Title:

Dominance of either physicochemical or biological phosphorus cycling processes in temperate forest soils of contrasting phosphate availability

Authors:

Bünemann EK^{1,2}, Augstburger S¹ and Frossard E¹

Abstract

The importance of organic phosphorus (P) mineralization for forest P nutrition may depend on the P status of the soil. Using an isotopic dilution technique, we measured organic P mineralization rates in two contrasting Cambisols under beech forest in Germany which had developed either on basalt, resulting in a silty-clayey soil high in available inorganic and total P, or on pleistocene sand, resulting in a sandy soil low in available inorganic and total P. To investigate if soil mixing during labeling causes artefacts in the assessment of microbial P immobilization and organic P mineralization we conducted a 38-day-long incubation experiment with labeling of pre-incubated soils either by mixing or by injection. Gross and net organic P mineralization were negligible and non-detectable against the great inorganic P availability in the soil developed on basalt, while biological and biochemical processes dominated P transformations by far in the sandy soil. A significant but transient pulse in soil respiration caused by mixing occurred in the silty-clayey soil, and it was accompanied by a transient increase in microbial P. Mixing of the sandy soil induced a small but persistent increase in respiration but a decrease in microbial P. Despite these effects on the microbial pool and activity, no evidence for an alteration of P transformation rates by soil mixing was found. This study suggests that labeling by injection might be feasible in P isotopic dilution studies, especially in low-P-sorbing sandy soils.

Keywords

Isotopic dilution; P-33; microbial activity; immobilization; texture; beech forest

1. Introduction

In view of decreasing foliar phosphorus (P) concentrations of the main European tree species over the last 20 years (Jonard et al., 2015; Talkner et al., 2015), a better understanding of forest P nutrition is needed, and a major knowledge gap concerns biologically mediated soil P transformations. The mineralization of soil organic P can make an important contribution to potentially plant available P, especially in ecosystems with low availability of inorganic P and high amounts of soil organic carbon (C) such as forests (Bünemann, 2015). Since phosphate released by mineralization reacts quickly with the soil solid phase, organic P mineralization can in most mineral soils only be assessed using isotopic dilution principles in incubation experiments with ^{32}P - or ^{33}P -labeled soils.

So far, only a few studies have used isotopic dilution approaches to assess the relative and absolute contribution of physicochemical and biological/biochemical processes to P availability in forest ecosystems. For acid organic soils, a pioneering isotopic dilution study indicated net transfer from unlabeled organic P into both available inorganic and microbial P (Walbridge and Vitousek, 1987). The methodology was subsequently further developed (Oehl et al., 2001) and applied to arable soils, in which P availability was found to be dominated (70-80%) by physicochemical processes (Oehl et al., 2004). In forest soils, however, P dynamics may be largely controlled by microbial activity (Olander and Vitousek, 2004; 2005). Indeed, in a low-P sorbing Podzol with very low concentrations of total P (31 mg P kg^{-1}) under pine plantation forest in France, gross organic P mineralization contributed 70-90% of total isotopically exchangeable P, with the proportion increasing over the duration of the experiment (Achat et al., 2009). The majority of this flux was attributed to remineralization of microbial P. In mineral

topsoils under forest in central Germany, biological/biochemical processes contributed 36-86% of total isotopically exchangeable P, depending on the soil type (Spohn et al., 2013). Further assessment of organic P mineralization rates by isotopic dilution on a range of soil types under forest is warranted, especially to clarify the effect of inorganic P availability on biological/biochemical processes.

Isotopic dilution studies to assess soil P transformation rates rely on a short-term isotopic exchange kinetics (IEK) experiment from which the baseline of isotopic exchange due to physicochemical processes only is extrapolated, and a parallel incubation experiment in which both physicochemical and biological/biochemical processes contribute to the isotopic dilution of phosphate in the soil solution (Oehl et al., 2001). The inverse of the specific activity ($^{33}\text{P}/^{31}\text{P}$) of phosphate in solution yields isotopically exchangeable P (the so-called *E*-value, in mg P kg⁻¹). The difference between *E*-values measured in the incubation experiment and *E*-values extrapolated from the IEK experiment corresponds to gross organic P mineralization. In addition, net organic P mineralization can be derived by subtracting microbial P immobilization from gross organic P mineralization (Bünemann et al., 2007).

Such experiments are conducted under steady-state conditions, i.e. with soil respiration rates and concentrations of phosphate in the soil solution being constant, and therefore render basal mineralization rates, i.e. in the absence of flush effects (Oehl et al., 2001). Typically, intensive soil mixing during labeling is used in order to reach a homogeneous distribution of the added tracer in the soil. This mixing can result in transient pulses in soil respiration (Achat et al., 2009; Bünemann et al., 2012). At the same time, very rapid microbial P immobilization has been observed in grassland soils with low availability of inorganic P and a large microbial P pool

(Bünemann et al., 2012). This has raised the question whether the intensive soil mixing during labeling may stimulate microbial P uptake, inflating estimates of microbial P immobilization and leading to a transient deviation from steady-state conditions. This idea is supported by the fact that very small amounts of labile C substrates in the order of 5-15 $\mu\text{g g}^{-1}$ can activate soil microorganisms, causing an up to fivefold release of C compared to the amount added (de Nobili et al., 2001). If microorganisms soon return to a state of resting or very low activity, this may explain why the recovery of the added P tracer in the microbial biomass has sometimes been found to be rather constant over time (Bünemann et al., 2012; Bünemann et al., 2004; Oberson et al., 2001). In addition, the potential destruction of aggregates during soil mixing may release P or increase P sorption (Borda et al., 2014; Sinaj et al., 1997).

The biggest soil disturbance occurs already during sampling and homogenization, and this effect may be particularly large in forest soils which have remained undisturbed for decades. Therefore, ideally organic P mineralization would be assessed in undisturbed soil cores, as practiced in ^{15}N isotopic dilution studies (Rütting et al., 2011). For P, the feasibility of this approach may depend on the texture and P sorption properties of the soil. In a first step, this could be assessed on homogenized samples together with an evaluation of the potential for artefacts resulting from soil mixing at labeling.

Our main objectives were i) to assess the relative contribution of physicochemical and biological/biochemical processes to P availability in two forest soils of contrasting P status and ii) to assess the effect of soil mixing during labeling on soil P transformation rates. More specifically, we aimed to assess the potential for artefacts when assessing microbial P immobilization and organic P mineralization in soils labeled with ^{33}P by mixing, and to pre-

evaluate if it might be possible to conduct future ^{33}P isotopic dilution studies without soil disturbance. We conducted an incubation experiment with mineral topsoil from two sites under beech (*Fagus sylvatica* L.) forest on contrasting parent materials (basalt vs. pleistocene sand). We hypothesized that biological/biochemical processes would dominate P cycling in a low-P sorbing sandy soil containing little total P, in which assessment of P transformation rates by labeling without mixing would be possible. In contrast, we expected that labeling by injection would fail in a high-P sorbing clayey soil with a large total P stock due to inhomogeneous distribution of the label, and that physicochemical processes would be more important than biological/biochemical processes, at least in relative terms.

