



Leaching of Colloidal Phosphorus from Organic Layers and Mineral Topsoils of German Beech Forests in Response to Drought and Nutrient Inputs

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Abstract

Purpose The purpose of the study was to clarify the relevance of colloidal phosphorus (P_{coll}) to total P (TP) leaching and its link to dissolved organic P (DOP) leaching in forest topsoils under field conditions. Effects of heavy rainfall after a drought and the influence of P and N inputs to forest soils on P_{coll} leaching from the topsoil were assessed to better understand the causes of P_{coll} leaching.

Methods We carried out an irrigation experiment with zero-tension lysimeters collecting leachate from the organic forest floor layers and mineral topsoils of a sandy, low-P and a loamy, high-P soil.

Results The contribution of P_{coll} to TP concentrations was on average around $10 \pm 14\%$ irrespective of sampling date, season and soil. The highest P_{coll} concentrations were recorded upon irrigation after a summer dry period with the increase in concentrations being most pronounced for the 20–70 nm size fraction with low median C:P ratios (29 to 31) typical for microbial residues. Significant relationships between P_{coll} and DOP concentrations suggested that 27% (low P soil) and 17% (high P soil) of DOP were of colloidal nature. Independent of the soil P stock, the addition of P and P+N affected neither P_{coll} concentrations nor their share of TP significantly.

Conclusion While P_{coll} hardly affected the overall estimates of beech forest P budgets, it provides important further insights into processes governing the mobilization, transport, and cycling of P in forest ecosystems.

Keywords Forest · Colloid-facilitated transport · Dissolved organic phosphorus · Drought · Natural nanoparticles

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1 Introduction

Natural colloids and nanoparticles have been identified as important carriers of phosphorus (P) not only in agricultural soils (Haygarth et al. 1997; Hens and Merckx 2001; Jonge et al. 2004; Ilg et al. 2005; Rick and Arai 2011; Regelink et al. 2013; Ai Chen and Yuji Arai 2020; Gu et al. 2020), but also in forest and savannah soils (Jiang et al. 2015; Missong 2017; Missong et al. 2018b; Wang et al. 2020; Zhang et al. 2021, 2023; Jiang et al. 2023). These natural nanoparticles and colloids have typically been subdivided into three size fractions of <20 nm, 20–70 nm and 70–450 nm using asymmetric flow field fractionation (AF4 (Missong 2017). In temperate forest soils, the smallest size fraction (<20 nm) was mainly composed of organic material, the 20–70 nm fraction consists of organic matter (OM) associated with iron (Fe) and aluminum (Al) (hydr)oxides, and the largest

colloidal fraction (70–450 nm) mainly comprised clay mineral particles (Missong et al. 2018a, b).

The mobilization and displacement of colloids from the topsoil to the lower soil layers depends on multiple abiotic and biotic factors. Abiotic factors do include meteorological conditions (e.g. rainfall intensity and soil moisture fluctuations, see below) and soil properties (e.g. mineralogy, texture, porosity, elemental content and the ionic strength as well as composition of the soil solution). Biotic factors are related to such as micro-, meso- and macrofauna whom all play a role in the turnover of the soil structure and the related dynamics of colloids (Dang et al. 2023).

Laboratory experiments with soil columns and the Diffusive-Gradients-in-Thin-Films (DGT) technique demonstrated a high mobility of colloidal P (P_{coll}) in forest topsoils (Holzmann et al. 2016; Missong et al. 2018b; Konrad et al. 2021). In these experiments, P_{coll} accounted for 12–91% of total P (TP) leaching losses (Holzmann et al. 2016; Missong et al. 2016). The mobility of P_{coll} was particularly large for a sandy, podzolic soil with small overall P stocks compared to loamy soils with larger P stocks (Holzmann et al. 2016; Missong et al. 2016; Konrad et al. 2021). Forests on soils with small soil P stocks rely on close recycling of P within plants, organic forest floor layers, and the mineral topsoil (Lang et al. 2017). Therefore leaching of P_{coll} from organic layers and mineral topsoils into deeper subsoils with less roots or even export from soil via deep drainage are of relevance for the long-term P nutrition of such ecosystems. In agreement with these findings on P_{coll} mobility in soils, P transported in forest headwaters and rivers seems to be primarily associated with nanoparticles and colloids (Gottselig et al. 2017a, b, 2020). In addition, types and sizes of P-carrying nanoparticles and colloids transported with forest headwaters and rivers were similar to those leached from soils (Missong et al. 2018a). Nevertheless, the mobilization and leaching of P_{coll} in forest soils has rarely been studied under field conditions, and Bol et al. (2016) pinpointed the lacking assessments of leaching fluxes of P_{coll} under field conditions.

Variations of soil moisture due to drying–rewetting cycles can trigger the mobilization and leaching of colloids in porous media and soils by altering the water-filled pore volume and ionic strength (El-Farhan et al. 2000; DeNovio et al. 2004; Makselon et al. 2017). In arable and forest soils, colloid release during infiltration increased with an increasing duration of the preceding drying period (up to 11 days), but for longer drying periods colloid mobilization decreased (Majdalani et al. 2008; Mohanty et al. 2015). Mobilization and leaching of P from forest topsoil increased in drying–rewetting cycles during laboratory experiments, which was primarily related to microbial cell lysis releasing inorganic and organic P (Hoemberg and Matzner 2018; Brödlin et al. 2019).

Apart from stronger droughts and heavy rainfall events due to climate change, the inputs of nutrients (especially N) might trigger perturbations of nutrient cycles in forest ecosystems, because nutrient availability determines the plant and microbial uptake and competition. Fetzer et al. (2022) found that the effects of N and P inputs on P concentrations in leachates of beech forests depended on soil P stocks. For sandy, podzolic soils with small P stocks (test site Luess, see 2.1) the separate addition of either N or P strongly increased dissolved inorganic P (DIP) concentrations leached from the organic surface layers, while the combined addition of N+P had no effect. The authors explained these observations with a co-limitation of microorganisms in the sandy soil by N and P. The P addition increased the amount of available and mobile P that was not taken up by microorganisms due to N limitation. Whereas N additions promoted soil P leaching due to overcoming the N limitation, allowing the production of enzymes hydrolyzing organic P. Combined addition of N and P boosted microbial growth to such an extent that added P and P released by mineralization was completely immobilized in microbial biomass. At a site with large soil P stocks, only the combined addition of N+P increased concentrations of DIP significantly, while no significant effect was observed after separate additions of N or P, which Fetzer et al. (2022) explained with the absence of co-limitation in the nutrient rich soil. Holzmann et al. (2016) found in soil column experiments that concentrations of DOP were unaffected by nutrient addition.

Here, we examined the size properties and chemical composition of leached colloids from the beech forests lysimeter study of Fetzer et al. (2022) to test the following hypotheses:

- (I) Higher P_{coll} leaching from organic layer and mineral topsoil in sandy compared to loamy soil, because of larger and better connected pores supporting greater colloids mobility.
- (II) P_{coll} contributes substantially to DOP leaching, because the organic colloids are relatively P-rich compared to other soil P fractions.
- (III) Larger fertilizer-induced increase of leached P_{coll} at the low-P compared to high-P site, because of stronger P limitation of soil organisms.
- (IV) Heavy rainfall after drought enhances P_{coll} leaching, due to the leaching of drought-legacy residues of microbial biomass and overall disintegration of soil aggregates into colloidal particles during re-wetting.

