Biological processes dominate phosphorus dynamics under low phosphorus availability in organic horizons of temperate forest soils

Author(s):
Pistocchi, Chiara; Mészáros, Éva; Tamburini, Federica; Frossard, Emmanuel; Bünemann, Else K.

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[a] Institute of Agricultural Sciences, ETH Zurich, 8315 Lindau, Switzerland

[b] Dept. of Soil Sciences, Research Institute of Organic Agriculture FiBL, Ackerstrasse 113, CH-5070 Frick, Switzerland

Corresponding author information

* Phone: +33 (0)499613060, e-mail: Chiara.pistocchi@supagro.fr

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[1] Present address: Eco&Sols, Montpellier SupAgro, Univ Montpellier, CIRAD, INRA, IRD, Montpellier, France

Abstract

Understanding the mechanisms underlying phosphorus (P) availability is important to predict forest productivity in a changing environment. We quantified P fluxes and traced P from plant litter into inorganic and organic soil P pools in organic horizons from two contrasting temperate forest soils with low and high inorganic P availability, respectively. We incubated the two organic horizons with and without litter after labelling the soil solution with ^{33}P and performed sequential extractions at several time points in order to trace P dynamics in labile (water-extractable, available and microbial P) and non-labile (non-living organic P, P bound to iron and aluminium and P bound to calcium) pools. Under low P availability, P fluxes were dominated by gross P mineralization, and microbial P immobilization accounted for up to 95% of gross P mineralization. Additionally, labile P in plant litter was rapidly incorporated into microbial P and only a small fraction ended up in the non-labile inorganic P pools. In contrast, P fluxes under high P availability were dominated by abiotic processes, particularly by fast (within 10 days) sorption/desorption reactions between the available P and the P bound to aluminium. These findings support the hypothesis that under low P availability biological processes control P fluxes. The observed tight cycling of P, with little efflux due to net P mineralization, suggests that the mineralization of organic P is driven by microbial P demand, and that the microbial community could compete with plants for available P.

1. Introduction

Anthropogenic impacts are expected to affect phosphorus (P) cycling in temperate forest ecosystems. For example, a decline in foliar P concentration in European

48 forests has been observed in the past decade and ascribed to an increased tree demand
49 for this nutrient caused by intensified nitrogen depositions and atmospheric carbon
50 dioxide enrichment (Jonard et al., 2015). The magnitude and possible consequences
51 of this trend are still under debate; however, P is expected to become progressively
52 more limiting (Talkner et al., 2015). A deeper understanding of the underlying
53 mechanisms, e.g. the processes governing P availability, speciation and fluxes in
54 soils, is needed to predict the effects on net primary production by changing
55 environmental conditions.
56 Plants mostly access P from the soil solution, which represents usually a small
57 proportion of the total P in soil. Multiple chemical equilibria with the mineral and
58 sorbed phases regulate the replenishment of the soil solution (Helfenstein et al.,
59 2018). Additionally, microbial processes can strongly influence the availability of P in
60 soil (Achat et al., 2016; Bünemann, 2015). Microbes mineralize organic P (P_o) from
61 plant litter and non-living soil organic matter, and the newly mineralized P is
62 incorporated into the microbial biomass (immobilization), sorbed to the solid phase or
63 remains in solution. Upon cell death or predation, the microbial P, which is not
64 remineralized, enters the non-living soil organic P pool. The extent to which these
65 processes influence P availability for plants varies widely, depending on factors such
66 as land-use and inorganic P availability (Becquer et al., 2014; Bünemann, 2015).
67 In forest soils, organic horizons are essential for the recycling of nutrients coming
68 from plant inputs such as leaf litter. Studies tracing P uptake by selected forest species
69 have shown that the contribution of the organic horizon to plant P supply can be as
70 high as 99% (Brandtberg et al., 2004; Jonard et al., 2009).
71 Our understanding of such dynamics is hampered by the difficulty of quantifying P
72 fluxes. These are challenging to measure, because they often occur without net or

detectable changes in pool size. However, the use of P radiotracers (^{33}P or ^{32}P) helps circumventing this issue. P radiotracers can be used to quantify gross P mineralization and immobilization rates (Bünemann, 2015), or the fate of P added with plant residues (Daroub et al., 2000), and hence they can allow assessing the relevance of such processes to P availability.

A handful of studies have applied P radiotracers to assess P dynamics in forest soils (Achat et al., 2010, 2009b, 2009a; Bünemann et al., 2016; Heuck et al., 2015; Mooshammer et al., 2012; Spohn et al., 2013) or fluxes from soil to plants (Jonard et al., 2009). Most of these studies targeted P dynamics in labile P pools, i.e. inorganic P in solution and microbial P, not directly assessing the contribution of less labile pools, particularly the mineral or sorbed P and the non-living organic P pool. Sequential extractions are commonly used to characterize inorganic and organic soil P pools. Such procedures yield operationally-defined pools, assuming an inverse relationship between P availability in a given pool and the strength of the extractants (Tiessen and Moir, 1993). Unless coupled with other techniques, sequential extractions alone do not provide any information about the availability or fluxes of P (Frossard et al., 1996). The recovery of a radiotracer in sequentially-extracted P pools was used to compare soils under different land-use or tillage systems (Buehler et al., 2002; Daroub et al., 2000), soil types (Vu et al., 2010) or crop rotations (Bünemann et al., 2004b). In highly weathered and unfertilized tropical soils a shift toward microbial P and organic P was observed, with higher recovery of the tracer in these pools (Buehler et al 2002), which points to a high importance of biological P transformations under limited inorganic P availability.

Despite its potential in identifying the relevance of different processes in influencing P dynamics and recycling, such a combined approach using a radiotracer and sequential extractions has never been applied to forest soils.

In this study, we aimed at elucidating P dynamics in inorganic and organic pools in two forest organic horizons (Oe) differing in P content and availability (low *vs* high). We also aimed at following the fate of P added with fresh plant litter into soil P pools. Labile pools in mineral top soils from these forests were already investigated by Bünemann and co-workers (2016), who showed that under low P availability microbial processes accounted for up to 90% of isotopically exchangeable P fluxes, while this contribution reduced to almost nil in a mineral topsoil with the same vegetation but, very high P availability. Our objectives were to: i) assess which inorganic and organic P pools participate in exchange reactions with the available P and to which extent; ii) quantify P fluxes related to physico-chemical (sorption/desorption, precipitation/dissolution) and microbiological processes (gross mineralization/immobilization) in the presence or absence of litter inputs. To do so, we adopted an isotopic dilution approach (Oehl et al., 2001) and followed the tracer into P pools extracted with a sequential extraction (Tiessen and Moir, 1993).

Our hypotheses were that: under low inorganic P availability, I) biological processes dominate P dynamics and II) there is a faster incorporation of P from the litter into soil inorganic and organic pools due to a higher microbial activity.

2. Materials and methods

2.1 Site and sampling description

120 The organic horizons used in this study were collected from two 100 to 120 years old
121 beech (*Fagus sylvatica* L.) forest sites. The site Bad Brückenau (BBR), is located at
122 about 800 m asl in Northern Bavaria, Germany (50°21'7.26"N, 9°55'44.53"E). The
123 soil is classified as Dystric Skeletic Cambisol (Hyperhumic, Loamic)
124 (FAO/ISRIC/ISSS, 1998) and developed on basalt. The site Lüss (LUE) is located at
125 100 m asl in Lower Saxony, Germany (52°50'21.77"N, 10°16'2.37"E). The soil in
126 LUE is developed on Pleistocene sand and is classified as a Hyperdystric Folic
127 Cambisol (Arenic, Loamic, Nechic, Protospodic). This two sites represent the
128 extremes of a geosequence covering a wide range of total and available soil P. Their
129 characteristics are described in detail in Lang et al. (2017).
130 At each site recent beech litter, i.e. litter deposited during the previous autumn, was
131 first collected. Then, after removing the litter layer, 5 to 6 subsamples from the Oe
132 horizon (0-12 cm and 0-5 cm at BBR and LUE, respectively) were taken and pooled
133 to form a composite sample. Samples from the LUE site were collected in April, and
134 samples from BBR site in May 2015.
135 The soil was sieved moist to < 5 mm. The litter was dried at 35°C, manually crushed
136 and sieved twice to collect the fraction between 20 mm and 5 mm. Both materials
137 were stored at 4°C for a period of two weeks (BBR) to one month (LUE) before the
138 experiment.
139
140 *2.2 Soil and litter characteristics*
141 Maximum water holding capacity (WHC) of the Oe horizons was determined
142 gravimetrically by placing the saturated soils in tared cylinders and letting them drain
143 on a sand bath for 4 h. pH was measured on settled 1:2 soil-water suspensions after 90

