

# Soil phosphorus cycling is modified by carbon and nitrogen fertilization in a long-term field experiment

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## Abstract

**Background and aims:** Phosphorus (P) is an essential element for crop growth. However, while links of P turnover in soils to carbon (C) and nitrogen (N) availability have been described, it remains to be clarified how combinations of fertilizer C and N additions affect stocks and cycling of distinct P fractions at different soil depths. The objectives of our study were (1) to assess how soil total P stocks are affected by organic amendments and N fertilization, (2) to evaluate how different soil P fractions respond to N fertilization, and (3) to verify whether N fertilization increases soil biological P cycling.

**Methods:** We collected soil samples from a long-term field experiment established in 1984 in Rauschholzhausen, Germany. The soil is a Haplic Luvisol and received either no organic fertilizer (NOF), farmyard manure (FYM) or a combination of organic and mineral N fertilizer (OMF). Each treatment additionally received three levels of mineral N: 0 kg ha<sup>-1</sup> y<sup>-1</sup> (N0), 100 kg ha<sup>-1</sup> y<sup>-1</sup> (N100), and 200 kg ha<sup>-1</sup> y<sup>-1</sup> (N200). The organic fertilizers were applied by a manure spreader and the N fertilizer (calcium ammonium nitrate) was applied in spring as top dressing by a plot fertilizer machine. We estimated stocks of P in fractions isolated by sequential P fractionation, and assessed the oxygen isotopic composition of 1 M HCl-extractable phosphate ( $\delta^{18}\text{O}_\text{P}$ ).

**Results:** We found that increased organic matter (OM) addition and mineral N inputs caused significant decreases in the stocks of resin- and NaHCO<sub>3</sub>-extractable P in the topsoil (0–30 cm). Mineral N fertilization alone resulted in significant increases in stocks of resin-, NaHCO<sub>3</sub>-, and NaOH-extractable P in the upper subsoil (30–50 cm). These changes occurred for both inorganic and organic P. The subsoil  $\delta^{18}\text{O}_\text{P}$  values were closer to expected equilibrium values in soil fertilized with mineral N, indicative of more intensive biological P cycling than in the treatments without mineral N inputs.

**Conclusions:** These findings suggest that long-term OM and mineral N fertilization promotes topsoil P losses from labile fractions by crop uptake with an enrichment of these P forms in the subsoil, and an overall increase in biological P cycling in both top- and subsoil horizons upon N fertilization.

**Key words:** nitrogen / organic matter / oxygen isotopes in phosphate ( $\delta^{18}\text{O}_\text{P}$ ) / sequential P fractionation / soil P stocks

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## 1 Introduction

Phosphorus (P) is an essential element to sustain high crop yields in agricultural systems (Cordell et al., 2009). Minimizing P inputs and losses is a prerequisite for sustainable land management and calls for improved knowledge on efficient P cycling in soils (Withers et al., 2018).

There are several tons per hectare of P in soil, and 25–70% of total P is stored in subsoil below 30 cm depth, beneath the tilled soil horizon (Kautz et al., 2013). However, P is still a limiting element for crop growth as most soil P is in stable and non-easily available forms (Barej et al., 2014; Bauke et al.,



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2018). Most of inorganic P (Pi) is strongly bound to Al and Fe oxides and Ca minerals, while organic P (Po) is present within biomolecules, such as nucleic acids, phosphoproteins, sugar phosphates, and inositol phosphates, which are not directly available to crops. The sequential fractionation scheme suggested by Hedley et al. (1982) distinguishes resin-P,  $\text{NaHCO}_3\text{-P}$ ,  $\text{NaOH-P}$ ,  $\text{HCl-P}$ , and residual-P fractions. While the assignment of P to different fractions provides information on its potential availability to plants, the fractionation scheme does not provide information on whether P in these fractions has been biologically recycled or not.

The microbial recycling of organic P in soils is regulated by phosphatase enzymes. When these enzymes are exuded into soil, plant-available P can be released from P-containing organic compounds (Arruda et al., 2018). As the synthesis of phosphatases requires carbon and energy, supplementing soils with labile carbon substrates can potentially boost microbial growth and, thus, the synthesis of phosphatase enzymes (Richardson et al., 2011). In addition, synthesis of phosphatase enzymes produced by plants and microbes requires N (Olander and Vitousek, 2000) and, thus, their synthesis is limited by low amounts of available N in the soil (Wang et al., 2007). Adding N could stimulate phosphatase activities in ecosystems and increase contents of plant available P in soils (Olander and Vitousek, 2000). As N addition stimulates crop growth, available P will increasingly be taken up by the crop which ultimately reduces soil P stocks, especially the labile P (resin-P,  $\text{NaHCO}_3\text{-P}$ ) stocks (Chen et al., 2018). In turn, larger P stocks were found in soils where crop growth was limited by long-term N deficiency, especially in the subsoil (30–100 cm) (Bauke et al., 2018). However, it remains to be clarified how the type of fertilizer and rates of N fertilization affect P stocks and fractions at different soil depths.

Biological P cycling in the soil–plant system can be traced by the oxygen isotopic composition of phosphate ( $\delta^{18}\text{O}_\text{P}$ ) in soil (Tamburini et al., 2010, 2012; Angert et al., 2011, 2012). The P–O bond in phosphate is stable and, thus, no oxygen atoms are exchanged between water and phosphate under ambient environmental conditions as long as the phosphate is not microbially processed (O’Neil et al., 2003). However, when organic P compounds become hydrolyzed by extracellular phosphatases, such as phosphomonoesterases and phosphodiesterases, the  $\delta^{18}\text{O}_\text{P}$  of the phosphate is altered as the released molecule inherits 3 to 2 oxygen atoms from the original organic compound, while 1 to 2 oxygen atoms are incorporated from water with a fractionation factor characteristic of the respective type of enzyme (Liang and Blake, 2006, 2009; von Sperber et al., 2014, 2015). Further, when Pi is assimilated into cells, the  $\delta^{18}\text{O}_\text{P}$  value also changes because the intracellular enzyme pyrophosphatase induces repeated oxygen exchange between Pi and water, resulting in a temperature-dependent equilibration of oxygen isotopes between the phosphate and water (Chang and Blake, 2015; von Sperber et al., 2017a). This process overprints the  $\delta^{18}\text{O}_\text{P}$  of the initial primary phosphate. Thus, the soil  $\delta^{18}\text{O}_\text{P}$  value relative to the expected isotopic equilibrium value can provide information on the extent of biological recycling of P in the plant–soil system. Specifically, the  $\delta^{18}\text{O}$  value of phosphate extracted with 1 M HCl can provide integrated information of biologically

recycled P over long time periods (Angert et al., 2012; Bauke et al., 2017) because this fraction includes phosphate from the continuous precipitation of biologically cycled P into secondary Ca-P minerals (Amelung et al., 2015; Bauke et al., 2018). In arable topsoils, the  $\delta^{18}\text{O}_\text{P}$  value of  $\text{HCl-P}$  is usually at isotopic equilibrium with the soil water, whereas in the subsoil the  $\delta^{18}\text{O}_\text{P}$  values are often lower and close to signals expected from loess, igneous or metamorphic primary minerals (Tamburini et al., 2014; Amelung et al., 2015).

Sequential P fractionation and the oxygen isotope composition of phosphate ( $\delta^{18}\text{O}_\text{P}$ ) in  $\text{HCl-P}$  were used here to resolve how different types of organic amendment and rates of mineral N application affect P stocks at different soil depths, and whether the pool of biologically recycled P has been increased or not. We hypothesized that (1) continuous inputs of labile C sources (by organic fertilization) and mineral N results in enhanced P utilization and thus decline in P stocks, especially in the topsoil; (2) the labile P fractions (resin-P and  $\text{NaHCO}_3\text{-P}$ ) respond more sensitively to N inputs than other P fractions, and (3) mineral N fertilization shifts the  $\delta^{18}\text{O}_\text{P}$  values of  $\text{HCl-P}$  closer to the theoretical equilibrium value because of more intensive biological P cycling. To test these hypotheses, we sampled a long-term field experiment with different organic matter and mineral N fertilization.