2. Material and Methods

2.1 Site description and soil sampling

Two sites under beech forest which are permanently monitored (BML, 1995) were selected for this study. Bad Brückenau (BBR) is located at about 800 m asl in Northern Bavaria, Germany (50°21'7.26"N, 9°55'44.53"E). Mean annual temperature is 5.5°C and mean annual precipitation 1000 mm. The Dystric Skeletic Cambisol (Hyperhumic, Loamic) (FAO/ISRIC/ISSS, 1998) has developed on basalt, and the vegetation consists of a more than 120 year-old pure beech stand, with a diverse herbal layer (up to 31 species on an area of 400 m²; Isaak Rieger, personal communication). Lüss (LUE) is located at 100 m asl in Lower Saxony, Germany (52°50'21.77"N, 10°16'2.37"E). Mean annual temperature is 8.4°C and mean annual precipitation 775 mm. The Hyperdystric Folic Cambisol (Arenic, Loamic, Nechic, Protospodic) has developed on pleistocene sand. The vegetation is dominated by beech trees which are between 100 and 120 years old and shrubs or herbs are almost completely absent. The current

annual N deposition at the two sites is 6-8 kg N ha⁻¹ at BBR (Schönthaler and von Andrian-Werburg, 2008) and approximately 13 kg N ha⁻¹ at LUE (NWFVA, 2008).

Soil samples were taken in October 2014 from the Ah1 horizon (0-7 cm) at BBR and from the Ahe horizon (0-7 cm) at LUE at several locations within an area of 9 m², sieved moist at 2 mm and combined to one composite sample per site. Small portions of these samples were dried at 40°C or frozen at -20°C, while the remainder was stored at 4°C for a few days before starting the preincubation. The Ah1 from BBR contained 80, 550 and 370 g kg⁻¹ of sand, silt and clay, respectively, while the corresponding numbers for the Ahe at LUE were 750, 190 and 60 g kg⁻¹ (Jaane Krüger, personal communication). The maximum water holding capacity (WHC) of the sieved soils determined by placing the saturated soils on filter paper in funnels and letting the water drain for 24 h was 1.16 g H₂O g⁻¹ dry soil for BBR, while it was 0.37 g H₂O g⁻¹ dry soil for LUE.

2.2 Experimental design and set-up

An incubation experiment with ³³P labeling of soil from both sites was conducted. It had a two-factorial design with two soils (BBR and LUE) and two levels of soil mixing at labeling (mixed and unmixed). The incubation experiment was complemented by IEK experiments in order to determine the baseline of isotopic exchange due to physicochemical processes only. The incubation experiment lasted six weeks, with determination of ³¹P and ³³P in water-extractable phosphate, resin-extractable and microbial P at seven sampling dates. In parallel to the incubation experiment, soil respiration was determined on similarly treated but unlabeled soils in weekly intervals.

In more detail, the gravimetric water content of the sieved soils was at 78% and 66% of the maximum WHC for BBR and LUE, respectively. Since the samples were taken neither immediately after rainfall events nor after a drought, we decided to conduct the incubation experiment at 75% of the WHC for both soils, which is in the range of the typical soil moisture in the field. In order to be able to add the labeling solution, the soils were air-dried slightly before pre-incubation until reaching 68% and 52% of the WHC for BBR and LUE, respectively. At these water contents, soil equal to 75 g dry soil was placed in plastic petri dishes (5 cm height, 10 cm diameter) at densities of 0.5 and 1 g dry soil cm⁻³ for BBR and LUE, respectively, and pre-incubated at room temperature in the dark for 14 and 24 days for BBR and LUE, respectively. We considered 14 days of pre-incubation sufficient to reach steady-state conditions and the longer pre-incubation at LUE was due only to the earlier sampling at this site.

Two days before soil labeling, the IEK experiments were conducted in triplicate, subsampling soil from one petri-dish per soil. On the day of soil labeling (day 0), the soil-specific ³³P labeling solutions (made from carrier-free phosphoric acid) were added in 6 ml solution per 75 g dry soil. For the mixed samples, the 6 ml were spread on top and the samples were immediately mixed for five minutes with a spatula directly in the petri dishes. For the unmixed samples, 3 ml were added by injection with a pipette tip, pulling the tip upwards while releasing the solution to the soil (6 injections of 0.5 ml each which were evenly distributed over the surface area) and the other 3 ml were spread on the surface as evenly as possible. This labeling procedure was chosen after a pre-test with the color tracer Brilliant Blue had shown that with injection, the color concentrated mainly in the bottom layer, whereas it did not infiltrate much with addition at the top, especially in BBR (supplementary information, Figure S1). Therefore, the combination of the two approaches yielded the most homogeneous distribution of the tracer. To reach 75% of the

WHC, BBR samples additionally received 0.5 ml H₂O. The resulting soil label was 45.035 kBq g⁻¹ soil for BBR and 7.044 kBq g⁻¹ soil for LUE. The labeled soils in the petri dishes (seven per soil and treatment combination) were incubated at room temperature in the dark and sampled destructively throughout the experiment, with one petri dish removed at each sampling date (1, 4, 11, 18, 25, 32 and 38 days after labeling) and subsamples taken after thorough mixing of the soil in the petri dish for determination of water-extractable phosphate and microbial P. The water content was checked gravimetrically every week and evaporation losses (maximum 0.04 g H₂O g⁻¹ soil) were replaced by adding H₂O.

For the determination of soil respiration, a separate set of samples of 20 g dry weight equivalent was pre-incubated in aluminum containers (3 cm height, 5.5 cm diameter) under the same conditions as above, with four replicates per soil and treatment. On day 0, the water content was raised to 75% of the WHC by adding H₂O either by injection or by mixing. The samples were then incubated under the same conditions as above, but in tightly closed jars which included alkaline traps. Traps were changed and titrated in weekly intervals, starting on day 2.

2.3 Analytical methods

2.3.1 General soil characteristics

Soil pH was measured on moist soil in a soil-water suspension (1:25 soil:water ratio) after horizontal shaking for 90 min. After letting the soil settle for 20 min, the pH was measured in the supernatant using an electrode (ORION, model 720A). Total C (C_{tot}) and N (N_{tot}) were determined on finely ground samples by dry combustion (ThermoFisher Flash EA 1112 series). Microbial C (C_{mic}) and microbial N (N_{mic}) were determined on moist soils by 24 h chloroform fumigation and extraction of fumigated and non-fumigated subsamples with 0.5 M K₂SO₄ in a

1:4 soil:solution ratio (Vance et al., 1987). The frozen filtrates were analyzed with a TOC/TN analyzer (Formacs^{SERIES}, Skalar, The Netherlands). The difference of total organic C extracted from fumigated minus non-fumigated subsamples was taken as C_{mic} , and N_{mic} was calculated accordingly from total dissolved N. No conversion factors were applied, since they are soil-specific and have not been determined for these soils.

2.3.2 Total soil P pools

Total P (P_{tot}) was determined on finely ground subsamples by digestion with H_2SO_4 and H_2O_2 (Anderson and Ingram, 1993), followed by colorimetric P determination using malachite green (Ohno and Zibilske, 1991) in a photometer (UV-1800, Shimadzu). Soil organic P was assessed by the ignition method of Saunders and Williams (1955) as described by Kuo (1996). In detail, 1 g of ground soil was shaken for 16 h with 25 ml of 0.5 M H_2SO_4 before or after ignition at 550°C for 1 h, followed by colorimetric P measurement in the filtrates (0.2 μm) as above. Organic P (H_2SO_4 - P_o) was calculated as the difference between P extracted from ignited minus non-ignited samples (H_2SO_4 - P_i).