2 Materials and Methods

2.1 Study Sites

The study was conducted in two beech forest stands in Germany dominated by 120–140-year-old *Fagus sylvatica* on soils derived from contrasting parent material of soil formation and P availability (Lang et al. 2017). The soil with high P stock at Bad Brückenau (809 m a.s.l., 50.35° N, 9.27° E, referred to “BBR/high-P site”) is a loamy Cambisol developed on basalt and the organic forest floor layer is a mull-like moder (Lang et al. 2017). The soil with the lower P stock at Unterlüss (115 m a.s.l., 52.8° N, 10.3° E, referred to “LUE/low-P site”) is a sandy Cambisol that developed from glacial till, featuring a thicker organic layer and indications of initial podzolization. The organic forest floor layer is a mor-like moder (Lang et al. 2017). The organic layers and mineral topsoils have greater dissolved organic carbon (DOC)-to-DOP ratios (C:P ratios) at the low-P site than at the high-P site, but the C:N ratios are similar. The C:P ratio is an indicator for the soil P availability and P cycling intensity. Small C:P ratios suggest a high availability and intense microbial cycling of P. Vice versa, large C:P ratios indicate a low availability and limited cycling of soil P.

Compared to the LUE low-P site, the BBR high-P site has a slightly higher pH of 3.3 vs. 2.5 (measured in 1 M KCl at a soil (air dried)-to-solution ratio of 1:2.5 (w/v), higher cation exchange capacity (371 vs. 108 meq kg⁻¹), and higher contents of Al and Fe in pedogenic oxides (29.3 and 8.4 vs. 0.9 and 0.3 g kg⁻¹ in the mineral topsoil; Lang et al. 2017). More details on the two sites can be found in Lang et al. (2017).

2.2 Fertilization Experiment

At each site, plots of 20 m × 20 m were established and fertilized with either +P, +N, or unfertilized (control plots).

They had a distance of 20 m from each other and were replicated three times in blocks, resulting in a total of 12 plots per site (details on experimental set-up are given in, e.g. Hauenstein et al. 2020). Five g P m⁻² were applied as KH₂PO₄ in a single dose in April to May 2016 (representing 0.6 and 3.0% of TP stock at the high-P and the low-P site). To compensate for the K input to the P fertilized plots, 6.3 g K m⁻² were applied as KCl at the N fertilized and control plots. Nitrogen was added as NH₄NO₃ in five equal doses from 2016 to 2018, amounting to a total of 15 g N m⁻² (equivalent to 1.2 and 2.1% of total N stock at the high-P and the low-P site, Table 1) in spring, summer, and autumn to account for seasonal demand of trees and to reduce leaching losses.

2.3 Lysimeters

The samples analyzed for P_{coll} concentrations were aliquots of samples collected by Fetzer et al. (2022) using zero-tension lysimeters installed beneath the litter layer (Oi), the fermented/humified horizon (Oe/Oa), and the mineral topsoil in November 2017 at both study sites. The two uppermost lysimeters were 20 cm × 20 cm acrylic glass plates with a mesh and three holes ensuring contact with the soil underneath. The third lysimeter beneath the mineral topsoil (A horizon) was a 19.5 cm × 25.5 cm pod with a 3.3 cm high rim filled with three layers of acid-washed quartz sand of different grain sizes forming a hydrological continuum to ensure proper drainage of the overlying mineral soil. More details and figures of the lysimeter design are given in the supplementary material (SI2) of Fetzer et al. (2022). This lysimeter design is similar to the ones used by Makowski et al. (2020). The lysimeters at the three depths were installed adjacent to each other. They were slightly inclined and connected to polyethylene (PE) bottles. Following installation, the plots were left to recover from disturbance for five months.

Table 1 Soil organic carbon (SOC), soil total nitrogen (N), soil totalphosphorus (P), water dispersible colloidal organic C (OC), andwater-dispersible colloidal P concentrations in Oi, Oe/Oa, and A horizons fromeach the control treatment (unfertilized) at the high-P (Bad Brückenau) and thelow-P site (Unterlüss).

Site	Horizon	Horizon thickness ¹	SOC ¹	Total N ¹	Total P ¹	Water-dispersible colloidal OC ²	Water-dispersible colloidal P ²
			g C kg ⁻¹	g N kg ⁻¹	g P kg ⁻¹		
high-P site	Oir	2	451 (± 1)	17 (± 0.1)	0.96 (± 0.08)	192 (± 12)	1.9 (± 0.1)
high-P site	Oe/Oa	2.5	352 (± 17)	19 (± 0.3)	2.3 (± 0.32)	204 (± 12)	5.3 (± 0.3)
high-P site	A	5	178 (± 20)	12 (± 1.3)	3.02 (± 0.28)	156 (± 9)	5.3 (± 0.3)
low-P site	Oi	4.5	391 (± 9)	14 (± 0.3)	0.79 (± 0.08)	1728 (± 104)	3.4 (± 0.2)
low-P site	Oe/Oa	6	230 (± 25)	11 (± 1.5)	0.53 (± 0.08)	792 (± 48)	8.1 (± 0.5)
low-P site	A	5	70 (± 14)	3.4 (± 0.7)	0.17 (± 0.02)	384 (± 23)	11.2 (± 0.7)

¹Samples were taken in July 2019; values represent means ± standard error of three field replicates, horizon thickness measured above the lysimeters (Fetzer et al. 2022)

²Determined for samples collected in autumn 2013 (Missong et al. 2018b)

2.4 Irrigation and Leachate Sampling

In order to collect leachates with minimal storage time in the collection bottles, leaching events were artificially induced by irrigating an area of 1 m² above the installed lysimeters in July 2018, October 2018 and March 2019. The irrigation water was P- and N-free artificial rainwater matching pH (5.5) and electrical conductivity (25 $\mu\text{S cm}^{-1}$) of the average local canopy throughfall at both sites. The artificial rainwater was applied at a constant rate of 20 mm h⁻¹ using an Accu-Power sprayer (Birchmeier Sprühtechnik AG, Switzerland). This volume corresponds to 71% and 61% of the pore volume above the lysimeters in the mineral topsoil at the low-P and the high-P site, respectively. The application rate of 20 mm h⁻¹ mimics maximum rainfall intensities at the study sites. The amount of water added with irrigation corresponds to the average weekly precipitation at the high-P site and exceeds it by 33% at the low-P site. The total volume of irrigation water equaled roughly 8% of total yearly throughfall at the high-P site and 16% of throughfall at the low-P site.

Leachate was collected over a period of 1.5 h from the start of the irrigation. Therefore, the leachates includes the ‘first flush’ transporting ‘loosely bound’ colloids, which had a high organic content compared to the soil (Lægdsmand et al. 1999). This ‘first flush’ may only be caused already within the first few mm of percolation, as shown in the experiments of Vendelboie et al. (2011) using agricultural soils of varying clay content. Therefore, the colloid (size) composition and P_{coll} concentrations we measured for the period of 1.5 h after the simulated heavy rainfall event should also cover colloids which would naturally be released over longer percolation period. The first flush, typically contributes most to the leaching of P during rainfall events (Bol et al. 2016). Leachates were collected in PE bottles, stored in cooling boxes, and aliquots shipped cooled within 2–3 days to the Agrosphere Institute (IBG-3) of the German Research Center Jülich. There, the samples were stored at 8 °C and analyzed within 7 days using AF4. In total 119 aliquots of leachate samples were analyzed.

