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

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2 **phosphorus availability in organic horizons of temperate forest soils**

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21 **Abstract**

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22   Understanding the mechanisms underlying phosphorus (P) availability is important to

23   predict forest productivity in a changing environment. We quantified P fluxes and

24   traced P from plant litter into inorganic and organic soil P pools in organic horizons

25   from two contrasting temperate forest soils with low and high inorganic P availability,

26   respectively. We incubated the two organic horizons with and without litter after

27   labelling the soil solution with ^{33}P and performed sequential extractions at several

28   time points in order to trace P dynamics in labile (water-extractable, available and

29   microbial P) and non-labile (non-living organic P, P bound to iron and aluminium and

30   P bound to calcium) pools. Under low P availability, P fluxes were dominated by

31   gross P mineralization, and microbial P immobilization accounted for up to 95% of

32   gross P mineralization. Additionally, labile P in plant litter was rapidly incorporated

33   into microbial P and only a small fraction ended up in the non-labile inorganic P

34   pools. In contrast, P fluxes under high P availability were dominated by abiotic

35   processes, particularly by fast (within 10 days) sorption/desorption reactions between

36   the available P and the P bound to aluminium. These findings support the hypothesis

37   that under low P availability biological processes control P fluxes. The observed tight

38   cycling of P, with little efflux due to net P mineralization, suggests that the

39   mineralization of organic P is driven by microbial P demand, and that the microbial

40   community could compete with plants for available P.

41

42   **1.   Introduction**

43   Anthropogenic impacts are expected to affect phosphorus (P) cycling in temperate

44   forest ecosystems. For example, a decline in foliar P concentration in European

45   forests has been observed in the past decade and ascribed to an increased tree demand

46   for this nutrient caused by intensified nitrogen depositions and atmospheric carbon

47 dioxide enrichment (Jonard et al., 2015). The magnitude and possible consequences

48 of this trend are still under debate; however, P is expected to become progressively

49 more limiting (Talkner et al., 2015). A deeper understanding of the underlying

50 mechanisms, e.g. the processes governing P availability, speciation and fluxes in

51 soils, is needed to predict the effects on net primary production by changing

52 environmental conditions.

53 Plants mostly access P from the soil solution, which represents usually a small

54 proportion of the total P in soil. Multiple chemical equilibria with the mineral and

55 sorbed phases regulate the replenishment of the soil solution (Helfenstein et al.,

56 2018). Additionally, microbial processes can strongly influence the availability of P in

57 soil (Achat et al., 2016; Bünemann, 2015). Microbes mineralize organic P (P_o) from

58 plant litter and non-living soil organic matter, and the newly mineralized P is

59 incorporated into the microbial biomass (immobilization), sorbed to the solid phase or

60 remains in solution. Upon cell death or predation, the microbial P, which is not

61 remineralized, enters the non-living soil organic P pool. The extent to which these

62 processes influence P availability for plants varies widely, depending on factors such

63 as land-use and inorganic P availability (Becquer et al., 2014; Bünemann, 2015).

64 In forest soils, organic horizons are essential for the recycling of nutrients coming

65 from plant inputs such as leaf litter. Studies tracing P uptake by selected forest species

66 have shown that the contribution of the organic horizon to plant P supply can be as

67 high as 99% (Brandtberg et al., 2004; Jonard et al., 2009).

68 Our understanding of such dynamics is hampered by the difficulty of quantifying P

69 fluxes. These are challenging to measure, because they often occur without net or

70 detectable changes in pool size. However, the use of P radiotracers (^{33}P or ^{32}P) helps

71 circumventing this issue. P radiotracers can be used to quantify gross P mineralization

72   and immobilization rates (Bünemann, 2015), or the fate of P added with plant residues

73   (Daroub et al., 2000), and hence they can allow assessing the relevance of such

74   processes to P availability.

75   A handful of studies have applied P radiotracers to assess P dynamics in forest soils

76   (Achat et al., 2010, 2009b, 2009a; Bünemann et al., 2016; Heuck et al., 2015;

77   Mooshammer et al., 2012; Spohn et al., 2013) or fluxes from soil to plants (Jonard et

78   al., 2009). Most of these studies targeted P dynamics in labile P pools, i.e. inorganic P

79   in solution and microbial P, not directly assessing the contribution of less labile pools,

80   particularly the mineral or sorbed P and the non-living organic P pool. Sequential

81   extractions are commonly used to characterize inorganic and organic soil P pools.

82   Such procedures yield operationally-defined pools, assuming an inverse relationship

83   between P availability in a given pool and the strength of the extractants (Tiessen and

84   Moir, 1993). Unless coupled with other techniques, sequential extractions alone do

85   not provide any information about the availability or fluxes of P (Frossard et al.,

86   1996). The recovery of a radiotracer in sequentially-extracted P pools was used to

87   compare soils under different land-use or tillage systems (Buehler et al., 2002;

88   Daroub et al., 2000), soil types (Vu et al., 2010) or crop rotations (Bünemann et al.,

89   2004b). In highly weathered and unfertilized tropical soils a shift toward microbial P

90   and organic P was observed, with higher recovery of the tracer in these pools (Buehler

91   et al 2002), which points to a high importance of biological P transformations under

92   limited inorganic P availability.

93   Despite its potential in identifying the relevance of different processes in influencing

94   P dynamics and recycling, such a combined approach using a radiotracer and

95   sequential extractions has never been applied to forest soils.

96   In this study, we aimed at elucidating P dynamics in inorganic and organic pools in

97   two forest organic horizons (Oe) differing in P content and availability (low *vs* high).

98   We also aimed at following the fate of P added with fresh plant litter into soil P pools.

99   Labile pools in mineral top soils from these forests were already investigated by

100   Bünemann and co-workers (2016), who showed that under low P availability

101   microbial processes accounted for up to 90% of isotopically exchangeable P fluxes,

102   while this contribution reduced to almost nil in a mineral topsoil with the same

103   vegetation but, very high P availability. Our objectives were to: i) assess which

104   inorganic and organic P pools participate in exchange reactions with the available P

105   and to which extent; ii) quantify P fluxes related to physico-chemical

106   (sorption/desorption, precipitation/dissolution) and microbiological processes (gross

107   mineralization/immobilization) in the presence or absence of litter inputs. To do so,

108   we adopted an isotopic dilution approach (Oehl et al., 2001) and followed the tracer

109   into P pools extracted with a sequential extraction (Tiessen and Moir, 1993).

110   Our hypotheses were that: under low inorganic P availability, I) biological processes

111   dominate P dynamics and II) there is a faster incorporation of P from the litter into

112   soil inorganic and organic pools due to a higher microbial activity.

113

114   **2.   Materials and methods**

115

116   *2.1 Site and sampling description*

117   The organic horizons used in this study were collected from two 100 to 120 years old

118   beech (*Fagus sylvatica* L.) forest sites. The site Bad Brückenau (BBR), is located at

119   about 800 m asl in Northern Bavaria, Germany (50°21'7.26"N, 9°55'44.53"E). The

120   soil is classified as Dystric Skeletic Cambisol (Hyperhumic, Loamic)

121 (FAO/ISRIC/ISSS, 1998) and developed on basalt. The site Lüss (LUE) is located at

122 100 m asl in Lower Saxony, Germany (52°50'21.77"N, 10°16'2.37"E). The soil in

123 LUE is developed on Pleistocene sand and is classified as a Hyperdystric Folic

124 Cambisol (Arenic, Loamic, Nechic, Protospodic). This two sites represent the

125 extremes of a geosequence covering a wide range of total and available soil P. Their

126 characteristics are described in detail in Lang et al. (2017).