144 min shaking using an ORION 720A pH-meter. The two Oe horizons had both acidic
145 pH, but differed in almost all considered variables (table 1).
146 Total carbon (C_{tot}) and nitrogen (N_{tot}) content were determined on ground soil and
147 litter samples by dry combustion on an elemental analyzer (Variopyro Cube,
148 Elementar Analysensysteme GmbH, Germany). Total inorganic (H_2SO_4-P_i) and
149 organic (H_2SO_4-P_o) P content in the samples were determined according to Saunders
150 and Williams (1955) after ignition at 550°C for 1 h and successive extraction of
151 ignited and non-ignited subsamples with 0.5 M H_2SO_4 for 16h. H_2SO_4-P_o was then
152 calculated as P in ignited samples minus P in non-ignited samples. P in the litter was
153 done by incineration at 550°C for 1 h followed by extraction with concentrated hot
154 HNO_3 (Nanzer et al., 2014).
155 Inorganic P determination in all extracts was made with the malachite green method
156 (Ohno and Zibilske, 1991) using a UV-VIS spectrophotometer (UV-1800, Shimadzu).
157 Microbial carbon (C_{mic}) and nitrogen (N_{mic}) were determined by chloroform
158 fumigation and subsequent extraction with 0.5 M K_2SO_4 (Vance et al., 1987)(Fig.1).
159 The extracts were analyzed with a TOC/TN analyzer (Formacs^{SERIES}, Skalar, The
160 Netherlands).
161

Table1. Initial (day 0) and final (day 93) characteristics of the Of horizon of Lüss (LUE) and Bad Brückenau (BBR). NL = incubation without litter addition, L = incubation with litter addition, E_{24h} = phosphorus isotopically exchangeable in 24h, lowercase letters indicate significant differences among the initial and final values of one soil, capital letters indicate significant differences between the initial values of the two soils ($p < 0.05$), nd = not determined

		day 0		day 93				day 0		day 93			
	unit	BBR		BBR NL		BBR L		LUE		LUE NL		LUE L	
Humus type	-	Mull-like Moder						Mor-like Moder					
pH_{H2O}	-	3.70	A	nd	-	nd	-	3.55	A	nd	-	nd	-
WHC	g g^{-1}	3.26	A	nd	-	nd	-	2.92	B	nd	-	nd	-
C_{org}	g C kg^{-1}	237	Aa	220	a	234	a	364	Ba	317	a	317	a
N_{org}	g N kg^{-1}	14.8	Aa	14.6	a	13.6	b	16.7	Aa	14.3	a	15.0	a
P_o§	mg P kg^{-1}	1523	A	nd	-	nd	-	371	B	nd	-	nd	-
C_{mic}	mg C kg^{-1}	844	Aa	859	a	875	a	1047	Ba	758	b	725	b
N_{mic}	mg N kg^{-1}	152	Aa	161	a	150	a	238	Ba	161	b	154	b
P_{mic}	mg P kg^{-1}	93.6	Aa	60.4	b	78.1	c	53.4	Ba	52.2	a	58.6	a
C_{org} : N_{org}	mol/mol	18.7	Aa	17.6	a	20.1	a	25.4	Ba	25.9	a	24.7	a
C_{mic} : N_{mic}	mol/mol	6.5	Aa	6.2	a	6.8	a	5.1	Ba	5.5	a	5.5	a
C_{mic} : P_{mic}	mol/mol	23.3	Aa	36.7	b	28.9	c	50.6	Ba	37.5	b	31.9	c
C_{org} : P_o	mol/mol	426.4	A	nd	-	nd	-	2949	B	nd	-	nd	-
E_{24h}	mg P kg^{-1}	140.0	A	nd	-	nd	-	4.2	B	nd	-	nd	-
P_w	mg P kg^{-1}	5.8	Aa	6.8	a	7.3	b	1.1	Ba	3.7	b	3.1	b
Total P_i§	mg P kg^{-1}	1041	A	nd	-	nd	-	114	B	nd	-	nd	-

		texture of the mineral fraction in BBR£	texture of the mineral fraction in LUE£
Sand	%	8	75
Silt	%	55	19
Clay	%	36	6

Notes: § Total organic and inorganic P according to Saunders and Williams (1955), £ after Lang et al. 2017.

2.3 Experimental principle and design

The isotopic dilution approach relies on the combination of short (80-100 min) batch experiments, the so-called isotopic exchange kinetics (IEKs), and long-term soil incubations (weeks to months). In both, the soil inorganic P in solution is labelled with a radioactive P isotope (^{33}P or ^{32}P) and then the isotopic dilution, i.e. the decrease in concentration of the radioisotope, is followed over time. During the IEKs,

177 due to the short duration, the isotopic dilution is assumed to be affected only by
178 physico-chemical processes, i.e. sorption, desorption, precipitation and dissolution.
179 IEK-derived parameters enable the extrapolation of the isotopic dilution to a longer
180 time span, the so-called isotopic dilution baseline, and of the estimation of
181 isotopically exchanged P (Fardeau, 1993; Frossard and Sinaj, 1998). During
182 incubations, both physico-chemical and biological processes affect the isotopic
183 dilution, thus the contribution of biological processes to the P exchanged can be
184 calculated by difference with the isotopic baseline (Oehl et al 2001).
185 In our study, a 3-month incubation (section 2.6) of the two Oe horizons was combined
186 with IEKs (section 2.5), and with sequential extractions of the incubated soil (sections
187 2.7 and 2.8). The IEKs were conducted on subsamples of the two Oe materials a few
188 days before the beginning of the main incubation. A 3-week pre-incubation, during
189 which we monitored the respiration, was carried out to obtain constant soil
190 respiration, required to meet the assumption of steady state (Oehl et al 2001).
191 The experimental design of the incubation had two factors: the soil (BBR and LUE)
192 and the litter treatment, which included soil amended with litter (L) and non-amended
193 soil (NL). All treatments had four replicates. During incubation, concentrations of ^{31}P
194 and ^{33}P (r) were measured in water-extractable P (inorganic P in solution, P_w), resin-
195 extractable P (inorganic available P, P_{res}) and hexanol-labile P pools (microbial P,
196 P_{mic}) at day 1, 4, 11, 17, 29, 64 and 93 after labelling. Additionally, at day 4, 29 and
197 93 we performed a modified Hedley sequential extraction (Tiessen and Moir, 1993) to
198 follow the fate of ^{33}P beyond the hexanol-labile pool. Soil respiration was determined
199 at weekly intervals (Fig. 1).
200
201 <Figure 1>

202

203 *2.4 Calculations of P exchanged*

204 During IEKs, the simultaneous desorption of ^{31}P and sorption of ^{33}P determine the
205 progressive decline of the initially added radioactivity (R) in the soil solution, so that
206 the specific activity (SA) of the solution, i.e. the ratio $^{33}P/^{31}P$, decreases with time.
207 Since there is no isotopic discrimination between ^{31}P and ^{33}P, the specific activity of
208 the solution is equal to the specific activity of the entire mass of distribution called E-
209 value, or isotopically exchanged P:

210

211 $SA_{(t)} = {}^{r_{(t)}}/_{P_w} = {}^{R}/_{E_{(t)}}$ Equation 1

212

213 where $SA_{(t)}$ is the specific activity at time t in kBq g^{-1}/mg P kg^{-1}, $r_{(t)}$ is the residual
214 radioactivity in kBq g^{-1}, P_w is the concentration of inorganic P in solution (water-
215 extractable P) in mg P kg^{-1}, R is the added radioactivity in kBq g^{-1} and $E_{(t)}$ in mg P kg^{-1} is the E-value at the time t, which can be derived by rearranging Eq. 1.

217 The decline of the radioactivity in the soil solution due to physico-chemical processes,
218 $r_{(t)}/R$, as a function of time can be described by the model proposed by Fardeau et al
219 (1991) (see Supplementary Information and Eq. 1S).
220 The model (Eq. 1S) was fitted with the experimental data from the IEKs and then
221 used to extrapolate the $r_{(t)}/R$ for the time span of the incubation. The corresponding E-
222 values ($E_{mod(t)}$) represent the isotopic dilution baseline and were calculated with Eq. 1
223 using the extrapolated $r_{(t)}/R$ and the P_w measured during the IEKs.
224 During the incubation, the amount of isotopically exchanged P ($E_{meas(t)}$) was
225 calculated with Eq. 1, but using the $r_{(t)}/R$ and P_w measured at each sampling point of

the incubation. The cumulated gross organic P mineralization at time t ($GPM_{(t)}$) was then derived by difference (Oehl et al. 2001):

$$GPM_{(t)} = E_{meas(t)} - E_{mod(t)} \quad \text{Equation 2}$$

where $E_{meas(t)}$ and $E_{mod(t)}$ are the isotopically exchanged P measured during the incubation or extrapolated with Eq. 1S, respectively, both in mg P kg^{-1}.