2.5 Sample Analysis

The colloids were size separated using the AF4 technique (Postnova Analytics, Landsberg, Germany), a technique to separate particles between 1 nm and 1 μm size. The AF4 was coupled online to an UV-vis detector (Postnova Analytics), a dynamic light scattering detector (DLS; Malvern Instruments), an organic carbon (OC) detector (OCD; DOC laboratory Dr. Huber, Germany), and an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7500, Agilent Technologies, Japan). The setup allows for determining the

nanoparticle composition depending on the size. The P concentration was analyzed using the ICP-MS. Details on the AF4 technique and analytical element determination are given elsewhere (Giddings 1993; Nischwitz et al. 2016).

The samples were gently sonicated (360 J) to prevent secondary aggregations before analysis. The parameters of the separation are nearly same as in the separation method of Gottselig et al. (2014). We used a 1-kDa polyethersulfone (PES) membrane and a carrier solution of 25 μM NaCl. For the ICP-MS-coupled measurements, a volume of 4 mL was injected, the focus time was 25 min at a cross flow of 3 ml min⁻¹ to also separate the small particles. After focusing, the particles were separated by a linear cross flow decrease for 40 min. The largest particles were released at a constant flow of 0.5 ml min⁻¹ detector flow at 0 ml min⁻¹ cross flow for 30 min. For the OCD measurement, smaller sample volumes of 1 ml were injected and the focus time was set to 15 min. Three colloidal size fractions were identified (<20 nm, 20–70 nm, and 70–450 nm). The particle size resolution of the applied FFF separation were also checked by analysis of latex standards of 21 nm, 64 nm, 100 nm, and 600 nm size (from Postnova Analytics) under the same conditions as the samples. Quantification using ICP-MS and OCD was based on external multipoint calibration (4 up to 8 concentration levels) and linear regression.

A comparison of TP concentrations measured using ICP-OES at the Institute of Soil Science of the Martin Luther University Halle and using ICP-MS at the Agrosphere laboratory of the Research Center Jülich revealed roughly 25% smaller TP concentrations for the ICP-MS compared to the ICP-OES (Figure S1, online supporting material). This means that P concentrations reported here might be underestimated and conservative as compared to the P concentrations reported by (Fetzer et al. 2022). In order to ensure data consistency, we used the TP concentrations measured by ICP-MS only to calculate the share of P_{coll} to TP. For 6 samples collected in October 2018, TP concentrations determined using ICP-MS in the Agrosphere laboratory were 2 to 14 times smaller than concentrations determined using ICP-OES. In order to avoid a strong overestimation of the P_{coll} share of TP, we used ICP-OES TP concentrations multiplied with a factor of 0.75 for calculating the P_{coll} fraction of these 6 samples (see online supporting material for details).

2.6 Data Analysis and Statistics

Since the P_{coll} samples were aliquots of the samples analyzed by Fetzer et al. (2022), we used their water fluxes and TP fluxes to derive P_{coll} fluxes. The effects of season (month of sample collection), size fraction, and nutrient addition on P concentrations were tested using the Tukey Honest Significant Difference Test for unequal number of samples, using

the STATISTICA 13.3 software (TIBCO Software Inc. Palo Alto, USA).

3 Results

The study year 2018 was exceptionally dry. Annual precipitation amounted to 772 mm at the BBR high-P and 463 mm at the LUE low-P site, which corresponded to 68% and 60% of the long-term mean annual precipitation (Figure S2). This rainfall deficit was even more pronounced for the period May to October, amounting only to 50% of the long-term average at BBR and 40% at LUE. Maximum soil temperatures in summer were 18 °C at both sites (Figure S1; Fetzer et al. 2022).

The addition of P and P+N did neither affect the P_{coll} concentrations nor the P_{coll} fraction of TP concentrations in leachates significantly ($p > 0.05$). Therefore, the P_{coll} data were combined without differentiation according to fertilization. Concentrations of P_{coll} in leachates differed

significantly between months for both sites, with the largest concentrations occurring upon the irrigation after the summer drought (Fig. 1; July vs. October: $p = 0.005$; July vs. March: $p = 0.0001$; October vs. March: $p = 0.02$; HSD test for unequal n). During the sampling campaign in July, P_{coll} concentrations reached $79 \mu\text{g P l}^{-1}$ at the LUE low-P site (Fig. 1). The July concentrations at BBR significantly exceeded those in March by a factor of 5. At LUE, the July concentrations were 2.5 times larger than the October and 8 times larger than the March concentrations (Fig. 1). The increase in leachate P_{coll} concentrations after the summer drought was mainly due to increased concentrations of P in the medium sized (20–70 nm) fraction (Fig. 2). Leachate P_{coll} concentrations, as well as P_{coll} proportion of TP did not change significantly with increasing sampling depth from the Oi layer down to the lower boundary of the mineral topsoil (Table 2).

While the absolute P_{coll} concentrations in leachates differed strongly between July on the one hand and October and March on the other hand, the P_{coll} share of TP

