and ammonium sulfate) and K (potassium chloride and potassium sulfate) for one 4-year rotation were applied at the rate of 75 and 100 $kg\,ha^{-1}\,year^{-1}$. P (triple superphosphate) and Ca (calcium carbonate or dolomitic lime) treatments received 24 and 125 $kg\,ha^{-1}\,year^{-1}$, respectively (Table 1).

All soil samples were collected as undisturbed soil cores in March 2016 by core sampler (sheath probe, inner diameter: 6 cm). Cores were then cut into depth increments of 0–15, 15–30, 30–40, 40–50, 50–70, and 70–100 cm for Dahlem, 0–30, 30–43, 43–50, 50–70, 70–84, and 84–100 cm for Dikopshof and 0–24, 24–30, 30–50, 50–70, and 70–100 cm for Thyrow, according to the observed soil horizons. Linear interpolation was used for the correction of soil depths between 30 and 100 cm when drilling compaction occurred (Walter et al. 2016). All samples were dried (40°C) and weighed, visible stones and plant residues were removed, and the sample was passed through a sieve of 2 mm mesh size. In our results, the individual soil depths were summarized to 0–30, 30–50, and 50–100 cm for better visualization of effects across sites.

2.2 | General Soil Parameters

Soil bulk density was calculated from the weight of air-dried samples before sieving relative to the volume of the respective depth increment in the soil core (Walter et al. 2016). Soil pH was measured in 0.01 M $CaCl_2$ suspension at a soil-solution ratio of 1:2.5 (w/v). Soil clay content was predicted from visible near infrared light reflectance spectroscopy (Hobley and Prater 2019). Soil C and N concentrations were measured by dry combustion (HEKAtech EuroEA 3000, Hekatech, Wegberg, Germany) after grinding a subsample to fine powder. Soil C and N concentrations were converted to stocks according to the bulk density of the respective depth increments.

2.3 | Sequential P Fractionation

According to the modified protocol by Tiessen and Moir (1993), 0.5 g air-dried soil was sequentially extracted by anion resin

TABLE 1 | Fertilization rates (kg ha^{-1} year^{-1}) per crop rotation in long-term field experiments in Dahlem, Dikopshof and Thyrow, Germany.

		NKPCa				NKCa				NKP				NK or none			
	Crops	**N**	**K**	**P**	**Ca**	**N**	**K**	**P**	**Ca**	**N**	**K**	**P**	**Ca**	**N**	**K**	**P**	**Ca**
Dahlem	Fodder beet	120	149	44	0	120	149	0	0	120	149	44	0	120	149	0	0
	Winter wheat	100	100	28	400	100	100	0	400	100	100	28	0	100	100	0	0
	Potato	100	149	35	0	100	149	0	0	100	149	35	0	100	149	0	0
	Winter wheat	100	100	28	400	100	100	0	400	100	100	28	0	100	100	0	0
	Rotation mean per year	105	125	34	200	105	125	0	200	105	125	34	0	105	125	0	0
Dikopshof	Sugar beet	80	116	31	0	80	116	0	0	80	116	31	0	0	0	0	0
	Winter wheat	60	116	31	0	60	116	0	0	60	116	31	0	0	0	0	0
	Winter rye	40	116	31	0	40	116	0	0	40	116	31	0	0	0	0	0
	Persian clover	0	116	31	1143	0	116	0	1143	0	116	31	0	0	0	0	0
	Potato	50	116	31	0	50	116	0	0	50	116	31	0	0	0	0	0
	Rotation mean per year	46	116	31	229	46	116	0	229	46	116	31	0	0	0	0	0
Thyrow	Spring barley	60	100	24	125	60	100	0	125	60	100	24	0	60	100	0	0
	Potato	90	100	24	125	90	100	0	125	90	100	24	0	90	100	0	0
	Spring barley	60	100	24	125	60	100	0	125	60	100	24	0	60	100	0	0
	Maize	90	100	24	125	90	100	0	125	90	100	24	0	90	100	0	0
	Rotation mean per year	75	100	24	125	75	100	0	125	75	100	24	0	75	100	0	0

strips, 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, and hot aqua regia (3:1 [v/v] mixture of concentrated HCl and concentrated HNO_3). Total P concentration in each fraction was measured by ICP-OES (Ultima 2, HORIBA Jobin Yvon, Longjumeau, France). Inorganic P (Pi) concentration was measured by spectrophotometer (Tecan infinite M200pro, Grödig, Austria) using the malachite green method (Ohno and Zibilske 1991) and determining light absorption at 630 nm wavelength. Organic P (Po) was calculated as the difference of total P and Pi. P concentration was transferred to P stocks using the respective bulk density and soil depth increment.

2.4 | $\delta^{18}O_P$

Thirty grams of all soil samples were successively extracted by 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl at a 1:10 soil-solution ratio (Amelung et al. 2015). The alkaline solutions were used to remove most of the organic P compounds and polyphosphates and only the 1 M HCl extract was kept for further purification. Based on the procedure by Tamburini et al. (2010), phosphate was first precipitated to ammonium phosphomolybdate (APM) and magnesium ammonium phosphate (MAP), then washed by cation exchange resin (Dowex 50X8, 200–400 mesh, Sigma-Aldrich, Darmstadt, Germany) and finally precipitated to silver phosphate (Ag_3PO_4). After drying at 50°C, Ag_3PO_4 crystals were stored in a desiccator. The Ag_3PO_4 samples were pyrolyzed in a carbon-based reactor (1450°C) with a trap chromatography system (PYRO Cube, Elementar, Hanau, Germany) connected to an isotope ratio mass spectrometer (Isoprime 100, Isoprime, Manchester, UK) for the analysis of oxygen isotope ratios. All oxygen isotope ratios were calibrated against the Vienna Standard Mean Oceanic Water (VSMOW) and are reported in the conventional delta notation ($\delta^{18}O$).

Theoretical equilibrium values of soil $\delta^{18}O_P$ were calculated according to Equation (1) by Chang and Blake (2015):

$$\delta^{18}O_p = e^{\left(\frac{14.43}{T} - \frac{26.54}{1000}\right)} \times \left(\delta^{18}O_w + 1000\right) - 1000 \tag{1}$$

where T is the soil ambient temperature in K, and $\delta^{18}O_P$ and $\delta^{18}O_w$ are the standardized ^{18}O:^{16}O ratios of phosphate and water in ‰, respectively. There was no information about the soil water and precipitation $\delta^{18}O$ values in Dikopshof. Hence, we used the average of observed annual $\delta^{18}O_w$ values at the nearest GNIP (Global Network Isotopes in Precipitation) stations in Koblenz (50.36° N, 7.59° E) and Emmerich (51.83° N, 6.24° E), with a $\delta^{18}O_w$ value of −6.96‰. The mean, minimum and maximum temperature were calculated for monthly data from 1987 to 2016 as 10.9°C, 4.9°C and 17.4°C (Dikopshof Meteorological Station), respectively. Thus, the mean, minimum and maximum $\delta^{18}O_P$ equilibrium values in Dikopshof were calculated as 17.43‰, 16.27‰ and 18.54‰, respectively. The site of Dahlem is close to Thyrow. For both sites, we used mean, minimum and maximum $\delta^{18}O_P$ equilibrium values of 16.7‰, 12.1‰ and 20.2‰ as given by Bauke et al. (2018) for Thyrow. The minimum and maximum $\delta^{18}O_P$ equilibrium values of the three sites (12.1‰ and 20.2‰) were used to compute the maximum range of expected equilibrium $\delta^{18}O_P$ values given in Figure 2.

2.5 | Statistics

Statistical analysis was done using R (version 3.4.1). Data are presented as the mean and standard error (SE) of field replicates in Dahlem and Thyrow, but there is only one value for each treatment at Dikopshof due to missing replication. Thus, we did not conduct data analysis for P stocks and $\delta^{18}O_P$ values among treatments in Dikopshof. Due to missing randomization in the long-term experiment in Dahlem, a multiple regression model was used to analyze treatment effects across the experimental replicate blocks (statistical model results provided in Tables S1, S4, and S7) for soil P stocks and $\delta^{18}O_P$ values. In Thyrow, where experimental plots were arranged in a fully randomized block design, two-way ANOVA for P fertilization and liming was used to analyze soil P stocks and $\delta^{18}O_P$ values and differences among treatments were assessed with a least significant difference (LSD) test ($p < 0.05$). The results of the ANOVA model for the effects of P fertilization, liming and interaction of P fertilization and liming are given in Tables S2, S5, and S8. Linear regression was also used to analyze the effects of soil clay content on soil N, C, and P stocks among three long-term experimental fields (Tables S3 and S6).