The microbial P immobilization was calculated assuming P_w is the sole source of P for microbes (Bünemann et al., 2007):

$$\text{Immobilization}_{(t)} = SA_{Pmic} / SA_{Pw} * P_{mic(t)} \quad \text{Equation 3}$$

where SA_{Pmic} and SA_{Pw} are the specific activities of microbial and water-extractable P, respectively, both in kBq g^{-1}/mg P kg^{-1}, and P_{mic} is the microbial P at time t (in mg P kg^{-1} soil), taken from Eq. 2S (Supplementary Information). However, we calculated the immobilization only when SA_{Pmic} and SA_{Pw} were significantly different ($p<0.05$), since further exchange between the microbial and the water-extractable pools cannot be detected when both have reached a complete equilibrium. Finally, net organic P mineralization (NPM) can be calculated by subtracting microbial P immobilization from GPM (Bünemann et al., 2007). Mineralization and immobilization were calculated at each sampling time and divided by the corresponding number of days to obtain the daily rates.

Additionally, gross C mineralization rate was estimated as net C respired divided by 0.6, assuming a microbial C use efficiency of 0.4 (Murphy et al., 2003).

The fate of litter P in soil pools was calculated analogously to the proportion of a non-labeled fertilizer introduced in a soil-plant system (Fardeau et al., 1995):

$$\%Pdl = 100(1 - SA_L/SA_{NL}) \qquad \text{Equation 4}$$

where %Pdl is the percentage of P in a given pool that is derived from the litter, SA_L is the specific activity of the pool in the soil amended with litter and SA_{NL} is the specific activity of the same pool in the non-amended soil. This calculation was done only when significant differences ($p<0.05$) were detected between SA_L and SA_{NL}.

2.5 Isotopic exchange kinetics (IEKs)

The possible effect of microbial P uptake during the IEK was checked with the use of a microbial inhibitor, Bronopol PESTANAL (1 ml 0.025M, Sigma Aldrich, analytical grade).

Four replicates of 5 g equivalent dry soil were shaken overnight with 98 ml of ultrapure H_2O (accounting for water contained in the soil) to reach steady state, i.e. constant P concentration in solution, and therefore equal Pi sorption and desorption rates. The microbial inhibitor was added 20 minutes before the samples were placed on a magnetic stirrer. At t0, 1 ml carrier-free ^{33}P solution (474 kBq ml^{-1}) was added to each sample. Aliquots were collected from each replicate with a plastic syringe at 1, 4, 10, 30, 50 and 100 minutes after the ^{33}P addition and filtered through syringe filters (0.2 µm, Minisart, Sigma-Aldrich). The radioactivity in these aliquots ($r_{(t)}$) was measured by scintillation counting, while P concentration (P_w) was determined with the malachite green method.

2.6 Incubation experiment

After sieving, soils were dried down slightly at room temperature before the pre-incubation to lower the moisture content and enable subsequent addition of the labeling solution. The pre-incubation was conducted at approximately 40% of the maximum WHC in plastic containers kept at room temperature in the dark for 25 days.

After pre-incubation, soil was weighed in polyethylene zip lock bags (equivalent of 65 g dry soil each), and a labeling solution was prepared with carrier-free phosphoric acid (Hartmann Analytic, Braunschweig, Germany). Four ml of the labeling solution (244.4 kBq ml^{-1}) was added to each bag, spreading on the top by pipetting and mixing with a whisk for 1 minute. This operation was repeated for a total of 8 ml of labeling solution. To reach the desired WHC, each bag additionally received 9 and 18.6 ml of ultrapure H_2O in the case of BBR and LUE, respectively. Finally, litter was added to half of the bags, at a rate of 10 mg per g of dry soil, corresponding to 4.6 mg C g^{-1} soil. This amount is very close to natural litter inputs at the two sites as recalculated from Lang et al. (2017). Each replicate was mixed again for 1 minute and placed slightly open in a plastic tray with cover and incubated in the dark at 19°C. During the incubation, the gravimetric water content of the soils was kept at 50% and 53% of water holding capacity (WHC), respectively for BBR and LUE. A beaker filled with water was added to each tray in order to keep air moisture as constant as possible. The final soil label (R) was 30.077 kBq g^{-1} soil for both soils.

For soil respiration measurements, a separate set of samples including all the treatments, each one of 10 g dry weight equivalent, was prepared on the day of labeling adding ultrapure H_2O instead of the labeling solution. Each sample was placed in a tightly closed jar (1 L volume) together with an alkaline trap made of 20

ml 0.2M NaOH solution, including four blanks without soil. The jars were then incubated together with the labeled samples. Soil respiration was measured by trapping the CO_2 liberated from the soil followed by back titration (Alef, 1995).

2.7 ^{31}P and r in resin-extractable, water-extractable and hexanol-labile P during incubation

Water-extractable P (P_w), was extracted by shaking 5 g equivalent dry soil in 100 ml ultrapure H_2O (accounting for water contained in the soil) for 16 h on an overhead shaker. Samples were filtered directly after the shaking using 0.2 µm syringe filters (Millipore).

In the case of resin-extractable and hexanol-labile P (P_{res} and P_{mic}), we followed the method proposed by Kouno and co-workers (1995) and modified by Bünemann and co-workers (2004). In detail, three subsamples constituted by 1:15 soil suspensions with 2 g equivalent dry soil were prepared. In each, a resin membrane (BDH #55164, 6 cm x 2 cm) in the carbonate form was added. The first subsample had no additional treatment (P_{res}), the second subsample received 1 ml of 1-hexanol (P_{hex}), and the third subsample received a P spike (P_{spike}). The latter simulates P release from cell lysis after hexanol addition and allows estimating the possible sorption onto the solid phase of the newly released P. A single P spike of about 50 mg P kg^{-1} was used, since the relationship between recovered and added P was shown to be linear in the range of 10 to 50 mg P kg^{-1} on the mineral horizon of these soils (Bergkemper et al., 2016). No spike of radioactivity was included because Bünemann and co-workers (2016) showed that in these soils the recovery of added radioactivity is similar to that of the P spike.

The samples and blanks were shaken horizontally for 16 h. Then the resins were eluted with 0.1 M NaCl/HCl for 2 h, after rinsing with ultrapure H_2O. P_{mic} concentration in mg kg^{-1} of soil was calculated by the difference between the hexanol and the P_{res} subsamples accounting for sorption (Eq. 2S in Supplementary Information). No conversion factor (Kp) was used to correct for possible inefficiency of the fumigant, i.e. incomplete recovery of microbial P, since this is soil-specific and has not been determined for these soils. Estimated Kp ranges between 0.3 and 1 (Oberson and Joner, 2005; Achat et al., 2009b), therefore the underestimation of the microbial P may exceed 100%.

The recovery of radioactivity in the microbial mass (r_{mic} in percent of total radioactivity) had to be corrected for possible ^{33}P release from the labeled soil due to replacement with ^{31}P liberated from microbial cells, which would lead to an overestimation of r_{mic} (Oehl et al, 2001). Therefore, we corrected r_{mic} as reported in Bünemann and co-workers (2016), using the radioactivity recovered from the spiked samples (see supplementary information and Eq. 3S and 4S)

2.8 ^{31}P and r in sequentially-extracted P pools during incubation

The sequentially extracted pools were the 0.25M NaOH/0.05M EDTA-extractable P, representing the inorganic and organic P bound to Fe and Al oxides (hereafter P_{iNa} and P_{oNa}), and the HCl-extractable P (P bound to Ca, P_{Cl}).

The subsample extracted with hexanol was used for the subsequent steps of the sequential extraction. After removing the resins, NaOH and EDTA disodium salt were added to the soil suspensions in solid form to reach the wanted concentration. After 16 h shaking, the samples were centrifuged (5300 *g* for 15 minutes), filtered through Millipore nylon filters (0.8 μm), and the filtrates were collected for P_{iNa} and P_{oNa}

349 determination. Subsequently, 30 ml of 1 M HCl were added to the same samples and
350 the extracts collected after shaking overnight and filtering using glass fiber filters (0.8
351 μm, Millipore).
352 To separate P_{iNa} and P_{oNa} and measure the radioactivity in the NaOH-EDTA extracts,
353 two methods were tested: separation by isobutanol (Jayachandran et al., 1992) and by
354 acidification-centrifugation (Tiessen and Moir, 1993). This latter gave better Pi
355 recovery (>80%) and was preferred. The NaOH-EDTA extracts were therefore
356 acidified to pH 1.5 with 0.5 M H_2SO_4 to induce the precipitation of organic
357 substances. The ^{31}P and ^{33}P measured in the acidified supernatant after centrifugation
358 represent the inorganic fraction. The organic fractions, P_{oNa} and r_{oNa}, were then
359 determined by difference with the total P concentration or total radioactivity,
360 respectively.
361 The radioactivity in all extracts was detected by liquid scintillation using a beta-
362 emission counter (Tri-carb 2500 TR, Packard Instruments, Meriden, CT) after
363 thoroughly mixing the samples with Ultima Gold or Ultima Gold AB for acid
364 extracts. Quenching effects in colored extracts, e.g. NaOH-EDTA extracts, were
365 corrected by comparing the counts per minute of a ^{33}P spike in water, a ^{33}P spike in
366 the sample and a water spike in the sample.
367 Radioactivity measurements were recalculated to t = 0 using the equation of
368 radioactive decay. Radioactivity recovery (r_w, r_{hex}, r_{res}, r_{iNa}, r_{oNa} and r_{Cl}) of a given
369 pool is expressed in percentage of the total introduced radioactivity R (r/R*100).
370
371 *2.9 Statistical analysis*
372 A 2-way factorial ANOVA (1st factor = litter application, 2nd factor = date) was used
373 to analyze the variables measured during the incubation for each soil separately

except the respiration rates. These latter results were analyzed using a mixed model, where the litter amendment was the fixed factor and the time of the measurement (weekly) was a random factor with the replicate nested in it. The Tukey test was used for post hoc comparison. The whole data set was analyzed with a 3-way ANOVA including the soil as a factor. However, we discuss mostly the results of the 2-way ANOVA as the two very contrasting soils (table 1) resulted in constantly significant differences. The Student's paired t-test was used when comparing single dates and cumulative values, after checking for homogeneity of variances. The Shapiro-Wilk test was used to assess normality of the data. All analyses were performed in R 3.1.1 (R version 3.1.1, R Core Team).