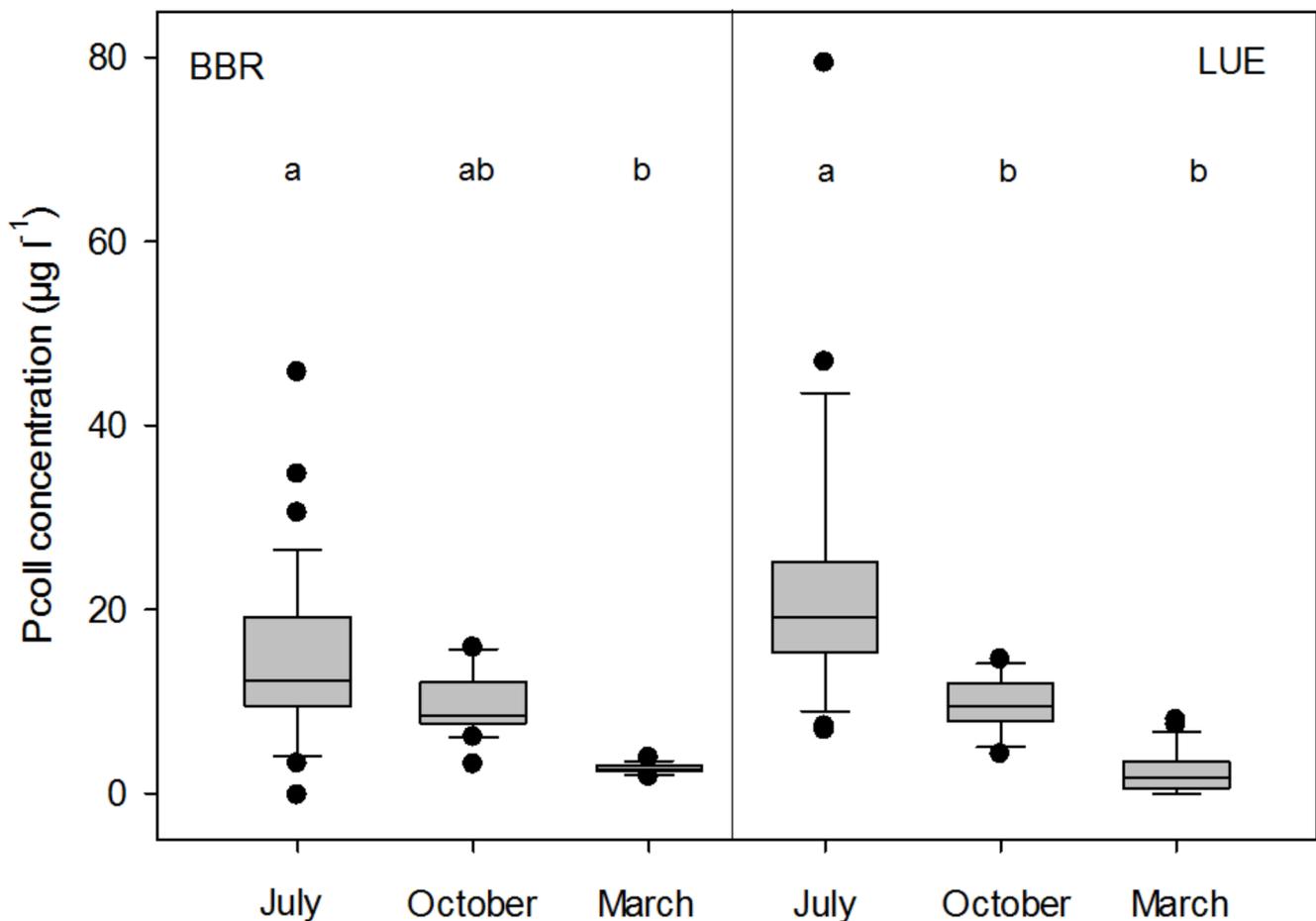


Fig. 1 Concentrations of colloidal phosphorus (P_{coll}) at the BBR high-P and LUE low-P sites in July, October and March are shown. Monthly medians are calculated from P_{coll} concentrations across all horizons from both fertilization and control plots. The median is represented by

the horizontal line in the grey box representing the 25–75% interval of the data. Whiskers indicate the 5–95% interval, and dots show extreme values. Different letters denote significant differences in concentrations across months at each site

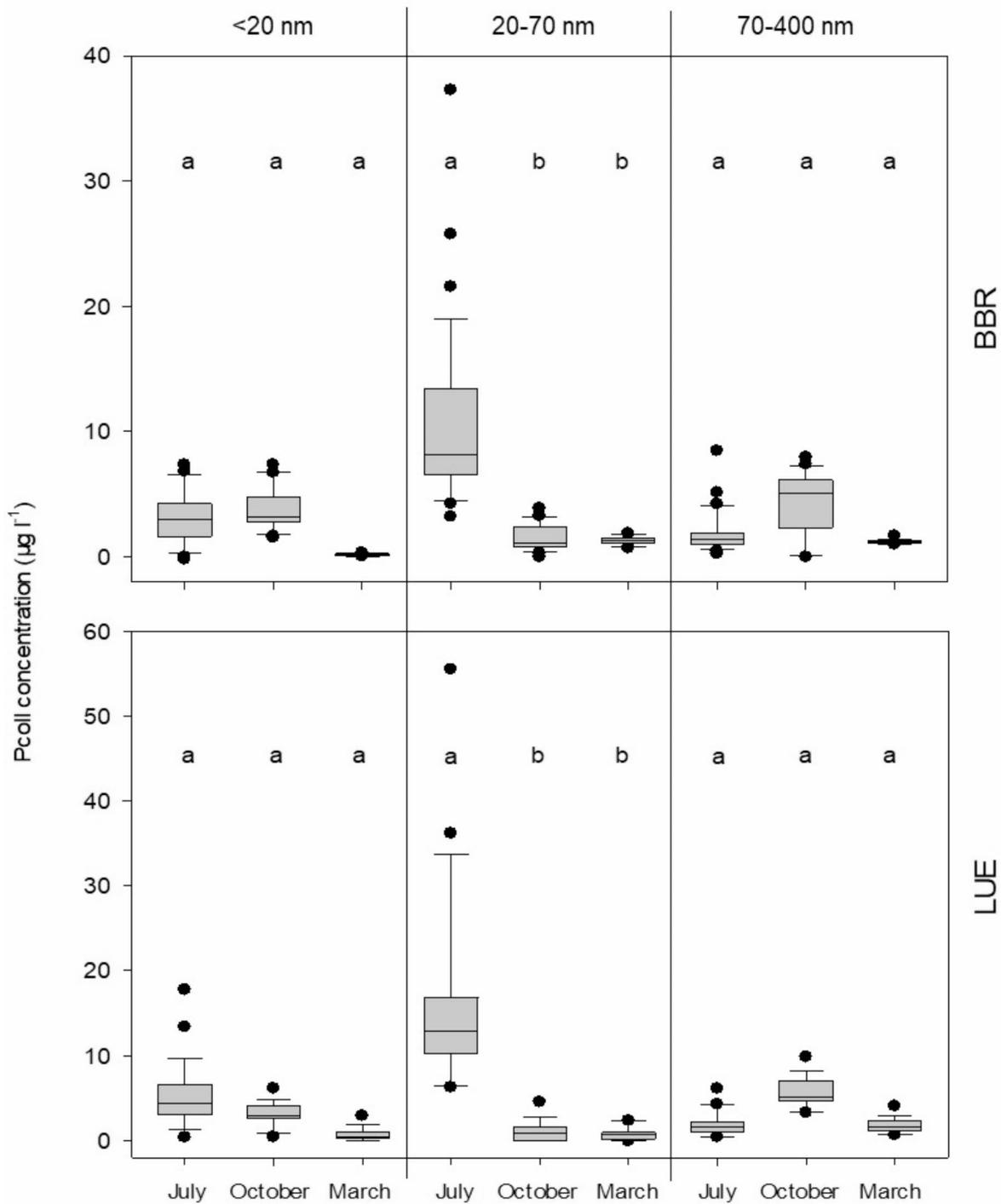


Fig. 2 Concentrations of P in different colloid size fractions. Different letters indicate significant differences between P_{coll} concentrations (calculated from P_{coll} concentrations across all horizons and fertilisa-

tion plots) within each size fraction for samples collected in different months. Size fractions are as follows: 1st fraction: <20 nm, 2nd fraction: 20–70 nm, and 3rd fraction: 70–400 nm

Table 2 Concentrations of P below litter layers (Oi), Oe/Oa horizons, and a horizons of the two study sites Bad Brückenau (BBR) and Unterlüss (LUE). Arithmetic means of all samples \pm standard error and median concentrations. P_{coll} : colloid-bound P, DIP: dissolved inorganic P, DOP: dissolved organic P. DIP and DOP concentrations refer to sample aliquots used in the study of Fetzer et al. (2022)

Site	Soil horizon	P_{coll} mean \pm SE ($\mu\text{g l}^{-1}$)	P_{coll} median ($\mu\text{g l}^{-1}$)	DIP mean \pm SE ($\mu\text{g l}^{-1}$)	DIP median ($\mu\text{g l}^{-1}$)	DOP mean \pm SE ($\mu\text{g l}^{-1}$)	DOP median ($\mu\text{g l}^{-1}$)
BBR	Oi	10 (± 3)	10	207 (± 53)	115	60 (± 11)	48
	Oe/Oa	11 (± 3)	10	256 (± 44)	291	60 (± 9)	60
	A	12 (± 3)	10	211 (± 47)	152	65 (± 8)	54
LUE	Oi	11 (± 2)	10	175 (± 39)	116	39 (± 6)	38
	Oe/Oa	16 (± 3)	9	282 (± 71)	115	44 (± 5)	46
	A	10 (± 3)	7	174 (± 65)	46	35 (± 7)	33