2.3.3 Phosphatase activity

Potential acid phosphatase activity was determined on frozen soils in a microplate assay with 4-methylumbelliferon phosphate (MUF-P) as substrate as developed by Marx et al. (2001) and modified by Poll et al. (2006). In detail, 50 μl of a soil suspension (1:100 w:v, suspended using ultrasound for 2 min) was pipetted with wide-opening tips into each well, buffered with 0.1 M MES buffer at pH 6.1 and incubated with the substrate at 30°C. The linear increase in fluorescence over 180 min was measured in a microplate reader (Biotek, FLx800) and converted

into hourly rates of substrate release using soil-specific standard curves with MUF in order to account for soil autofluorescence and quenching.

2.3.4 Water-extractable phosphate and microbial P

Water-extractable phosphate (P_w) was extracted by shaking moist soil equivalent to 10 g dry soil in 100 ml H_2O (accounting for water contained in the soil) for 16 h on an overhead shaker. In the incubation experiment, the samples were then put on magnetic stirring plates at 200 rpm for 10 min in order to create conditions similar to the IEK experiment. A subsample was taken with a plastic syringe, filtered at 0.2 μm (Minisart, Sartorius) and split into two aliquots. The first aliquot was used for the colorimetric determination of the concentration of phosphate ($^{31}P_w$) using malachite green (Ohno and Zibilske, 1991) in a 1 cm cell (UV-1800, Shimadzu) with a detection limit of 8 $\mu g P l^{-1}$ (Randriamanantsoa et al., 2013). In the second aliquot, the radioactivity ($^{33}P_w$) was measured by liquid scintillation counting using Ultima Gold in a beta-emission counter (Tri-carb 2500 TR, Packard Instruments, Meriden, CT).

Microbial P (P_{mic}) was determined after Kouno et al. (1995) by liquid hexanol fumigation and simultaneous extraction with anion exchange resin membranes. Moist soil equivalent to 2 g dry soil was shaken for 16 h in 30 ml H_2O together with a resin membrane (BDH #55164, 6 cm x 2 cm) previously saturated with carbonate. Four different subsamples were included: 1) non-fumigated subsamples rendering resin-extractable P (P_{resin}), 2) fumigated subsamples receiving 1 ml of 1-hexanol, 3) non-fumigated subsamples receiving 1 ml of a ^{31}P spike in the order of 35 and 6 mg $P kg^{-1}$ for BBR and LUE, respectively, and 4) non-fumigated subsamples receiving 1 ml of a ^{33}P spike which added 5 kBq g^{-1} soil and contained the same amount of ^{31}P as the ^{31}P spike. A single P spike was considered sufficient since a pre-test with three P spikes between 10

219 and 50 mg P kg⁻¹ had indicated a linear relationship between P added and P recovered in both
 220 soils. The resin membranes were rinsed with H₂O and eluted with 0.1 M NaCl/HCl. The
 221 concentration of ³¹P and the activity of ³³P in the eluates were determined colorimetrically and
 222 by scintillation counting, respectively.

223 The concentration of ³¹P_{mic} in mg kg⁻¹ was calculated as

$$224 \quad {}^{31}\text{P}_{\text{mic}} = ({}^{31}\text{P}_{\text{fum}} - {}^{31}\text{P}_{\text{resin}}) / {}^{31}\text{P}_{\text{rec}} \quad \text{Equation 1}$$

225 where ³¹P_{fum} and ³¹P_{resin} are the concentrations of ³¹P (in mg P kg⁻¹) extracted from fumigated and
 226 non-fumigated subsamples, respectively, and ³¹P_{rec} is the fraction of the added P spike that is
 227 recovered on the resin membranes, which is calculated as

$$228 \quad {}^{31}\text{P}_{\text{rec}} = ({}^{31}\text{P}_{\text{Pspike}} - {}^{31}\text{P}_{\text{resin}}) / {}^{31}\text{P spike} \quad \text{Equation 2}$$

229 where ³¹P_{Pspike} is the concentration of ³¹P measured in the P-spiked subsample 3 and ³¹P spike is
 230 the amount of ³¹P added with the spike (both in mg P kg⁻¹).

231 The release of ³¹P from microbial cells during fumigation increases the amount of ³³P from the
 232 surfaces of a ³³P labeled soil that is recovered on the resin membranes. Therefore, the recovery of
 233 ³³P in P_{mic} cannot be calculated from the difference of ³³P recovered in fumigated and non-
 234 fumigated subsamples, as this would lead to an overestimation of ³³P_{mic}. Rather, the linear
 235 relationship between ³¹P added to subsamples 1 and 3 (0 mg P kg⁻¹ in non-fumigated subsample
 236 1, and 35 and 6 mg P kg⁻¹ for BBR and LUE, respectively, in ³¹P spiked subsample 3) and ³³P
 237 recovered on the resin membranes in these subsamples was established for each soil and at each
 238 sampling point in order to calculate the ³³P extracted (in kBq g⁻¹) depending on the amount of ³¹P
 239 added (in mg P kg⁻¹):

240 $^{33}\text{P}_{\text{extracted}} = a * ^{31}\text{P spike} + b$ Equation 3

241 The concentration of $^{31}\text{P}_{\text{mic}}$ calculated according to equation 1 was then inserted as $^{31}\text{P spike}$ into
 242 equation 3. The resulting value of extracted ^{33}P was inserted as $^{33}\text{P}_{\text{resin corrected}}$ into equation 4 to
 243 calculate the incorporation of ^{33}P in P_{mic} ($^{33}\text{P}_{\text{mic}}$ in kBq g^{-1}) as

244 $^{33}\text{P}_{\text{mic}} = (^{33}\text{P}_{\text{fum}} - ^{33}\text{P}_{\text{resin corrected}}) / ^{33}\text{P}_{\text{rec}}$ Equation 4

245 where $^{33}\text{P}_{\text{fum}}$ is the ^{33}P extracted from fumigated subsamples and $^{33}\text{P}_{\text{rec}}$ the recovery of the
 246 separate ^{33}P spike containing the same amount of ^{31}P as the $^{31}\text{P spike}$. This $^{33}\text{P}_{\text{rec}}$ is calculated as

247 $^{33}\text{P}_{\text{rec}} = (^{33}\text{P}_{33\text{P spike}} - ^{33}\text{P}_{31\text{P spike}}) / ^{33}\text{P spike}$ Equation 5

248 where $^{33}\text{P}_{33\text{P spike}}$ is the ^{33}P extracted from subsample 4 receiving the $^{33}\text{P spike}$, $^{33}\text{P}_{31\text{P spike}}$ is the ^{33}P
 249 extracted from subsample 3 receiving the $^{31}\text{P spike}$, and $^{33}\text{P spike}$ is the radioactivity added with
 250 the $^{33}\text{P spike}$ (all in kBq g^{-1}).

251 Only in cases where the slope of equation 3 was nil or negative, equations 3 and 4 were not used
 252 and $^{33}\text{P}_{\text{mic}}$ was derived as

253 $^{33}\text{P}_{\text{mic}} = (^{33}\text{P}_{\text{fum}} - ^{33}\text{P}_{\text{resin}}) / ^{33}\text{P}_{\text{rec}}$ Equation 6

254 where $^{33}\text{P}_{\text{fum}}$ and $^{33}\text{P}_{\text{resin}}$ are the amounts of ^{33}P (in kBq g^{-1}) extracted from fumigated and non-
 255 fumigated subsamples, respectively.

256 In this study, $^{33}\text{P}_{\text{rec}}$ and $^{31}\text{P}_{\text{rec}}$ were similar, averaging 0.72 ± 0.17 in BBR and 0.91 ± 0.05 in LUE
 257 (calculated for mixed samples only because of greater variation in unmixed samples).
 258 Occasionally, the calculation of $^{33}\text{P}_{\text{mic}}$ according to equation 4 (or equation 6, if applicable)
 259 rendered negative values. If this occurred with one or two replicates, then $^{33}\text{P}_{\text{mic}}$ in these

replicates was set to 0. If more than two replicates were negative, then $^{33}\text{P}_{\text{mic}}$ at this sampling date was considered as not detected.