127 At each site recent beech litter, i.e. litter deposited during the previous autumn, was

128 first collected. Then, after removing the litter layer, 5 to 6 subsamples from the Oe

129 horizon (0-12 cm and 0-5 cm at BBR and LUE, respectively) were taken and pooled

130 to form a composite sample. Samples from the LUE site were collected in April, and

131 samples from BBR site in May 2015.

132 The soil was sieved moist to < 5 mm. The litter was dried at 35°C, manually crushed

133 and sieved twice to collect the fraction between 20 mm and 5 mm. Both materials

134 were stored at 4°C for a period of two weeks (BBR) to one month (LUE) before the

135 experiment.

136

137 *2.2 Soil and litter characteristics*

138 Maximum water holding capacity (WHC) of the Oe horizons was determined

139 gravimetrically by placing the saturated soils in tared cylinders and letting them drain

140 on a sand bath for 4 h. pH was measured on settled 1:2 soil-water suspensions after 90

141 min shaking using an ORION 720A pH-meter. The two Oe horizons had both acidic

142 pH, but differed in almost all considered variables (table 1).

143 Total carbon (C_{tot}) and nitrogen (N_{tot}) content were determined on ground soil and

144 litter samples by dry combustion on an elemental analyzer (Variopyro Cube,

145 Elementar Analysensysteme GmbH, Germany). Total inorganic (H_2SO_4-P_i) and

146   organic (H_2SO_4-P_o) P content in the samples were determined according to Saunders

147   and Williams (1955) after ignition at 550°C for 1 h and successive extraction of

148   ignited and non-ignited subsamples with 0.5 M H_2SO_4 for 16h. H_2SO_4-P_o was then

149   calculated as P in ignited samples minus P in non-ignited samples. P in the litter was

150   done by incineration at 550°C for 1 h followed by extraction with concentrated hot

151   HNO_3 (Nanzer et al., 2014).

152   Inorganic P determination in all extracts was made with the malachite green method

153   (Ohno and Zibilske, 1991) using a UV-VIS spectrophotometer (UV-1800, Shimadzu).

154   Microbial carbon (C_{mic}) and nitrogen (N_{mic}) were determined by chloroform

155   fumigation and subsequent extraction with 0.5 M K_2SO_4 (Vance et al., 1987)(Fig.1).

156   The extracts were analyzed with a TOC/TN analyzer (Formacs[SERIES], Skalar, The

157   Netherlands).

158

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

	unit	day 0 BBR		day 93 BBR NL		day 93 BBR L		day 0 LUE		day 93 LUE NL		day 93 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,

174 due to the short duration, the isotopic dilution is assumed to be affected only by

175 physico-chemical processes, i.e. sorption, desorption, precipitation and dissolution.

176 IEK-derived parameters enable the extrapolation of the isotopic dilution to a longer

177 time span, the so-called isotopic dilution baseline, and of the estimation of

178 isotopically exchanged P (Fardeau, 1993; Frossard and Sinaj, 1998). During

179 incubations, both physico-chemical and biological processes affect the isotopic

180 dilution, thus the contribution of biological processes to the P exchanged can be

181 calculated by difference with the isotopic baseline (Oehl et al 2001).

182 In our study, a 3-month incubation (section 2.6) of the two Oe horizons was combined

183 with IEKs (section 2.5), and with sequential extractions of the incubated soil (sections

184 2.7 and 2.8). The IEKs were conducted on subsamples of the two Oe materials a few

185 days before the beginning of the main incubation. A 3-week pre-incubation, during

186 which we monitored the respiration, was carried out to obtain constant soil

187 respiration, required to meet the assumption of steady state (Oehl et al 2001).

188 The experimental design of the incubation had two factors: the soil (BBR and LUE)

189 and the litter treatment, which included soil amended with litter (L) and non-amended

190 soil (NL). All treatments had four replicates. During incubation, concentrations of ^{31}P

191 and ^{33}P (r) were measured in water-extractable P (inorganic P in solution, P_w), resin-

192 extractable P (inorganic available P, P_{res}) and hexanol-labile P pools (microbial P,

193 P_{mic}) at day 1, 4, 11, 17, 29, 64 and 93 after labelling. Additionally, at day 4, 29 and

194 93 we performed a modified Hedley sequential extraction (Tiessen and Moir, 1993) to

195 follow the fate of ^{33}P beyond the hexanol-labile pool. Soil respiration was determined

196 at weekly intervals (Fig. 1).

197

198 <Figure 1>

199

200 *2.4 Calculations of P exchanged*

201 During IEKs, the simultaneous desorption of ^{31}P and sorption of ^{33}P determine the

202 progressive decline of the initially added radioactivity (R) in the soil solution, so that

203 the specific activity (SA) of the solution, i.e. the ratio $^{33}P/^{31}P$, decreases with time.

204 Since there is no isotopic discrimination between ^{31}P and ^{33}P, the specific activity of

205 the solution is equal to the specific activity of the entire mass of distribution called E-

206 value, or isotopically exchanged P:

207

208 $$SA_{(t)} = {r_{(t)}}/{P_w} = R/{E_{(t)}} \qquad \text{Equation 1}$$

209

210 where $SA_{(t)}$ is the specific activity at time t in kBq g^{-1}/mg P kg^{-1}, $r_{(t)}$ is the residual

211 radioactivity in kBq g^{-1}, P_w is the concentration of inorganic P in solution (water-

212 extractable P) in mg P kg^{-1}, R is the added radioactivity in kBq g^{-1} and $E_{(t)}$ in mg P kg^{-}

213 ^{1} is the E-value at the time t, which can be derived by rearranging Eq. 1.

214 The decline of the radioactivity in the soil solution due to physico-chemical processes,

215 $r_{(t)}$/R, as a function of time can be described by the model proposed by Fardeau et al

216 (1991) (see Supplementary Information and Eq. 1S).

217 The model (Eq. 1S) was fitted with the experimental data from the IEKs and then

218 used to extrapolate the $r_{(t)}$/R for the time span of the incubation. The corresponding E-

219 values ($E_{mod(t)}$) represent the isotopic dilution baseline and were calculated with Eq. 1

220 using the extrapolated $r_{(t)}$/R and the P_w measured during the IEKs.

221 During the incubation, the amount of isotopically exchanged P ($E_{meas(t)}$) was

222 calculated with Eq. 1, but using the $r_{(t)}$/R and P_w measured at each sampling point of

223   the incubation. The cumulated gross organic P mineralization at time t ($GPM_{(t)}$) was

224   then derived by difference (Oehl et al. 2001):

225

226      $GPM_{(t)} = E_{meas(t)} - E_{mod(t)}$ Equation 2

227

228   where $E_{meas(t)}$ and $E_{mod(t)}$ are the isotopically exchanged P measured during the

229   incubation or extrapolated with Eq. 1S, respectively, both in mg P kg^{-1}.