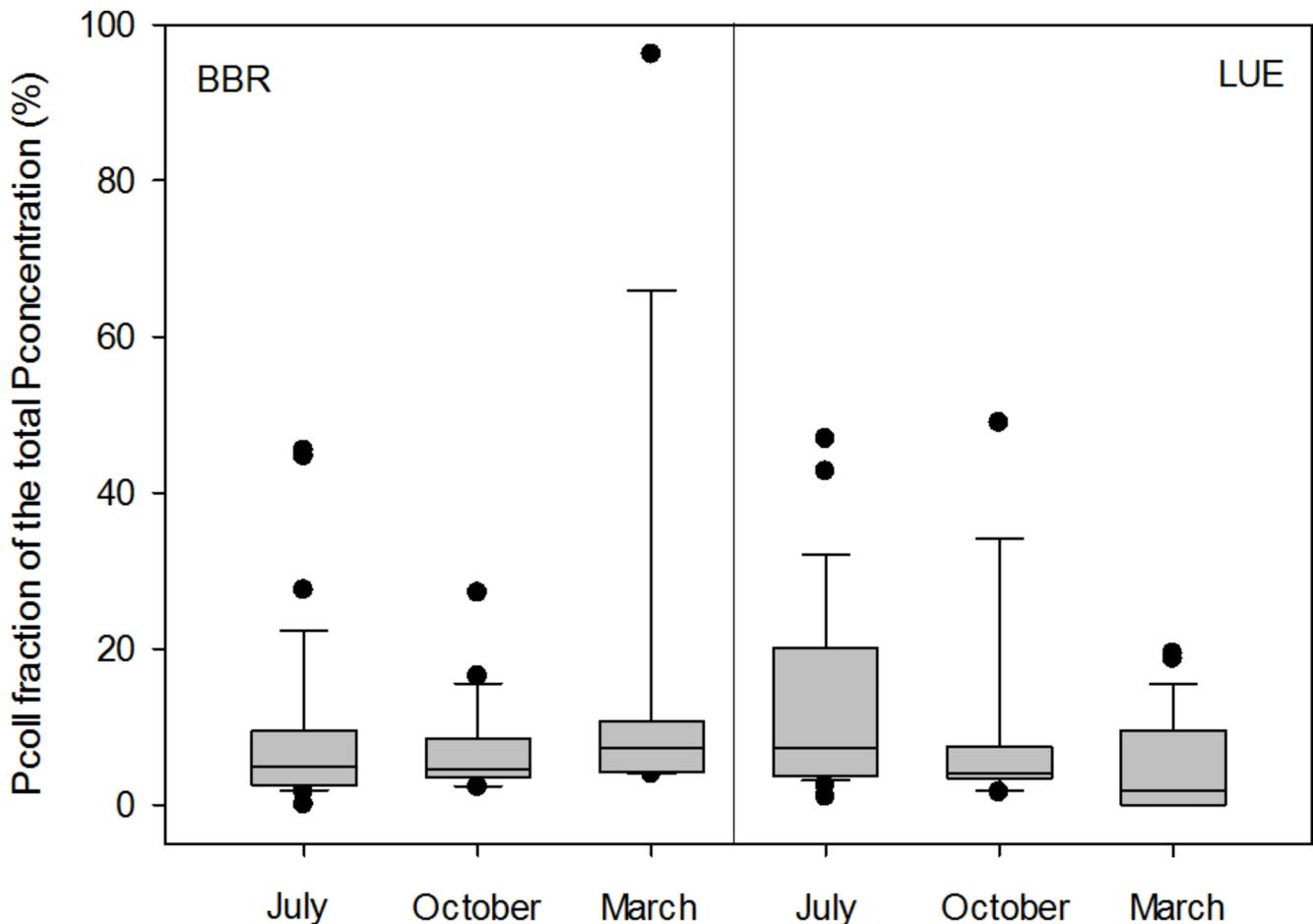


Fig. 3 Contribution of P_{coll} to total phosphorus (TP) concentrations at the BBR high-P site and LUE low-P site, based on P_{coll} concentrations across all horizons from fertilization and control plots

concentrations changed little across sampling periods, as TP concentrations varied proportionally to P_{coll} concentrations (Fig. 3). There were no significant differences between the P_{coll} shares of TP between the two sites and across the three sampling periods. The maximum contribution of P_{coll} to TP in few leachates was up to 96%, but in most samples the P_{coll} fraction was far smaller. The mean contribution was $10\% \pm 14\%$ (mean \pm standard deviation; median: 5%) for BBR and $13\% \pm 26\%$ (median: 4%) for LUE across all months (see Fig. 3 for distribution).

We found no significant differences between the C:P ratios of the total colloids and nanoparticles (organic C:P of particles as w: w) in leachates from October and July and across Oi, Oe/Oa, and A horizons with median values of 109 to 163 (Fig. 4). Interestingly, the three size fractions had different C:P ratios, i.e. the C:P ratios of the 20–70 nm fraction were significantly smaller than those of the <20 and the 70–450 nm fraction. The C:P ratios of colloids of the 20–70 nm fraction that were leached during irrigation after the drought period in July were slightly smaller (median

values of 29 for LUE and 31 for BBR) than those in October (LUE: 39; BBR: 34).

Correlating P_{coll} concentrations against DOP concentrations from the study of Fetzer et al. (2022) revealed that P_{coll} concentrations increased significantly with increasing DOP concentrations (Fig. 5). This increase was stronger for leachates sampled at the LUE low-P site than for samples collected at the BBR high P site. The regressions between P_{coll} and DOP concentrations suggested that P_{coll} contributed on average 27% of DOP at LUE, while at the BBR site P_{coll} made up 17% of DOP on average (Fig. 5).

4 Discussion

4.1 Relative Contribution of P_{coll} to TP Leaching in Forest Soils

The constant contribution of P_{coll} to TP concentrations with no significant differences between sites and sampling periods (Fig. 2) suggests that the mobilization and transport of both TP and P_{coll} were driven by the same factors. The average proportion of roughly 10% of TP being leached as P_{coll} was comparable with the results from the column experiment using soil from the LUE site (Missong et al., 2018). The size distributions of colloids leached from the LUE soil columns and field experiment were similar over the three

colloid size fractions, with the majority of P_{coll} recovered in the 20–70 nm fraction (<20 fraction: 0.5, 20–70 nm fraction: 0.9, 70–450 nm fraction: 0.4 $\mu\text{g l}^{-1}$; (Missong et al. 2018b)). However, in the column experiment with BBR soil, the P_{coll} proportion was substantially greater (54%) than for the field leaching experiment, probably because of much smaller TP concentrations in the former. Although, the P_{coll} fraction of TP observed in field lysimeter and in soil column leachates (Holzmann et al. 2016; Missong et al. 2018b) were smaller than the detected P_{coll} shares of TP in forest stream headwaters (Gottselig et al. 2017b), the order of magnitude of P_{coll} concentrations observed here was in the range of values reported for stream waters and topsoil eluates (Gottselig et al. 2017a, b; Missong et al. 2018a). Additionally, C:P ratio of leachate and stream water colloids are smaller than C:P ratios of bulk soils, demonstrating the enrichment of the colloidal soil fraction with P. The forest stream waters colloidal C:P ratios in a European transects (Gottselig et al. 2017b) were larger than our lysimeter leachates, but the distribution of C:P ratios among colloid size fractions was similar, i.e., the 20–70 nm fraction featuring smallest ratios among the three fractions. The soil leachate C:P ratios of the 20–70 nm fraction from LUE and BBR were in range of ratios reported for soil microbial biomass (e.g., Griffiths et al. 2012). Therefore might also be indicative of some contribution of microbial residues to the colloids of particularly that fraction.