We present $^{31}\text{P}_{\text{mic}}$ and $^{33}\text{P}_{\text{mic}}$ without the use of a conversion factor (K_p) which could account for incomplete extraction of microbial P since this factor is method- and soil-dependent (Oberson and Joner, 2005) but was not determined in our study. Nevertheless, we discuss the consequences of applying a commonly used K_p factor of 0.4 (Brookes et al., 1982).

2.3.5 Soil respiration

Soil respiration was measured by trapping CO_2 in NaOH, followed by precipitation and backtitration (Alef, 1995). Moist soil equivalent to 20 g dry soil was incubated together with a beaker containing 10 ml of 0.2 M NaOH in a closed jar of 1 l volume. Blanks without soil were included to account for the CO_2 in the 1 l airspace. At each measuring time point, the traps were exchanged, the CO_2 in the used traps was precipitated as BaCO_3 by adding an excess of 1.5 M BaCl_2 , and phenolphthalein was added as color indicator before titration with 0.2 M HCl prepared from standardized ampoules (Titrisol, Merck). The amount of CO_2 released from the soil was derived from the difference in HCl used for titration of blanks and samples.

2.3.6 Isotopic exchange kinetics (IEK) experiment

An IEK experiment (Frossard and Sinaj, 1998) was conducted in triplicate with each soil to determine the parameters required to extrapolate the baseline of isotopic dilution due to physicochemical processes. Moist soil equivalent to 10 g dry soil was shaken with 99 ml H_2O (accounting for water contained in the soil) for 16 h on an overhead shaker. The samples were stirred at 200 rpm for 10 min before addition of 1 ml carrier-free ^{33}P solution (approximately 100

281 kBq ml⁻¹). Under continuous stirring, 1-2 ml of the soil suspension was sampled after 1, 4, 10,
 282 30, 60 and 90 min with a plastic syringe and filtered at 0.2 µm. The radioactivity remaining in
 283 the soil solution (³³P_w = $r(t)$) was determined by scintillation counting at each sampling point t ,
 284 while the concentration of ³¹P (C_p , in mg l⁻¹) was determined colorimetrically at the end of the
 285 IEK experiment as described above.

286 To check the assumption that microbial processes do not affect parameters of isotopic exchange,
 287 1 ml of a microbial inhibitor (1.355 mg HgCl₂ ml⁻¹ as in Bünemann et al. 2012) was added to an
 288 additional three replicates 10 min before introduction of the isotope. To keep the final
 289 soil:solution ratio constant, these samples had been equilibrated with 98 ml H₂O.

290 The development of added radioactivity (R) remaining in the soil solution ($r(t)$) over time t (in
 291 min) can be described by the following equation (Fardeau et al., 1991):

$$292 \quad \frac{r(t)}{R} = m \cdot \left(t + (m)^{1/n} \right)^n + r_{inf}/R \quad \text{Equation 7}$$

293 where the fitting parameter m accounts for fast physicochemical processes and is typically
 294 similar to the fraction of radioactivity remaining in the soil solution after 1 min (r_{1min}/R) as
 295 determined from the log-log relationship of $r(t)/R$ and t during the IEK experiment. The
 296 parameter n describes the decrease in radioactivity in solution beyond 1 min, and the maximum
 297 possible dilution of the added radioactivity r_{inf}/R is approximated by the ratio of phosphate in the
 298 soil solution to total inorganic P in the soil (both in mg P kg⁻¹). For both soils, the difference
 299 between P_{tot} and H₂SO₄-P_o was used as an estimate of total inorganic P to derive r_{inf}/R .

300 2.4 Calculations of P transformation rates

301 Since ^{31}P and ^{33}P behave similarly in soil/solution systems (Randriamanantsoa et al., 2013),
 302 isotopically exchanged P (the so-called E -value) can be calculated as the inverse of the specific
 303 activity ($^{33}\text{P}/^{31}\text{P}$) of phosphate in the soil solution according to

$$304 \quad E(t) = 10 * \frac{C_p}{r(t)/R} \quad \text{Equation 8}$$

305 where C_p is the concentration of phosphate in the soil solution (in mg P l^{-1}) and the factor 10
 306 accounts for the ratio of water to soil to allow calculation of the $E(t)$ -value in mg P kg^{-1} soil.
 307 Thus $10 * C_p$ corresponds to $^{31}\text{P}_w$ as defined above (2.3.4), and $r(t)$ corresponds to $^{33}\text{P}_w$ as
 308 determined in the incubation experiment.

309 Gross organic P mineralization is derived from the difference between measured and
 310 extrapolated E -values (Oehl et al., 2001). Measured E -values are calculated from $^{33}\text{P}_w$ and $^{31}\text{P}_w$
 311 measured at each sampling time during the incubation experiment, whereas extrapolated E -
 312 values are derived for a given time t by calculating $r(t)/R$ based on equation 7 and using $10 * C_p$
 313 determined during the IEK experiment. Net organic P mineralization can be assessed by
 314 subtracting microbial P immobilization from gross organic P mineralization (Bünemann et al.,
 315 2007). Microbial P immobilization is calculated based on the assumption that P_w is the sole
 316 source of microbial P uptake. Using the specific activities of the source (P_w) and the sink (P_{mic})
 317 as well as the size of the sink, the amount of microbial P derived from the source can be
 318 calculated, analogous to P in other soil pools or plants that is derived from a certain source such
 319 as P_w or P fertilizer (Fardeau et al., 1996):

$$320 \quad \text{P immobilized (mg P kg}^{-1} \text{ soil)} = \text{SA}_{\text{Pmic}} / \text{SA}_{\text{Pw}} * ^{31}\text{P}_{\text{mic}} \quad \text{Equation 9}$$

where $SA_{P_{mic}}$ and SA_{P_w} are the specific activities of P_{mic} and P_w , respectively, both in the same unit, and $^{31}P_{mic}$ (in mg P kg⁻¹) is taken from equation 1.

Gross C mineralization was estimated by dividing net C release measured during the incubation experiment (2.4.5) by 0.6, thus assuming a microbial C use efficiency of 0.4 (Murphy et al., 2003).

2.5 Statistical analysis

After testing for normal distribution and homogeneity of the variances, a two-factorial ANOVA with the factors “day” and “treatment” was done for each soil in R 3.0.2 (RStudio version 0.98.1062), with effects considered significant for $p < 0.05$. Likewise, a three-factorial ANOVA (site, treatment, day) was done for all measurements on P pools in the incubation experiment. The treatment effect was additionally analyzed at each time point using a t-test. A t-test was also used to compare general soil characteristics and IEK parameters between sites. The IEK fitting parameters m and n were derived by a non-linear fitting procedure using an in-house R script.

3. Results

3.1 Soil characteristics, total soil P pools and IEK parameters

The two soils BBR and LUE had a similar acidic pH and similar total C and N concentrations, but the soil at BBR supported about three times more microbial C and N (Table 1). Likewise, potential acid phosphatase activity at BBR exceeded that at LUE by a factor of 6. The difference between the two soils was even more pronounced for total, inorganic and organic P pools, which were 30-80 times greater at BBR than at LUE.