## 2 Material and methods

### 2.1 Study site and samples

The study area is located in Rauischholzhausen (50°45' N, 8°52' E) in the Amöneburger Becken, central Germany. The mean annual precipitation is 576 mm and mean annual temperature is 8.5°C. The soil is a Haplic Luvisol (*IUSS Working Group WRB*, 2014), which developed from quaternary loess deposits. It has a loamy texture with average clay content ranging from 32% in the topsoil to 39% in the subsoil (Tab. 1), a pH of about 7.7, and bulk density ranging from 1.5 g cm<sup>-3</sup> in the topsoil to 1.8 g cm<sup>-3</sup> in the subsoil. Soil structure is subangular blocky and soil carbonate content is 10% in the depth of 70–100 cm (Tab. 1). According to previous research at Mardorf (Heine, 1970), located approx. 4 km from Rauischholzhausen, soil clay minerals are dominated by illite, kaolinite, and hydrous mica. In addition, more than half of the total N and total C stocks summed up down to 100 cm are stored in the topsoil (Tab. 2).

The long-term fertilization trial was set up with a block design in 1984. In a crop rotation of sugar beet (*Beta vulgaris* L.) [replaced by maize (*Zea mays* L.) in 2009], winter wheat (*Triticum aestivum* L. ssp. *aestivum*) and winter barley (*Hordeum vulgare* L.) each crop was grown for one year. Sugar beet was sown using pelleted seeds coated with insecticides (against insect pests) in April with a density of 10–18 pellets m<sup>-2</sup> resulting in plant distance of 18–22 cm within the rows and 45 cm between the rows. Maize was sown in the second half of April with 8–10 seeds m<sup>-2</sup> and 75 cm row distance. Depending on the cultivar, winter wheat was sown in October with 320–450 seeds m<sup>-2</sup>, and winter barley was sown in mid to end of September with sowing

**Table 1:** Soil characteristics of the long-term fertilizer trial in Rauschholzhausen, Germany.<sup>a</sup>

| Depth (cm) | Soil horizon | Bulk density (g cm <sup>-3</sup> ) | pH  | Aggregate         | Carbonate content (%) | Sand content (%) | Silt content (%) | Clay content (%) |
|------------|--------------|------------------------------------|-----|-------------------|-----------------------|------------------|------------------|------------------|
| 0–30       | Ap 1         | 1.58                               | 7.7 | Subangular blocky | 0                     | 3.02             | 64.24            | 32.00            |
| 30–50      | Ap 2         | 1.53                               | 7.7 | Subangular blocky | 0                     | 1.65             | 58.45            | 38.84            |
| 50–60      | Bt           | 1.58                               | 7.7 | Subangular blocky | 0                     | 1.67             | 59.15            | 37.51            |
| 60–70      | Bw           | 1.64                               | 7.7 | Subangular blocky | 0                     | 1.21             | 60.39            | 37.14            |
| 70–100     | C            | 1.85                               | 7.8 | Subangular blocky | 10                    | 1.37             | 64.04            | 33.92            |

<sup>a</sup>Soil horizons were classified according to the World Reference Base for Soil Resource (IUSS Working Group WRB, 2014).

**Table 2:** Soil N and C stocks of the long-term fertilizer trial with no organic fertilizer (NOF), farmyard manure (FYM), and a combination of organic and mineral N fertilizer (OMF) addition were given as mean and standard error (SE) ( $n = 3$ ); each organic fertilizer treatment was multiplied with three levels of mineral N addition: 0 kg ha<sup>-1</sup> y<sup>-1</sup> (N0), 100 kg ha<sup>-1</sup> y<sup>-1</sup> (N100), and 200 kg ha<sup>-1</sup> y<sup>-1</sup> (N200).

| Depth (cm) |     |      | N stocks (tons ha <sup>-1</sup> ) |      |      | C stocks (tons ha <sup>-1</sup> ) |       |       |
|------------|-----|------|-----------------------------------|------|------|-----------------------------------|-------|-------|
|            |     |      | N0                                | N100 | N200 | N0                                | N100  | N200  |
| 0–30       | NOF | Mean | 3.90                              | 4.63 | 4.83 | 39.16                             | 42.97 | 43.57 |
|            |     | SE   | 0.13                              | 0.13 | 0.24 | 1.24                              | 0.98  | 1.77  |
|            | FYM | Mean | 4.40                              | 5.10 | 4.90 | 41.49                             | 45.68 | 44.59 |
|            |     | SE   | 0.26                              | 0.07 | 0.16 | 2.49                              | 0.58  | 1.27  |
|            | OMF | Mean | 4.12                              | 4.83 | 4.55 | 42.17                             | 43.06 | 45.05 |
|            |     | SE   | 0.17                              | 0.12 | 0.34 | 1.37                              | 1.00  | 3.10  |
| 30–50      | NOF | Mean | 1.26                              | 1.34 | 1.63 | 11.13                             | 11.12 | 12.40 |
|            |     | SE   | 0.06                              | 0.04 | 0.05 | 0.77                              | 0.33  | 0.37  |
|            | FYM | Mean | 1.10                              | 1.63 | 1.44 | 8.99                              | 12.60 | 10.59 |
|            |     | SE   | 0.06                              | 0.13 | 0.02 | 0.60                              | 1.32  | 0.12  |
|            | OMF | Mean | 1.10                              | 1.59 | 1.21 | 8.76                              | 11.78 | 10.56 |
|            |     | SE   | 0.16                              | 0.05 | 0.02 | 1.41                              | 0.76  | 0.31  |
| 50–100     | NOF | Mean | 3.06                              | 2.81 | 3.13 | 26.70                             | 20.26 | 21.22 |
|            |     | SE   | 0.04                              | 0.01 | 0.11 | 5.55                              | 0.57  | 1.01  |
|            | FYM | Mean | 2.75                              | 3.01 | 2.99 | 25.93                             | 26.26 | 20.26 |
|            |     | SE   | 0.10                              | 0.10 | 0.14 | 7.84                              | 3.73  | 1.24  |
|            | OMF | Mean | 2.74                              | 3.34 | 2.80 | 29.39                             | 25.76 | 19.16 |
|            |     | SE   | 0.10                              | 0.24 | 0.06 | 9.60                              | 4.41  | 0.61  |

rates between 320–400 seeds m<sup>-2</sup>. Weed control was carried out on all plants using herbicides. In addition, winter wheat and winter barley were treated with growth regulators and fungicides. There were three replicates for each treatment and each replicate plot had an area of 30 m<sup>2</sup> but only a core area of 12.5 m<sup>2</sup> was used for harvest. As shown in the field layout (Fig. 1S), the experiment design is not randomized. There

might have been soil fertility gradients crosswise or lengthwise at the time when the experiment started, which could be mixed with the effects from the experimental treatment. We assume that possible original spatial fertility trends will be superseded and dominated by the experimental treatments after more than 30 years of continuous field management (Macholdt et al., 2019). Thus, this long-term experiment still

gives valuable information despite limitations in statistical evaluation due to the missing randomization.