3. Results

3.1 Soil respiration

The soil respiration in LUE was almost the double of that in BBR. The litter amendment resulted in a significant increase of the cumulative amount of C released in both soils, with an additional C release in the amended treatments of 5.98 and 7.30 % for LUE and BBR, respectively (Table 2).

Compared with the pre-incubation period there was a 50% increase in respiration immediately after labelling in LUE, compared with a 10% increase in BBR (fig. 1S). During the incubation, soil respiration showed two distinct phases. During the first four weeks after labelling the respiration was higher and then decreased by about 20 and 25% in LUE and BBR, respectively. From five-six weeks onwards, it remained approximately stable. During the first phase, the differences between the litter amendments were more pronounced, with higher respiration in the litter-amended soils (fig. 1S).

399

400 Table 2. Cumulative respiration in LUE and BBR (mean ± standard deviation of 4
401 replicates), L= litter-amended, NL= non-amended

Site	Treatment	Cumulative respiration	Additional C release	Statistics (paired t-test)
		mg C kg soil^{-1}	increase in %	P-value
LUE	NL	9607.7 ± 202.2	5.98	0.006382
LUE	L	10182.3 ± 194.1		
BBR	BL	4850.5 ± 120.9	7.30	0.007551
BBR	L	5204.8 ± 131.8		

402
403

404 *Radioactivity recovery in the resin-extractable and the sequentially extracted P pools*

405 The radioactive tracer was distributed very differently over various P pools in the two
406 soils (fig. 2). In BBR, the recovery of radioactivity in the microbial pool (r_{mic}) was
407 very low, fluctuating around 2%, and for some replicates below the detection limit
408 (fig. 2b and d). In contrast, it reached 30% in LUE after only four days of incubation
409 (fig. 2a). Similarly, a consistent recovery of radioactivity in LUE was found in the
410 organic P pool already at day 4 (r_{oNa} = 18.6% as average of the two litter treatments, L
411 and NL), increasing significantly to 23% at day 93 (fig. 2a and c), while in BBR the
412 corresponding values were initially close to 0 and increased to about 6% at day 93
413 (fig. 2b and d). Most of the radioactivity in BBR was recovered in the inorganic P
414 extracted with NaOH-EDTA (r_{iNa} = 43.2% to 53.5% at day 4 and 93, respectively)
415 and in the resin-extractable P (r_{res} = 34.5% to 7.5% at day 1 and 93, respectively).
416 Differences due to the litter addition were found only in the recoveries of water-
417 extractable, microbial and HCl-extractable P of LUE, where the recovery of
418 radioactivity was slightly lower in the litter-amended treatment.
419 The tracer could not be recovered entirely, with the non-recovered fraction
420 representing 17 to 27% in BBR and 15 to 26% in LUE. Losses during the
421 manipulation were estimated to be around 5% of the total radioactivity. The

422 remainder was likely transferred into the residual P pool, which was not extracted and
423 quantified.
424
425 <Figure 2>
426
427 *Changes in pool sizes and specific activities: labile pools*
428 In LUE, the water-extractable P (P_w) increased at day 1 compared to the initial value
429 in both litter treatments (L and NL, Table 1 and fig. 3), i.e. 1.1 mg P kg^{-1} *vs* 2.9 and
430 3.2 mg P kg^{-1} for the non-amended and litter-amended treatment, respectively. Then it
431 dropped to around 1 mg P kg^{-1}, remained constant until day 29 and increased again in
432 the last two sampling dates.
433 In BBR, P_w increased steadily from the initial value of 4.2 to 6.5 mg P kg^{-1} (as
434 average of the treatments). The litter addition caused statistically significant
435 differences in BBR at day 93, with a slightly higher concentration in the amended (7.3
436 mgP kg^{-1}) compared to the non-amended treatment (6.8 mgP kg^{-1}).
437 The specific activity of this pool (SA_{Pw}) decreased over time in both soils and it
438 remained approximately stable after day 29 (LUE) or 64 (BBR). Significant
439 differences between the treatments were found only at the initial stage of the
440 incubation in LUE (day 1 to 11).
441 <Figure 3>
442 <Figure 4>
443 <Figure 5>
444
445 As for P_w, the resin-extractable P (P_{res}) increased during incubation: from 6.4 to 13.0
446 mg P kg^{-1} as average of the litter treatments in LUE and from 28.3 to 50.0 mg P kg^{-1}

447 in BBR. The corresponding specific activities (SA_{Pres}) decreased steadily until the end
448 of the incubation (Fig. 4). The specific activities of water and resin pools were similar
449 both in LUE and BBR. No significant differences due to the litter addition were
450 observed.
451 As for the radioactivity recovery, the dynamics of the microbial P (P_{mic}) were very
452 different in the two soils (Fig. 5). In LUE, the P_{mic}, after a slight, but significant
453 increase between day 4 and 11 likely caused by soil mixing, remained rather stable for
454 the duration of the experiment and was not affected by the litter amendment.
455 Conversely, in BBR, P_{mic} declined from the initial value of 93 mg P kg^{-1} down to 78.1
456 and 60.4 mg P kg^{-1} for the litter-amended and non-amended treatment, respectively, at
457 the final sampling. Hence, the litter addition led to significantly higher P_{mic}
458 concentrations.
459 The specific activity in the microbial P (SA_{Pmic}) in LUE decreased until the middle of
460 the incubation as a consequence of constant P_{mic} and decreasing radioactivity
461 recovery, then remained approximately constant. Overall, litter addition caused
462 slightly lower SA_{Pmic} values compared to the untreated soil.
463 In BBR, because of the very low tracer recovery, the SA_{Pmic} fluctuated around very
464 low values without differences due to the litter amendment.
465
466 *Changes in pool sizes and specific activity: sequentially extracted pools*
467 In BBR, the NaOH-EDTA-extractable inorganic and organic P (P_{iNa} and P_{oNa}) and the
468 HCl-extractable P remained stable over time, while the specific activity of those three
469 pools increased slightly but significantly from day 4 to day 93 as a consequence of the
470 increase of radioactivity recovery (table 3 and fig. 2b and d). For the P_{iNa} this
471 translated in a specific activity (SA_{PiNa}) of 0.066 and 0.079 $(mgP\ kg^{-1})^{-1}$ at day 4 and

93, respectively (average of L and NL treatments, table 3). The little radioactivity recovery in the organic pool translated in a specific activity (SA_{NaPo}) of about 0.005 $(mgP\ kg^{-1})^{-1}$ at the end of the incubation. These pools were not affected by the litter amendment.

In LUE, only the HCl-extractable P showed a temporal trend, as it increased from day 4 to day 29 and decreased again at the last sampling time. Conversely, its specific activity (SA_{Cl}) was nearly constant, though it was affected by the litter addition, with a lower specific activity in the litter-amended treatment (table 3).