3 | Results

3.1 | Basic Soil Properties

In all three experiments, mineral P fertilization and liming hardly affected soil bulk density in different soil depths (Table 2, statistical models given in Tables S1 and S2). Soil pH had significantly increased with liming in the topsoil of all experiments by 0.4–2.1 units ($p < 0.01$). This increase was also observed in the deeper subsoil (50–100 cm). Nevertheless, slightly acidic conditions prevailed in all experiments and treatments.

Soil clay content in Dikopshof was greater than that in Dahlem and Thyrow, and at all sites, clay content tended to increase with increasing soil depth (Table 2). Soil N and C stocks increased with increasing soil clay contents in the soil profile in Dikopshof compared to Dahlem and Thyrow (Figure S2). In Thyrow, the topsoil N and C stocks of the treatments with P fertilization and liming were significantly greater than those in the treatment without fertilization ($p < 0.05$; Table 2), and a similar trend was also observed at Dikopshof. However, in Dahlem, the topsoil (0–30 cm) N and C stocks were smaller after liming compared to the other treatments ($p < 0.01$). For the subsoil, P fertilization and liming did not show significant effects on N and C stocks of the three field experiments (Table 2, statistical models given in Tables S1 and S2).

3.2 | Sequential P Fractionation

According to the averaged percentage across the three field experiments for different P fractions, the proportions of resin- and $NaHCO_3$-P decreased with soil depth, while the proportions of NaOH-, HCl- and residual-P increased in deeper soil layers (Figure 1). Further, long-term P fertilization resulted in greater proportions of resin- and $NaHCO_3$-P in comparison with the treatments without P fertilization, whereas liming increased stable soil P proportions (HCl- and residual-P).

In the topsoil (0–30 cm), long-term P fertilization increased Pi stocks in all fractions of the NKPCa and NKP treatments relative to those of the NKCa and NK/None treatments, respectively. This effect was significant at Dahlem ($p < 0.01$) and Thyrow (Table 3, statistical models given in Tables 4S and 5S). Compared to Pi stocks, soil Po stocks were less sensitive to P fertilization. In addition, resin-P stocks significantly decreased with liming in Dahlem ($p < 0.05$, statistical model given in Table S4), whereas the opposite effect was observed in Thyrow and as a trend in Dikopshof. With lime application, both $NaHCO_3$-Pi and NaOH-Pi stocks were significantly smaller than those without Ca input in Dahlem and Thyrow, and as a tendency also at Dikopshof (Table 3). Moreover, liming significantly decreased labile Pi (sum of resin-P and $NaHCO_3$-Pi) stocks in Dahlem ($p < 0.05$), and the same trend was evident at Dikopshof and in Thyrow. The decline of the easily extractable Pi forms upon liming went along with greater stocks of organic P pools. The effect was significant in $NaHCO_3$-Po stocks ($p < 0.05$ in Dahlem) but in tendency also evident for NaOH- and HCl-Po stocks (Tables 3 and S4). Although the interaction effect of P fertilization and liming was only significant in HCl-Po stocks ($p < 0.05$) at the site of Thyrow, HCl-Pi stocks in NKPCa treatment were the largest among all treatments (Table 3).

Compared to the topsoil, the difference in P stocks among treatments was smaller in the upper subsoil (30–50 cm) for all fractions (Table 3). Nevertheless, P fertilization had significant effects on subsoil resin-P and $NaHCO_3$-Pi stocks in Dahlem ($p < 0.01$) and Thyrow, as well as on NaOH-Pi stocks in Dahlem ($p < 0.05$). In contrast, liming resulted in smaller stocks of the resin-, $NaHCO_3$-, and NaOH-Pi pools at Dahlem and Dikopshof, and NaOH-Pi pools in Thyrow (Table 3). The decrease of labile Pi stocks by liming was significant only in Dahlem ($p < 0.01$) and as a trend at Dikopshof. Effects on P

TABLE 2 | Soil properties of the long-term fertilizer trials in Dahlem, Dikopshof and Thyrow, Germany.

	Depth (cm)	Treatments	Bulk density ($g\,cm^{-3}$)	pH	Clay content (%)	N stocks ($t\,ha^{-1}$)	C stocks ($t\,ha^{-1}$)
Dahlem ($n=3$)	0–30	NKPCa	1.48 ± 0.02	5.1 ± 0.1	7.39 ± 0.82	1.55 ± 0.06	17.60 ± 0.76
		NKCa	1.52 ± 0.02	5.3 ± 0.1	8.25 ± 0.74	1.71 ± 0.07	19.56 ± 1.07
		NKP	1.49 ± 0.02	4.7 ± 0.1	7.04 ± 0.76	2.00 ± 0.15	23.53 ± 1.24
		NK	1.48 ± 0.04	4.6 ± 0.1	5.63 ± 0.15	2.19 ± 0.07	25.07 ± 0.67
	30–50	NKPCa	1.81 ± 0.09	5.4 ± 0.2	11.36 ± 5.19	0.60 ± 0.10	4.70 ± 0.39
		NKCa	1.76 ± 0.09	6.1 ± 0.5	16.49 ± 2.27	0.75 ± 0.11	6.07 ± 1.22
		NKP	1.81 ± 0.13	4.2 ± 0.1	9.38 ± 3.10	0.71 ± 0.10	6.71 ± 1.33
		NK	1.80 ± 0.07	4.2 ± 0.1	10.49 ± 0.81	0.68 ± 0.03	5.68 ± 0.34
	50–100	NKPCa	1.79 ± 0.07	6.5 ± 0.5	16.14 ± 1.45	1.26 ± 0.14	32.26 ± 13.30
		NKCa	1.83 ± 0.06	6.8 ± 0.5	20.11 ± 0.59	1.49 ± 0.08	38.03 ± 15.02
		NKP	1.82 ± 0.07	5.3 ± 0.6	16.86 ± 5.05	1.18 ± 0.32	12.76 ± 6.70
		NK	1.74 ± 0.04	5.8 ± 0.5	20.92 ± 1.19	1.42 ± 0.05	25.00 ± 8.67
Dikoshof ($n=1$)	0–30	NKPCa	1.39	6.5	22.38	3.38	36.83
		NKCa	1.37	6.9	22.26	3.04	32.05
		NKP	1.43	5.8	19.34	3.11	33.69
		None	1.43	6.0	20.88	2.77	29.13
	30–50	NKPCa	1.44	6.8	34.36	0.89	7.32
		NKCa	1.44	6.9	31.35	0.85	6.77
		NKP	1.48	6.1	34.77	0.97	8.17
		None	1.56	6.0	30.22	0.90	7.52
	50–100	NKPCa	1.47	7.0	35.72	1.09	7.63
		NKCa	1.52	7.0	33.31	0.98	6.43
		NKP	1.61	6.5	40.00	1.03	6.90
		None	1.77	6.1	35.85	0.91	6.04
Thyrow ($n=4$)	0–30	NKPCa	1.68 ± 0.03 a	6.0 ± 0.1 b	5.09 ± 0.59 a	1.29 ± 0.18 b	15.59 ± 0.66 b
		NKCa	1.67 ± 0.02 a	6.0 ± 0.2 b	5.38 ± 0.50 a	1.24 ± 0.06 b	13.79 ± 0.62 ab

(Continues)

TABLE 2 | (Continued)

Depth (cm)	Treatments	Bulk density ($g\,cm^{-3}$)	pH	Clay content (%)	N stocks ($t\,ha^{-1}$)	C stocks ($t\,ha^{-1}$)
	NKP	1.66 ± 0.02 a	3.9 ± 0.1 a	5.04 ± 0.23 a	1.44 ± 0.08 b	16.74 ± 0.74 b
	NK	1.68 ± 0.02 a	4.1 ± 0.1 a	6.48 ± 0.76 a	0.71 ± 0.17 a	12.44 ± 1.41 a
30–50	NKPCa	1.85 ± 0.01 a	6.2 ± 0.2 b	5.17 ± 1.00 a	0.22 ± 0.03 a	3.89 ± 0.57 a
	NKCa	1.85 ± 0.04 a	6.4 ± 0.2 b	5.94 ± 0.35 a	0.16 ± 0.03 a	3.14 ± 0.50 a
	NKP	1.84 ± 0.04 a	4.0 ± 0.0 a	6.15 ± 0.94 a	0.20 ± 0.03 a	3.69 ± 0.84 a
	NK	1.85 ± 0.06 a	4.0 ± 0.0 a	7.16 ± 1.91 a	0.18 ± 0.02 a	3.40 ± 0.36 a
50–100	NKPCa	1.75 ± 0.05 a	6.1 ± 0.3 b	11.34 ± 1.44 a	0.81 ± 0.14 a	6.65 ± 0.68 b
	NKCa	1.85 ± 0.07 a	6.0 ± 0.2 b	14.34 ± 2.09 ab	0.57 ± 0.08 a	5.08 ± 0.61 ab
	NKP	1.84 ± 0.08 a	4.5 ± 0.1 a	13.80 ± 0.97 ab	0.61 ± 0.11 a	4.45 ± 0.78 ab
	NK	1.77 ± 0.05 a	4.8 ± 0.2 a	16.24 ± 1.33 b	0.71 ± 0.14 a	3.95 ± 0.90 a