Most IEK parameters differed strongly between the two soils (Table 2). While the concentration of P_w was twice as high at LUE, r_{Imin}/R was close to 0 and 1 for BBR and LUE, respectively. The parameter m was similar to r_{Imin}/R for BBR and > 1 for LUE, while the parameter n was three times greater at BBR than at LUE. Resulting E_{Imin} -values at BBR surpassed those at LUE by a factor of 22. Presence of the microbial inhibitor $HgCl_2$ did not decrease extrapolated E -values in either soil (data not shown). Therefore, results from the IEK without microbial inhibitor were used for all further calculations.

3.2 Soil respiration

Soil mixing at labeling resulted in a significant but transient increase in CO_2 release at BBR and a non-significant but persistent increase at LUE (Figure 1a, b). Daily respiration rates decreased over time in both soils and were 2-4 times greater at BBR than at LUE.

Cumulative respiration was increased by soil mixing only at LUE, even though this was significant only on two dates (Figure 1c, d). The additional C release after 37 days in the mixed treatment at LUE was 28 mg C kg^{-1} which was 15% more than in the unmixed treatment (190 mg C kg^{-1}). At BBR, both treatments released approximately 600 mg C kg^{-1} over the entire incubation.

3.3 Water-extractable phosphate and microbial P

Soil mixing at labeling did not affect the concentration of P_w at BBR, but it increased it by about 20% at the first two sampling dates at LUE (Figure 2a, b). This was followed by a transient decrease of P_w in mixed samples below that observed in unmixed samples, but at the end of the incubation the concentration was again slightly higher in mixed samples. At the first sampling

date, concentrations of P_w in the unmixed samples were similar in both soils (0.6 mg P kg^{-1}), but the concentrations declined over time to 0.3 mg P kg^{-1} at BBR, whereas they increased to about 1.0 mg P kg^{-1} at LUE.

The mixing at soil labeling had no significant effect on the fraction of ^{33}P recovered in the water extract of either soil (Figure 2c, d). At the first sampling date, only 0.2% of the added radioactivity was recovered in the soil solution at BBR, compared to approximately 10% at LUE. At the end of the incubation, these values had declined to 0.03% and 3% for BBR and LUE, respectively.

At BBR, P_{mic} was initially affected by soil mixing, being significantly greater in mixed compared to unmixed samples at day 1 (Figure 3a). However, the opposite effect was observed on day 4, and thereafter, no significant effect of soil mixing on P_{mic} was found at BBR. At LUE, P_{mic} was consistently greater in the unmixed compared to the mixed samples by about 1 mg P kg^{-1} , although the difference was not significant at day 25 and 38 (Figure 3b). Despite some significant fluctuations over time, P_{mic} was rather constant in the two soils, averaging 38 and 6 mg P kg^{-1} in BBR and LUE, respectively.

In the mixed samples of BBR, the recovery of ^{33}P in microbial P varied between 1-9%, with a tendency to decrease over time (Figure 3c). In the unmixed samples, it was more variable and could not be detected at all on three sampling dates (day 11, 25 and 32). In LUE, slightly more ^{33}P in microbial P tended to be found in the unmixed samples, in which it was also more variable at each sampling point and over time (Figure 3d). In the mixed samples of LUE, a constant recovery of ^{33}P in microbial P of $23\% \pm 1\%$ was observed.

3.4 Extrapolated and measured E -values

In BBR, extrapolated *E*-values reached $> 300 \text{ mg P kg}^{-1}$ already after one day (Figure 4a, Table 3). The relative variation of extrapolated *E*-values of 4-6% translated into absolute standard deviations of 19-44 mg P kg^{-1} at the different sampling dates. Measured *E*-values likewise showed a high variation by often $> 10\%$, especially in the unmixed soil. Apart from day 4 when the *E*-value was greater in the unmixed soil (Table 3), no significant effect of soil mixing on measured *E*-values was observed. Importantly, measured *E*-values were sometimes lower but never significantly greater than extrapolated *E*-values, except for day 38 in the mixed treatment.

In LUE, extrapolated *E*-values increased little beyond day 1 (1.9 mg P kg^{-1}), reaching 3.1 mg P kg^{-1} by the end of the incubation (Figure 4b, Table 4). Measured *E*-values were not affected by soil mixing, apart from an increased variation in the unmixed treatment, and were significantly greater than extrapolated *E*-values on all sampling dates.

3.5 Gross and net organic P mineralization

In BBR, gross organic P mineralization could only be calculated for the last sampling date of the mixed treatment, in which measured *E*-values were significantly greater than extrapolated *E*-values (Table 3). The resulting cumulative gross organic P mineralization of 123 mg P kg^{-1} after 38 days translates into a daily rate of about 3 mg P kg^{-1} . Such a rate cannot be detected reliably against the large variation in extrapolated *E*-values. Microbial P immobilization was measurable on all dates in the mixed treatment but showed no consistent temporal trend, fluctuating between 6 and 32 mg P kg^{-1} . When it could be measured in the unmixed treatment (4 out of 7 sampling dates), it was not significantly different from the mixed treatment, but more variable. For the one sampling date on which gross organic P mineralization could be assessed, microbial P

immobilization contributed 19% to it. This would suggest a net organic P mineralization rate of 2.6 mg P kg⁻¹ d⁻¹.

In LUE, cumulative gross organic P mineralization was not affected by soil mixing at labeling, increasing from 6 mg P kg⁻¹ after 1 day to about 28 mg P kg⁻¹ after 38 days (Table 4). Microbial P immobilization was also similar in both treatments, except on day 4 when it was significantly lower in the unmixed treatment. Microbial P immobilization increased from about 2 mg P kg⁻¹ after one day to 7 mg P kg⁻¹ at the end of the incubation. Daily rates of net organic P mineralization decreased over time from 4 mg P kg⁻¹ after one day to 0.5 mg P kg⁻¹ during the second half of the incubation.

Since gross organic P mineralization was rarely detected in BBR, C:P ratios of gross mineralization were calculated only for LUE (Table 4). There, they ranged between 3.5 after 1 day and 10-16 beyond 10 days of incubation. Likewise, C:P ratios of net mineralization in LUE ranged between 3 and 15.

4 Discussion

4.1 Assessment of organic P mineralization rates

Soil respiration rates declined over time in both soils, regardless of the mixing treatment (Figure 1), indicating that the soils were not strictly under steady-state conditions, despite pre-incubation for at least two weeks. Likewise, P_w was not constant over time, decreasing in BBR and increasing in LUE (Figure 2). Since the decrease in BBR occurred irrespective of soil mixing at labeling, it cannot be attributed to microbial stimulation or destruction of aggregates at labeling. Rather, it may still be an effect of soil disturbance during sample preparation which likely has

much greater effects than the mixing at labeling. An increase in P_w over time like in LUE has also been observed in other low-P sorbing soils (Achat et al., 2009; Grierson et al., 1998). While in the study of Achat et al. (2009) the increase in P_w was largely due to the release of P from the declining microbial pool, we observed no clear temporal trend in P_{mic} in LUE (Figure 3), suggesting mineralization of non-living organic P rather than remineralization of P_{mic} as the main source of the increase in P_w . The rather constant P_{mic} also supports the notion that the study was conducted in the absence of recent inputs of labile C which would cause changes in the microbial nutrient pool.