230   The microbial P immobilization was calculated assuming P_w is the sole source of P

231   for microbes (Bünemann et al., 2007):

232

233      $Immobilization_{(t)} = SA_{Pmic} / SA_{Pw} * P_{mic(t)}$ Equation 3

234

235   where SA_{Pmic} and SA_{Pw} are the specific activities of microbial and water-extractable

236   P, respectively, both in kBq g^{-1}/mg P kg^{-1}, and P_{mic} is the microbial P at time t (in mg

237   P kg^{-1} soil), taken from Eq. 2S (Supplementary Information). However, we calculated

238   the immobilization only when SA_{Pmic} and SA_{Pw} were significantly different ($p<0.05$),

239   since further exchange between the microbial and the water-extractable pools cannot

240   be detected when both have reached a complete equilibrium. Finally, net organic P

241   mineralization (NPM) can be calculated by subtracting microbial P immobilization

242   from GPM (Bünemann et al., 2007). Mineralization and immobilization were

243   calculated at each sampling time and divided by the corresponding number of days to

244   obtain the daily rates.

245   Additionally, gross C mineralization rate was estimated as net C respired divided by

246   0.6, assuming a microbial C use efficiency of 0.4 (Murphy et al., 2003).

247   The fate of litter P in soil pools was calculated analogously to the proportion of a non-

248   labeled fertilizer introduced in a soil-plant system (Fardeau et al., 1995):

249

250   %Pdl=100(1-SA$_L$/SA$_{NL}$)   Equation 4

251

252   where %Pdl is the percentage of P in a given pool that is derived from the litter, SA$_L$

253   is the specific activity of the pool in the soil amended with litter and SA$_{NL}$ is the

254   specific activity of the same pool in the non-amended soil. This calculation was done

255   only when significant differences ($p < 0.05$) were detected between SA$_L$ and SA$_{NL}$.

256

257   *2.5 Isotopic exchange kinetics (IEKs)*

258   The possible effect of microbial P uptake during the IEK was checked with the use of

259   a microbial inhibitor, Bronopol PESTANAL (1 ml 0.025M, Sigma Aldrich, analytical

260   grade).

261   Four replicates of 5 g equivalent dry soil were shaken overnight with 98 ml of

262   ultrapure H$_2$O (accounting for water contained in the soil) to reach steady state, i.e.

263   constant P concentration in solution, and therefore equal Pi sorption and desorption

264   rates. The microbial inhibitor was added 20 minutes before the samples were placed

265   on a magnetic stirrer. At t0, 1 ml carrier-free ^{33}P solution (474 kBq ml^{-1}) was added to

266   each sample. Aliquots were collected from each replicate with a plastic syringe at 1,

267   4, 10, 30, 50 and 100 minutes after the ^{33}P addition and filtered through syringe filters

268   (0.2 µm, Minisart, Sigma-Aldrich). The radioactivity in these aliquots ($r_{(t)}$) was

269   measured by scintillation counting, while P concentration (P$_w$) was determined with

270   the malachite green method.

271

2.6 Incubation experiment

273   After sieving, soils were dried down slightly at room temperature before the pre-

274   incubation to lower the moisture content and enable subsequent addition of the

275   labeling solution. The pre-incubation was conducted at approximately 40% of the

276   maximum WHC in plastic containers kept at room temperature in the dark for 25

277   days.

278   After pre-incubation, soil was weighed in polyethylene zip lock bags (equivalent of

279   65 g dry soil each), and a labeling solution was prepared with carrier-free phosphoric

280   acid (Hartmann Analytic, Braunschweig, Germany). Four ml of the labeling solution

281   (244.4 kBq ml^{-1}) was added to each bag, spreading on the top by pipetting and mixing

282   with a whisk for 1 minute. This operation was repeated for a total of 8 ml of labeling

283   solution. To reach the desired WHC, each bag additionally received 9 and 18.6 ml of

284   ultrapure H_2O in the case of BBR and LUE, respectively. Finally, litter was added to

285   half of the bags, at a rate of 10 mg per g of dry soil, corresponding to 4.6 mg C g^{-1}

286   soil. This amount is very close to natural litter inputs at the two sites as recalculated

287   from Lang et al. (2017). Each replicate was mixed again for 1 minute and placed

288   slightly open in a plastic tray with cover and incubated in the dark at 19°C. During the

289   incubation, the gravimetric water content of the soils was kept at 50% and 53% of

290   water holding capacity (WHC), respectively for BBR and LUE. A beaker filled with

291   water was added to each tray in order to keep air moisture as constant as possible. The

292   final soil label (R) was 30.077 kBq g^{-1} soil for both soils.

293   For soil respiration measurements, a separate set of samples including all the

294   treatments, each one of 10 g dry weight equivalent, was prepared on the day of

295   labeling adding ultrapure H_2O instead of the labeling solution. Each sample was

296   placed in a tightly closed jar (1 L volume) together with an alkaline trap made of 20

297   ml 0.2M NaOH solution, including four blanks without soil. The jars were then

298   incubated together with the labeled samples. Soil respiration was measured by

299   trapping the CO_2 liberated from the soil followed by back titration (Alef, 1995).

300

301   *2.7 ^{31}P and r in resin-extractable, water-extractable and hexanol-labile P during*

302   *incubation*

303   Water-extractable P (P_w), was extracted by shaking 5 g equivalent dry soil in 100 ml

304   ultrapure H_2O (accounting for water contained in the soil) for 16 h on an overhead

305   shaker. Samples were filtered directly after the shaking using 0.2 µm syringe filters

306   (Millipore).

307   In the case of resin-extractable and hexanol-labile P (P_{res} and P_{mic}), we followed the

308   method proposed by Kouno and co-workers (1995) and modified by Bünemann and

309   co-workers (2004). In detail, three subsamples constituted by 1:15 soil suspensions

310   with 2 g equivalent dry soil were prepared. In each, a resin membrane (BDH #55164,

311   6 cm x 2 cm) in the carbonate form was added. The first subsample had no additional

312   treatment (P_{res}), the second subsample received 1 ml of 1-hexanol (P_{hex}), and the third

313   subsample received a P spike (P_{spike}). The latter simulates P release from cell lysis

314   after hexanol addition and allows estimating the possible sorption onto the solid phase

315   of the newly released P. A single P spike of about 50 mg P kg^{-1} was used, since the

316   relationship between recovered and added P was shown to be linear in the range of 10

317   to 50 mg P kg^{-1} on the mineral horizon of these soils (Bergkemper et al., 2016). No

318   spike of radioactivity was included because Bünemann and co-workers (2016)

319   showed that in these soils the recovery of added radioactivity is similar to that of the P

320   spike.