Table 3. Concentrations and corresponding specific activities (SA) of stable pools: NaOH-EDTA extractable inorganic (P_{iNa}) and organic P (P_{oNa}) and HCl extractable P (P_{Cl}). R= radioactivity introduced, r = radioactivity in solution. Letters indicate significant differences among the three time points as average of NL and L treatments, asterisks indicate significant differences between non-amended (NL) and litter-amended (L) treatments according to two-way ANOVA, ** = p<0.005

day after labelling		4		29		93		4		29		93	
		mgP kg^{-1}						r/R (mgP kg^{-1}) $^{-1}$					
		P_{iNa}						SAP_{iNa}					
LUE	NL	81.0 ±9.2		71.6 ±16.5		87.4 ±6.2		0.283 ±0.016		0.335 ±0.086		0.298 ±0.048	
LUE	L	82.3 ±15.3		86.3 ±7.6		84.5 ±10.3		0.296 ±0.065		0.268 ±0.018		0.270 ±0.044	
BBR	NL	650.4 ±37.0		586.0 ±127.1		685.1 ±51.0		0.065 ±0.006		0.073 ±0.008		0.077 ±0.011	
BBR	L	668.3 ±53.0		620.3 ±41.6		676.0 ±20.0		0.067 ±0.011	a	0.061 ±0.012	a	0.081 ±0.007	b
		P_{oNa}						SAP_{oNa}					
LUE	NL	187.6 ±14.5		213.5 ±24.9		177.8 ±19.5		0.105 ±0.010		0.100 ±0.027		0.138 ±0.033	
LUE	L	161.9 ±41.8		202.4 ±33.0		212.7 ±37.1		0.122 ±0.027		0.102 ±0.037		0.104 ±0.009	
BBR	NL	1347.9 ±183.0		1477.0 ±162		1631.7 ±306.6		0.000 ±0.000		0.002 ±0.003		0.004 ±0.002	
BBR	L	1360.3 ±159.0		1502.0 ±333		1449.0 ±241.8		0.000 ±0.001	a	0.006 ±0.007	ab	0.005 ±0.002	b
		P_{Cl}						SAP_{Cl}					
LUE	NL	5.8 ±0.082		10.7 ±0.59		7.3 ±0.93		0.423 ±0.027		0.498 ±0.038		0.474 ±0.108	
LUE	L	6.1 ±0.19	a	11.1 ±1.50	b	7.9 ±0.34	c	0.382 ±0.021		0.409 ±0.030		0.389 ±0.014	**
BBR	NL	262.3 ±40.3		279.0 ±16.9		256.7 ±23.8		0.042 ±0.007		0.053 ±0.005		0.048 ±0.005	
BBR	L	282.0 ±48.23		292.0 ±22.8		279.8 ±52.1		0.042 ±0.006	a	0.049 ±0.005	b	0.047 ±0.010	ab

E-values: P exchanged by physico-chemical and microbial processes

In LUE, the estimated isotopically exchangeable P values, $E_{mod(t)}$, were extremely low, attaining 11.7 (±3) mgP kg^{-1} at the end of the incubation period, which corresponds to 10% of the total inorganic P (H_2SO_4-P_i, Table 1). The corresponding value in BBR was 568 (±76) mg P kg^{-1}, which corresponds to 55% of the total inorganic P (table 4 and 5).

In LUE, the measured E values ($E_{meas(t)}$) were always much higher than the estimated ones ($E_{mod(t)}$). In contrast, in BBR the $E_{meas(t)}$ were always lower than $E_{mod(t)}$, preventing the calculation of the gross P mineralization (GPM) with equation 3.

In LUE, the litter addition resulted in higher $E_{meas(t)}$ values at the beginning of the incubation (day 4-11), whereas in BBR no effect of the litter was visible (table 4 and 5).

The resulting $GPM_{(t)}$ values in LUE were rather high. The daily rates decreased over time, from 60.1 mg P kg^{-1} day^{-1}, as average of the litter treatments, at day 1 to 2 mg P kg^{-1} day^{-1} at day 93. At day 4-11, $GPM_{(t)}$ values were higher where the litter was added (table 4).

Microbial immobilization and net mineralization ($NPM_{(t)}$) were calculated for the time points in which the specific activities of microbial and water-extractable P were significantly different (see section 2.4 and Eq. 3). In LUE, after day 11, the two specific activities were indeed very close, masking any further exchange between P_{mic} and P_w. During this period, the $NPM_{(4\text{-}11)}$ was significantly higher in the litter-amended treatment, accounting for 62% and 70% of the $GPM_{(t)}$ in the non-amended and litter-amended treatment, respectively.

In BBR, the specific activities of the microbial and water-extractable P never converged; therefore, we could calculate the immobilization. This fluctuated around

low values (2.1-9.7 mg P kg^{-1}) without clear trends and the effect of the litter was weak or not detectable (table 5).

It is important to highlight that both the immobilization and $NPM_{(t)}$ are derived from P_{mic} and are therefore potentially affected by the error associated with the fumigation efficiency.

Table 4. Measured (E_{meas}) and extrapolated E-values (E_{mod}) (mean ± standard deviation of 4 replicates), mineralization and immobilization rates (GPM: gross P mineralization, NPM: net mineralization, IMM: microbial immobilization) in LUE, nd: not determined. * indicates significant differences (p>0.05) between litter amended (L) and non-amended (NL) treatment

		Treatment	Day after labeling						
			1.5	4	11	17	29	64	93
E_{mod}	mg P kg^{-1}	-	4.2±0.4	6.0±0.9	7.4±1.3	8.1±1.5	9.1±1.8	10.8±2.4	11.7±2.7
E_{meas}	mg P kg^{-1}	NL	78.7±1.4	99.9±3.9*	126.0±12.3*	181.1±20.3	218.8±37.5	178.8±3.1	185.6±9.2
		L	110.0±27.9	135.7±17.8	187.0±16.8	173.3±18.1	201.7±22.4	180.7±11.6	195.8±5.2
GPM	mg P kg^{-1}	NL	74.4±1.4	93.9±4.0*	118.6±12.3*	173.0±20.3	209.7±37.5	168.0±3.1	173.9±9.2
		L	105.8±28.0	129.7±17.7	179.5±16.8	165.3±18.1	192.5±22.4	169.9±11.6	184.1±5.2
NPM	mg P kg^{-1}	NL	49.3±5.6	61.3±5.8*	79.1±15.0*	nd	nd	nd	nd
		L	73.8±30.1	94.5±21.5	129.8±16.3	nd	nd	nd	nd
IMM	mg P kg^{-1}	NL	25.1±3.8	32.6±2.6	39.5±1.8*	nd	nd	nd	nd
		L	32.1±5.3	35.2±6.3	49.7±2.8	nd	nd	nd	nd
Daily GPM	mg P kg^{-1} d^{-1}	NL	49.6±0.9	23.5±1.0*	10.8±1.1*	10.2±1.2	7.2±1.3	2.6±0.1	1.9±0.1
		L	70.6±18.7	32.4±4.4	16.3±1.5	9.7±1.0	6.6±0.7	2.7±0.2	2.0±0.1
Daily NPM	mg P kg^{-1} d^{-1}	NL	32.9±3.6	15.3±1.4*	7.2±1.4*	nd	nd	nd	nd
		L	49.2±20.0	23.6±5.3	11.8±1.5	nd	nd	nd	nd
Daily NPM§	mg P kg^{-1} d^{-1}	NL	nd	nd	0.08	0.15	0.17	0.08	0.07
		L	nd	0.05	0.19	0.10	0.16	0.09	0.07
Daily IMM	mg P kg^{-1} d^{-1}	NL	16.8±2.8*	8.2±0.5	3.6±0.3*	nd	nd	nd	nd
		L	21.4±3.6	8.8±1.6	4.5±0.3	nd	nd	nd	nd
C:P of GPM	mol/mol	NL	17	28	55	56	78	195	269
	mol/mol	L	14	23	40	66	95	208	270

§ calculated as the net change of the available P, $(P_{res(t)}-P_{res(t0)})/t$

522 Table 5. Measured (E_{meas}) and extrapolated E-values (E_{mod}) (mean ± standard deviation of 4 replicates), mineralization and immobilization rates
523 (GPM: gross P mineralization, NPM: net mineralization, IMM: microbial immobilization) in BBR. * indicates significant difference (p>0.05)
524 between litter amended (L) and non-amended (NL) treatment

		Treatment	Day after labeling						
			1.5	4	11	17	29	64	93
E_{mod}	mg P kg^{-1}	-	153.6±29	261.3±51	355.7±60	382.2±69	440.8±75	522.3±80	568.0±76
E_{meas}	mg P kg^{-1}	NL	140.0±3.8	175.2±14.8	269.7±12.2	293.8±27.9	341.0±25.3	389.4±31.2	471.7±29.3
		L	136.4±1.5	164.8±22.2	272.0±9.0	316.1±24.9	343.4±9.5	379.3±15.5	449.7±22.0
IMM	mg P kg^{-1}	NL	8.7±8.5	2.1±1.2*	9.1±1.9	3.3±3.7	6.0±1.7	8.3±9.0	9.7±3.0
		L	2.2±1.9	5.3±2.2	5.5±4.1	8.6±1.8	6.9±4.4	5.8±1.4	7.9±3.9
Daily NPM§	mg P kg^{-1} d^{-1}	NL	nd	0.73	0.63	0.49	0.35	0.28	0.22
		L	nd	0.65	0.68	0.38	0.38	0.20	0.24
Daily IMM	mg P kg^{-1} d^{-1}	NL	5.7±4.8	0.5±0.3*	0.8±0.2	0.2±0.2	0.2±0.1	0.1±0.1	0.1±0.0
		L	1.5±1.5	1.3±0.3	0.5±0.4	0.5±0.1	0.2±0.2	0.1±0.0	0.1±0.1

525 § calculated as the net change of the available P, $(P_{res(t)}-P_{res(t0)})/t$

4. Discussion

4.1 Dynamics of P in inorganic and organic pools

The large differences observed in the tracer recovery among P pools between the two organic horizons showed that exchanges between organic pools dominated under low P availability (in LUE), and exchanges between inorganic pools under high P availability (in BBR).