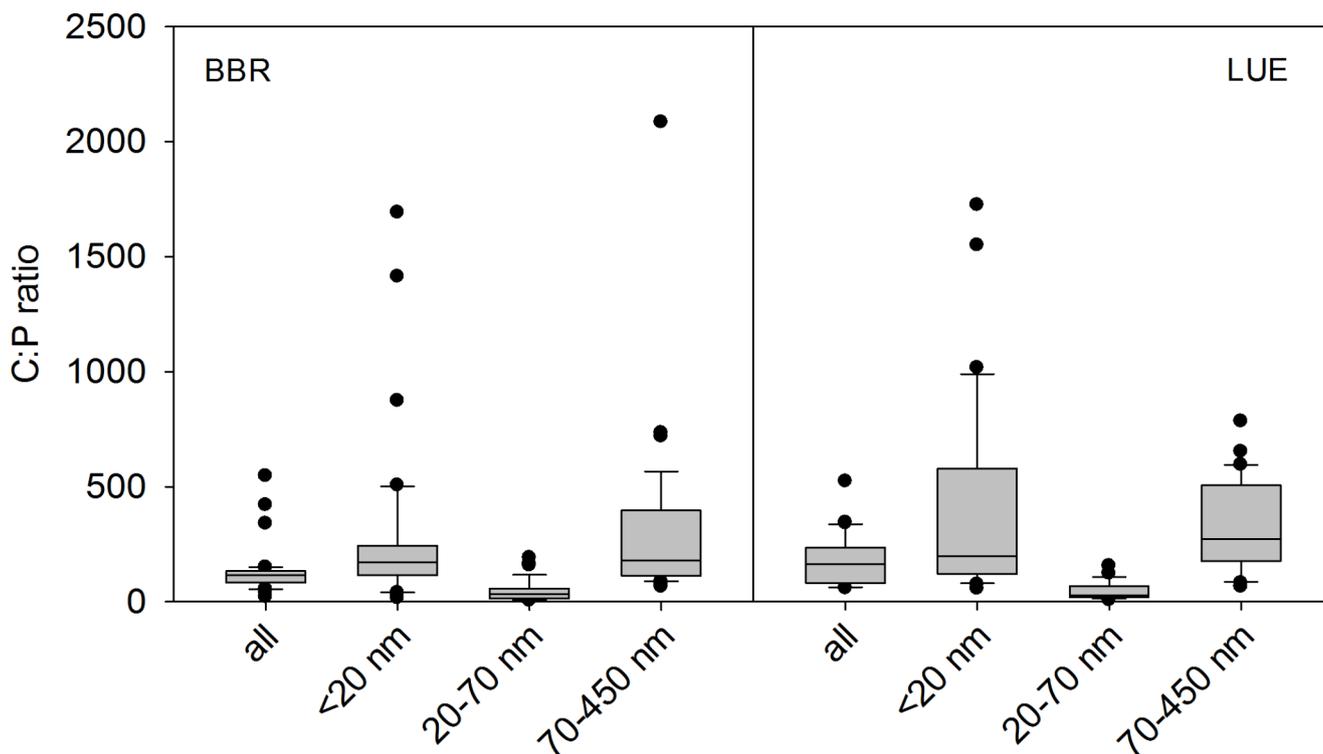


Fig. 4 The C:P ratio (w:w) of P_{coll} and its different size fractions in leachates collected in July and October 2018 Data are from samples across all horizons from both fertilization and control plots

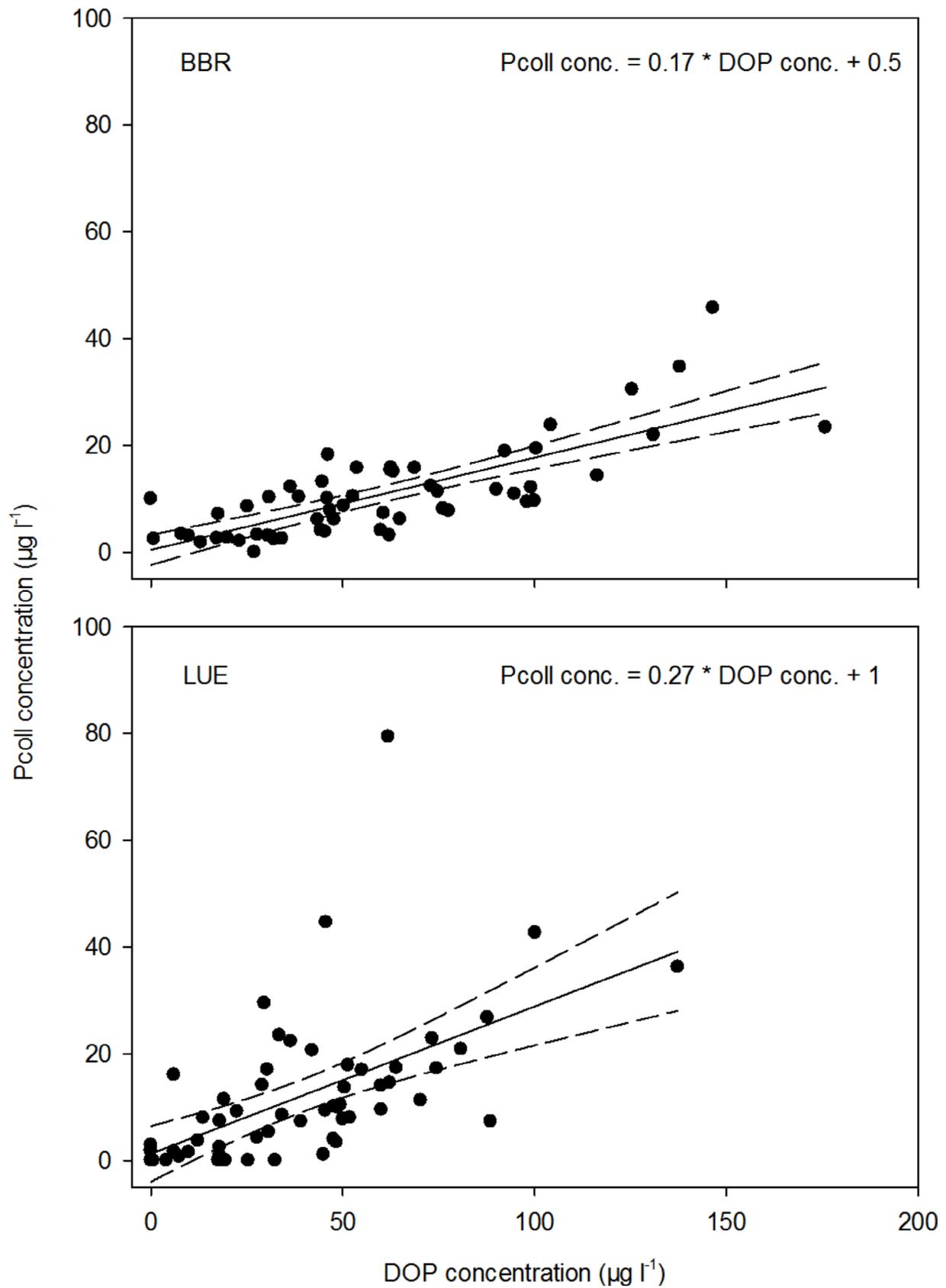


Fig. 5 Relationship between P_{coll} concentrations and DOP concentrations as determined in the study of Fetzer et al. (2022). P_{coll} and DOP concentration data are from samples across all horizons, including both fertilization and control plots, collected across all three months