321 The samples and blanks were shaken horizontally for 16 h. Then the resins were

322 eluted with 0.1 M NaCl/HCl for 2 h, after rinsing with ultrapure H_2O. P_{mic}

323 concentration in mg kg^{-1} of soil was calculated by the difference between the hexanol

324 and the P_{res} subsamples accounting for sorption (Eq. 2S in Supplementary

325 Information). No conversion factor (Kp) was used to correct for possible inefficiency

326 of the fumigant, i.e. incomplete recovery of microbial P, since this is soil-specific and

327 has not been determined for these soils. Estimated Kp ranges between 0.3 and 1

328 (Oberson and Joner, 2005; Achat et al., 2009b), therefore the underestimation of the

329 microbial P may exceed 100%.

330 The recovery of radioactivity in the microbial mass (r_{mic} in percent of total

331 radioactivity) had to be corrected for possible ^{33}P release from the labeled soil due to

332 replacement with ^{31}P liberated from microbial cells, which would lead to an

333 overestimation of r_{mic} (Oehl et al, 2001). Therefore, we corrected r_{mic} as reported in

334 Bünemann and co-workers (2016), using the radioactivity recovered from the spiked

335 samples (see supplementary information and Eq. 3S and 4S)

336

337 *2.8 ^{31}P and r in sequentially-extracted P pools during incubation*

338 The sequentially extracted pools were the 0.25M NaOH/0.05M EDTA-extractable P,

339 representing the inorganic and organic P bound to Fe and Al oxides (hereafter P_{iNa}

340 and P_{oNa}), and the HCl-extractable P (P bound to Ca, P_{Cl}).

341 The subsample extracted with hexanol was used for the subsequent steps of the

342 sequential extraction. After removing the resins, NaOH and EDTA disodium salt were

343 added to the soil suspensions in solid form to reach the wanted concentration. After

344 16 h shaking, the samples were centrifuged (5300 g for 15 minutes), filtered through

345 Millipore nylon filters (0.8 μm), and the filtrates were collected for P_{iNa} and P_{oNa}

346   determination. Subsequently, 30 ml of 1 M HCl were added to the same samples and

347   the extracts collected after shaking overnight and filtering using glass fiber filters (0.8

348   μm, Millipore).

349   To separate P_{iNa} and P_{oNa} and measure the radioactivity in the NaOH-EDTA extracts,

350   two methods were tested: separation by isobutanol (Jayachandran et al., 1992) and by

351   acidification-centrifugation (Tiessen and Moir, 1993). This latter gave better Pi

352   recovery (>80%) and was preferred. The NaOH-EDTA extracts were therefore

353   acidified to pH 1.5 with 0.5 M H_2SO_4 to induce the precipitation of organic

354   substances. The ^{31}P and ^{33}P measured in the acidified supernatant after centrifugation

355   represent the inorganic fraction. The organic fractions, P_{oNa} and r_{oNa}, were then

356   determined by difference with the total P concentration or total radioactivity,

357   respectively.

358   The radioactivity in all extracts was detected by liquid scintillation using a beta-

359   emission counter (Tri-carb 2500 TR, Packard Instruments, Meriden, CT) after

360   thoroughly mixing the samples with Ultima Gold or Ultima Gold AB for acid

361   extracts. Quenching effects in colored extracts, e.g. NaOH-EDTA extracts, were

362   corrected by comparing the counts per minute of a ^{33}P spike in water, a ^{33}P spike in

363   the sample and a water spike in the sample.

364   Radioactivity measurements were recalculated to t = 0 using the equation of

365   radioactive decay. Radioactivity recovery (r_w, r_{hex}, r_{res}, r_{iNa}, r_{oNa} and r_{Cl}) of a given

366   pool is expressed in percentage of the total introduced radioactivity R (r/R*100).

367

368   *2.9 Statistical analysis*

369   A 2-way factorial ANOVA (1st factor = litter application, 2nd factor = date) was used

370   to analyze the variables measured during the incubation for each soil separately

371   except the respiration rates. These latter results were analyzed using a mixed model,

372   where the litter amendment was the fixed factor and the time of the measurement

373   (weekly) was a random factor with the replicate nested in it. The Tukey test was used

374   for post hoc comparison. The whole data set was analyzed with a 3-way ANOVA

375   including the soil as a factor. However, we discuss mostly the results of the 2-way

376   ANOVA as the two very contrasting soils (table 1) resulted in constantly significant

377   differences. The Student's paired t-test was used when comparing single dates and

378   cumulative values, after checking for homogeneity of variances. The Shapiro-Wilk

379   test was used to assess normality of the data. All analyses were performed in R 3.1.1

380   (R version 3.1.1, R Core Team).

381

382   **3. Results**

383   *3.1 Soil respiration*

384   The soil respiration in LUE was almost the double of that in BBR. The litter

385   amendment resulted in a significant increase of the cumulative amount of C released

386   in both soils, with an additional C release in the amended treatments of 5.98 and 7.30

387   % for LUE and BBR, respectively (Table 2).

388   Compared with the pre-incubation period there was a 50% increase in respiration

389   immediately after labelling in LUE, compared with a 10% increase in BBR (fig. 1S).

390   During the incubation, soil respiration showed two distinct phases. During the first

391   four weeks after labelling the respiration was higher and then decreased by about 20

392   and 25% in LUE and BBR, respectively. From five-six weeks onwards, it remained

393   approximately stable. During the first phase, the differences between the litter

394   amendments were more pronounced, with higher respiration in the litter-amended

395   soils (fig. 1S).

396

Table 2. Cumulative respiration in LUE and BBR (mean ± standard deviation of 4
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		

399
400

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

The radioactive tracer was distributed very differently over various P pools in the two

soils (fig. 2). In BBR, the recovery of radioactivity in the microbial pool (r_{mic}) was

very low, fluctuating around 2%, and for some replicates below the detection limit

(fig. 2b and d). In contrast, it reached 30% in LUE after only four days of incubation

(fig. 2a). Similarly, a consistent recovery of radioactivity in LUE was found in the

organic P pool already at day 4 (r_{oNa} = 18.6% as average of the two litter treatments, L

and NL), increasing significantly to 23% at day 93 (fig. 2a and c), while in BBR the

corresponding values were initially close to 0 and increased to about 6% at day 93

(fig. 2b and d). Most of the radioactivity in BBR was recovered in the inorganic P

extracted with NaOH-EDTA (r_{iNa} = 43.2% to 53.5% at day 4 and 93, respectively)

and in the resin-extractable P (r_{res} = 34.5% to 7.5% at day 1 and 93, respectively).

Differences due to the litter addition were found only in the recoveries of water-

extractable, microbial and HCl-extractable P of LUE, where the recovery of

radioactivity was slightly lower in the litter-amended treatment.

The tracer could not be recovered entirely, with the non-recovered fraction

representing 17 to 27% in BBR and 15 to 26% in LUE. Losses during the

manipulation were estimated to be around 5% of the total radioactivity. The

18

419   remainder was likely transferred into the residual P pool, which was not extracted and

420   quantified.

421

422   <Figure 2>

423

424   *Changes in pool sizes and specific activities: labile pools*

425   In LUE, the water-extractable P (P_w) increased at day 1 compared to the initial value

426   in both litter treatments (L and NL, Table 1 and fig. 3), i.e. 1.1 mg P kg^{-1} *vs* 2.9 and

427   3.2 mg P kg^{-1} for the non-amended and litter-amended treatment, respectively. Then it

428   dropped to around 1 mg P kg^{-1}, remained constant until day 29 and increased again in

429   the last two sampling dates.