Note: Data for Dahlem and Thyrow are represented as mean ± standard error. Values with different letters within a column show significant differences among treatments in the respective experiment. Multiple regression model was used for data analysis due to missing randomization in Dahlem. Two-way ANOVA was used for data analysis in Thyrow and differences among treatments were assessed with least significant difference (LSD) test ($p < 0.05$).

stocks in the deeper subsoil (50–100 cm) were not significant except for significant increases of $NaHCO_3$- and HCl-Pi, and HCl-Po stocks by liming in Thyrow (Table 3). Differences in texture with depth or among the three sites had only minor effects on observed differences in P stocks; only HCl-P and residual-P stocks increased with soil clay contents over the soil profile (Figure S2 and Table S6).

3.3 | $\delta^{18}O_P$

Throughout the soil profiles, $\delta^{18}O_P$ values showed a decreasing trend with soil depth when averaged across all three experiments (Figure 2). The $\delta^{18}O_P$ values in the topsoil were in the range of the expected equilibrium values, while full fertilization (i.e., the NKPCa treatment) resulted in the greatest $\delta^{18}O_P$ values among all treatments also at deeper depth within the soil profiles. Similar depth profiles were also observed for $\delta^{18}O_P$ values for the NPK and None/NK treatments, whereas the profile for the NKCa treatment was shifted to smaller $\delta^{18}O_P$ values, which were below the equilibrium range at > 50 cm soil depth (Figure 2). When looking at the individual long-term experimental fields (Figure 3S), this effect was largely driven by the site Dahlem (statistical model in Table S7); nevertheless, the smallest $\delta^{18}O_P$ values in the subsoil also occurred in the NKCa treatment at the sites Dikopshof and Thyrow.

4 | Discussion

Long-term liming at all our sites increased soil pH as desired (Sumner and Yamadu 2002; Huang et al. 2021; Tiecher et al. 2023), but effects on C and N stocks varied among the three sites (Table 2). On the one hand, Ca and P input increased topsoil C and N stocks at the sites Dikopshof and Thyrow, which can be explained by better conditions for crop growth and presumably more root residue return to the soil, enhancing soil organic matter storage. On the other hand, previous studies also observed that elevated pH values increased microbial mineralization of soil organic matter (Nachimuthu et al. 2009; Achat et al. 2010; Wang, Yao, et al. 2021), which may account for smaller C and N stocks as observed here at the site in Dahlem. Further, as discussed in previous publications for the same sites (Gocke et al. 2023; Skadell et al. 2023), the data suggest that P and Ca fertilization can increase or decrease organic matter storage in soils depending on the quantity of inputs relative to the stimulation of mineralization processes. It should be considered here that the experimental design of the long-term field experiments in Dahlem and Dikopshof was deficient in randomization and/or replication. Hence, effects that explain variations in P cycling among the individual sites should be interpreted with caution. The general trends, however, were consistent within the three long-term experiments, which also suggests that analyzing comparable treatments across different long-term agricultural research trials may circumvent limitations from lacking randomization within one single experiment.

4.1 | Effects of Long-Term P Fertilization

Long-term P fertilization increased soil P stocks at all long-term experiment sites. P supply from fertilizer is often in excess of

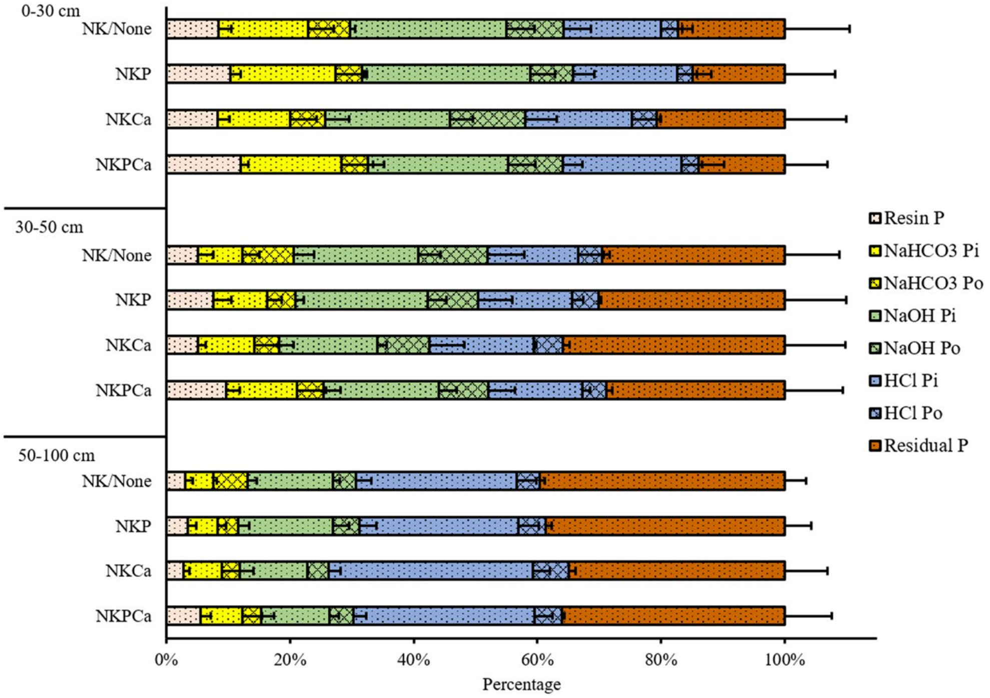

FIGURE 1 | Percentages of P stocks in fractions determined by sequential extraction. Bars and whiskers indicate the mean and standard error across the three long-term trials in Dahlem, Dikopshof and Thyrow, Germany. Pi refers to inorganic P forms, Po refers to organic P forms; resin and residual P fractions were only determined as total P without separation of inorganic and organic P forms. This figure summarizes the differences in P pools across the three field experiments as displayed in Table 3, which indicates significant differences in P stocks to the $p < 0.05$ level for the individual experiments.

the amount of P required by crops (Zhang et al. 2004; Buczko et al. 2017), which results in reduced fertilizer P use efficiency (McBeath et al. 2012; Tiecher et al. 2023). Indeed, besides labile P (resin- and NaHCO$_3$-P) stocks, the stocks of stable soil P (HCl- and residual-P) pools also increased with P fertilization, indicating that continuous P fertilization went along with a transformation from labile to stable P forms, for example, via precipitation and sorption processes (Zhang et al. 2004; Gichangi et al. 2009). Similar findings have been reported from other long-term P fertilization field experiments (Campbell et al. 1986; Shen et al. 2004; Bauke et al. 2018) as well as in incubation experiments (Malik et al. 2012).

Noteworthy, not only P stocks in the topsoil but also those in the upper subsoil were consistently larger than P stocks in the controls without P fertilizer, especially in labile P fractions. Phosphorus can reach deeper soil horizons via leaching of dissolved inorganic P, organic matter or soil colloids along soil pores (Kang et al. 2011). Eghball et al. (2004) observed that the leaching of plant available P in a silty clay loam soil can reach a depth of 60 cm within 4 years and leaching rates of up to 10 kg P ha^{-1} have been reported for fertilized arable topsoils (Djodjic et al. 2004; Fortune et al. 2005; Riddle et al. 2018). However, we suggest that leaching alone can hardly account for differences of about 100 kg P ha^{-1} in the subsoil of plots with or without fertilization, for example, at the site in Thyrow (Table 3), which receives very limited amounts of precipitation to initiate leaching.