Table 3 Estimates of colloidal P (P_{coll}) leaching flux from organic layers and a horizon to deeper soil horizons. TDP: total dissolved P; other abbreviations as in table 2

Site	Horizon	TDP flux of control ¹	DOP flux of control ¹	P_{coll} flux ^{2/3}	P_{coll} flux ^{2/4}
		mg m ⁻² yr ⁻¹	mg m ⁻² yr ⁻¹	mg m ⁻² yr ⁻¹	mg m ⁻² yr ⁻¹
BBR	Oi	49.7 (±4.6)	22.1 (±2.4)	5 / 3	4
BBR	Oe/Oa	59.9 (±8.1)	19.9 (±2.3)	6 / 3	3
BBR	A	39.2 (±14.5)	13.6 (±3)	4 / 2	2
LUE	Oi	43.4 (±2.1)	10.5 (±1.5)	6 / 2	3
LUE	Oe/Oa	28.2 (±13.2)	5.8 (±1.3)	4 / 1	2
LUE	A	12.2 (±3.8)	3.6 (±0.6)	2 / 1	1

⁽¹⁾ fluxes reported by Fetzer et al. 2022; Mean±standard error

⁽²⁾ Estimated P_{coll} leaching losses by multiplying the fluxes reported by Fetzer et al. (2022) with the P_{coll} fraction of total P or DOP

⁽³⁾ based on mean/median P_{coll} fraction of total P

⁽⁴⁾ based on P_{coll} fraction of DOP derived from regressions between P_{coll} and DOP concentrations of Fig. 4

Fetzer et al. (2022) reported annual mean fluxes of total dissolved P of 12–60 mg m⁻² y⁻¹ with leachates at the sites of this study (Table 3). Our estimates of P_{coll} fluxes of 2 to 6 mg m⁻² y⁻¹ from the organic forest floor layer and mineral topsoils at BBR and LUE (Table 3) should be taken as first approximations as they are based on few sampling dates and the rather constant P_{coll} shares in TP. Nevertheless, to the best of our knowledge, these are the first estimates of P_{coll} fluxes ever published for forests, and therefore provide first quantitative information on the potential role of P_{coll} in the P cycling in forests. The estimated P_{coll} fluxes in the organic layers and mineral topsoils are in the same order of magnitude as the 2–9 mg m⁻² y⁻¹ annual P export rates with runoff water in forest catchments in Germany (Sohrt et al. 2017). They are substantially smaller than the reported P inputs with throughfall in a beech forest in the Black Forest of about 60 mg m⁻² y⁻¹ (Sohrt et al. 2017). Generally, average estimates for above canopy atmospheric P deposition mostly range from 10 to 100 mg P m⁻² y⁻¹ and discharge losses are typically in the range of 1–10 mg P m⁻² y⁻¹ (Cole and Rapp 1981; Sohrt et al. 2017). Therefore, P_{coll} exports at these estimated quantities are unlikely to contribute much to the long-term P depletion of the studied forest ecosystems and their soils (e.g., Hedin et al. 2003). Even on the longer time scale, the P_{coll} fluxes appear small as compared to the P stocks. For instance, the organic layers at the two studied forest sites hold between 5000 and 7000 mg P m⁻². Relative to P stocks in the topsoils, the P_{coll} fluxes were more relevant for the low-P site LUE than for the high-P site BBR (Table 3). At both studied forest sites, given that the P stock of the organic layer is replenished by annual litter fall, P_{coll} export of less than 10 mg m⁻² seems marginal, but it contributes to translocation of P from organic layers and mineral topsoils into deeper soil compartments, where the accessibility of P for the vegetation is lower. Our results clearly show that despite its high reactivity and strong sorption, DIP (i.e. ortho P) dominates TP leaching from organic layers and topsoils of the forests investigated.

4.2 Contribution of P_{coll} to DOP

We consider OM as the major source of colloids and natural nanoparticles in leachates given the high OC content of natural nanoparticles and the small amount of nanoparticles and colloids found in water-dispersible colloids from OM poor subsoils (Missong et al. 2018b). Therefore, it can be expected that the operationally defined DOP (<450 nm, TP minus molybdate-reactive P) comprises a part of P_{coll} .

Indeed, P_{coll} concentrations correlated linearly with DOP concentrations (Fig. 5), which supports the idea that part of the operationally defined DOP is made up by colloids and nanoparticles carrying P as also shown by Gu et al. (2018) for drying-wetting cycles of two riparian soils. The slope of the regression suggests that the colloidal contribution varies between 17% at the high-P site BBR and 27% at the low-P site LUE. The larger colloidal contribution to DOP in the sandy soils corresponds to the large mobility of colloids in the soil in column experiments (Holzmann et al. 2016; Missong et al. 2016) and DGT experiments (Konrad et al. 2021). Konrad et al. (2021) hypothesized that the mobility of colloidal organic matter-Fe/Al-P complexes in the sandy soil was linked to the podsolization.

The regression between P_{coll} and DOP concentrations at both sites implies that although the majority of the operationally defined DOP is truly dissolved, colloids made up a considerable portion of the leached operationally defined DOP.

4.3 Consequences of Artificial Heavy Rainfall after Drought

Our irrigation study suggests that drought periods prior to heavy rainfall events trigger the leaching of P_{coll} . The drying-wetting effect was more pronounced for the low-P LUE site than for the high-P BBR site (Fig. 1). Missong et al. (2018b) reported slightly larger concentrations of water-dispersible colloidal P (WDC-P) for LUE than for BBR topsoil

samples despite bulk WDC concentrations being comparable from LUE and BBR topsoil. In line, Gerhard et al. (2021) found higher concentrations of molybdate-unreactive P (MUP), including WDC-P, in LUE topsoil leachates than in BBR eluates in a soil column experiment. Furthermore, the experiments of Konrad et al. (2021) showed that WDC isolated from the LUE topsoil have a particularly high diffusive mobility under water-unsaturated conditions. These findings suggest that the WDC-P fraction might be particularly relevant for the redistribution of P within the soil profile and for P leaching at LUE. However, the higher WDC-P stocks and greater diffusive mobility of WDC-P at LUE did not translate into overall higher P_{coll} concentrations in lysimeter leachates from organic layers or mineral topsoils than at BBR under field conditions. This suggests that effective boundary conditions in the field, such as variations in water contents or uptake and release of P by biota, might be more relevant for P_{coll} leaching than the total WDC stock and the diffusive mobility of WDC. Similarly, Siemens et al. (2008) concluded from the results of column experiments with sandy agricultural soils that increasing accumulation of soil P does not necessarily promote enhanced leaching of colloids and P_{coll} , because a multitude of factors other than soil P status exert control on the mobility of colloids.