430   In BBR, P_w increased steadily from the initial value of 4.2 to 6.5 mg P kg^{-1} (as

431   average of the treatments). The litter addition caused statistically significant

432   differences in BBR at day 93, with a slightly higher concentration in the amended (7.3

433   mgP kg^{-1}) compared to the non-amended treatment (6.8 mgP kg^{-1}).

434   The specific activity of this pool (SA_{Pw}) decreased over time in both soils and it

435   remained approximately stable after day 29 (LUE) or 64 (BBR). Significant

436   differences between the treatments were found only at the initial stage of the

437   incubation in LUE (day 1 to 11).

438   <Figure 3>

439   <Figure 4>

440   <Figure 5>

441

442   As for P_w, the resin-extractable P (P_{res}) increased during incubation: from 6.4 to 13.0

443   mg P kg^{-1} as average of the litter treatments in LUE and from 28.3 to 50.0 mg P kg^{-1}

444   in BBR. The corresponding specific activities (SA_{Pres}) decreased steadily until the end

445   of the incubation (Fig. 4). The specific activities of water and resin pools were similar

446   both in LUE and BBR. No significant differences due to the litter addition were

447   observed.

448   As for the radioactivity recovery, the dynamics of the microbial P (P_{mic}) were very

449   different in the two soils (Fig. 5). In LUE, the P_{mic}, after a slight, but significant

450   increase between day 4 and 11 likely caused by soil mixing, remained rather stable for

451   the duration of the experiment and was not affected by the litter amendment.

452   Conversely, in BBR, P_{mic} declined from the initial value of 93 mg P kg^{-1} down to 78.1

453   and 60.4 mg P kg^{-1} for the litter-amended and non-amended treatment, respectively, at

454   the final sampling. Hence, the litter addition led to significantly higher P_{mic}

455   concentrations.

456   The specific activity in the microbial P (SA_{Pmic}) in LUE decreased until the middle of

457   the incubation as a consequence of constant P_{mic} and decreasing radioactivity

458   recovery, then remained approximately constant. Overall, litter addition caused

459   slightly lower SA_{Pmic} values compared to the untreated soil.

460   In BBR, because of the very low tracer recovery, the SA_{Pmic} fluctuated around very

461   low values without differences due to the litter amendment.

462

463   *Changes in pool sizes and specific activity: sequentially extracted pools*

464   In BBR, the NaOH-EDTA-extractable inorganic and organic P (P_{iNa} and P_{oNa}) and the

465   HCl-extractable P remained stable over time, while the specific activity of those three

466   pools increased slightly but significantly from day 4 to day 93 as a consequence of the

467   increase of radioactivity recovery (table 3 and fig. 2b and d). For the P_{iNa} this

468   translated in a specific activity (SA_{PiNa}) of 0.066 and 0.079 (mgP kg^{-1})$^{-1}$ at day 4 and

20

469   93, respectively (average of L and NL treatments, table 3). The little radioactivity

470   recovery in the organic pool translated in a specific activity (SA_{NaPo}) of about 0.005

471    $(mgP\ kg^{-1})^{-1}$ at the end of the incubation. These pools were not affected by the litter

472   amendment.

473   In LUE, only the HCl-extractable P showed a temporal trend, as it increased from day

474   4 to day 29 and decreased again at the last sampling time. Conversely, its specific

475   activity (SA_{Cl}) was nearly constant, though it was affected by the litter addition, with

476   a lower specific activity in the litter-amended treatment (table 3).

477

478 Table 3. Concentrations and corresponding specific activities (SA) of stable pools: NaOH-EDTA extractable inorganic (P_{iNa}) and organic P (P_{oNa})
479 and HCl extractable P (P_{Cl}). R= radioactivity introduced, r = radioactivity in solution. Letters indicate significant differences among the three time
480 points as average of NL and L treatments, asterisks indicate significant differences between non-amended (NL) and litter-amended (L) treatments
481 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

482
483

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

485   In LUE, the estimated isotopically exchangeable P values, $E_{mod(t)}$, were extremely

486   low, attaining 11.7 ($\pm$3) mgP kg^{-1} at the end of the incubation period, which

487   corresponds to 10% of the total inorganic P (H_2SO_4-P_i, Table 1). The corresponding

488   value in BBR was 568 ($\pm$76) mg P kg^{-1}, which corresponds to 55% of the total

489   inorganic P (table 4 and 5).

490   In LUE, the measured E values ($E_{meas(t)}$) were always much higher than the estimated

491   ones ($E_{mod(t)}$). In contrast, in BBR the $E_{meas(t)}$ were always lower than $E_{mod(t)}$,

492   preventing the calculation of the gross P mineralization (GPM) with equation 3.

493   In LUE, the litter addition resulted in higher $E_{meas(t)}$ values at the beginning of the

494   incubation (day 4-11), whereas in BBR no effect of the litter was visible (table 4 and

495   5).

496   The resulting $GPM_{(t)}$ values in LUE were rather high. The daily rates decreased over

497   time, from 60.1 mg P kg^{-1} day^{-1}, as average of the litter treatments, at day 1 to 2 mg P

498   kg^{-1} day^{-1} at day 93. At day 4-11, $GPM_{(t)}$ values were higher where the litter was

499   added (table 4).

500   Microbial immobilization and net mineralization ($NPM_{(t)}$) were calculated for the

501   time points in which the specific activities of microbial and water-extractable P were

502   significantly different (see section 2.4 and Eq. 3). In LUE, after day 11, the two

503   specific activities were indeed very close, masking any further exchange between P_{mic}

504   and P_w. During this period, the $NPM_{(4-11)}$ was significantly higher in the litter-

505   amended treatment, accounting for 62% and 70% of the $GPM_{(t)}$ in the non-amended

506   and litter-amended treatment, respectively.

507   In BBR, the specific activities of the microbial and water-extractable P never

508   converged; therefore, we could calculate the immobilization. This fluctuated around

509   low values (2.1-9.7 mg P kg^{-1}) without clear trends and the effect of the litter was

510   weak or not detectable (table 5).

511   It is important to highlight that both the immobilization and NPM$_{(t)}$ are derived from

512   P$_{mic}$ and are therefore potentially affected by the error associated with the fumigation

513   efficiency.

514

515 Table 4. Measured (E_{meas}) and extrapolated E-values (E_{mod}) (mean ± standard deviation of 4 replicates), mineralization and immobilization rates
516 (GPM: gross P mineralization, NPM: net mineralization, IMM: microbial immobilization) in LUE, nd: not determined. * indicates significant
517 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

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

519 Table 5. Measured (E_{meas}) and extrapolated E-values (E_{mod}) (mean ± standard deviation of 4 replicates), mineralization and immobilization rates

520 (GPM: gross P mineralization, NPM: net mineralization, IMM: microbial immobilization) in BBR. * indicates significant difference (p>0.05)

521 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

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

523

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

548   were detected in the Ah horizon of BBR (Prietzel et al., 2016), and material from this

549   horizon might be transferred to the Oe horizon by bioturbation or during the

550   sampling. More likely the 1M HCl extracted some P bound to Fe and Al

551   oxyhydroxides by surface precipitation (Werner et al 2017), which could not be

552   entirely extracted by the NaOH-EDTA step.