In BBR, the decline of radioactivity in the available P (P_{res}) corresponded to an increase of radioactivity in the P_{iNa} pool without significant change in the size of the pool (Fig. 2b and d and table 3). This reflected the occurrence of exchange processes between the tracer and the inorganic P associated with the solid phase (Fardeau, 1996). The largest decrease in the radioactivity recovery in the P_{res} (71%) was between day 1 and 11, suggesting the predominance of fast exchange reactions, such as sorption/desorption reactions. Fast exchange reactions have been observed with incubated tropical soils rich in Fe and Al oxides in the absence of organic or inorganic P inputs (Buehler et al., 2002) and with some temperate soils (Daroub et al., 2000). P bound to Al-saturated organic matter seems to be the dominant inorganic P form in the O horizon of this soil (Prietzel et al., 2016), and we suggest that this is the pool in rapid (1-10 days) equilibrium with the available P.

The HCl-extractable P seemed to have a longer equilibration time with the available P as indicated by the lowest specific activity, suggesting the occurrence of slower reactions such as precipitation/dissolution. This agrees with observations made on soils and organic materials such as sewage sludge indicating turnover times of this fraction longer than 3 months (Frossard et al., 1996). The 1M HCl extraction is assumed to target the P bound to Ca. Indeed, monocalcium phosphate and apatite

551 were detected in the Ah horizon of BBR (Prietzel et al., 2016), and material from this
552 horizon might be transferred to the Oe horizon by bioturbation or during the
553 sampling. More likely the 1M HCl extracted some P bound to Fe and Al
554 oxyhydroxides by surface precipitation (Werner et al 2017), which could not be
555 entirely extracted by the NaOH-EDTA step.
556 Overall, the contribution of living and dead organic P pools to the exchanges with the
557 available P was marginal in BBR, but visible in the progressive increase in available
558 P, which correlated with the decrease in microbial P (Fig. 3S). Therefore, we
559 conclude that the dynamics of the available P pool in this horizon were mainly
560 controlled by abiotic processes. This is rather surprising for an organic horizon, as this
561 pattern is more typical of mineral top- or subsoils (Bünemann, 2015).
562 The picture differed drastically in LUE, where the highest radioactivity recovery
563 found in the microbial pool (r_{mic} of 30% after 1 day) suggested a rapid P uptake by the
564 microbes. Accordingly, we observed a very fast convergence between the specific
565 activities of the water-extractable P and the microbial P: within 11-20 and 4-11 days
566 for non-amended and litter-amended treatments, respectively. The time until these
567 two specific activities converge can indeed be interpreted as an estimation of the
568 microbial P turnover time (Oehl et al 2001).
569 The relative stability of the r_{mic} after the first month of incubation suggested either a
570 tight P cycling within the microbial community with little P efflux upon death or the
571 return to a dormant state of the community after the initial activation by soil mixing
572 (Bünemann et al., 2016). The trend in the respiration (fig. 1S) seems to support this
573 second explanation.
574 Additionally, in LUE we observed high and rapid tracer incorporation into the non-
575 living organic P pool (NaOH-EDTA-labile P_o, fig. 2a and c). In the absence of

576 labelled P_o sources, the labelled P_o can only be of microbial origin, suggesting a rapid
577 release of organic P forms through microbial turnover. This, however, could be the
578 result of experimental artefacts since part of the microbial P_o might have remained in
579 solution after the hexanol fumigation, and cell lysis could then have been be carried
580 over in the subsequent NaOH-EDTA extraction. The precipitation of inorganic P
581 along with the organic matter upon acidification (Tiessen and Moir, 1993) could also
582 bias the separation between the inorganic and organic fractions. The fact that we
583 observed a temporal trend in the recovery of both microbial P (from 30 to 20%) and
584 non-living P_o (from 18 to 23%) supports, at least partially, the assumption of an active
585 process rather than an experimental artefact.
586 Bünemann and co-workers (2004b) and Buehler and co-workers (2002) measured
587 recoveries of 10 to 15% in the organic P pool of weathered tropical soils after 7 and
588 10 days of incubation, respectively. Vu and co-workers (2010) found up to 6-7%
589 recovery in 0.1 M NaOH-labile P_o after 4 days of incubation of a Chromosol. Overall,
590 in LUE, the sum of recoveries in microbial and non-living organic P accounted for 40
591 to 50% of the introduced radioactivity, which is higher than the sum of recovery in P_o
592 fractions in the aforementioned studies. At the same time, the size of the non-living P_o
593 pool remained approximately constant, indicating no net accumulation of the newly
594 produced P_o and pointing to the importance of recycling.
595 Altogether, these results support our first hypothesis about a biologically dominated P
596 cycling under low inorganic P availability, characterized by a rapid microbial P
597 turnover and a significant recycling of organic P. This is highlighted by the difference
598 in respiration of the two organic horizons, which was much higher in LUE than in
599 BBR (Table 2).
600

1. *P fluxes related to physico-chemical and microbial processes*

In BBR, we observed a very high baseline of isotopic dilution ($E_{mod(93)}$ = 568 mg P kg^{-1}), which impeded the calculation of gross P mineralization rates with equation 3. The same was found in the mineral topsoil of BBR and was attributed to its very high inorganic P availability (Bünemann et al., 2016). This explanation agrees with the predominance of fast exchanges observed between the available P and the P bound to Al. If estimated from the carbon release (Achat et al., 2009b), the cumulated gross P mineralization in BBR at day 93 would range between 55 and 60 mg P kg^{-1}, which confirms the dominance of physico-chemical processes.

In LUE, gross P mineralization accounted for more than 94% of P isotopically exchanged over the incubation for both treatments (table 4), clearly showing the dominance of microbial processes. This proportion is slightly higher than the one measured on the mineral topsoil of LUE (74 to 90% in the Ahe horizon, Bünemann et al 2016) and on few other forest soils (Achat et al., 2009b; Spohn et al., 2013).

Gross P mineralization rates ($GPM_{(t)}$) derived from 7 to 10-days incubations of different soils range from 0.8 to 12.6 mg P kg^{-1} d^{-1} as reviewed by Bünemann, (2015). The corresponding $GPM_{(11)}$ daily rates measured in LUE were in the upper end of this range or higher: 10.8 and 16.3 mg P kg^{-1} d^{-1} for non-amended and litter-amended treatments, respectively (table 4). However, in our experiment, the rates measured in the first four weeks were likely not under steady-state conditions, because the respiration rate increased after soil mixing and was not constant (Oehl et al., 2001). The values measured in the subsequent period, ranging from 7.2 to 1.9 mg P kg^{-1} d^{-1} (table 4), represent a more realistic estimation of the basal P mineralization rate. These are, however, rather high as compared to the mineral topsoil under forest (cfr.

625 Table 1 in Bünemann 2015 and Bünemann et al 2016), and confirm the relevance of
626 the organic horizon for the recycling of P_o under P limiting conditions.
627 Further partitioning of the gross mineralization rates in net mineralization ($NPM_{(t)}$)
628 and immobilization is complicated by the lack of a specific correction factor
629 accounting for fumigation efficiency (Kp). In case of incomplete microbial P
630 recovery, the net P mineralization rate is overestimated and the immobilization is
631 underestimated (see section 2.4).
632 An estimation of net P mineralization rate is obtained for a low-sorbing organic layer
633 by calculating the net change in inorganic P in solution over an incubation period
634 (Jonard et al., 2010). In LUE, the net change in the available P would result in a
635 $NPM_{(93)}$ of about 0.07 mg P kg^{-1} d^{-1} (table 4) and account for 3-4% of the gross P
636 mineralization over the incubation period. This would indicate that most of the
637 mineralized P was immobilized rather than released to the soil solution. In accordance
638 with this, the dominance of microbial immobilization over net mineralization was
639 observed in P-deficient soils with a large proportion of fumigant-labile P (Achat et al.,
640 2009b; Bünemann et al., 2012).
641 The $NPM_{(93)}$ estimated for BBR (Table 5) would be instead about 40% of the gross P
642 mineralization estimated by C release, suggesting that the gross P mineralization was
643 in this case rather driven by the need of carbon (Ali et al., 2014; Heuck et al., 2015).
644
645 ***2. Fate of litter P in BBR***
646 In BBR, the litter addition was not producing a net change in soil inorganic or organic
647 P pools, except for microbial P, which showed a net increase of about 18 $mgPkg^{-1}$ by
648 day 93. Microbial P indeed declined in both treatments, but more in the non-amended
649 treatment, suggesting that litter addition delayed the return of the microbial