The total amount of P stored in top- and subsoils of the three field experiments was not only related to the amount of fertilizer application, but also to soil clay content and the amount of P fertilizer applied at each site. Average amounts of P fertilizer application in Dahlem, Dikopshof and Thyrow ranged from 24 to 34 kg ha^{-1} year^{-1} (Table 1). After long-term P fertilization, the sum of total P stocks from sequential extractions in Thyrow was smaller than those in Dikopshof (Table 3), due to the lesser amount of P fertilizer applied in Thyrow (24 kg ha^{-1} year^{-1}) than at Dikopshof (31 kg ha^{-1} year^{-1}), and due to smaller inherent P stocks at Thyrow with lesser clay content (Figure S2). In line with these findings, total P stocks in the sandy topsoil at Dahlem were close to those of the sandy topsoils in Thyrow, whereas slightly greater clay content in the subsoil also resulted in larger subsoil total P stocks at Dahlem compared to Thyrow (Tables 2 and 3; Figure S2). The positive relationships of P stocks and clay content were especially evident in larger stable P stocks (i.e., HCl-P and residual-P), whereas labile P pools such as resin-P, NaHCO$_3$-P, and NaOH-P stocks responded more variably (Figure S2) to different amounts of P fertilizer input and crop P uptake (Maranguit et al. 2017).

Phosphorus may also be redistributed within the soil profile by plant roots growing into the subsoil (Bauke, Landl, et al. 2017), which could then be recovered as root-derived Po in the deeper soil layers (Campbell et al. 1993). Nevertheless, the rates of such processes for P redistribution within the soil are still unclear, and should primarily result in an accumulation of organic P, yet we did not find elevated stocks of Po in the subsoil after long-term P fertilization (Table 3). Root-derived or microbial P can be converted to Pi by biochemical mineralization and hydrolysis (Laboski and John 2003) and prolonged duration of arable cropping was shown to promote a shift from organic to inorganic soil P forms (von Sperber et al. 2017). In line with these studies,

TABLE 3 | Soil P stocks (t ha^{-1}) of sequential P fractionation, sum of labile Pi (resin- and NaHCO$_3$-Pi) and sum of total P stocks in the three long-term fertilizer trials, Dahlem, Dikopshof, and Thyrow, Germany.

	Depth (cm)	Treatments	Resin P stocks	NaHCO$_3$ Pi stocks	NaHCO$_3$ Po stocks	NaOH Pi stocks	NaOH Po stocks	HCl Pi stocks	HCl Po stocks	Residual P stocks	Sum of labile Pi stocks	Sum of total P stocks
Dahlem ($n=3$)	0–30	NKPCa	0.20 ± 0.01	0.31 ± 0.03	0.16 ± 0.01	0.52 ± 0.05	0.20 ± 0.01	0.18 ± 0.00	0.06 ± 0.00	0.13 ± 0.03	0.51 ± 0.04	1.77 ± 0.08
		NKCa	0.13 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.38 ± 0.02	0.22 ± 0.01	0.16 ± 0.01	0.06 ± 0.01	0.14 ± 0.02	0.34 ± 0.01	1.48 ± 0.02
		NKP	0.26 ± 0.02	0.38 ± 0.01	0.09 ± 0.01	0.56 ± 0.04	0.18 ± 0.02	0.22 ± 0.04	0.07 ± 0.01	0.15 ± 0.01	0.65 ± 0.02	1.91 ± 0.08
		NK	0.18 ± 0.01	0.27 ± 0.01	0.11 ± 0.03	0.38 ± 0.01	0.22 ± 0.02	0.14 ± 0.01	0.06 ± 0.01	0.10 ± 0.01	0.44 ± 0.01	1.44 ± 0.07
	30–50	NKPCa	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.21 ± 0.02	0.06 ± 0.02	0.12 ± 0.02	0.05 ± 0.00	0.18 ± 0.09	0.17 ± 0.02	0.87 ± 0.13
		NKCa	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.16 ± 0.01	0.04 ± 0.01	0.15 ± 0.02	0.05 ± 0.01	0.26 ± 0.02	0.12 ± 0.02	0.85 ± 0.06
		NKP	0.14 ± 0.01	0.14 ± 0.01	0.05 ± 0.03	0.28 ± 0.02	0.06 ± 0.02	0.12 ± 0.01	0.05 ± 0.01	0.20 ± 0.05	0.28 ± 0.01	1.03 ± 0.09
		NK	0.08 ± 0.00	0.10 ± 0.00	0.04 ± 0.03	0.21 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.05 ± 0.01	0.17 ± 0.06	0.18 ± 0.00	0.80 ± 0.04
	50–100	NKPCa	0.17 ± 0.05	0.12 ± 0.04	0.18 ± 0.03	0.36 ± 0.10	0.06 ± 0.02	0.91 ± 0.28	0.11 ± 0.01	0.69 ± 0.11	0.29 ± 0.09	2.61 ± 0.14
		NKCa	0.13 ± 0.05	0.11 ± 0.03	0.20 ± 0.02	0.30 ± 0.09	0.07 ± 0.01	1.06 ± 0.20	0.13 ± 0.07	0.78 ± 0.09	0.23 ± 0.08	2.78 ± 0.12
		NKP	0.14 ± 0.03	0.17 ± 0.02	0.03 ± 0.02	0.43 ± 0.05	0.06 ± 0.02	0.59 ± 0.30	0.14 ± 0.01	0.77 ± 0.29	0.30 ± 0.05	2.32 ± 0.59
		NK	0.12 ± 0.01	0.13 ± 0.01	0.10 ± 0.06	0.38 ± 0.05	0.05 ± 0.02	0.69 ± 0.13	0.11 ± 0.00	0.79 ± 0.06	0.25 ± 0.02	2.36 ± 0.12
Dikopshof ($n=1$)	0–30	NKPCa	0.24	0.16	0.09	0.34	0.06	0.77	0.04	0.65	0.40	2.38
		NKCa	0.07	0.05	0.06	0.19	0.03	0.38	0.07	0.58	0.12	1.43
		NKP	0.19	0.17	0.08	0.46	0.00	0.66	0.06	0.74	0.35	2.32
		None	0.08	0.10	0.08	0.28	0.01	0.42	0.03	0.62	0.17	1.62
	30–50	NKPCa	0.05	0.04	0.03	0.13	0.01	0.15	0.03	0.40	0.09	0.85
		NKCa	0.02	0.03	0.03	0.12	0.01	0.13	0.02	0.44	0.05	0.81
		NKP	0.05	0.06	0.02	0.18	0.00	0.19	0.04	0.54	0.11	1.08
		None	0.03	0.04	0.05	0.15	0.02	0.24	0.03	0.50	0.07	1.07
	50–100	NPKCa	0.05	0.07	0.05	0.26	0.03	0.63	0.09	1.24	0.13	2.41
		NKCa	0.05	0.07	0.04	0.28	0.01	0.75	0.12	1.25	0.12	2.57
		NPK	0.04	0.07	0.06	0.29	0.02	0.90	0.13	1.35	0.11	2.85
		None	0.08	0.10	0.09	0.33	0.01	0.77	0.06	1.19	0.18	2.66

(Continues)

TABLE 3 | (Continued)