In LUE, gross organic P mineralization was well quantifiable against the baseline of isotopic dilution resulting from physicochemical processes only (Figure 4), and microbial P immobilization on a given day varied by no more than 11% in the mixed treatment (Table 4). Even though the variation of measured E -values and microbial P immobilization was greater in the unmixed treatment of LUE, gross and net organic P mineralization could still be assessed. Several factors contributed to the successful assessment of organic P mineralization in LUE even without soil mixing at labeling: i) the low availability of inorganic P as indicated by very low extrapolated E -values, resulting in a large difference to measured E -values, ii) reasonable infiltration (Figure S1) and thus homogeneous distribution of the labeling solution, resulting in a variation in measured E -values of <28%, and iii) significant incorporation of ^{33}P (always >20%) into the microbial pool (Figure 3d).

In BBR, gross organic P mineralization was not quantifiable except on day 38 in the mixed treatment (Table 3). The main reason was the very high availability of inorganic P as indicated by extrapolated E -values, which are in agreement with the assessment of P pools by sequential P

fractionation (sum of resin-extractable, bicarbonate- and sodium hydroxide-extractable inorganic P; Bauhus, personal communication). We did not perform the IEK experiment with a sufficiently high number of replicates to calculate a limit of detection or quantification based on the variation in extrapolated E -values as suggested by Bünemann et al. (2007). Nevertheless, assuming that cumulative gross organic P mineralization would need to be at least three times the standard deviation of the extrapolated E -values in order to be detected renders values for gross organic P mineralization of 57 and 96 mg P kg⁻¹ soil after 1 and 4 days, respectively, which is much greater than values reported in the literature (Bünemann, 2015). Based on the cumulative net respiration in the mixed treatment of 26 and 88 mg C kg⁻¹ soil after 1 and 4 days, respectively, and a microbial C use efficiency of 0.4 (Murphy et al., 2003), the C:P ratio (mass:mass) of gross mineralization would be <1 on day 1 and approximately 1.5 on day 4, which is not possible considering the C:P ratios of mineralizable substrates in soil. In addition to the high availability of inorganic P in BBR, labeling without mixing gave highly variable results in soil P pools which can be attributed to the observed low infiltration (Figure S1) and the high P sorption of BBR as indicated by the IEK parameters of 0.03 for r_{1min}/R and 0.49 for n (Frossard et al., 1993; Tran et al., 1988). Finally, a much smaller percentage of ³³P was recovered in the microbial P pool of BBR compared to LUE, even though this translated into greater microbial P immobilization rates at BBR on most dates due to the low specific activity of P_w (³³P_w/³¹P_w). In the absence of soil mixing, however, microbial P immobilization could not always be quantified in BBR (Table 3). Our results thus suggest that the assessment of gross and net organic P mineralization rates without soil mixing at labeling may be difficult but not impossible in a high-P-sorbing soil, but failed in our study because of the very high availability of inorganic P in BBR.

4.2 Contribution of biological/biochemical processes to soil P availability

The two soils studied here differed substantially in P pools, not only in absolute, but also in relative terms. For example, isotopically exchangeable phosphate by the end of the incubation experiment was 29% of total P in BBR compared to 3% in LUE, whereas the proportion of total P held in the microbial pool was 6% in LUE compared to 1% in BBR. Consequently, all measurements on P pools conducted during the incubation experiment were significantly different between the two sites when examining the entire dataset by three-factorial ANOVA (not shown).

In LUE, the relative contribution of physicochemical processes to total isotopically exchangeable P decreased from 25% after 1 day to about 10% at the last two sampling dates (Table 4). This is in a similar range as in a sandy Podzol under pine forest (Achat et al., 2009) and a silty-clayey Leptosol developed from shell limestone under mixed deciduous forest (Spohn et al., 2013). In contrast, physicochemical processes dominated isotopically exchangeable P in BBR to the point that the contribution of biological/biochemical processes became non-detectable. This contrast is also illustrated by the recovery of ^{33}P in the microbial P pool (mixed treatment only) which averaged 23% in LUE, compared to 4% in BBR (Figure 3). In the study of Achat et al. (2009), the microbial P pool was of a similar size as in LUE, whereas the resulting P immobilization was somewhat greater at LUE (7 mg P kg^{-1} after 38 days compared to 4 mg P kg^{-1} in Achat et al. 2009). In both studies, this immobilization occurred at constant microbial P and must thus have been accompanied by a similar flux of P out of the microbial pool, i.e. by remineralization *sensu lato* (Bünemann, 2015). To fully assess the relative contribution of biological/biochemical processes to supply plant-available inorganic P to the forest ecosystem, the contribution of other soil layers needs to be assessed as well (Achat et al., 2013; Jonard et al., 2009).

While gross and net organic P mineralization could only be assessed for LUE (Table 4), the potential acid phosphatase activity at BBR surpassed that at LUE by a factor of 6 (Table 1) and the cumulative C-release by a factor of almost 3 (Figure 1). The greater phosphatase activity at BBR might be attributed to the stabilization of enzymes on clay surfaces and sesquioxides (Burns et al., 2013), but as a potential activity it does not necessarily translate into greater actual mineralization rates. However, if P mineralization was linked to C mineralization (Gressel and McColl, 1997), similar proportions of organic C and organic P might be mineralized, as shown by Achat et al. (2010) for gross mineralization during a long-term incubation (> 317 days). At BBR, this would result in a cumulative net organic P mineralization of 20 mg P kg⁻¹ after 37 days, which is low relative to extrapolated *E*-values at BBR.

More importantly, the proportion of C respired relative to total organic C differed between the sites, amounting to 0.4% at LUE and 1.1% at BBR. This supports the notion that organic matter is less rapidly degradable at LUE, in line with the observed prolonged effect of soil mixing on respiration. In line with this, the proportion of total organic C held in the microbial pool was greater at BBR (0.7%) than at LUE (0.2%), suggesting a greater availability of organic matter to soil microorganisms at BBR (Anderson and Domsch, 1989). However, the metabolic quotient (C respired per unit microbial C) was in the same range in both soils (1.6-2.0), pointing to a similar substrate use efficiency (Hartman and Richardson, 2013).

In LUE, C:P ratios of both gross and net organic P mineralization ranged between about 3 and 15 and were thus in the same range as in other studies, as reviewed by Bünemann (2015). However, a cumulative net organic P mineralization of about 20 mg P kg⁻¹ within 38 days was indicated (Table 4), equal to 27% of total organic P. Since such a great decrease in organic P over such a

short time is quite unlikely, microbial P immobilization may indeed be underestimated because no K_p factor was applied. Recalculation assuming a K_p of 0.4 resulted in non-detectable net organic P mineralization at the first four sampling dates, depending on the mixing treatment, and rendered a cumulative net organic P mineralization of about 10 mg P kg^{-1} at the end of the incubation (Table S2). In comparison, estimating net organic P mineralization from C mineralization renders only 0.3 mg P kg^{-1} by the end of the incubation, which is similar to the observed increase in P_w and is therefore probably an underestimation. Evaluation of the data from the incubation experiment using a numerical modeling approach can estimate nine concomitant P transformation rates (Müller and Bünemann, 2014) and may therefore be better suited to partition gross organic P mineralization into its various components.

4.3 Effect of soil mixing on respiration, P pools and organic P mineralization

Mixing of the two soils during labeling affected soil respiration as a measure of microbial activity in different ways, with a significant but transient pulse observed in BBR and a non-significant but persistent increase in LUE (Figure 1). A transient pulse in respiration after soil mixing has been observed before (Achat et al., 2009; Bünemann et al., 2012) and can be explained by additional C substrates becoming available which can be respired, but which can also cause additional usage of other substrates, the so-called priming effect (Kuzyakov, 2010). The longer duration of elevated respiration after mixing in LUE may point to microorganisms remaining active beyond the exhaustion of the substrate which activated them (Blagodatsky et al., 2010).