Blackwell et al. (2009) reported that drying–rewetting and freezing–thawing cycles are two of the most common forms of soil abiotic perturbations resulting in P solubilization. The degree of solubilization depends on many variables, such as intensity, duration, and temporal patterns of stress, as well as the soil type. The mobilization of total and molybdate-reactive P from dried samples generally decreased as duration of rewetting increases from 0.5 to 24 h (Blackwell et al. 2013). Gu et al. (2018) reported an increased release of P_{coll} after soil drying in riparian OM rich soils. They suggest that the physically- and biologically-driven processes (soil aggregates disruption and microbial cell lysis, respectively) co-exist in soils during drying and rewetting cycles and enhance P_{coll} release after drying. Kjaergaard et al. (2004) noted in a column experiment that intense drying of a mineral arable soil strongly reduced the mobilization and leaching of colloids. They argued that the intense drying caused a strong association of particles hampering their dispersion upon subsequent rewetting. A reduced colloid mobility after drying periods exceeding 2.5–11 days has also been found by Majdalani et al. (2008) and Mohanty et al. (2015). The strong association of particles upon intense drying as observed in arable soils with low OC may not necessarily hold true for organic matter-rich surface layers and mineral topsoils in forests. Interestingly, Gerhard et al. (2021) observed no effect of drying–rewetting on molybdate-unreactive P concentrations (analogous to DOP) leached from the LUE and BBR topsoils. They argued that the drying period

of 3 to 5 days in their experiment might have been too short to cause die-off and lysis of microbial cells. This die-off and lysis of microbial cells likely caused the pronounced pulses of DOP and DIP observed in the laboratory experiments of Brödlin et al. (2019) and Hoemberg and Matzner (2018) as well as the field experiment of Fetzer et al. (2022). The latter revealed an increase in DOP (or molybdate-unreactive P) concentrations by a factor of 2.5 after the 2018 drought. Since DOP and molybdate-unreactive P may also comprise P_{coll} , these results support the idea that drying–rewetting can trigger the leaching of not only TP, but also P_{coll} . Hoemberg and Matzner (2018) and Fetzer et al. (2022) attributed the increase in P leaching upon drying–rewetting to the release of P from microbial residues, as indicated by the strongly decreasing C:P ratios. In our lysimeter study on P_{coll} , we found that C:P ratios in the 20–70 nm fractions were in the range of C:P ratios of microbial biomass. Together with the finding that only the P_{coll} of the 20–70 nm fraction increased in July, this supports the idea that lysis of bacterial cells might have strongly contributed to the increase of P_{coll} concentrations in July during rewetting after drought. Hoemberg and Matzner (2018) also mentioned disruption of soil aggregates as another possible source of leached P. Buenemann et al. (2013) investigated the release of molybdate-reactive and unreactive P ($<0.2 \mu\text{m}$) upon drying–rewetting in sterilized and unsterilized grassland soils in relation to changes in soil aggregation. They found that drying–rewetting induced disruption of aggregates, resulting in a reduction of mean aggregate diameter that was linked to an increase of extractable molybdate-unreactive P in sterilized as well as unsterilized soils. Since sterilization suppressed the release of organic C to lesser extent than that of P, Buenemann et al. (2013) concluded that the P released upon drying–rewetting was also from non-microbial sources. Most P_{coll} released upon irrigation after the summer drought in 2018 was in the 20–70 nm fraction. This fraction typically contains also larger amounts of Fe and Al than the smallest size fraction (Missong et al. 2018a; Wang et al. 2020), which supports the idea that some of the P_{coll} released upon rewetting derived from non-microbial sources. However, this does not preclude that mobilized microbial residues, such as cell envelopes, can be associated with minerals surfaces, and therefore show up in the 20–70 nm size fraction.

It is unlikely that reducing conditions during or following the artificial rainfall events caused a mobilization of P_{coll} , since no hydromorphic features were observed in the soils and because leached P_{coll} concentrations of the highly permeable sandy soil and the less permeable loamy soil were similar. Also, the time period of the sampling after the simulated rainfall events was short enough to prevent establishment of reducing conditions.

4.4 Effects of N and P Inputs into Forest Soils on P_{coll} Concentrations and Leaching

We hypothesized that the nutrient additions facilitate mobilization and leaching of P_{coll}, at least transiently under favorable soil moisture conditions. However, neither the addition of P nor P+N significantly ($p > 0.05$) affected P_{coll} concentrations. The addition of readily available P and P+N was expected to activate the soil microbial community, causing shifts in P_{coll} concentrations and the P_{coll} distribution across different size classes. Given the large surface area and reactivity of inorganic colloids, we also expected that the addition of P could induce the mobilization of such colloids upon excessive P sorption to them. Additions of P can induce mobilization of P_{coll} if resulting in P accumulations at the surfaces of Fe and Al (hydr)oxides that cause their surface potential to shift to values more negative than ~ -30 mV (Ilg et al. 2008). The fact that the addition of P and/or N neither changed the concentrations of P_{coll} nor its size distribution, suggests that any possible effects of P and N additions on P_{coll} were overridden by other drivers than microbial nutrient turnover or P sorption, such as variations in soil water, i.e., by drought. In contrast, in soil column experiments of Holzmann et al. (2016), an N addition equivalent to $10 \text{ g N m}^{-2} \text{ yr}^{-1}$ increased the concentrations of molybdate-reactive P (MRP) and molybdate-unreactive P in eluates, with the effect being much weaker for molybdate-unreactive P than for MRP. In that study, N addition did not change the P_{coll} fraction of TP concentrations in leachates, but increased the OC content and, in turn, decreased the Fe and Al contents of colloids. While the combination of increased MRP concentrations and unchanged P_{coll} fraction of TP could in principle result in increased absolute P_{coll} concentrations after N addition, no such increases were observed neither in the soil column experiment nor in our field lysimeter experiment.

5 Conclusions

Colloidal P accounts for about 10% of the TP leaching fluxes from the organic layers and mineral topsoils of two soils under beech forests. Significant relationships between P_{coll} and DOP concentrations indicate that a considerable part of the operationally-defined DOP is of colloidal nature. Similar to DIP and DOP, P_{coll} leaching is highly sensitive to rainfall events after droughts. The increase in P_{coll} leaching was approximately proportional to the increase in TP leaching because the contribution of P_{coll} to TP did not significantly change after drought. Related to P stocks, P_{coll} leaching was higher at the low-P site, indicating that low-P forest ecosystems are more sensitive to perturbations by dry spells. The P leaching losses in beech forest soils are mainly determined

by DIP and less by P_{coll}. Nevertheless, despite being a minor component of overall forest P budgets, P_{coll} still provides important further insight into processes governing the mobilization, transport, and cycling of P in forest ecosystems.

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Declarations

Conflict of Interest The authors declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in the manuscript “Leaching of colloidal phosphorus from organic layers and mineral topsoils of German beech forests in response to drought and nutrient inputs”.

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