	Depth (cm)	Treatments	Resin P stocks	$NaHCO_3$ Pi stocks	$NaHCO_3$ Po stocks	NaOH Pi stocks	NaOH Po stocks	HCl Pi stocks	HCl Po stocks	Residual P stocks	Sum of labile Pi stocks	Sum of total P stocks
Thyrow ($n=4$)	0–30	NKPCa	0.26 ± 0.01 c	0.44 ± 0.02 c	n.d.	0.43 ± 0.01 b	0.23 ± 0.01 a	0.26 ± 0.01 b	0.06 ± 0.01 b	0.12 ± 0.00 ab	0.70 ± 0.03 b	1.80 ± 0.06 b
		NKCa	0.14 ± 0.02 a	0.22 ± 0.03 a	n.d.	0.27 ± 0.03 a	0.24 ± 0.01 a	0.18 ± 0.01 a	0.04 ± 0.01 b	0.15 ± 0.01 b	0.35 ± 0.05 a	1.24 ± 0.10 a
		NKP	0.20 ± 0.01 b	0.51 ± 0.02 c	0.10 ± 0.02 a	0.70 ± 0.01 d	0.24 ± 0.02 a	0.24 ± 0.02 b	0.02 ± 0.00 a	0.12 ± 0.02 a	0.71 ± 0.03 b	2.10 ± 0.07 c
		NK	0.13 ± 0.01 a	0.31 ± 0.02 b	0.13 ± 0.01 a	0.54 ± 0.04 c	0.20 ± 0.02 a	0.19 ± 0.01 ab	0.04 ± 0.01 ab	0.11 ± 0.01 a	0.44 ± 0.03 a	1.64 ± 0.10 b
	30–50	NKPCa	0.06 ± 0.01 b	0.09 ± 0.01 c	n.d.	0.07 ± 0.01 ab	0.07 ± 0.01 a	0.06 ± 0.01 a	0.01 ± 0.00 a	0.08 ± 0.01 a	0.15 ± 0.02 b	0.45 ± 0.05 a
		NKCa	0.02 ± 0.01 a	0.06 ± 0.01 b	n.d.	0.05 ± 0.01 a	0.07 ± 0.01 a	0.06 ± 0.01 a	0.02 ± 0.00 a	0.08 ± 0.01 a	0.08 ± 0.01 a	0.35 ± 0.04 a
		NKP	0.02 ± 0.01 a	0.03 ± 0.01 ab	0.03 ± 0.01 a	0.09 ± 0.02 b	0.08 ± 0.01 a	0.07 ± 0.00 a	0.02 ± 0.01 a	0.09 ± 0.01 a	0.05 ± 0.02 a	0.42 ± 0.04 a
		NK	0.01 ± 0.00 a	0.02 ± 0.00 a	0.06 ± 0.01 b	0.08 ± 0.01 ab	0.09 ± 0.01 a	0.05 ± 0.01 a	0.01 ± 0.00 a	0.08 ± 0.00 a	0.03 ± 0.01 a	0.40 ± 0.02 a
	50–100	NKPCa	0.09 ± 0.08 a	0.15 ± 0.01 b	n.d.	0.10 ± 0.02 a	0.09 ± 0.01 a	0.31 ± 0.04 ab	0.06 ± 0.02 ab	0.35 ± 0.04 a	0.24 ± 0.08 b	1.16 ± 0.15 a
		NKCa	0.02 ± 0.01 a	0.15 ± 0.02 b	n.d.	0.14 ± 0.02 ab	0.09 ± 0.01 a	0.40 ± 0.06 b	0.10 ± 0.02 b	0.35 ± 0.05 a	0.17 ± 0.02 ab	1.25 ± 0.15 a
		NKP	0.03 ± 0.01 a	0.05 ± 0.01 a	0.07 ± 0.02 a	0.18 ± 0.03 b	0.10 ± 0.01 a	0.21 ± 0.04 a	0.03 ± 0.01 a	0.37 ± 0.09 a	0.08 ± 0.02 a	1.04 ± 0.20 a
		NK	0.01 ± 0.00 a	0.04 ± 0.01 a	0.08 ± 0.01 a	0.12 ± 0.02 ab	0.08 ± 0.01 a	0.18 ± 0.05 a	0.04 ± 0.01 a	0.37 ± 0.09 a	0.05 ± 0.01 a	0.93 ± 0.18 a

Note: Pi refers to inorganic P forms, Po refers to organic P forms; resin and residual P fractions were only determined as total P without separation of inorganic and organic P forms. Data for Dahlem and Thyrow are represented as mean ± standard error. 'n.d.' indicates that the respective P fraction was not detected in these samples. Values with different letters within a column show significant differences among treatments in the respective experiment. Multiple regression model was used for data analysis due to missing randomization in Dahlem. Two-way ANOVA was used for data analysis in Thyrow and differences among treatments were assessed with least significant difference (LSD) test ($p < 0.05$).

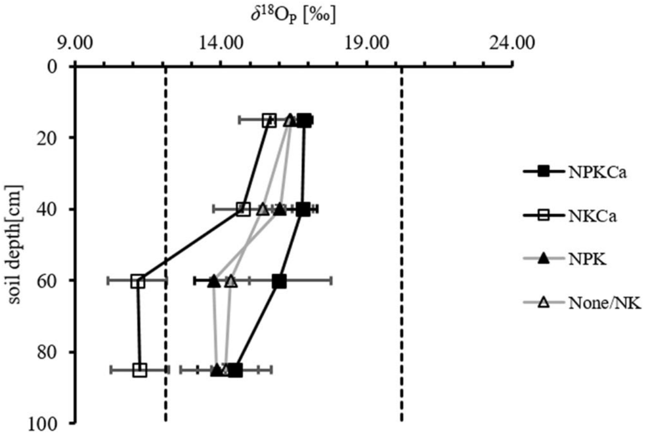

FIGURE 2 | $\delta^{18}O_P$ values of HCl-extractable P across three long-term fertilizer trials in Dahlem, Dikopshof and Thyrow, Germany. Symbols and whiskers indicate the mean and standard error per soil depth for each treatment combined across all three experiments. The dashed vertical lines represent the minimum and maximum expected equilibrium values across all three experiments.

in our field experiments effects of long-term P fertilization were mainly observed in inorganic P fractions.

Soil $\delta^{18}O_P$ values may inform on biological P turnover during long-term P fertilization. When fertilizer P accumulates in soils without biological utilization, $\delta^{18}O_P$ values are retained. The $\delta^{18}O_P$ value in mineral P fertilizers is expected to be around 20‰ (Amelung et al. 2015; Sun et al. 2020). However, the soil $\delta^{18}O_P$ values in the topsoil of the P fertilizer treatments were smaller than the value reported for fertilizer and ranged between the minimum and maximum expected equilibrium values (Figure 2). The finding suggests that most of the mineral P included in the HCl fraction had been biologically cycled and was subsequently precipitated, for example, as secondary Ca-P minerals (Amelung et al. 2015; Joshi et al. 2016; Bauke et al. 2018). Hence, topsoil P cycling was evident in all treatments. The continuous precipitation of secondary minerals with biologically cycled P even led to greater $\delta^{18}O_P$ values in the upper subsoil at all sites in fertilized treatments relative to treatments without P fertilization (Figure 2). As we did not observe significant differences in total HCl-P stocks in the subsoil, we assume that these changes in subsoil $\delta^{18}O_P$ values were not related to direct accumulation of fertilizer P in stable P fractions. Instead, we suggest that long-term P fertilization stimulated biological P cycling—not only in the topsoil, but also in the subsoil of all three trials.

4.2 | Effects of Long-Term Combined Liming and P Fertilization

Liming decreased topsoil labile Pi stocks in the three field experiments (Table 3). This is due to, on the one hand, increased soil pH and less soil acidity after liming, which stimulated plant growth and increased plant P uptake (Holford and Crocker 1994; Curtin and Syers 2001; Qaswar et al. 2020; Tiecher et al. 2023). On the other hand, with increasing soil pH, adsorbed Pi could be released into the soil solution as adsorption surfaces of soil particles become more negatively charged (Barrow 1984; Curtin and Syers 2001) or as Ca^{2+} ions partially displace Fe and Al ions from the adsorption sites (Hao et al. 2002). In addition, increased inputs of Ca^{2+} by fertilization will react with the released Pi and continuously precipitate it as Ca-P minerals (Curtin and Syers 2001). These processes are consistent with our observation that $NaHCO_3$-Pi and NaOH-Pi stocks were smaller and HCl-Pi stocks were larger in treatments with liming than in those without lime application in both topsoil and subsoil (Table 3). The combined application of lime and P fertilization further reinforced these processes, resulting in larger HCl-Pi stocks both in the topsoil and in the upper subsoil than in the treatments that did not receive lime and P fertilization (NK/none treatment).

Liming is also expected to stimulate plant growth (Qaswar et al. 2020; Tiecher et al. 2023) and microbial activity in soils (Wang, Yao, et al. 2021), which may result in increased concentrations of Po due to immobilization in plant and microbial biomass. Previous studies reported an accumulation of soil Po by lime application in grassland (Quin et al. 1984) and agricultural fields (Murphy and Stevens 2010). In our study, we observed significantly greater Po contents only in alkaline extractable Po pools at Dahlem, and, as a trend, at Dikopshof (Table 3; Figure 1). Although the $NaHCO_3$- and NaOH-Po stocks in Thyrow were hardly affected by liming, Po stocks in the HCl pool were larger in treatments with than in treatments without liming. These results indicate that at all three sites, liming enhanced Po accumulation in the soil profile, likely resulting from increased biomass production after lime application. It should also be noted that Po stocks in the deeper subsoil in Dahlem were largest in the NKCa treatment, which points to intensive Po assimilation from root growth as well as to potential for subsequent re-mineralization of Po to Pi by microorganisms (Oehl et al. 2004).