In line with the transient increase in soil respiration in BBR, we also observed a transient increase in P_{mic} in this soil after mixing (Figure 3). Possibly, activated cells took up P to build P-

rich molecules such as RNA which are needed in greater concentrations in active compared to resting cells (Elser et al., 2003), but reverted to a resting state again within four days. In contrast, soil mixing reduced P_{mic} persistently in LUE. Soil disturbance is typically more detrimental to fungi than to bacteria (Jansa et al., 2006), and the microbial community at LUE has been shown to change more after nutrient additions than that at BBR (Heuck et al., 2015). In line with this, soil mixing reduced the microbial P pool at LUE, but not at BBR.

Measured E -values were not significantly affected by mixing except on day 4 in BBR (Table 3, Table 4), suggesting that the observed effects of mixing on microbial activity and biomass did not affect gross organic P mineralization, or that such an effect was undetectable due to the high availability of inorganic P. Importantly, there was also little indication that microbial P immobilization as derived from the recovery of ^{33}P in microbial P (equations 4 and 9) is altered by soil mixing, except on day 4 at LUE (Table 3, Table 4). Thus, the concern that the stimulation of microbial activity by the mixing of soils during labeling might create artefacts in the assessment of microbial immobilization and thus net organic P mineralization (Bünemann, 2015) can be defeated, at least for the soils studied here, although for BBR the large variation in all measurements may have prevented the detection of significant effects. Since P_{mic} in LUE was rather small, even though it accounted for 6% of total P, it would still be important to verify that mixing does not affect P transformations in a soil low in P_w and high in P_{mic} . The P-limited soil studied by Bünemann et al. (2012), for example, reached a specific P immobilization (cumulative microbial P immobilization / microbial P) > 1 after 8 days, compared to 32 days in LUE. Thus, a soil with faster microbial P immobilization than LUE should be used to test the effect of mixing on P transformations.

5 Conclusions

Biological/biochemical processes largely dominated the availability of inorganic P in a low-P-sorbing sandy forest soil with low total P, whereas they were hardly detectable against the great availability of inorganic P in a high-P-sorbing clayey-silty forest soil with very high total P. Strategies of ecosystem P nutrition may thus vary greatly in soils developed on different parent materials.

Mixing soil during labeling with a radioactive tracer decreased the variation in ^{31}P and ^{33}P in various soil P pools compared to labeling by injection, but it did not affect organic P mineralization and microbial P immobilization rates, even though some effects on microbial activity and microbial P were present. We showed that the assessment of organic P mineralization is possible by injecting the radioactive P tracer rather than mixing it into the soil, especially in a low-P-sorbing sandy soil with good infiltration. The next logical step is to assess organic P mineralization in intact soil cores in order to omit even the soil disturbance during sieving, but avoiding soils with very high levels of available P and/or high P sorption capacity.

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729 **Tables**

730 Table 1: General characteristics, total soil P pools and acid phosphatase activity of the soils Bad
 731 Brückenau (BBR) and Löss (LUE). Averages of four analytical replicates except for pH (n=3),
 732 total P (n=2) and acid phosphatase activity (n=3). p-values indicate the outcome of a t-test.

	unit	BBR	LUE	p-value
pH _{H2O}		3.8	3.6	0.088
C _{tot}	g C kg ⁻¹	54.3	59.1	0.255
N _{tot}	g N kg ⁻¹	2.9	2.5	0.138
C _{mic}	mg C kg ⁻¹	374	106	< 0.001
N _{mic}	mg N kg ⁻¹	53.2	19.4	< 0.001
P _{tot}	mg P kg ⁻¹	3377	100	0.047
H ₂ SO ₄ -P _i	mg P kg ⁻¹	1025	13.3	< 0.001
H ₂ SO ₄ -P _o	mg P kg ⁻¹	1878	76.1	< 0.001
Acid phosphatase activity	μmol g ⁻¹ h ⁻¹	15.0	2.4	< 0.001

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734 Table 2: Mean IEK parameters of the soils Bad Brückenau (BBR) and Lüss (LUE) determined
 735 with three analytical replicates. p-values indicate the outcome of a t-test.

	unit	BBR	LUE	p-value
C_p	mg l ⁻¹	0.042	0.076	0.012
r_{lmin}/R ^a		0.032	0.962	<0.001
m ^b		0.031	1.101	<0.001
n ^b		0.490	0.150	<0.001
E_{lmin}	mg kg ⁻¹	13.2	0.6	0.004

736 ^a derived from the log-log relationship of $r(t)/R$ and t

737 ^b derived by a non-linear fitting procedure

Table 3: Measured and extrapolated *E*-values, cumulative and daily rates of gross and net organic P mineralization, and cumulative P immobilization of the Ah1 horizon in Bad Brückenau as affected by soil mixing. Means $\pm$ standard deviations of four replicates (except extrapolated *E*-values: n=3). * indicates a significant difference between mixed and unmixed at a given sampling date at $p < 0.05$; p-values are derived from a t-test comparing measured and extrapolated *E*-values. No Kp factor was applied for calculation of microbial P immobilization, which may thus be underestimated, resulting in a potential overestimation of net organic P mineralization.

		Treat- ment	Day after labeling						
			1	4	11	18	25	32	38
Extrapolated <i>E</i> -value	mg P kg ⁻¹	n/a	355 $\pm$ 19	569 $\pm$ 32	749 $\pm$ 40	840 $\pm$ 43	898 $\pm$ 43	941 $\pm$ 44	970 $\pm$ 44
Measured <i>E</i> -value	mg P kg ⁻¹	mixed	239 $\pm$ 72	355 $\pm$ 29	537 $\pm$ 66	671 $\pm$ 26	1011 $\pm$ 75	1011 $\pm$ 91	1094 $\pm$ 70
p-value (measured vs. extrapolated)			0.043	<0.001	0.004	0.008	0.056	0.242	0.036
Measured <i>E</i> -value	mg P kg ⁻¹	unmixed	355 $\pm$ 123	406 $\pm$ 2*	885 $\pm$ 490	672 $\pm$ 105	1171 $\pm$ 237	1021 $\pm$ 215	920 $\pm$ 314
p-value (measured vs. extrapolated)			0.997	0.013	0.619	0.042	0.103	0.516	0.771
Gross organic P mineralization	mg P kg ⁻¹	mixed	nd	nd	nd	nd	nd	nd	123 $\pm$ 70
		unmixed	nd	nd	nd	nd	nd	nd	nd
Microbial P immobilization	mg P kg ⁻¹	mixed	17 $\pm$ 6	31 $\pm$ 17	10 $\pm$ 8	32 $\pm$ 5	24 $\pm$ 15	6 $\pm$ 9	23 $\pm$ 4
		unmixed	54 $\pm$ 62	41 $\pm$ 48	nd	41 $\pm$ 57	nd	nd	35 $\pm$ 47
Net organic P mineralization	mg P kg ⁻¹	mixed	nd	nd	nd	nd	nd	nd	100 $\pm$ 70
		unmixed	nd	nd	nd	nd	nd	nd	nd

n/a not applicable, nd not detected

Table 4: Measured and extrapolated *E*-values, cumulative and daily rates of gross and net organic P mineralization, cumulative P immobilization, and C:P ratios of mineralization of the Ahe horizon in Lüss as affected by soil mixing. Means $\pm$ standard deviations of four replicates (except extrapolated values: n=3). ** indicates a significant difference between mixed and unmixed at a given sampling date at $p < 0.01$; p-values are derived from a t-test comparing measured and extrapolated *E*-values. No Kp factor was applied to calculate microbial P immobilization, which may thus be underestimated, resulting in a potential overestimation of net organic P mineralization.