The experiment included two treatments with organic fertilizers. The first treatment (FYM) was fertilized with  $3 \times 10^4$  kg ha<sup>-1</sup> farmyard manure (water content: 75%) from cattle farming every third year before maize cultivation. After harvesting winter barley (as previous crop to maize) in July, cattle manure was spread by a manure spreader on the plots and incorporated flat into the surface soil with a disc harrow a few days later. The second treatment received a combination of organic (straw, rapeseed green manure, and biogas residue) and mineral N (calcium ammonium nitrate) fertilizers (OMF). For this treatment, chopped wheat straw ( $5 \times 10^3$  kg ha<sup>-1</sup>, water content 14%) and mineral N fertilizer (50 kg ha<sup>-1</sup>) were applied separately and mechanically one day after the winter wheat was harvested (July), and later the field was cultivated with a disc harrow. Chopped barley straw ( $5 \times 10^3$  kg ha<sup>-1</sup>, water content 14%) and mineral N (50 kg ha<sup>-1</sup>) were then applied after winter barley (before maize) in July and mixed with the soil by disc harrow. Then rapeseed was sown as green manure. The rapeseed plants were mechanically mulched at the end of October or beginning of November after three months of growth with an average biomass yield of  $3 \times 10^3$  kg ha<sup>-1</sup> y<sup>-1</sup> (85% water content). In April of the following year, biogas residues (20 m<sup>-3</sup> ha<sup>-1</sup>, 90% water content) were applied on the same plots, spread manually and homogeneously with a watering can and then incorporated into soil with subsequent maize planting. A third treatment without organic fertilizer (NOF) was used as control.

Each of these three organic fertilization treatments was combined with three levels of mineral N fertilization (calcium ammonium nitrate): no mineral N (N0), 100 (added as two applications of 50 kg ha<sup>-1</sup> y<sup>-1</sup> mineral N (N100) and 200 (added as three applications of 100, 50, and 50) kg ha<sup>-1</sup> y<sup>-1</sup> mineral N (N200) for maize and winter wheat or 180 (added as three applications of 80, 60, and 40) kg ha<sup>-1</sup> y<sup>-1</sup> mineral N for winter barley (the lower application rate for winter barley is nevertheless designated as N200 treatment for a better visualization). All mineral N fertilizer were applied in spring with a plot fertilizer machine. In case of maize, the first amount of mineral N fertilizer was applied after sowing (end of April), the second at four-leaf stage and the third at six-to-eight-leaf stage (the third was only applied for N200 treatments). Mineral N fertilizer was applied as top dressing except the first time before maize emergence. For winter wheat and winter barley, the first amount of mineral N fertilizer was applied in March at the beginning of growing cycle, the second at the end of the tillering stage and the third during stem elongation as top dressing (the third was only applied for N200 treatments). Superphosphate was used as the main P fertilizer, which contains calcium dihydrogen phosphate and calcium sulfate. All plots have received 30 kg ha<sup>-1</sup> of P from granulated superphosphate fertilizer applied by a fertilizer spreader every three years (after maize cultivation) since 1984.

In April 2016, soil samples were collected with an auger of 6 cm inner diameter at the end of tillering of winter barley (experimental crop in 2016). Shortly before sampling, each treat-

ment received the first mineral N fertilization for the year 2016 (applied according to the split application schedule outlined above, i.e., 0 kg ha<sup>-1</sup> for N0, 50 kg ha<sup>-1</sup> for N100, and 80 kg ha<sup>-1</sup> for N200). The latest applications of P fertilizer and farmyard manure were in 2014, and chopped wheat straw and mineral N fertilizer in OMF treatment were applied in August 2015 after harvest of the previous crop. All soil cores were divided according to observed soil horizons (0–30, 30–50, 50–60, 60–70, 70–100 cm). In case of drilling compaction, a correction by linear interpolation of soil depth separation between 30 cm and 100 cm depth was used (Walter et al., 2016). All samples were air-dried and sieved to < 2 mm grain size. For a better visualization of the results, data for P fractions were summarized (weighted sum) and analyzed for 0–30, 30–50, and 50–100 cm depth increments.

## 2.2 Basic characterization of soil and organic material

Bulk density was determined from the soil cores, with the weight of each oven-dried (40°C) sample divided by the volume of the respective core fraction (Walter et al., 2016). Soil pH was determined in water suspension at a soil:water ratio of 1:5 (w/v). Total soil P (P<sub>T XRF</sub>) concentrations were analyzed by X-ray fluorescence (XRF) spectroscopy (SRS 3000, Siemens AG, Germany, wavelength-dispersive with Rh tube) of pressed pellets.

Soil C and N concentrations were measured on the air-dried samples after sieving at 2 mm and removal of visible roots. Then, approx. 3 g of each bulk sample was finely ground using a ball-mill prior to duplicate total C and N analysis by dry combustion (Hekatech, Germany). Inorganic C (IC) was determined in bulk samples *via* calcimetry upon reaction with 4 M HCl (ISO, 1995). Soil organic C was calculated as the difference between total C and IC. The total P, C and N concentrations were converted to stocks using the observed bulk densities and depth increments.

For the determination of plant P contents, 5 g of whole biomass samples were combusted at 550°C overnight, and the ashes were dissolved in 1 M HNO<sub>3</sub>. Then the solution was mixed with vanadate molybdate reagent and the extinction of the yellow-colored solution was measured at 430 nm using a spectrophotometer (Analytik Jena AG Germany, Specord 205 220–240V). The P contents in farmyard manure and biogas residue were first extracted by calcium acetate and calcium lactate solution (pH 4.1), which extracts plant-available P, then they were measured at 767 nm using the same spectrophotometer after colorization with molybdate reagent (VDLUF, 1976, 1991). The P input was calculated as the total amount of P added as mineral P fertilizer, FYM and OMF (Tab. 3S), and P output was calculated as P removed with harvest products (for the cereals both grain and straw were removed) from 1984 to 2016. The difference between P input and output was denoted as the balance  $\Delta P$ .

### 2.3 Sequential P fractionation

Soil P concentrations of different fractions were determined by sequential extraction according to the modified method by *Tiessen and Moir* (1993). For this purpose, 0.5 g of air-dried soil were sequentially extracted by anion resin strips, 0.5 M NaHCO<sub>3</sub>, 0.1 M NaOH, and 1 M HCl. Finally, residual P was extracted by hot aqua regia digestion (45°C for 20 min, 65°C for 20 min, and then 95°C for 120 min). The total P (Pt) concentrations of each extract were measured by ICP-OES (Ultima 2, HORIBA Jobin Yvon, Longjumeau, France). The concentration of Pi was determined using the malachite green method (*D'Angelo et al.*, 2001), based on light absorption at 630 nm (Tecan infinite M200pro spectrophotometer, Grödig, Austria). Concentrations of Po were calculated as the difference between Pt and Pi. Then Pt, Pi and Po concentrations in each fraction were converted to stocks using the bulk density and soil depth increments. Stocks of total P extractable by sequential P fractionation (Pt<sub>seq</sub>) were calculated by summing up the stocks in each soil depth.

### 2.4 Oxygen isotopic composition of phosphate ( $\delta^{18}\text{O}_p$ )

For all soil samples, 30 g of soil were sequentially extracted with 0.5 M NaHCO<sub>3</sub>, 0.1 M NaOH, and 1 M HCl at a 1:10 soil solution ratio (*Amelung et al.*, 2015). The aim of the two alkaline extractions was to remove the organic compounds and polyphosphates. The HCl-extracts were further processed for phosphate purification following the protocol of *Tamburini et al.* (2010). Phosphates were first precipitated as ammonium phosphomolybdate (APM) and then as magnesium ammonium phosphate (MAP), then passed through a cation exchange resin (Dowex 50X8, 200–400 mesh, Sigma-Aldrich, Darmstadt, Germany) and finally precipitated as silver phosphate (Ag<sub>3</sub>PO<sub>4</sub>). The Ag<sub>3</sub>PO<sub>4</sub> crystals were dried (50°C) and stored in a desiccator. The  $\delta^{18}\text{O}_p$  values were measured after pyrolysis in a carbon-based reactor with trap and purge chromatography system (PYRO Cube, Elementar, Hanau, Germany) connected in continuous flow to an isotope ratio mass spectrometer (Isoprime 100, Isoprime, Manchester, UK). After calibration, all values were reported in the conventional delta notation based on the Vienna Standard Mean Oceanic Water (VSMOW).