In contrast to P immobilization in organic matter, enhanced microbial growth may also increase mineralization of organic matter with subsequent release of phosphate into the soil solution. When Pi from biological cycling re-precipitates in Ca minerals, soil $\delta^{18}O_P$ values should approach the theoretical equilibrium value. However, $\delta^{18}O_P$ values in NKCa treatments were smaller than those in the NK treatments or in the unfertilized controls (Figure 2). Pfahler et al. (2020) also found that $\delta^{18}O_P$ values in HCl extracts were up to 3.7‰ smaller when lime was applied at Rothamsted Research Station, which the authors attributed to impurities in the lime. As an alternative explanation, $\delta^{18}O_P$ values within the range of the expected equilibrium mainly result from the activity of the intracellular pyrophosphatase, while the initial enzymatic reaction releasing Po moieties from organic matter by phosphomono- and -diesterases is associated with strong negative isotopic fractionation (von Sperber et al. 2023). Thus, in principle $\delta^{18}O_P$ values that are smaller than the equilibrium value could also result from direct precipitation of P after enzymatic mineralization from organic matter, without further P turnover in microbial cells. This explanation can, however, not account for observations at our sites where the deviation of $\delta^{18}O_P$ values from the expected equilibrium was observed especially in the subsoil of the NKCa treatments (Figure 2).

The small subsoil $\delta^{18}O_P$ values were mainly related to $\delta^{18}O_P$ values of the site in Dahlem (Figure 4S). The soil at this site has developed from substrate deposited during the Weichselian Late Glacial period and is reported to show strong heterogeneity of chemical and physical properties in the subsoil (Chmielewski and Koehn 1999; Wu et al. 2020). For this type of soil parent material, phosphate in primary minerals shows $\delta^{18}O_P$ values of around 10‰ (Tamburini et al. 2014; Sun et al. 2020). In combination with increased HCl-Pi contents, the observed $\delta^{18}O_P$ values in the subsoil of the NKCa treatment in Dahlem point to a large contribution of P derived from primary minerals, unaffected by biological cycling. Nevertheless, additional P fertilization shifted (NPKCa treatment) $\delta^{18}O_P$ values closer to the expected equilibrium values in the soil profile, indicating that improved nutrient supply, and presumably deeper crop root growth, enhanced P cycling and eventually led to $\delta^{18}O_P$ values close to equilibrium (Bauke et al. 2018).

The $\delta^{18}O_P$ depth profiles of treatments receiving combined Ca and P fertilization were similar for Thyrow and Dahlem, while subsoil $\delta^{18}O_P$ values in Dikopshof were slightly smaller (Figure S3). The soil at Dikopshof is characterized by greater clay contents in the subsoil compared to the soil in Thyrow and Dahlem. Based on observations in other loess-derived soil profiles (Barej et al. 2014; Bauke, von Sperber, et al. 2017) we suggest that P cycling at Dikopshof mainly occurred in soil pores, with a larger contribution of P derived from parent material in the bulk soil contributing to the overall smaller $\delta^{18}O_P$ values. Thus, while full Ca and P fertilization generally enhanced P cycling in the subsoil compared to nonfertilized treatments, the extent of biological P cycling at different soil depths varied with soil texture.

5 | Conclusions

We analyzed soil P stocks in sequentially extracted P fractions as well as the oxygen isotopic composition of 1 M HCl-extractable phosphate ($\delta^{18}O_P$) in three German arable long-term field experiments. Our data show that the combined application of lime and P fertilizer shifted the distribution of P among sequential fractions, but also increased total P contents and biological P cycling relative to the treatments where liming, P fertilizer, or both were missing. These effects were not limited to the topsoil, but extended into the subsoil, although not to the same extent across the different textures at the sites. Our study thus highlights that an integrated management of soil nutrient status and soil pH is critical in creating improved conditions for crop growth and nutrient turnover throughout the soil profile in loamy to sandy arable soils under temperate climatic conditions.

Author Contributions

Ye Wang: investigation, writing – original draft, visualization, formal analysis, validation. **Sara L. Bauke:** conceptualization, writing – original draft, supervision, methodology, investigation, validation. **Martina I. Gocke:** writing – review and editing, investigation. **Christian von Sperber:** writing – review and editing, conceptualization, methodology, supervision. **Julien Guigue:** formal analysis, validation, writing – review and editing. **Kathlin Schweitzer:** validation, writing – review and editing. **Sabine J. Seidel:** validation, writing – review and editing. **Federica Tamburini:** formal analysis, writing – review and editing. **Wulf Amelung:** conceptualization, funding acquisition, writing – review and editing, supervision, resources.

Acknowledgements

We thank the staff at the research stations in Dahlem, Dikopshof, and Thyrow for their continued dedication in maintaining the long-term field experiments. Further we thank the team of the Thünen Institute for Climate-Smart Agriculture, Braunschweig, for support during soil sampling. Open Access funding enabled and organized by Projekt DEAL.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Achat, D. L., M. R. Bakker, B. Zeller, S. Pellerin, S. Bienaime, and C. Morel. 2010. "Long-Term Organic Phosphorus Mineralization in Spodosols Under Forests and Its Relation to Carbon and Nitrogen Mineralization." *Soil Biology and Biochemistry* 42: 1479–1490.

Amelung, W., P. Antar, I. Kleeberg, et al. 2015. "The $\delta^{18}O$ Signatures of HCl-Extractable Soil Phosphates: Methodological Challenges and Evidence of the Cycling of Biological P in Arable Soil." *European Journal of Soil Science* 66: 965–972.

Azeez, M. O., J. T. Christinsen, S. Ravnskov, et al. 2020. "Phosphorus in an Arable Coarse Sandy Soil Profile After 74 Years With Different Lime and P Fertilizer Applications." *Geoderma* 376: 114555.

Barej, J. A. M., S. Pätzold, U. Perkons, and W. Amelung. 2014. "Phosphorus Fractions in Bulk Subsoil and Its Biopore Systems." *European Journal of Soil Science* 65: 553–561.

Barrow, N. J. 1984. "Modelling the Effects of pH on Phosphate Sorption by Soils." *Journal of Soil Science* 35: 283–297.

Bauke, S. L. 2021. "Game Changer in Soil Science: Perspectives From the Fritz-Scheffer Awardee Oxygen Isotopes in Phosphate—The Key to Phosphorus Tracing?" *Journal of Plant Nutrition and Soil Science* 184: 12–19.

Bauke, S. L., M. Landl, M. Koch, et al. 2017. "Macropore Effects on Phosphorus Acquisition by Wheat Roots – A Rhizotron Study." *Plant and Soil* 416: 67–82.

Bauke, S. L., C. von Sperber, N. Siebers, F. Tamburini, and W. Amelung. 2017. "Biopore Effects on Phosphorus Biogeochemistry in Subsoils." *Soil Biology and Biochemistry* 111: 157–165.

Bauke, S. L., C. von Sperber, F. Tamburini, et al. 2018. "Subsoil Phosphorus Is Affected by Fertilization Regime in Long-Term Agricultural Experimental Trials." *European Journal of Soil Science* 69: 103–112.

Beck, M. A., and P. A. Sanchez. 1994. "Soil Phosphorus Fraction Dynamics During 18 Years of Cultivation on a Typic Paleudult." *Soil Science Society of America Journal* 58: 1424–1431.

Blake, R. E., J. R. O'Neil, and A. V. Surkov. 2005. "Biogeochemical Cycling of Phosphorus: Insights From Oxygen Isotope Effects of Phosphoenzymes." *American Journal of Science* 305: 596–620.

Boschetti, N. G., C. E. Quintero, and L. Giuffre. 2009. "Phosphorus Fractions of Soils Under *Lotus corniculatus* as Affected by Different Phosphorus Fertilizers." *Biology and Fertility of Soils* 45: 379–384.

Buczko, U., M. van Laak, B. Eichler-Löbermann, et al. 2017. "Re-Evaluation of the Yield Response to Phosphorus Fertilization Based on Meta-Analyses of Long-Term Field Experiments." *Ambio* 47, no. 1: 50–61.

Campbell, C. A., G. P. Lafond, V. O. Biederbeck, and G. E. Winkleman. 1993. "Influence of Legumes and Fertilization on Deep Distribution of Available Phosphorus (Olsen-P) in a Thin Black Chernozemic Soil." *Canadian Journal of Soil Science* 73: 555–565.

Campbell, C. A., M. Schnitzer, J. W. B. Stewart, V. O. Biederbeck, and F. Selles. 1986. "Effect of Manure and P Fertilizer on Properties of a Black Chernozem in Southern Saskatchewan." *Canadian Journal of Soil Science* 66: 601–614.