650 community to a dormant state. The higher respiration rate in the amended treatment
651 during the first four weeks agrees with this explanation (fig. 1S). The P released in the
652 non-amended treatment from the microbial pool was not recovered in the available
653 pool and presumably ended up in the pool of P bound to Al, although not detectable
654 against the amount of P present in this pool.
655 The lack of differences in specific activities between amended and non-amended
656 treatments precluded the estimation of P derived from the litter in soil P pools
657 (equation 4). As the amount of P added with the litter was rather small compared to
658 the soil P pools in BBR, a small isotopic dilution would be hard to detect against the
659 error of measurement. For example, the entire amount of inorganic P added with the
660 litter (Pi in table 2S) would dilute the specific activity of the available P by only 5%,
661 which corresponds to the coefficient of variation of replicates.
662 The decline in P_{mic} was decoupled from the microbial C (C_{mic}), which instead
663 remained stable over time and between treatments (Table 1). Such decoupling can be
664 attributed to a change in the activity of microbial cells with the build-up of P rich
665 compounds (Bünemann, 2015) after the addition of fresh plant inputs, or to a shift in
666 the microbial community composition in response to different environmental
667 conditions (Fanin et al., 2013; Mooshammer et al., 2014).
668 The second interpretation is supported by the parallel study of Mészáros and co-
669 workers (2016), who analysed the microbial community composition at different time
670 points of our incubation and found significant differences. Additionally, the similar
671 metabolic quotient (C respired per unit of microbial C) between litter-amended and
672 non-amended treatment (2.4 and 2.5 mg $C\text{-}CO_2$ $mg^{-1}C_{mic}$ h^{-1}) suggests a similar
673 substrate use efficiency (Hartman and Richardson, 2013).
674

3. Fate of litter P in LUE

Although the litter did not induce any detectable change in P pool sizes in LUE, we detected isotopic dilution, i.e. lower specific activity, in the water-extractable (P_w), microbial (P_{mic}) and HCl-extractable P (P_{Cl}) during the first period of incubation (day 1 to 29). Our interpretation is that the inorganic and labile P_o, e.g. litter phosphomonoesters, were initially released from the litter and/or mineralized by extracellular enzymes and went into solution, thus explaining the initial isotopic dilution in P_w. According to equation 5, the released quantities corresponded to 0.8 (±0.2) to 0.3 (±0.05) mg P kg^{-1} (Table 1S), about 10 and 4% of the added P. Such a small amount could not be detected as a net increase in pool size.

After day 11, we could not detect any significant differences between treatments in the specific activity of P_w, meaning that no further release from the litter occurred or that the newly released P was rapidly taken up by the microbes. Accordingly, the significantly lower specific activity of microbial P in the litter-amended treatment (fig. 5c) suggests that microbial uptake from an unlabelled source occurred. The threefold increase of the C:P ratio of the litter sampled at the end of the incubation agrees with this finding (Table 2S). However, the estimated quantity of P in the microbial pool derived from the litter (Eq. 4) calculated for day 29 was 14.4 (±2.7) mg P kg^{-1} (Table 1S). This amount exceeds the total P added with the litter. A possible explanation is that the litter addition stimulated the mineralization of other P_o substrates in soil, i.e. priming effect (Kuzyakov, 2010). The higher net mineralization rate in the 4-10 day interval points to that explanation. However, a corresponding decrease of the P_o pool could not be detected against the error affecting this measurement.

The significant isotopic dilution observed at day 29 in the P_{Cl} pool of the litter-amended treatment (table 3) suggests also that a small part of P released was transferred to this pool.

The lack of a net increase in P_{mic} following litter addition might be explained by the recalcitrant nature of this plant material. Indeed, P immobilization occurring after the addition of fresh substrates is less pronounced if the carbon source is less labile (Bünemann et al., 2004a). Beech litter contains more recalcitrant compounds, especially lignin, than other broadleaf trees litter (Mooshammer et al., 2012; Steffens et al., 2015). In agreement with this, the additional carbon release corresponded to only 14% of the added carbon, and no differences in gross or net P mineralization were recorded after day 11. Our results indicate that the microbial communities supplied their P demand from different sources in the presence or absence of fresh litter. The slightly different C:P ratio of the microbial pool between non-amended and litter-amended treatments at the end of the incubation (Table 1) indicates a possible shift in the community.

We conclude that the most labile P forms in plant litter were rapidly cycled through the biota and only a small amount was transferred to inorganic P pools in LUE. Given the impossibility to trace the fate of litter P in BBR, we could not confirm our second hypothesis.

Conclusions and perspectives

Under low P availability, we observed that the majority of P fluxes were biologically dominated, with a pivotal importance of microbial and non-living organic P, and a rapid turnover of microbial P (around 4-11 days). Additionally, labile P in plant litter

723 was rapidly cycled through the biota and only a small amount was transferred to
724 inorganic P pools.
725 Under high P availability, fast exchange dynamics were observed between available
726 and sorbed P pools, which accounted for the highest tracer recovery. Under these
727 conditions, trees can rely upon P desorption fluxes to cover their P demand. In
728 contrast, in LUE the flux of isotopically exchangeable P was very low so that plants
729 would have to rely on recycling of organic P. However, our results indicated that
730 microbes are very efficient in immobilizing P, i.e. the flux due to net mineralization
731 was small, which suggests that the microbial community could compete with plants
732 for available P. For this reason, the mechanisms underlying the microbial P pool
733 contribution to plant nutrition under low P availability remain to be elucidated.
734

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741

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Figure

Fig. 1 Overview of the experimental schedule

	Preincubation			Incubation														
Days	-21	-15	-7	1	4	11	17	29					64					93
Water content		40%										50% (LUE)	53% (BBR)					
Soil mixing	x																	
Soil labelling																		
Litter addition																		
IEK experiments			x															
P_w, P_{res}, P_{mic}				x	x	x	x	x					x					x
Complete seq. extraction					x			x										x
Respiration	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Microbial C and N			x															x

Fig. 2 Radioactivity recovery (radioactivity detected in a pool divided by the total introduced radioactivity, in %) in the P pools in the non-amended treatment, NL (a) and litter-amended treatment, L, (c) in LUE; in NL treatment (b) and L treatment (d) in BBR, bars represent the standard deviation (n=4). rres = recovery in resin-extractable P, rmic= recovery in hexanol-labile P, riNa= recovery in inorganic P extracted with NaOH-EDTA, roNa= recovery in organic P extracted with NaOH-EDTA, rCl = recovery in HCl-extractable P. D = number of days after labelling. Water-extractable P is not shown, as it is part of the resin-extractable pool

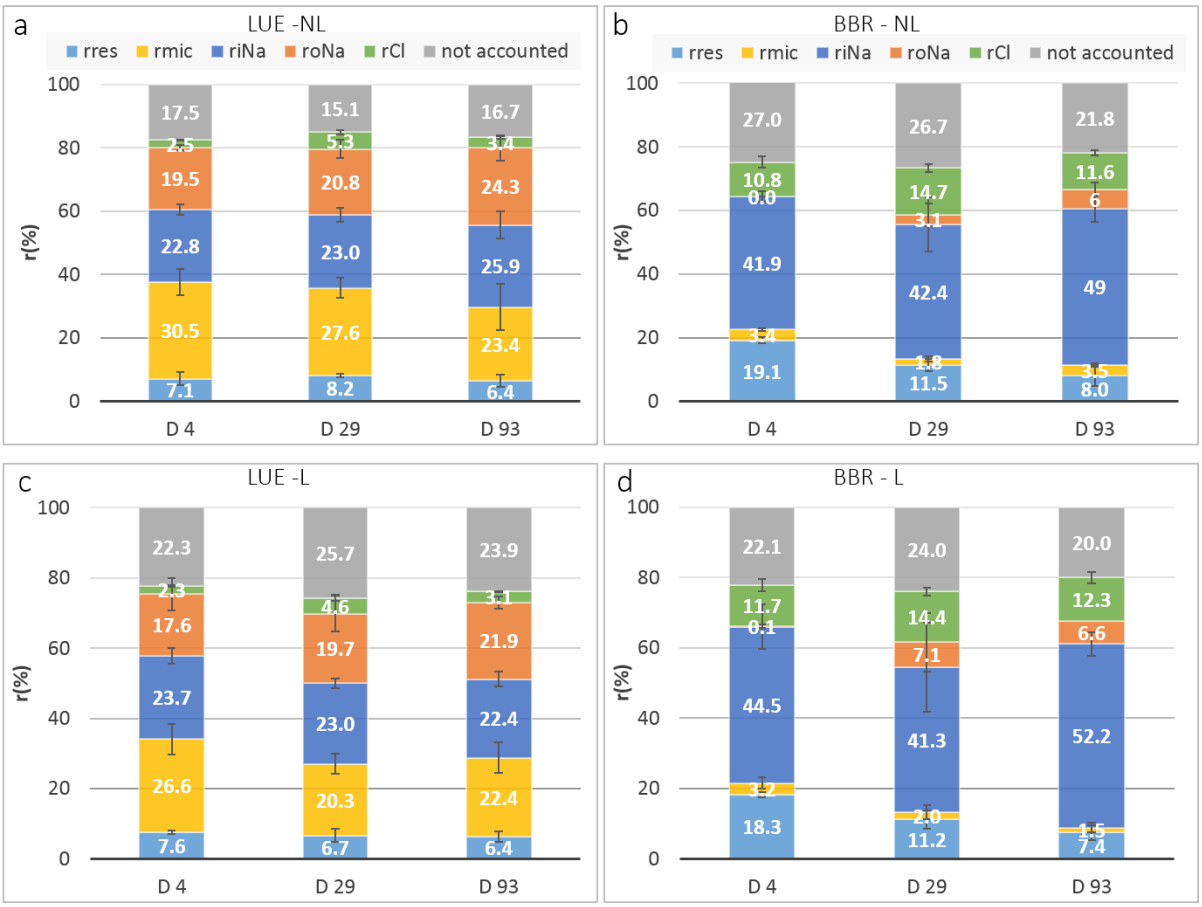

Fig. 3 Mean water-extractable P (P_w) concentration in LUE (a) and BBR (b) and corresponding specific activities in LUE (c) and BBR (d) during incubation, error bars represent the standard deviation (n=4), * above/below a single time point indicates significant differences between non-amended (NL) and litter-amended (L) treatments according to t-test. P-values from the two-factors ANOVA are shown on the figure.