Theoretical equilibrium values of  $\delta^{18}\text{O}_p$  in the soils were calculated from Eq. (1) (*Chang and Blake*, 2015):

$$\delta^{18}\text{O}_p = e^{\left(\frac{14.43}{T} - \frac{26.54}{1000}\right)} \times (\delta^{18}\text{O}_w + 1000) - 1000, \quad (1)$$

where  $T$  is the temperature in K and  $\delta^{18}\text{O}_p$  and  $\delta^{18}\text{O}_w$  are the standardized <sup>18</sup>O:<sup>16</sup>O ratios of phosphate and water, respectively. For  $T$  we used mean, minimum and maximum temperatures averaged from air temperature for every month from 1978 to 2013 (5.4°C, -1.2°C and 12.5°C, respectively). There were no soil water or precipitation  $\delta^{18}\text{O}_w$  values available for Rauschholzhausen. Therefore, the  $\delta^{18}\text{O}_w$  in precipitation was estimated as -9.3‰ by averaging the observed  $\delta^{18}\text{O}_w$  values at the GNIP (Global Network of Isotopes in Precipitation) stations in Kahler Asten (50°10' N, 8°29' E) and Wasserkuppe

(Rhön) (50°30' N, 9°56' E) close to Rauschholzhausen, resulting in mean, minimum and maximum  $\delta^{18}\text{O}_p$  equilibrium values of 16.1‰, 14.8‰, and 17.3‰.

### 2.5 Statistics

Data analysis has been conducted using R software (version 3.4.1). Graphs show the mean and standard error (SE) across three replicate blocks. The long-term field experiment as sampled lacked randomization. Yet, there was neither a systematic gradient in soil group, soil texture data or inclination among treatments, thus rendering this experiment still meaningful for understanding of long-term field management. In order to have a criterion for data evaluation, we thus performed two-way ANOVA as a reference despite missing randomization to assess the effects of OM treatments and N treatments, the interaction of OM and N treatments on soil P stocks and  $\delta^{18}\text{O}_p$  values in different soil layers. The differences among OM and N treatments were tested by least significant difference (LSD) test ( $p < 0.05$ ). The results from such analyses are similar to evaluating the data using linear regression approaches when no underlying gradient in other soil properties exists.

## 3 Results

### 3.1 Pt<sub>seq</sub> and Pt<sub>XRF</sub> in soil profile

There was no difference in Pt<sub>seq</sub> stocks (0–100 cm) of NOF, FYM and OMF treatments when no mineral N was applied (Tab. 3). However, the Pt<sub>seq</sub> stocks only accounted for 21% to 27% of Pt<sub>XRF</sub> stocks (Tab. 3). Different OM fertilization did not alter Pt<sub>XRF</sub> stocks, although the FYM treatment resulted in slightly enhanced overall P extractability (Tab. 3). In all NOF, FYM and OMF treatments, however, both methods resulted in smaller total P stocks in N200 compared to N0 for the soil profile (0–100 cm) (Tab. 3). This decrease was most pronounced for the OMF treatment. Soil N:P ratio (total N: XRF P) was around 0.28–0.35 with the lowest value (0.28–0.29) in N0 treatment compared to N100 and N200 in all OM treatments (Tab. 3).

### 3.2 P balance

P uptake by crops increased by 7–273% in all organic fertilizer treatments with N addition (Tab. 4). Compared to NOF, the addition of OM accounted for total P input from 1984 to 2016 of 0.40–0.63 t ha<sup>-1</sup> in the FYM treatments, and of 0.39–0.41 t ha<sup>-1</sup> in the OMF treatments. The balance  $\Delta P$  in OM treatments increased by 0.11–0.56 t ha<sup>-1</sup> in comparison with NOF treatment (Tab. 5). In addition, the elevated amounts of N added with mineral fertilizer resulted also in larger P output with the harvest, which was higher in the N200 fertilization treatment by 151.4% relative to N0 in the NOF treatment, and by 79.3% and 54.0% relative to the N0 fertilization level in the FYM and OMF treatments, respectively. Thus, the balance  $\Delta P$  from 1984 to 2016 was lower by 0.27–0.53 t ha<sup>-1</sup> in treatments with increased N input compared to N0 in all organic fertilizer treatments (Tab. 5).

**Table 3:** Total P stocks (soil layer: 0–100 cm) measured by sequential P fractionation ( $P_{seq}$ ) and XRF ( $P_{XRF}$ , X-ray fluorescence) as well as soil N:P ratio for no organic fertilizer (NOF), farmyard manure (FYM), and a combination of organic and mineral N fertilizer (OMF) treatments; each organic fertilizer treatment was multiplied with three levels of mineral N addition: 0 kg ha<sup>-1</sup> y<sup>-1</sup> (N0), 100 kg ha<sup>-1</sup> y<sup>-1</sup> (N100), and 200 kg ha<sup>-1</sup> y<sup>-1</sup> (N200).

|                                |      | Extractable P in soils by sequential P fractionation (tons ha <sup>-1</sup> ) | Total P in soils by XRF (tons ha <sup>-1</sup> ) | P stocks ratio (% $P_{seq} / P_{XRF}$ ) | N:P ratio (total N / XRF P) |
|--------------------------------|------|---|--|---|-----------------------------|
| No organic fertilizer (NOF)    | N0   | 6.55 ± 0.21   | 28.80  | 22.74                                   | 0.29                        |
|                                | N100 | 6.74 ± 0.22   | 25.40  | 26.54                                   | 0.35                        |
|                                | N200 | 6.13 ± 0.22   | 27.30  | 22.45                                   | 0.35                        |
| Farmyard manure (FYM)          | N0   | 6.40 ± 0.14   | 29.30  | 21.84                                   | 0.28                        |
|                                | N100 | 7.37 ± 0.16   | 28.20  | 26.13                                   | 0.35                        |
|                                | N200 | 6.00 ± 0.28   | 26.50  | 22.64                                   | 0.35                        |
| Organomineral fertilizer (OMF) | N0   | 6.34 ± 0.07   | 27.60  | 22.97                                   | 0.29                        |
|                                | N100 | 6.85 ± 0.03   | 27.80  | 24.64                                   | 0.35                        |
|                                | N200 | 5.33 ± 0.33   | 25.80  | 20.66                                   | 0.33                        |

**Table 4:** The average P uptake by harvested products per hectare and year from 1984 to 2016 for no organic fertilizer (NOF), farmyard manure (FYM), and a combination of organic and mineral N fertilizer (OMF) treatments; each organic fertilizer treatment was multiplied with three levels of mineral N addition: 0 kg ha<sup>-1</sup> y<sup>-1</sup> (N0), 100 kg ha<sup>-1</sup> y<sup>-1</sup> (N100), and 200 kg ha<sup>-1</sup> y<sup>-1</sup> (N200). The crop rotation is sugar beet (replaced by maize in 2009), winter wheat and spring barley. Each crop is grown for one year.

|                                |      | Average from 1984 to 2009                         |       |        | Average from 2009 to 2016                    |       |        |
|--------------------------------|------|---|-------|--------|--|-------|--------|
|                                |      | Sugar beet (kg ha <sup>-1</sup> y <sup>-1</sup> ) | Wheat | Barley | Maize (kg ha <sup>-1</sup> y <sup>-1</sup> ) | Wheat | Barley |
| No organic fertilizer (NOF)    | N0   | 7.64  | 11.91 | 10.17  | 12.22  | 10.95 | 15.80  |
|                                | N100 | 10.65   | 26.88 | 25.48  | 16.98  | 29.15 | 30.55  |
|                                | N200 | 11.22   | 33.60 | 30.72  | 18.11  | 40.85 | 37.09  |
| Farmyard manure (FYM)          | N0   | 9.99  | 19.51 | 14.44  | 18.02  | 24.13 | 19.51  |
|                                | N100 | 11.70   | 33.08 | 28.02  | 19.24  | 39.75 | 34.61  |
|                                | N200 | 12.04   | 38.45 | 31.03  | 19.77  | 44.16 | 40.23  |
| Organomineral fertilizer (OMF) | N0   | 9.73  | 26.40 | 18.94  | 17.02  | 25.18 | 22.69  |
|                                | N100 | 11.30   | 37.09 | 29.37  | 18.72  | 40.67 | 37.92  |
|                                | N200 | 12.18   | 40.15 | 30.72  | 18.98  | 45.51 | 40.89  |