Chang, S. J., and R. E. Blake. 2015. "Precise Calibration of Equilibrium Oxygen Isotope Fractionations Between Dissolved Phosphate and Water From 3 to 37°C." *Geochimica et Cosmochimica Acta* 150: 314–329.

Chmielewski, F.-M., and W. Koehn. 1999. "The Long-Term Agrometeorological Field Experiment at Berlin-Dahlem, Germany." *Agricultural and Forest Meteorology* 96: 39–48.

Curtin, D., and J. K. Syers. 2001. "Lime-Induced Changes in Indices of Soil Phosphate Availability." *Soil Science Society of America Journal* 65: 147–152.

Djodjic, F., K. Börling, and L. Bergström. 2004. "Phosphorus Leaching in Relation to Soil Type and Soil Phosphorus Content." *Journal of Environmental Quality* 33: 678–684.

Eghball, B., D. Ginting, and J. E. Gilley. 2004. "Residual Effects of Manure and Compost Applications on Corn Production and Soil Properties." *Agronomy Journal* 96: 442–447.

Filipek, T. 2011. "Liming, Effects on Soil Properties." In *Encyclopedia of Agrophysics. Encyclopedia of Earth Sciences Series*, edited by ´. J. Glinski, J. Horabik, and J. Lipiec, 425–428. Springer.

Fortune, S., J. Lu, T. M. Addiscott, and P. C. Brookes. 2005. "Assessment of Phosphorus Leaching Losses From Arable Land." *Plant and Soil* 269: 99–108.

Gao, S., and T. H. Deluca. 2018. "Wood Biochar Impacts Soil Phosphorus Dynamics and Microbial Communities in Organically-Managed Croplands." *Soil Biology and Biochemistry* 126: 144–150.

Gichangi, E. M., P. N. S. Mnkeni, and P. C. Brookes. 2009. "Effects of Goat Manure and Inorganic Phosphate Addition on Soil Inorganic and Microbial Biomass Phosphorus Fractions Under Laboratory Incubation Conditions." *Soil Science & Plant Nutrition* 55: 764–771.

Gocke, M. I., A. Don, A. Heidkamp, F. Schneider, and W. Amelung. 2021. "The Phosphorus Status of German Cropland—An Inventory of Top- and Subsoils." *Journal of Plant Nutrition and Soil Science* 184: 51–64.

Gocke, M. I., J. Guigue, S. L. Bauke, et al. 2023. "Interactive Effects of Agricultural Management on Soil Organic Carbon Accrual: A Synthesis of Long-Term Field Experiments in Germany." *Geoderma* 438: 116616.

Gu, C., and A. J. Margenot. 2021. "Navigating Limitations and Opportunities of Soil Phosphorus Fractionation." *Plant and Soil* 459: 13–17.

Hao, X., C. M. Cho, G. J. Racz, and C. Chang. 2002. "Chemical Retardation of Phosphate Diffusion in an Acid Soil as Affected by Liming." *Nutrient Cycling in Agroecosystems* 64: 213–224.

He, Z. L., A. K. Alva, Y. C. Li, D. V. Calvert, and D. J. Banks. 1999. "Sorption-Desorption and Solution Concentration of Phosphorus in a Fertilized Sandy Soil." *Journal of Environmental Quality* 28, no. 6: 1804–1810.

Hedley, M. J., J. W. B. Stewart, and B. S. Chauhan. 1982. "Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations." *Soil Science Society of America Journal* 46: 970–976.

Helfenstein, J., F. Tamburini, C. von Sperber, et al. 2018. "Combining Spectroscopic and Isotopic Techniques Gives a Dynamic View of Phosphorus Cycling in Soil." *Nature Communications* 9: 3226.

Hobley, E. U., and I. Prater. 2019. "Estimating Soil Texture From Vis-NIR Spectra." *European Journal of Soil Science* 70: 83–95.

Holford, I. C. R., and G. J. Crocker. 1994. "Long-Term Effects of Lime on Pasture Yields and Response to Phosphate Fertilizers on Eight Acidic Soils." *Australian Journal of Agricultural Research* 45: 1051–1062.

Huang, J., L. H. Zhou, S. J. Liu, et al. 2021. "Vertical Distribution of Phosphorus Fractions and the Environmental Critical Phosphorus Level in Acidic Red Soil Under Long-Term Fertilizer and Lime Application in Southern China." *Journal of Plant Nutrition and Soil Science* 184: 585–595.

IUSS Working Group WRB. 2015. "World Reference Base for Soil Resources 2014, Update 2015, International Soil Classification System for Naming Soils and Creating Legends for Soil Maps." World Soil Resources Report 106. FAO, Rome.

Joshi, S. R., X. Li, and D. P. Jaisi. 2016. "Transformation of Phosphorus Pools in an Agricultural Soil: An Application of Oxygen-18 Labeling in Phosphate." *Soil Science Society of America Journal* 80: 69–78.

Kang, J., A. Amoozegar, D. Hesterberg, and D. L. Osmond. 2011. "Phosphorus Leaching in a Sandy Soil as Affected by Organic and Inorganic Fertilizer Sources." *Geoderma* 161: 194–201.

Klotzbücher, A., K. Kaiser, T. Klotzbücher, M. Wolff, and R. Mikutta. 2019. "Testing Mechanisms Underlying the Hedley Sequential Phosphorus Extraction of Soils." *Journal of Plant Nutrition and Soil Science* 182: 570–577.

Laboski, C. A. M., and A. John. 2003. "Changes in Soil Test Phosphorus Concentration After Application of Manure or Fertilizer." *Soil Science Society of America Journal* 67: 544–554.

Liang, Y. H., and R. E. Blake. 2007. "Oxygen Isotope Fractionation Between Apatite and Aqueous-Phase Phosphate: 20-45°C." *Chemical Geology* 238: 121–133.

Malik, M. A., P. Marschner, and K. S. Khan. 2012. "Addition of Organic and Inorganic P Sources to Soil-Effects on P Pools and Microorganisms." *Soil Biology & Biochemistry* 49: 106–113.

Maranguit, D., T. Guillaume, and Y. Kuzyakov. 2017. "Land-Use Change Affects Phosphorus Fraction in Highly Weathered Tropical Soils." *Catena* 149: 385–393.

McBeath, T. M., M. J. McLaughlin, J. K. Kirby, and R. D. Armstrong. 2012. "The Effect of Soil Water Status on Fertilizer, Topsoil and Subsoil Phosphorus Utilization by Wheat." *Plant and Soil* 358: 337–348.

Murphy, P. N. C. 2007. "Lime and Cow Slurry Application Temporarily Increases Organic Phosphorus Mobility in an Acid Soil." *European Journal of Soil Science* 58, no. 3: 794–801.

Murphy, P. N. C., and R. J. Stevens. 2010. "Lime and Gypsum as Source Measures to Decrease Phosphorus Loss From Soils to Water." *Water, Air, and Soil Pollution* 212: 101–111.

Nachimuthu, G., C. Guppy, P. Kristiansen, and P. Lockwood. 2009. "Isotopic Tracing of Phosphorus Uptake in Corn From ^{33}P Labelled Legume Residues and ^{32}P Labelled Fertilisers Applied to a Sandy Loam Soil." *Plant and Soil* 314: 303–310.

Naidu, R., J. K. Syers, R. W. Tillman, and J. H. Kirkman. 1990. "Effect of Liming on Phosphate Sorption by Acid Soils." *Journal of Soil Science* 41: 165–175.

Oehl, F., E. Frossard, A. Fliessbach, D. Dubois, and A. Oberson. 2004. "Basal Organic Phosphorus Mineralization in Soils Under Different Farming Systems." *Soil Biology and Biochemistry* 36: 667–675.

Ohno, T., and L. M. Zibilske. 1991. "Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green." *Soil Science Society of America Journal* 55: 892–895.

Penn, C. J., and J. J. Camberato. 2019. "Acritical Review on Soil Chemical Processes That Control How Soil pH Affects Phosphorus Availability to Plants." *Agriculture* 9: 120.

Pfahler, V., A. Macdonald, A. Mead, A. C. Smith, and F. Tamburini. 2020. "Changes of Oxygen Isotope Values of Soil P Pools Associated With Changes in Soil pH." *Scientific Reports* 10: 2065.

Qaswar, M., L. Dongchu, H. Jing, et al. 2020. "Interaction of Liming and Long-Term Fertilization Increased Crop Yield and Phosphorus Use

Efficiency (PUE) Through Mediating Exchangeable Cations in Acidic Soil Under Wheat–Maize Cropping System." *Scientific Reports* 10: 19828.