		Treat- ment	Day after labeling						
			1	4	11	18	25	32	38
Extrapolated <i>E</i> -value	mg P kg ⁻¹	n/a	1.9 $\pm$ 0.2	2.3 $\pm$ 0.2	2.6 $\pm$ 0.3	2.8 $\pm$ 0.3	2.9 $\pm$ 0.3	3.0 $\pm$ 0.3	3.1 $\pm$ 0.3
Measured <i>E</i> -value	mg P kg ⁻¹	mixed	7.7 $\pm$ 0.5	10.1 $\pm$ 0.5	13.0 $\pm$ 0.8	16.4 $\pm$ 0.6	24.0 $\pm$ 6.6	31.5 $\pm$ 1.3	30.7 $\pm$ 0.4
p-value (measured vs. extrapolated)			<0.001	<0.001	<0.001	<0.001	0.008	<0.001	<0.001
Measured <i>E</i> -value	mg P kg ⁻¹	unmixed	7.5 $\pm$ 1.8	9.3 $\pm$ 2.6	13.7 $\pm$ 0.9	17.9 $\pm$ 3.3	29.1 $\pm$ 5.0	31.7 $\pm$ 6.9	31.8 $\pm$ 6.2
p-value (measured vs. extrapolated)			0.008	0.013	<0.001	0.003	0.002	0.004	0.003
Gross organic P mineralization	mg P kg ⁻¹	mixed	5.8 $\pm$ 0.5	7.8 $\pm$ 0.5	10.4 $\pm$ 0.8	13.6 $\pm$ 0.6	21.1 $\pm$ 6.6	28.5 $\pm$ 1.3	27.6 $\pm$ 0.4
		unmixed	5.6 $\pm$ 1.8	7.0 $\pm$ 2.6	11.1 $\pm$ 0.9	15.1 $\pm$ 3.3	26.2 $\pm$ 5.0	28.6 $\pm$ 6.9	28.7 $\pm$ 6.2
Microbial P immobilization	mg P kg ⁻¹	mixed	1.8 $\pm$ 0.2	3.2 $\pm$ 0.1	3.2 $\pm$ 0.0	3.9 $\pm$ 0.2	5.4 $\pm$ 0.6	6.7 $\pm$ 0.2	6.9 $\pm$ 0.2
		unmixed	2.8 $\pm$ 1.1	1.9 $\pm$ 0.4**	4.2 $\pm$ 2.2	5.6 $\pm$ 2.2	5.5 $\pm$ 1.8	7.1 $\pm$ 2.7	8.7 $\pm$ 3.1
Net organic P mineralization	mg P kg ⁻¹	mixed	4.0 $\pm$ 0.5	4.7 $\pm$ 0.5	7.2 $\pm$ 0.8	9.6 $\pm$ 0.6	15.6 $\pm$ 6.6	21.8 $\pm$ 1.3	20.7 $\pm$ 0.4
		unmixed	2.8 $\pm$ 1.8	5.1 $\pm$ 2.6	6.8 $\pm$ 0.9	9.6 $\pm$ 3.3	20.6 $\pm$ 5.0	21.5 $\pm$ 6.9	20.1 $\pm$ 6.2
Daily gross organic P mineralization	mg P kg ⁻¹ d ⁻¹	mixed	5.8 $\pm$ 0.5	2.0 $\pm$ 0.1	0.9 $\pm$ 0.1	0.8 $\pm$ 0.0	0.8 $\pm$ 0.3	0.9 $\pm$ 0.0	0.7 $\pm$ 0.0
		unmixed	5.6 $\pm$ 1.8	1.7 $\pm$ 0.7	1.0 $\pm$ 0.1	0.8 $\pm$ 0.2	1.0 $\pm$ 0.2	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2
Daily net organic P mineralization	mg P kg ⁻¹ d ⁻¹	mixed	4.0 $\pm$ 0.5	1.2 $\pm$ 0.1	0.7 $\pm$ 0.1	0.5 $\pm$ 0.0	0.6 $\pm$ 0.3	0.7 $\pm$ 0.0	0.5 $\pm$ 0.0
		unmixed	2.8 $\pm$ 1.8	1.3 $\pm$ 0.7	0.6 $\pm$ 0.1	0.5 $\pm$ 0.2	0.8 $\pm$ 0.2	0.7 $\pm$ 0.2	0.5 $\pm$ 0.2
C:P ratio of gross organic P mineralization		mixed	3.3 $\pm$ 0.3	7.9 $\pm$ 0.5	13.6 $\pm$ 1.1	15.9 $\pm$ 0.7	15.5 $\pm$ 7.0	12.1 $\pm$ 0.6	13.9 $\pm$ 0.2
		unmixed	3.5 $\pm$ 1.3	8.8 $\pm$ 3.8	10.8 $\pm$ 0.8**	12.6 $\pm$ 2.7	9.8 $\pm$ 1.9	10.6 $\pm$ 3.0	11.7 $\pm$ 2.7
C:P ratio of net organic P mineralization		mixed	2.9 $\pm$ 0.3	8.0 $\pm$ 0.9	11.8 $\pm$ 1.3	13.5 $\pm$ 0.9	14.5 $\pm$ 10.6	9.5 $\pm$ 0.6	11.1 $\pm$ 0.2
		unmixed	8.0 $\pm$ 9.5	8.4 $\pm$ 5.6	10.5 $\pm$ 1.3	12.7 $\pm$ 4.4	7.6 $\pm$ 1.8	8.9 $\pm$ 3.6	10.5 $\pm$ 3.6

n/a not applicable

Figure captions

Figure 1: Daily soil respiration rates (a, b) and cumulative soil respiration (c, d) of the soils Bad Brückenau (BBR) and Lüss (LUE) with or without mixing during labeling. Error bars indicate standard deviation of four replicates, stars indicate significant effects of the mixing treatment at a given sampling date. The LSD is shown for the interaction between day and treatment. Note the different scale of the y-axis for cumulative respiration (c, d).

Figure 2: Concentration of ^{31}P (a, b) and fraction of ^{33}P ($r(t)/R$) (c, d) in water-extractable phosphate of the soils Bad Brückenau (BBR) and Lüss (LUE) with or without mixing during labeling. Error bars indicate standard deviation of four replicates, stars indicate significant effects of the mixing treatment at a given sampling date. The LSD is shown for the interaction between day and treatment. Note the different scale of the y-axis for $r(t)/R$ (c, d).

Figure 3: Concentration of ^{31}P (a, b) and fraction of ^{33}P ($r(t)/R$) (c, d) in microbial P of the soils Bad Brückenau (BBR) and Lüss (LUE) with or without mixing during labeling. Error bars indicate standard deviation of four replicates, stars indicate significant effects of the mixing treatment at a given sampling date. The LSD is shown for the interaction between day and treatment. Note the different scale of the y-axis for the concentration (a, b). nd = not detected (applies to BBR unmixed at three sampling dates in panel c).

Figure 4: Extrapolated and measured E -values of the soils Bad Brückenau (BBR) (a) and Lüss (LUE) (b) with or without mixing during labeling. Error bars indicate standard deviation of four replicates for measured and of three replicates for extrapolated E -values. Stars indicate significant effects of the mixing treatment at a given sampling date. The LSD is shown for the

interaction between day and treatment of the measured E -values only. Note the different scale of the y-axis in panels a and b.

Figure 1

Figure 2

Figure 3

Figure 4