### 3.3 Pt stocks in P fractions at different soil depths

Pt stocks in sequential fractions were similar for all OM treatments that received the same amount of mineral N (e.g., NOF-N0 vs. FYM-N0 vs. OMF-N0). However, HCl-Pt stocks significantly decreased with OM addition in 0–30 cm soil depth (Tab. 2S). Further, in the topsoil (0–30 cm) of the mineral N input treatments, resin-Pt and HCl-Pt stocks were significantly smaller than those without mineral N fertilization, whereas in the upper subsoil (30–50 cm), NaHCO<sub>3</sub>-Pt and NaOH-Pt stocks of N input treatments were significantly larger than those without mineral N fertilization (Tab. 6). There were no significant differences among mineral N treatments in resin-Pt, NaHCO<sub>3</sub>-Pt and NaOH-Pt stocks in the deeper

subsoil (50–100 cm), while HCl-Pt stocks were largest in soils with N0 and residual P stocks were largest in treatment of N100 (Tab. 6).

### 3.4 Pi and Po stocks at different soil depths

In the topsoil, FYM and OMF addition significantly decreased the stocks of NaOH-Pi, HCl-Pi, and residual Pi, as well as resin-Po and NaHCO<sub>3</sub>-Po. Stocks of resin-Pi and NaHCO<sub>3</sub>-Pi in the topsoil were significantly smaller for N addition treatments than for treatments without mineral N fertilization (Tab. 3S). In the upper subsoil, however, both resin-Po and NaHCO<sub>3</sub>-Po stocks of N addition treatments were greater

**Table 5:** Total P input by fertilizers, P output by harvested products, and  $\Delta P$  (difference between P input and output) per hectare from 1984 to 2016 for no organic fertilizer (NOF), farmyard manure (FYM), and a combination of organic and mineral N fertilizer (OMF) treatments; each organic fertilizer treatment was multiplied with three levels of mineral N addition: 0 kg ha<sup>-1</sup> y<sup>-1</sup> (N0), 100 kg ha<sup>-1</sup> y<sup>-1</sup> (N100), and 200 kg ha<sup>-1</sup> y<sup>-1</sup> (N200).

|                                |      | P input                  | P output | $\Delta P$    |
|--------------------------------|------|--------------------------|----------|---------------|
|                                |      | (tons ha <sup>-1</sup> ) |          |               |
| No organic fertilizer (NOF)    | N0   | 0.33                     | 0.35     | -0.02         |
|                                | N100 | 0.33                     | 0.73     | -0.40         |
|                                | N200 | 0.33                     | 0.88     | -0.55         |
| Farmyard manure (FYM)          | N0   | 0.73–0.96                | 0.53     | 0.20–0.43     |
|                                | N100 | 0.73–0.96                | 0.85     | -0.13–0.11    |
|                                | N200 | 0.73–0.96                | 0.95     | -0.23–0.01    |
| Organomineral fertilizer (OMF) | N0   | 0.72–0.74                | 0.63     | 0.09–0.11     |
|                                | N100 | 0.72–0.74                | 0.90     | -0.19–(-0.16) |
|                                | N200 | 0.72–0.74                | 0.97     | -0.25–(-0.22) |

than those without mineral N fertilization (Tab. 7). For NaOH-Pi, HCl-Pi and residual P, the treatment of N100 tended to have the largest P stocks among all N treatments. Moreover, mineral N addition resulted in significantly higher NaOH-Po and HCl-Po stocks in topsoil and upper subsoil (Tab. 7).

### 3.5 Oxygen isotopic composition of HCl-extractable phosphate ( $\delta^{18}O_P$ )

$\delta^{18}O_P$  values decreased with soil depth. The different types of organic amendment did not affect the  $\delta^{18}O_P$  values in soil significantly (Tab. 4S). However, throughout the soil profile, the  $\delta^{18}O_P$  values of mineral N input treatments were greater than those of the treatment without mineral N fertilization in all NOF, FYM and OMF treatments (Fig 1). Furthermore, the  $\delta^{18}O_P$  values of mineral N input treatments were closer to the theoretical equilibrium value in FYM and OMF treatments than in NOF treatments (Fig. 1).

## 4 Discussion

### 4.1 P stocks in soil profile

The  $Pt_{seq}$  stocks are recognized as an estimation of the maximum P availability to plants. However, the  $Pt_{seq}$  stocks did not match the  $Pt_{XRF}$  stocks, which is due to non-extractable mineral P, such as the P bound to silicate (Ivanov et al., 2012). In our study, OM input (FYM and OMF treatments) increased the P balance ( $\Delta P$ ) between the start of the experiment and the current sampling in comparison with the NOF treatment (Tab. 5). This was due to P addition upon application of organic fertilizers. However,  $\Delta P$  in N100 and N200 treatments was lower than that in the N0 treatment because of higher P output (Tabs. 4 and 5). In NOF and FYM treatments, the higher P output with yields in mineral N addition treatments almost equaled the decline in P stocks from se-

quential P fractionation with increasing mineral N addition. This indicates that enhanced P losses upon N fertilization occurred mainly in P pools that can be extracted with sequential P fractionation.

The soil N:P ratio increased with mineral N addition in each OM treatment because of the enhanced N fertilizer input and soil P uptake by harvest. In addition, the soil N:P ratio in this experiment was lower than in other studies (Cleveland and Liptzin, 2007; Tian et al., 2010) (Tab. 3), which was not only due to the higher  $Pt_{XRF}$  stocks being used for N:P ratio calculation rather than  $Pt_{seq}$  stocks, but also likely indicates N limitation. Thus, crop growth and microbial activity are boosted by N inputs (Marklein and Houlton, 2012; Fan et al., 2019), thereby decreasing P stocks of se-

quential P fractions with increasing N addition. In addition, elevated input of mineral N led to decreased total P stocks in the soil profile (0–100 cm) (Tab. 3), which was also observed in other long-term field experiments (Bauke et al., 2018). This was due to increased P export with elevated crop yields following N fertilization (Tab. 4). The increase in plant productivity with N fertilization could further result in the release of more organic acids through root exudation (Olander and Vitousek, 2000). Such root exudates promote P solubilization for crop uptake. Accordingly, in our experiment mainly the Pi fractions were affected by N fertilization.

Compared with N100, topsoil  $Pt$  stocks in sequential P fractions were smaller in N200. Again, we attribute this observation to higher crop P demand with higher mineral N supply. However, not only the available  $Pt$  stocks were depleted with increasing mineral N addition, but also the  $Pt$  stocks in fractions less available to plants (HCl-Pt stocks and residual P stocks). Probably, the labile P fractions were constantly replenished from moderately labile P (NaOH-Pt) or more stable P fractions such as HCl-P and residual P (Solomon and Lehmann, 2000; von Sperber et al., 2017b; Fan et al., 2019). Results of a short-term incubation study (Guo et al., 2000) and of a long-term arable field survey (von Sperber et al., 2017b) indicated that labile P fractions were more sensitive to plant uptake, but that release of P from stable P fractions can compensate these losses.