553   Overall, the contribution of living and dead organic P pools to the exchanges with the

554   available P was marginal in BBR, but visible in the progressive increase in available

555   P, which correlated with the decrease in microbial P (Fig. 3S). Therefore, we

556   conclude that the dynamics of the available P pool in this horizon were mainly

557   controlled by abiotic processes. This is rather surprising for an organic horizon, as this

558   pattern is more typical of mineral top- or subsoils (Bünemann, 2015).

559   The picture differed drastically in LUE, where the highest radioactivity recovery

560   found in the microbial pool (r_{mic} of 30% after 1 day) suggested a rapid P uptake by the

561   microbes. Accordingly, we observed a very fast convergence between the specific

562   activities of the water-extractable P and the microbial P: within 11-20 and 4-11 days

563   for non-amended and litter-amended treatments, respectively. The time until these

564   two specific activities converge can indeed be interpreted as an estimation of the

565   microbial P turnover time (Oehl et al 2001).

566   The relative stability of the r_{mic} after the first month of incubation suggested either a

567   tight P cycling within the microbial community with little P efflux upon death or the

568   return to a dormant state of the community after the initial activation by soil mixing

569   (Bünemann et al., 2016). The trend in the respiration (fig. 1S) seems to support this

570   second explanation.

571   Additionally, in LUE we observed high and rapid tracer incorporation into the non-

572   living organic P pool (NaOH-EDTA-labile P_o, fig. 2a and c). In the absence of

28

573   labelled P_o sources, the labelled P_o can only be of microbial origin, suggesting a rapid

574   release of organic P forms through microbial turnover. This, however, could be the

575   result of experimental artefacts since part of the microbial P_o might have remained in

576   solution after the hexanol fumigation, and cell lysis could then have been be carried

577   over in the subsequent NaOH-EDTA extraction. The precipitation of inorganic P

578   along with the organic matter upon acidification (Tiessen and Moir, 1993) could also

579   bias the separation between the inorganic and organic fractions. The fact that we

580   observed a temporal trend in the recovery of both microbial P (from 30 to 20%) and

581   non-living P_o (from 18 to 23%) supports, at least partially, the assumption of an active

582   process rather than an experimental artefact.

583   Bünemann and co-workers (2004b) and Buehler and co-workers (2002) measured

584   recoveries of 10 to 15% in the organic P pool of weathered tropical soils after 7 and

585   10 days of incubation, respectively. Vu and co-workers (2010) found up to 6-7%

586   recovery in 0.1 M NaOH-labile P_o after 4 days of incubation of a Chromosol. Overall,

587   in LUE, the sum of recoveries in microbial and non-living organic P accounted for 40

588   to 50% of the introduced radioactivity, which is higher than the sum of recovery in P_o

589   fractions in the aforementioned studies. At the same time, the size of the non-living P_o

590   pool remained approximately constant, indicating no net accumulation of the newly

591   produced P_o and pointing to the importance of recycling.

592   Altogether, these results support our first hypothesis about a biologically dominated P

593   cycling under low inorganic P availability, characterized by a rapid microbial P

594   turnover and a significant recycling of organic P. This is highlighted by the difference

595   in respiration of the two organic horizons, which was much higher in LUE than in

596   BBR (Table 2).

597

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

599	In BBR, we observed a very high baseline of isotopic dilution ($E_{mod(93)}$ = 568 mg P

600	kg^{-1}), which impeded the calculation of gross P mineralization rates with equation 3.

601	The same was found in the mineral topsoil of BBR and was attributed to its very high

602	inorganic P availability (Bünemann et al., 2016). This explanation agrees with the

603	predominance of fast exchanges observed between the available P and the P bound to

604	Al. If estimated from the carbon release (Achat et al., 2009b), the cumulated gross P

605	mineralization in BBR at day 93 would range between 55 and 60 mg P kg^{-1}, which

606	confirms the dominance of physico-chemical processes.

607	In LUE, gross P mineralization accounted for more than 94% of P isotopically

608	exchanged over the incubation for both treatments (table 4), clearly showing the

609	dominance of microbial processes. This proportion is slightly higher than the one

610	measured on the mineral topsoil of LUE (74 to 90% in the Ahe horizon, Bünemann et

611	al 2016) and on few other forest soils (Achat et al., 2009b; Spohn et al., 2013).

612	Gross P mineralization rates (GPM$_{(t)}$) derived from 7 to 10-days incubations of

613	different soils range from 0.8 to 12.6 mg P kg^{-1} d^{-1} as reviewed by Bünemann, (2015).

614	The corresponding GPM$_{(11)}$ daily rates measured in LUE were in the upper end of this

615	range or higher: 10.8 and 16.3 mg P kg^{-1} d^{-1} for non-amended and litter-amended

616	treatments, respectively (table 4). However, in our experiment, the rates measured in

617	the first four weeks were likely not under steady-state conditions, because the

618	respiration rate increased after soil mixing and was not constant (Oehl et al., 2001).

619	The values measured in the subsequent period, ranging from 7.2 to 1.9 mg P kg^{-1} d^{-1}

620	(table 4), represent a more realistic estimation of the basal P mineralization rate.

621	These are, however, rather high as compared to the mineral topsoil under forest (cfr.

622   Table 1 in Bünemann 2015 and Bünemann et al 2016), and confirm the relevance of

623   the organic horizon for the recycling of P_o under P limiting conditions.

624   Further partitioning of the gross mineralization rates in net mineralization ($NPM_{(t)}$)

625   and immobilization is complicated by the lack of a specific correction factor

626   accounting for fumigation efficiency (Kp). In case of incomplete microbial P

627   recovery, the net P mineralization rate is overestimated and the immobilization is

628   underestimated (see section 2.4).

629   An estimation of net P mineralization rate is obtained for a low-sorbing organic layer

630   by calculating the net change in inorganic P in solution over an incubation period

631   (Jonard et al., 2010). In LUE, the net change in the available P would result in a

632    $NPM_{(93)}$ of about 0.07 mg P kg^{-1} d^{-1} (table 4) and account for 3-4% of the gross P

633   mineralization over the incubation period. This would indicate that most of the

634   mineralized P was immobilized rather than released to the soil solution. In accordance

635   with this, the dominance of microbial immobilization over net mineralization was

636   observed in P-deficient soils with a large proportion of fumigant-labile P (Achat et al.,

637   2009b; Bünemann et al., 2012).

638   The $NPM_{(93)}$ estimated for BBR (Table 5) would be instead about 40% of the gross P

639   mineralization estimated by C release, suggesting that the gross P mineralization was

640   in this case rather driven by the need of carbon (Ali et al., 2014; Heuck et al., 2015).

641

642   *2.   Fate of litter P in BBR*

643   In BBR, the litter addition was not producing a net change in soil inorganic or organic

644   P pools, except for microbial P, which showed a net increase of about 18 mgPkg^{-1} by

645   day 93. Microbial P indeed declined in both treatments, but more in the non-amended

646   treatment, suggesting that litter addition delayed the return of the microbial

647   community to a dormant state. The higher respiration rate in the amended treatment

648   during the first four weeks agrees with this explanation (fig. 1S). The P released in the

649   non-amended treatment from the microbial pool was not recovered in the available

650   pool and presumably ended up in the pool of P bound to Al, although not detectable

651   against the amount of P present in this pool.