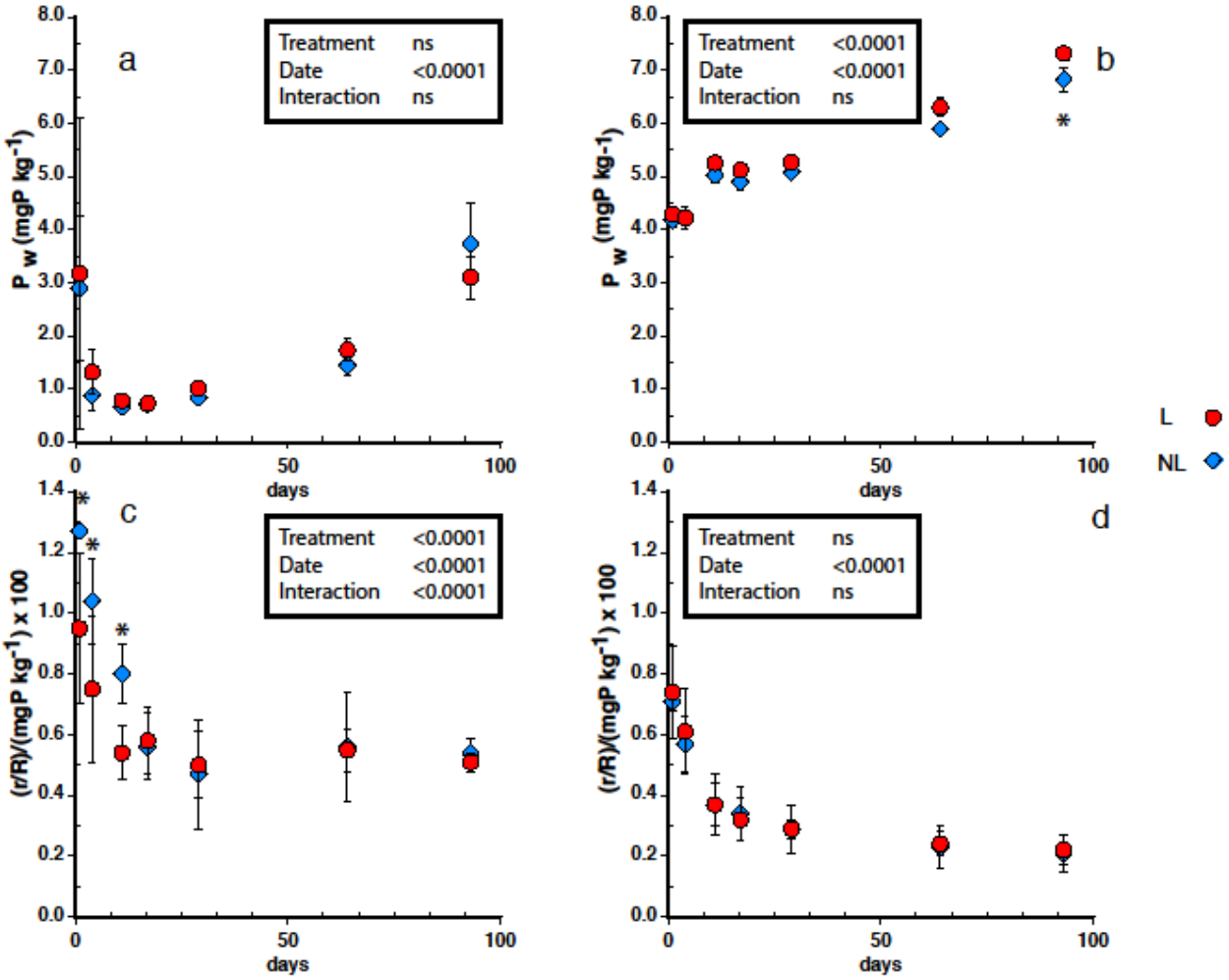

Fig. 4 Mean resin-extractable P (P_{res}) concentration in LUE (a) and BBR (b) and corresponding specific activities in LUE (c) and BBR (d) during incubation, error bars represent the standard deviation (n=4). P-values from the two-factors ANOVA are shown on the figure.

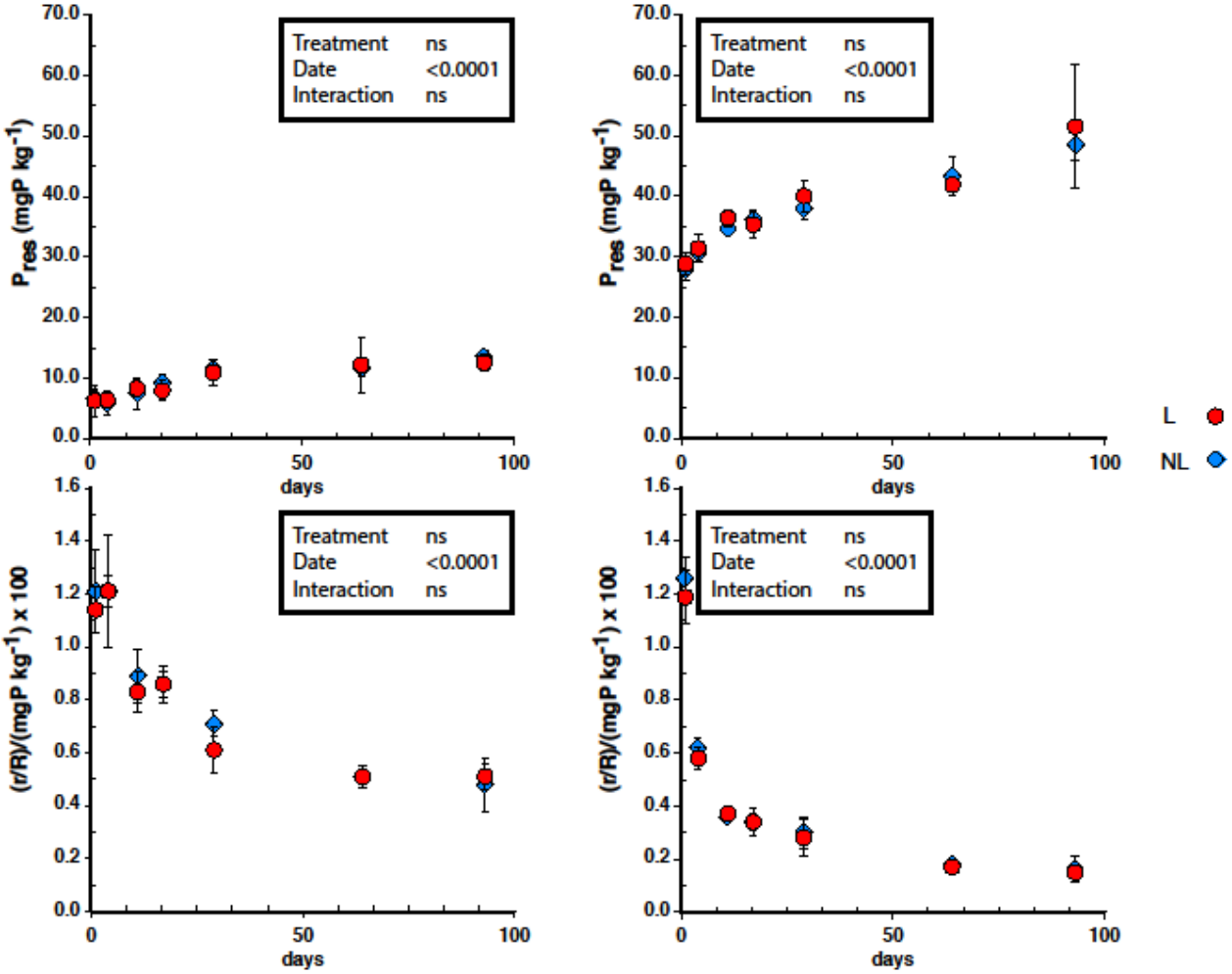

Fig. 5 Mean hexanol-labile P (P_{mic}) concentration in LUE (a) and BBR (b) and corresponding specific activities in LUE (c) and BBR (d) during incubation, error bars represent the standard deviation (n=4), * below a single time point indicates significant differences between non-amended (NL) and litter-amended (L) treatments according to t-test. P-values from the two-factors ANOVA are shown in the figure.