Amelung et al. (2015) outlined that plant P acquisition declines in subsoil, defining the upper subsoil as a transition zone from P acquisition from biological recycling to P acquisition from parent material. We found that in the upper subsoil at 30–50 cm depth stocks of more readily available fractions (resin-Pt and NaHCO<sub>3</sub>-Pt) and stocks of the moderately labile fraction (NaOH-Pt) were increased upon N input, whereas stable  $Pt$  stocks showed the opposite trend. This finding contrasts that of Bauke et al. (2018), who reported that elevated

**Table 6:** Phosphorus stocks of sequential P fractionation in no organic fertilizer (NOF), farmyard manure (FYM), and a combination of organic and mineral N fertilizer (OMF) treatments were given as mean with standard error (SE) ( $n = 3$ ); each organic fertilizer treatment was multiplied with three levels of mineral N addition: 0 kg ha<sup>-1</sup> y<sup>-1</sup> (N0), 100 kg ha<sup>-1</sup> y<sup>-1</sup> (N100), and 200 kg ha<sup>-1</sup> y<sup>-1</sup> (N200).<sup>a</sup>

| Depth (cm) |     |    | Resin P stocks (tons ha <sup>-1</sup> ) |             |            | NaHCO <sub>3</sub> P stocks (tons ha <sup>-1</sup> ) |            |             | NaOH P stocks (tons ha <sup>-1</sup> ) |             |             | HCl P stocks (tons ha <sup>-1</sup> ) |            |             | residual P stocks (tons ha <sup>-1</sup> ) |             |             |
|------------|-----|----|---|-------------|------------|--|------------|-------------|--|-------------|-------------|---------------------------------------|------------|-------------|--|-------------|-------------|
|            |     |    | N0                                      | N100        | N200       | N0   | N100       | N200        | N0                                     | N100        | N200        | N0                                    | N100       | N200        | N0   | N100        | N200        |
| 0–30       | NOF | Pt | 0.40<br>bA                              | 0.32<br>abA | 0.30<br>aA | 0.30<br>aA   | 0.30<br>aB | 0.27<br>aA  | 0.65<br>aB                             | 0.57<br>aA  | 0.64<br>aA  | 1.01<br>bA                            | 1.02<br>bC | 0.91<br>aA  | 0.82<br>aA                                 | 1.28<br>bB  | 0.81<br>aA  |
|            |     | SE | 0.03                                    | 0.02        | 0.03       | 0.01   | 0.01       | 0.01        | 0.03                                   | 0.01        | 0.04        | 0.02                                  | 0.02       | 0.02        | 0.05                                       | 0.04        | 0.09        |
|            | FYM | Pt | 0.43<br>bA                              | 0.36<br>abA | 0.32<br>aA | 0.29<br>aA   | 0.31<br>aB | 0.28<br>aA  | 0.58<br>aAB                            | 0.75<br>aA  | 0.60<br>aA  | 0.95<br>aA                            | 0.89<br>aB | 0.83<br>aA  | 0.81<br>aA                                 | 1.23<br>bAB | 0.82<br>aA  |
|            |     | SE | 0.03                                    | 0.02        | 0.03       | 0.00   | 0.01       | 0.02        | 0.01                                   | 0.09        | 0.04        | 0.04                                  | 0.01       | 0.06        | 0.01                                       | 0.03        | 0.14        |
|            | OMF | Pt | 0.37<br>bA                              | 0.33<br>aA  | 0.30<br>aA | 0.31<br>aA   | 0.25<br>aA | 0.26<br>aA  | 0.56<br>aA                             | 0.73<br>bA  | 0.61<br>aA  | 0.91<br>bA                            | 0.80<br>aA | 0.84<br>abA | 0.80<br>aA                                 | 1.08<br>bA  | 0.61<br>aA  |
|            |     | SE | 0.02                                    | 0.01        | 0.02       | 0.01   | 0.00       | 0.03        | 0.02                                   | 0.02        | 0.04        | 0.03                                  | 0.04       | 0.03        | 0.04                                       | 0.07        | 0.08        |
| 30–50      | NOF | Pt | 0.07<br>abA                             | 0.07<br>aA  | 0.09<br>bA | 0.09<br>aA   | 0.10<br>aA | 0.12<br>bB  | 0.26<br>abA                            | 0.24<br>aA  | 0.35<br>bA  | 0.32<br>aA                            | 0.17<br>aA | 0.32<br>aA  | 0.53<br>aA                                 | 0.74<br>bA  | 0.58<br>abB |
|            |     | SE | 0.01                                    | 0.00        | 0.00       | 0.01   | 0.00       | 0.00        | 0.01                                   | 0.01        | 0.05        | 0.10                                  | 0.02       | 0.05        | 0.04                                       | 0.03        | 0.01        |
|            | FYM | Pt | 0.05<br>aA                              | 0.06<br>aA  | 0.07<br>aA | 0.07<br>aA   | 0.10<br>bA | 0.11<br>bAB | 0.21<br>aA                             | 0.35<br>bAB | 0.29<br>abA | 0.41<br>aA                            | 0.28<br>aA | 0.27<br>aA  | 0.54<br>aA                                 | 0.75<br>bA  | 0.54<br>aB  |
|            |     | SE | 0.01                                    | 0.01        | 0.01       | 0.01   | 0.01       | 0.00        | 0.02                                   | 0.06        | 0.01        | 0.10                                  | 0.06       | 0.03        | 0.01                                       | 0.07        | 0.02        |
|            | OMF | Pt | 0.04<br>aA                              | 0.09<br>aA  | 0.07<br>aA | 0.08<br>aA   | 0.10<br>aA | 0.10<br>aA  | 0.23<br>aA                             | 0.37<br>bB  | 0.27<br>abA | 0.51<br>aA                            | 0.29<br>aA | 0.30<br>aA  | 0.54<br>bA                                 | 0.77<br>cA  | 0.41<br>aA  |
|            |     | SE | 0.01                                    | 0.01        | 0.01       | 0.02   | 0.00       | 0.01        | 0.05                                   | 0.01        | 0.01        | 0.17                                  | 0.05       | 0.05        | 0.01                                       | 0.01        | 0.02        |
| 50–100     | NOF | Pt | 0.05<br>aA                              | 0.04<br>aA  | 0.05<br>aA | 0.07<br>aA   | 0.08<br>aA | 0.09<br>aA  | 0.23<br>aA                             | 0.21<br>aA  | 0.27<br>aA  | 1.09<br>bA                            | 0.64<br>aA | 0.71<br>abA | 0.66<br>aA                                 | 0.96<br>bA  | 0.63<br>aB  |
|            |     | SE | 0.01                                    | 0.00        | 0.01       | 0.02   | 0.00       | 0.01        | 0.04                                   | 0.02        | 0.04        | 0.13                                  | 0.15       | 0.02        | 0.02                                       | 0.09        | 0.03        |
|            | FYM | Pt | 0.05<br>aA                              | 0.05<br>aA  | 0.06<br>aA | 0.06<br>aA   | 0.09<br>aA | 0.09<br>aA  | 0.19<br>aA                             | 0.31<br>aA  | 0.25<br>aA  | 1.06<br>aA                            | 0.87<br>aA | 0.81<br>aA  | 0.70<br>aA                                 | 0.96<br>bA  | 0.66<br>aB  |
|            |     | SE | 0.01                                    | 0.02        | 0.02       | 0.01   | 0.03       | 0.03        | 0.02                                   | 0.08        | 0.04        | 0.06                                  | 0.20       | 0.08        | 0.01                                       | 0.02        | 0.05        |
|            | OMF | Pt | 0.04<br>aA                              | 0.06<br>aA  | 0.05<br>aA | 0.07<br>aA   | 0.08<br>aA | 0.07<br>aA  | 0.21<br>aA                             | 0.26<br>aA  | 0.22<br>aA  | 0.95<br>aA                            | 0.78<br>aA | 0.80<br>aA  | 0.71<br>bA                                 | 0.87<br>bA  | 0.44<br>aA  |
|            |     | SE | 0.02                                    | 0.02        | 0.01       | 0.02   | 0.02       | 0.01        | 0.05                                   | 0.03        | 0.01        | 0.09                                  | 0.15       | 0.11        | 0.06                                       | 0.06        | 0.05        |

<sup>a</sup>Capital letters indicate significant differences among OM treatments; lower case letters indicate significant differences among N treatments.