652   The lack of differences in specific activities between amended and non-amended

653   treatments precluded the estimation of P derived from the litter in soil P pools

654   (equation 4). As the amount of P added with the litter was rather small compared to

655   the soil P pools in BBR, a small isotopic dilution would be hard to detect against the

656   error of measurement. For example, the entire amount of inorganic P added with the

657   litter (Pi in table 2S) would dilute the specific activity of the available P by only 5%,

658   which corresponds to the coefficient of variation of replicates.

659   The decline in P_{mic} was decoupled from the microbial C (C_{mic}), which instead

660   remained stable over time and between treatments (Table 1). Such decoupling can be

661   attributed to a change in the activity of microbial cells with the build-up of P rich

662   compounds (Bünemann, 2015) after the addition of fresh plant inputs, or to a shift in

663   the microbial community composition in response to different environmental

664   conditions (Fanin et al., 2013; Mooshammer et al., 2014).

665   The second interpretation is supported by the parallel study of Mészáros and co-

666   workers (2016), who analysed the microbial community composition at different time

667   points of our incubation and found significant differences. Additionally, the similar

668   metabolic quotient (C respired per unit of microbial C) between litter-amended and

669   non-amended treatment (2.4 and 2.5 mg C-CO_2 mg^{-1}C_{mic} h^{-1}) suggests a similar

670   substrate use efficiency (Hartman and Richardson, 2013).

671

3.   Fate of litter P in LUE

673   Although the litter did not induce any detectable change in P pool sizes in LUE, we

674   detected isotopic dilution, i.e. lower specific activity, in the water-extractable (P_w),

675   microbial (P_{mic}) and HCl-extractable P (P_{Cl}) during the first period of incubation (day

676   1 to 29). Our interpretation is that the inorganic and labile P_o, e.g. litter

677   phosphomonoesters, were initially released from the litter and/or mineralized by

678   extracellular enzymes and went into solution, thus explaining the initial isotopic

679   dilution in P_w. According to equation 5, the released quantities corresponded to 0.8

680   ($\pm$0.2) to 0.3 ($\pm$0.05) mg P kg^{-1} (Table 1S), about 10 and 4% of the added P. Such a

681   small amount could not be detected as a net increase in pool size.

682   After day 11, we could not detect any significant differences between treatments in

683   the specific activity of P_w, meaning that no further release from the litter occurred or

684   that the newly released P was rapidly taken up by the microbes. Accordingly, the

685   significantly lower specific activity of microbial P in the litter-amended treatment

686   (fig. 5c) suggests that microbial uptake from an unlabelled source occurred. The

687   threefold increase of the C:P ratio of the litter sampled at the end of the incubation

688   agrees with this finding (Table 2S). However, the estimated quantity of P in the

689   microbial pool derived from the litter (Eq. 4) calculated for day 29 was 14.4 ($\pm$2.7)

690   mg P kg^{-1} (Table 1S). This amount exceeds the total P added with the litter. A

691   possible explanation is that the litter addition stimulated the mineralization of other P_o

692   substrates in soil, i.e. priming effect (Kuzyakov, 2010). The higher net mineralization

693   rate in the 4-10 day interval points to that explanation. However, a corresponding

694   decrease of the P_o pool could not be detected against the error affecting this

695   measurement.

696   The significant isotopic dilution observed at day 29 in the P_{Cl} pool of the litter-

697   amended treatment (table 3) suggests also that a small part of P released was

698   transferred to this pool.

699   The lack of a net increase in P_{mic} following litter addition might be explained by the

700   recalcitrant nature of this plant material. Indeed, P immobilization occurring after the

701   addition of fresh substrates is less pronounced if the carbon source is less labile

702   (Bünemann et al., 2004a). Beech litter contains more recalcitrant compounds,

703   especially lignin, than other broadleaf trees litter (Mooshammer et al., 2012; Steffens

704   et al., 2015). In agreement with this, the additional carbon release corresponded to

705   only 14% of the added carbon, and no differences in gross or net P mineralization

706   were recorded after day 11. Our results indicate that the microbial communities

707   supplied their P demand from different sources in the presence or absence of fresh

708   litter. The slightly different C:P ratio of the microbial pool between non-amended and

709   litter-amended treatments at the end of the incubation (Table 1) indicates a possible

710   shift in the community.

711   We conclude that the most labile P forms in plant litter were rapidly cycled through

712   the biota and only a small amount was transferred to inorganic P pools in LUE. Given

713   the impossibility to trace the fate of litter P in BBR, we could not confirm our second

714   hypothesis.

715

716   **Conclusions and perspectives**

717   Under low P availability, we observed that the majority of P fluxes were biologically

718   dominated, with a pivotal importance of microbial and non-living organic P, and a

719   rapid turnover of microbial P (around 4-11 days). Additionally, labile P in plant litter

720   was rapidly cycled through the biota and only a small amount was transferred to

721   inorganic P pools.

722   Under high P availability, fast exchange dynamics were observed between available

723   and sorbed P pools, which accounted for the highest tracer recovery. Under these

724   conditions, trees can rely upon P desorption fluxes to cover their P demand. In

725   contrast, in LUE the flux of isotopically exchangeable P was very low so that plants

726   would have to rely on recycling of organic P. However, our results indicated that

727   microbes are very efficient in immobilizing P, i.e. the flux due to net mineralization

728   was small, which suggests that the microbial community could compete with plants

729   for available P. For this reason, the mechanisms underlying the microbial P pool

730   contribution to plant nutrition under low P availability remain to be elucidated.

731

738

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Figure

920   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
Microbial C and N			x							x

923 Fig. 2 Radioactivity recovery (radioactivity detected in a pool divided by the total

924 introduced radioactivity, in %) in the P pools in the non-amended treatment, NL (a)

925 and litter-amended treatment, L, (c) in LUE; in NL treatment (b) and L treatment (d)

926 in BBR, bars represent the standard deviation (n=4). rres = recovery in resin-

927 extractable P, rmic= recovery in hexanol-labile P, riNa= recovery in inorganic P