Quin, B. F., L. M. Condron, D. S. Rickard, and K. M. Goh. 1984. "Changes in Soil Organic Phosphorus Following Liming." In *Proceedings of the Workshop on Lime in New Zealand Agriculture*, 43–46. Ruakura Soil and Plant Research Station.

Riddle, M., L. Bergström, F. Schmieder, H. Kirchmann, L. Condron, and H. Aronsson. 2018. "Phosphorus Leaching From an Organic and a Mineral Arable Soil in a Rainfall Simulation Study." *Journal of Environmental Quality* 47: 487–495.

Rueda-Ayala, V., H. E. Ahrends, S. Siebert, T. Gaiser, H. Hueging, and F. Ewert. 2018. "Impact of Nutrient Supply on the Expression of Genetic Improvements of Cereals and Row Crops—A Case Study Using Data From a Long-Term Fertilization Experiment in Germany." *European Journal of Agronomy* 96: 34–46.

Seidel, S. J., T. Gaiser, H. E. Ahrends, et al. 2021. "Crop Response to P Fertilizer Omission Under a Changing Climate—Experimental and Modeling Results Over 115 Years of a Long-Term Fertilizer Experiment." *Field Crops Research* 268: 108174.

Shen, J., R. Li, F. Zhang, J. Fan, C. Tang, and Z. Rengel. 2004. "Crop Yields, Soil Fertility and Phosphorus Fractions in Response to Long-Term Fertilization Under the Rice Monoculture System on a Calcareous Soil." *Field Crops Research* 86: 225–238.

Skadell, L. E., F. Schneider, M. I. Gocke, et al. 2023. "Twenty Percent of Agricultural Management Effects on Organic Carbon Stocks Occur in Subsoils – Results of Ten Long-Term Experiments." *Agriculture, Ecosystems & Environment* 365: 108619.

Sumner, M. E., and T. Yamadu. 2002. "Farming With Acidity." *Communications in Soil Science and Plant Analysis* 33: 2467–2496.

Sun, Y. J., W. Amelung, B. Wu, et al. 2020. "Co-Evolution' of Uranium Concentration and Oxygen Stable Isotope in Phosphate Rocks." *Applied Geochemistry* 114: 104476.

Tamburini, F., S. M. Bernasconi, A. Angert, T. Weiner, and E. Frossard. 2010. "A Method for the Analysis of the $\delta\ ^{18}$O of Inorganic Phosphate Extracted From Soils With HCl." *European Journal of Soil Science* 61: 1025–1032.

Tamburini, F., V. Pfahler, C. von Sperber, and E. Frossard. 2014. "Oxygen Isotopes for Unraveling Phosphorus Transformation in the Soil-Plant System: A Review." *Soil Science Society of America Journal* 78: 38–46.

Tiecher, T., S. M. V. Fontoura, V. G. Ambrosini, et al. 2023. "Soil Phosphorus Forms and Fertilizer Use Efficiency Are Affected by Tillage and Soil Acidity Management." *Geoderma* 435: 116495.

Tiessen, H., and J. Moir. 1993. "Characterization of Available P by Sequential Extraction." In *Soil Sampling and Methods of Analysis*, edited by M. R. Carter and E. G. Gregorich, 75–86. Lewis Publisher.

Tiessen, H., J. W. B. Stewart, and J. O. Moir. 1983. "Changes in Organic and Inorganic Phosphorus Composition of Two Grassland Soils and Their Particle Size Fractions During 60-90 Years of Cultication." *Journal of Soil Science* 34: 815–823.

von Sperber, C., C. Pistocchi, M. Weiler, and F. Tamburini. 2023. "Oxygen Isotope Ratios of Phosphates in the Soil-Plant System: Limitations and Future Developments." *European Journal of Soil Science* 74: e13434.

von Sperber, C., R. Stallforth, C. C. du Preez, and W. Amelung. 2017. "Changes in Soil Phosphorus Pools During Prolonged Arable Cropping in Semi-Arid Grasslands." *European Journal of Soil Science* 68: 462–471.

Walter, K., A. Don, B. Tiemeyer, and A. Freibauer. 2016. "Determining Soil Bulk Density for Carbon Stock Calculation: A Systematic Method Comparison." *Soil Science Society of America Journal* 80: 579–591.

Wang, Y., S. L. Bauke, C. von Sperber, et al. 2021. "Soil Phosphorus Cycling Is Modified by Carbon and Nitrogen Fertilization in a Long-Term Field Experiment." *Journal of Plant Nutrition and Soil Science* 184: 282–293.

Wang, Y., Z. Yao, Y. Zhan, et al. 2021. "Potential Benefits of Liming to Acid Soils on Climate Change Mitigation and Food Security." *Global Change Biology* 27: 2807–2821.

Wu, B., Y. Wang, A. E. Berns, et al. 2020. "Iron Isotope Fractionation in Soil and Graminaceous Crops After 100 Years of Liming in the Long-Term Agricultural Experimental Site at Berlin-Dahlem, Germany." *European Journal of Soil Science* 72: 289–299.

Zhang, T. Q., A. F. Mackenzie, B. C. Liang, and C. F. Drury. 2004. "Soil Test Phosphorus and Phosphorus Fractions With Long-Term Phosphorus Addition and Depletion." *Soil Science Society of America Journal* 68: 519–528.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Results of multiple regression analysis given as estimate of intercept and slope, respectively, standard error of the estimate and *p* value for density, pH, N stocks, and C stocks at different soil depth in fertilizer treatments of the long-term agricultural research experiment in Dahlem, Germany (mineral calcium fertilizer [Ca], mineral phosphorus fertilizer [P], and for the interaction of Ca and P fertilizer). **Table S2:** Results of two-way ANOVA analysis given as df (degree of freedom), *f* value, and *p* value for density, pH, N stocks, and C stocks at different soil depth in fertilizer treatments of the long-term agricultural research experiment in Thyrow, Germany (mineral calcium fertilizer [Ca], mineral phosphorus fertilizer [P], and for the interaction of Ca and P fertilizer). **Table S3:** Results of linear regression analysis given as estimate of intercept and slope, respectively, standard error of the estimate, *p* value, and R^2 value for soil clay content and soil N and C stocks among three long-term agricultural research experiments. **Table S4:** Results of multiple regression analysis given as estimate of intercept and slope, respectively, standard error of the estimate and *p* value for the relationship of soil P stocks from sequential fractionation at different soil depth in fertilizer treatments of the long-term agricultural research experiment in Dahlem, Germany (mineral calcium fertilizer [Ca], mineral phosphorus fertilizer [P], and for the interaction of Ca and P fertilizer). **Table S5:** Results of two-way ANOVA analysis given as df (degree of freedom), *f* value, and *p* value for the relationship of soil P stocks from sequential fractionation at different soil depth in fertilizer treatments of the long-term agricultural research experiment in Thyrow, Germany (mineral calcium fertilizer [Ca], mineral phosphorus fertilizer [P], and for the interaction of Ca and P fertilizer). **Table S6:** Results of linear regression analysis given as estimate of intercept and slope, respectively, standard error of the estimate, *p*-value, and R^2 for soil clay content and soil P stocks from sequential fractionation among three long-term agricultural research experiments. **Table S7:** Results of multiple regression analysis given as estimate of intercept and slope, respectively, standard error of the estimate and *p*-value for $\delta^{18}O_P$ values of HCl-extractable P in fertilizer treatments of the long-term agricultural research experiment in Dahlem, Germany (mineral calcium fertilizer [Ca], mineral phosphorus fertilizer [P], and for the interaction of Ca and P fertilizer). **Table S8:** Results of two-way ANOVA analysis given as df (degree of freedom), *f* value, and *p* value for $\delta^{18}O_P$ values of HCl-extractable P in fertilizer treatments of the long-term agricultural research experiment in Thyrow, Germany (mineral calcium fertilizer [Ca], mineral phosphorus fertilizer [P], and for the interaction of Ca and P fertilizer). **Figure S1:** Relations of soil clay content and soil C and N stocks among three long-term trials in different soil layers at Dahlem, Dikopshof, and Thyrow. **Figure S2:** Relations of soil clay content and soil P stocks from sequential P fractions (as total P (Pt) per fraction) among three long-term trials in different soil layers at Dahlem, Dikopshof, and Thyrow. **Figure S3:** $\delta^{18}O_P$ values of HCl-extractable P in the three long-term fertilizer trials Dahlem, Dikopshof, and Thyrow, Germany. The solid line indicates the mean expected equilibrium value, and the dashed vertical lines represent the minimum and maximum expected equilibrium value.