N input promoted P uptake from the deeper soil layers. The main difference between the study of *Bauke et al.* (2018) and our research is that mineral N was added together with organic fertilizers. It has been reported that organic fertilizer addition stimulated microbial activity and microbial P turnover (*Deng et al.*, 2017), but the total and available P stocks in soil decreased because of a higher P budget deficit (*Oehl et al.*, 2001; *Maranguit et al.*, 2017). This is in line with our results that stocks of stable Pi and labile Po declined with organic fertilizer addition in the topsoil (Tab. 3S). The interactive effects of organic fertilizer and mineral N fertilizer was significant for moderately labile or stable P stocks, which indicated that P desorption and mineralization could be increased when other nutrients such as N and C are applied in sufficient

amounts. In turn, P associated with OM could be transferred to deeper soil layers, causing increased P content in subsoil (*Medinski et al.*, 2018). Several other processes potentially could be involved, such as:

- Input of labile OM could facilitate the mobilization of P from stable P pools to labile P pools in the upper subsoil where surface application of organic and mineral fertilizers did not replenish P taken up by the crops. The increase in labile Pi stocks in the upper subsoil (30–50 cm depth) with mineral N addition support this assumption.
- Besides Pi stocks, Po stocks in the upper subsoil in mineral N addition treatments were also larger than those in treatments without mineral N addition, indicating elevated input



**Table 7:** Stocks of Labile Pi (sum of resin-Pi and  $\text{NaHCO}_3$ -Pi) and non-labile Pi (sum of  $\text{NaOH}$ -Pi,  $\text{HCl}$ -Pi and residual-Pi) stocks, labile Po ( $\text{NaHCO}_3$ -Po) and non-labile Po (sum of  $\text{NaOH}$ -Po and  $\text{HCl}$ -Po) in no organic fertilizer (NOF), farmyard manure (FYM), and a combination of organic and mineral N fertilizer (OMF) treatments were given as mean with standard error (SE) ( $n = 3$ ); each organic fertilizer treatment was multiplied with three levels of mineral N addition:  $0 \text{ kg ha}^{-1} \text{ y}^{-1}$  (N0),  $100 \text{ kg ha}^{-1} \text{ y}^{-1}$  (N100), and  $200 \text{ kg ha}^{-1} \text{ y}^{-1}$  (N200).<sup>a</sup>

| Depth (cm) |     |    | Labile Pi stocks (tons $\text{ha}^{-1}$ ) |             |            | Non-labile Pi stocks (tons $\text{ha}^{-1}$ ) |            |             | Labile Po stocks (tons $\text{ha}^{-1}$ ) |             |              | Non-labile Po stocks (tons $\text{ha}^{-1}$ ) |             |             |
|------------|-----|----|---|-------------|------------|---|------------|-------------|---|-------------|--------------|---|-------------|-------------|
|            |     |    | N0  | N100        | N200       | N0  | N100       | N200        | N0  | N100        | N200         | N0  | N100        | N200        |
| 0–30       | NOF | P  | 0.62<br>bA                                | 0.53<br>abA | 0.48<br>aA | 2.20<br>aA                                    | 2.62<br>bC | 2.05<br>aA  | 0.08<br>aA                                | 0.08<br>aB  | 0.09<br>aA   | 0.27<br>aA                                    | 0.26<br>aA  | 0.30 aA     |
|            |     | SE | 0.04                                      | 0.02        | 0.04       | 0.09  | 0.05       | 0.12        | 0.01                                      | 0.01        | 0.01         | 0.02  | 0.02        | 0.02        |
|            | FYM | P  | 0.65<br>bA                                | 0.60<br>abB | 0.52<br>aA | 2.07<br>abA                                   | 2.46<br>bB | 1.96<br>aA  | 0.08<br>aA                                | 0.07<br>aAB | 0.07<br>aA   | 0.27<br>aA                                    | 0.41<br>aAB | 0.29 aA     |
|            |     | SE | 0.03                                      | 0.01        | 0.04       | 0.03  | 0.02       | 0.20        | 0.00                                      | 0.01        | 0.01         | 0.01  | 0.10        | 0.00        |
|            | OMF | P  | 0.60<br>aA                                | 0.52<br>aA  | 0.50<br>aA | 2.01<br>abA                                   | 2.14<br>bA | 1.78<br>aA  | 0.08<br>bA                                | 0.06<br>aA  | 0.06<br>abA  | 0.25<br>aA                                    | 0.47<br>bB  | 0.27 aA     |
|            |     | SE | 0.03                                      | 0.02        | 0.04       | 0.03  | 0.03       | 0.12        | 0.01                                      | 0.00        | 0.01         | 0.02  | 0.04        | 0.03        |
| 30–50      | NOF | P  | 0.14<br>abA                               | 0.14<br>aA  | 0.17<br>bB | 1.00<br>aA                                    | 1.05<br>aA | 1.07<br>aB  | 0.02<br>aA                                | 0.03<br>aA  | 0.04<br>aB   | 0.12<br>aA                                    | 0.10<br>aA  | 0.18 aA     |
|            |     | SE | 0.01                                      | 0.01        | 0.01       | 0.12  | 0.05       | 0.03        | 0.01                                      | 0.01        | 0.00         | 0.00  | 0.01        | 0.04        |
|            | FYM | P  | 0.10<br>aA                                | 0.12<br>aA  | 0.14<br>aA | 1.06<br>aA                                    | 1.19<br>aA | 0.96<br>aAB | 0.02<br>aA                                | 0.04<br>bA  | 0.04<br>bAB  | 0.10<br>aA                                    | 0.18<br>bB  | 0.13<br>abA |
|            |     | SE | 0.02                                      | 0.02        | 0.01       | 0.09  | 0.08       | 0.04        | 0.00                                      | 0.00        | 0.00         | 0.01  | 0.03        | 0.00        |
|            | OMF | P  | 0.10<br>aA                                | 0.16<br>aA  | 0.14<br>aA | 1.16<br>bA                                    | 1.21<br>bA | 0.86<br>aA  | 0.02<br>aA                                | 0.04<br>aA  | 0.03<br>aA   | 0.12<br>aA                                    | 0.22<br>bB  | 0.12 aA     |
|            |     | SE | 0.03                                      | 0.01        | 0.01       | 0.11  | 0.04       | 0.06        | 0.00                                      | 0.00        | 0.00         | 0.02  | 0.01        | 0.01        |
| 50–100     | NOF | P  | 0.24<br>aA                                | 0.22<br>aA  | 0.24<br>aA | 3.84<br>aA                                    | 3.66<br>aA | 3.20<br>aA  | 0.04<br>aA                                | 0.06<br>abA | 0.08<br>bB   | 0.37<br>aA                                    | 0.26<br>aA  | 0.36 aA     |
|            |     | SE | 0.05                                      | 0.02        | 0.02       | 0.15  | 0.50       | 0.03        | 0.01                                      | 0.00        | 0.00         | 0.03  | 0.01        | 0.07        |
|            | FYM | P  | 0.21<br>aA                                | 0.26<br>aA  | 0.29<br>aA | 3.85<br>abA                                   | 4.15<br>bA | 3.35<br>aA  | 0.03<br>aA                                | 0.08<br>bA  | 0.05<br>abAB | 0.39<br>abA                                   | 0.51<br>bB  | 0.33 aA     |
|            |     | SE | 0.04                                      | 0.10        | 0.07       | 0.05  | 0.33       | 0.20        | 0.01                                      | 0.01        | 0.02         | 0.04  | 0.05        | 0.01        |
|            | OMF | P  | 0.23<br>aA                                | 0.25<br>aA  | 0.23<br>aA | 3.69<br>bA                                    | 3.90<br>bA | 2.80<br>aA  | 0.06<br>aA                                | 0.06<br>aA  | 0.04<br>aA   | 0.33<br>aA                                    | 0.39<br>aAB | 0.30 aA     |
|            |     | SE | 0.09                                      | 0.06        | 0.03       | 0.02  | 0.21       | 0.33        | 0.01                                      | 0.02        | 0.00         | 0.02  | 0.05        | 0.03        |

<sup>a</sup>Capital letters indicate significant differences among OM treatments; lower case letters indicate significant differences among N treatments.

of Po, possibly related to increased root biomass. Elevated N supply in the topsoil improved overall crop growth, and thus increased the deposition of root-derived P. This idea is in line with earlier ecosystem research showing that N fertilization stimulated the growth of roots (Tu et al., 2006), while their subsequent decomposition increased the Po contents in the subsoil (Campbell et al., 1993).

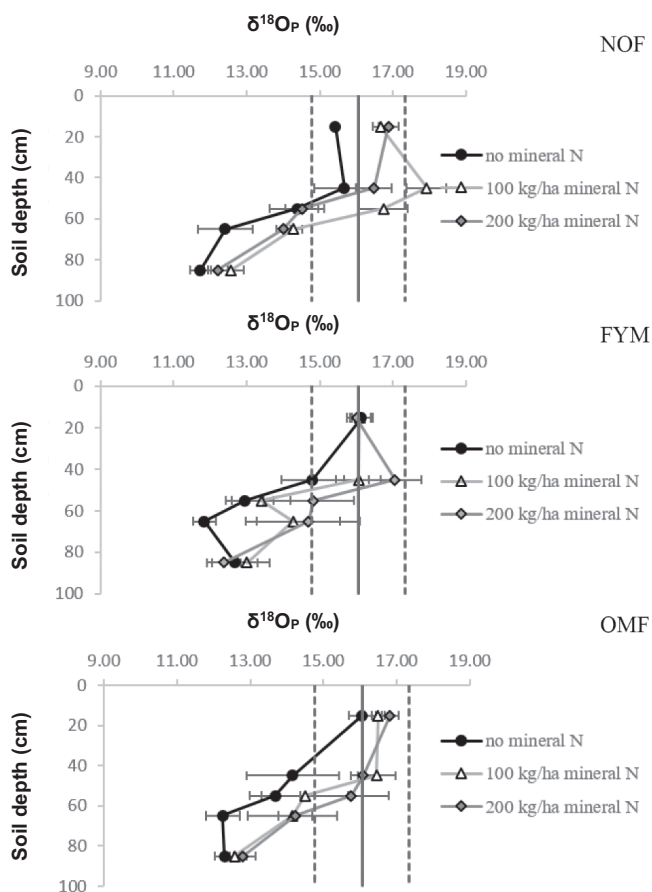
- Soil P sorption capacity decreased by long-term excessive P fertilization (Koch et al., 2018). When topsoil P sorption capacity declines due to continuous P fertilizer input, increasing leaching of P into deeper soil layers may occur (Medinski et al., 2018). In addition, it was reported that higher P stocks in upper subsoil due to P leaching occurred

in agricultural (Kang et al., 2011), grassland (Toor et al., 2005), and forest ecosystems (Frossard et al., 1989).

In the lower subsoil at 50–100 cm depth, P stocks did not show significant differences among N treatments in labile and moderately labile P stocks, likely because of less roots and lower microbial activity.

#### 4.2 Oxygen isotopic composition of $\text{HCl}$ -extractable phosphate ( $\delta^{18}\text{O}_\text{P}$ )

The above-mentioned changes in the P stocks of sequential fractions reflect alterations in the P balance with OM and min-



**Figure 1:**  $\delta^{18}\text{O}_P$  values of HCl-extractable P of the no organic fertilizer (NOF), farmyard manure (FYM), and a combination of organic and mineral N fertilizer (OMF) treatments; each organic fertilizer treatment was multiplied with three levels of mineral N addition: 0 kg ha<sup>-1</sup> y<sup>-1</sup> (NO), 100 kg ha<sup>-1</sup> y<sup>-1</sup> (N100), and 200 kg ha<sup>-1</sup> y<sup>-1</sup> (N200). The error bars indicate the standard error of the mean of field replicates (*n* = 3). The solid line indicates the mean expected equilibrium value, and the dashed vertical lines represent the minimum and maximum expected equilibrium value calculated with the monthly minimum and maximum temperature from the years 1978 to 2013.

eral N fertilization. In contrast, alterations of oxygen isotope ratios in phosphate result from P cycling and, thus, could be a more sensitive indicator of soil P dynamics than mere sequential fractionation. In topsoil, the  $\delta^{18}\text{O}_P$  values in the FYM and OMF treatments were closer to the expected equilibrium value than in the NOF treatments (Fig. 1). It was suggested that intracellular P cycling was the main reason for  $\delta^{18}\text{O}_P$  values close to equilibrium (Chang and Blake, 2015; Bauke et al., 2018). The intensive intracellular recycling of P was enhanced by substantial C and N input, leading to a larger degree of isotopic exchange between phosphate and ambient water. This intracellular microbial turnover of phosphate is subsequently incorporated into the isotopic signature of Ca-P minerals by secondary precipitation (Bauke et al., 2018; Helfenstein et al., 2018). Hence, the biological P recycling in arable soil seems affected by the addition of other nutrients, as previously shown in short-term incubation studies (Gross et al., 2015) and long-term fertilization experiments (Kaspari

et al., 2008). The  $\delta^{18}\text{O}_P$  values of NOF treatments partially retained the value of the fertilizer P (Amelung et al., 2015) or of phosphate released from OM (Tamburini et al., 2018), because of less intense enzymatic P recycling with insufficient C and N supply.

In subsoil, the  $\delta^{18}\text{O}_P$  values of treatments with mineral N addition were greater than those without mineral N addition and closer to the expected equilibrium value, which hinted at elevated microbial cycling upon N input (Oehl et al., 2001). In the layer of 30–50 cm, the  $\delta^{18}\text{O}_P$  values also could be still affected by leached fertilizer P. We did not analyze the isotope value of P fertilizers applied at the site but the isotopic value of triple superphosphate fertilizer was reported to be in the range of around 20‰ (Amelung et al., 2015). The fertilizer  $\delta^{18}\text{O}_P$  value is thus much higher than the  $\delta^{18}\text{O}_P$  values in soil phosphate. Leaching of fertilizer P may have caused the  $\delta^{18}\text{O}_P$  values of the N100 treatment in NOF exceeding the maximum expected equilibrium value.

Below 50 cm soil depth, the  $\delta^{18}\text{O}_P$  values were smaller than the expected equilibrium value. This is due to generally lower soil microbial biomass and root density in subsoil than in surface soil, resulting in lower biological turnover of P (Amelung et al., 2015; Bauke et al., 2017, 2018). In the soil layer of 70–100 cm, there were no differences in  $\delta^{18}\text{O}_P$  values among treatments. The most likely explanation is that most of the phosphate had not equilibrated with soil water due to the small amounts of microbial biomass, and thus, the  $\delta^{18}\text{O}_P$  values retained the value of the soil parent material (Angert et al., 2012; Tamburini et al., 2012; Amelung et al., 2015).

### 5 Conclusions

Nitrogen fertilization increased biological P cycling in top- and subsoil horizons, which emphasizes that fertilization effects extend beyond the tilled topsoil horizon. The long-term OM and mineral N fertilization promoted crop P uptake from labile fractions, whereas subsoil P stocks in labile fractions were increased, probably by enhanced input of root litter. Sustainable management of P stocks in arable soils should thus not only focus on direct effects of P fertilization but should also consider cross-cutting effects between P cycling and other nutrients such as C or N both in topsoil and in subsoil.

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## Data Availability Statement

The data that support the findings of this study are in the article itself.

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