928 extracted with NaOH-EDTA, roNa= recovery in organic P extracted with NaOH-

929 EDTA, rCl = recovery in HCl-extractable P. D = number of days after labelling.

930 Water-extractable P is not shown, as it is part of the resin-extractable pool
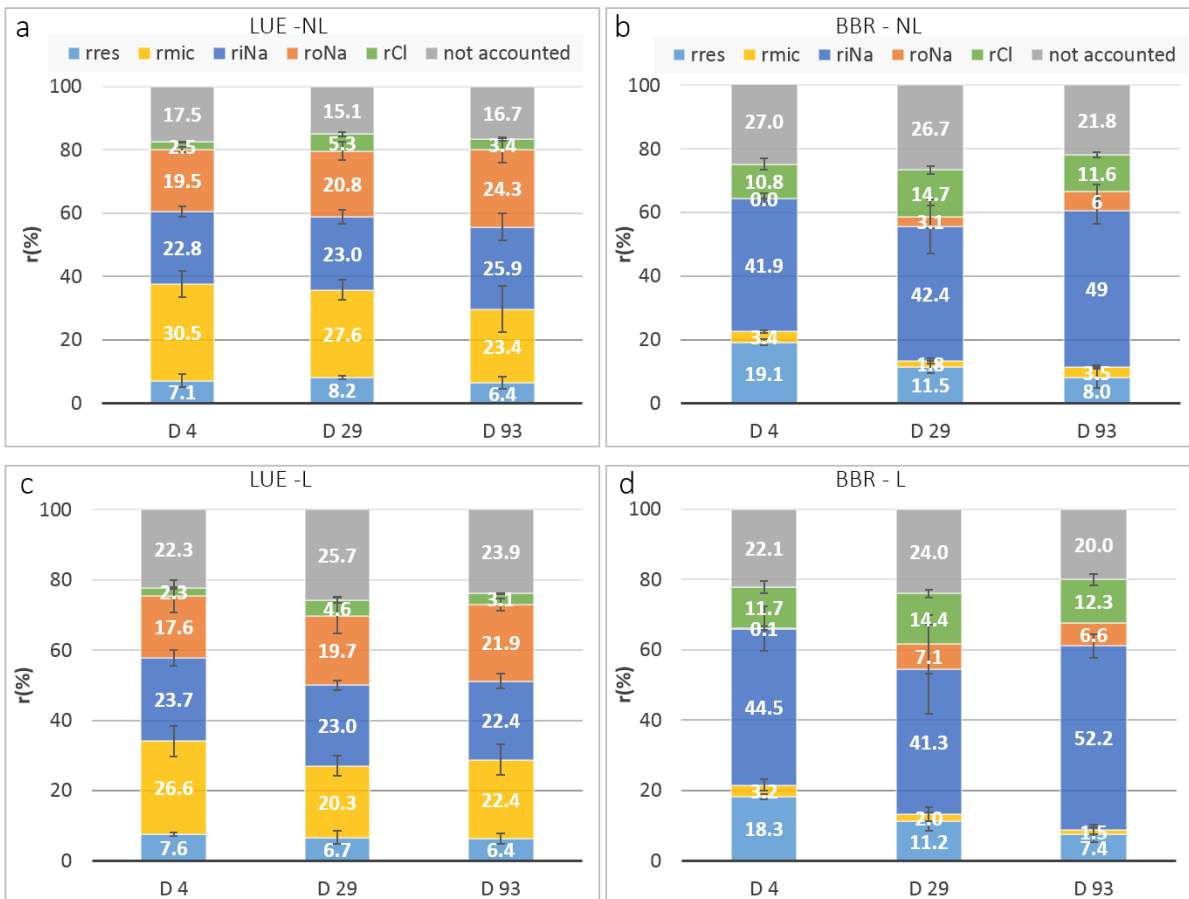
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933

934   Fig. 3 Mean water-extractable P (P_w) concentration in LUE (a) and BBR (b) and

935   corresponding specific activities in LUE (c) and BBR (d) during incubation, error bars

936   represent the standard deviation (n=4), * above/below a single time point indicates

937   significant differences between non-amended (NL) and litter-amended (L) treatments

938   according to t-test. P-values from the two-factors ANOVA are shown on the figure.
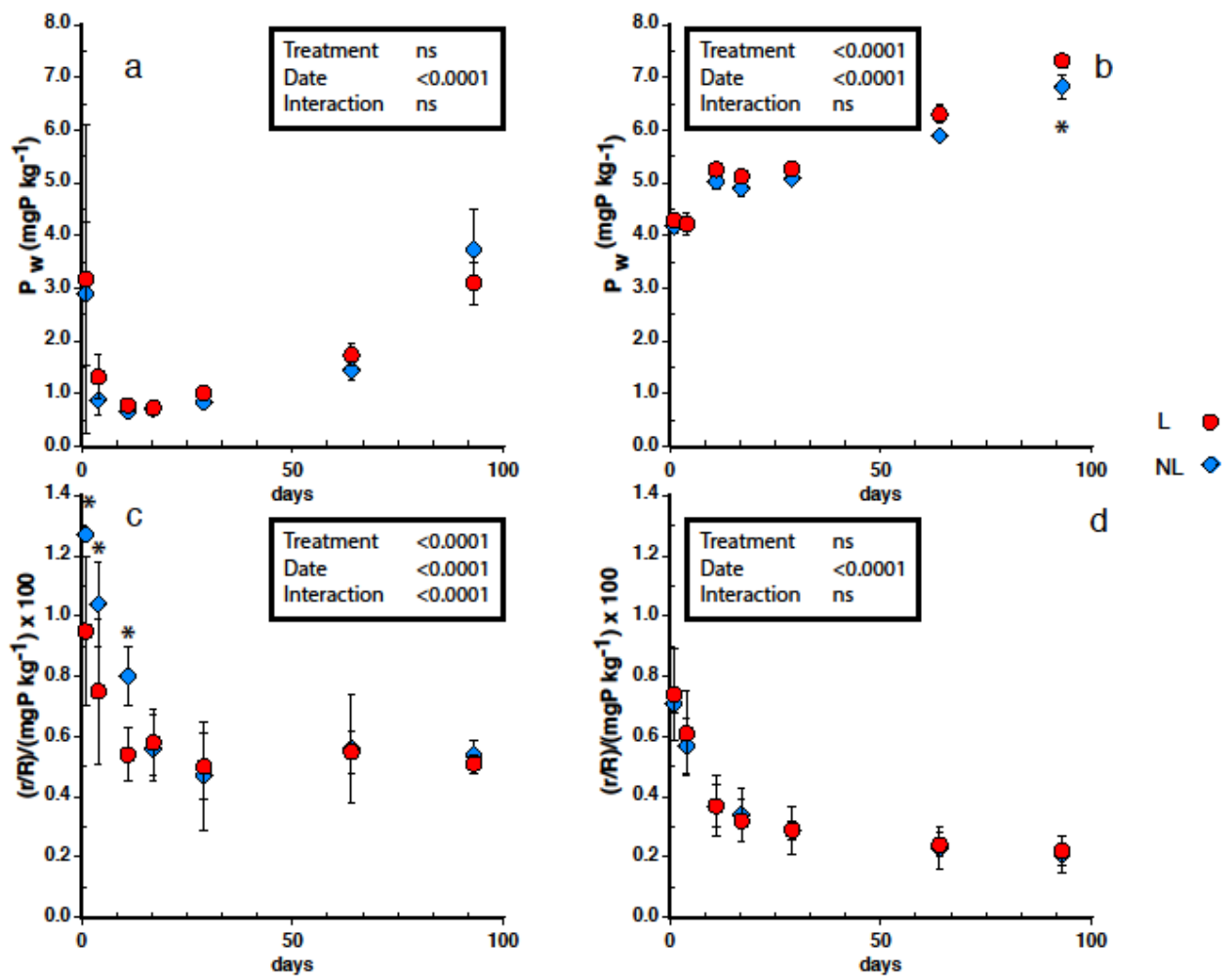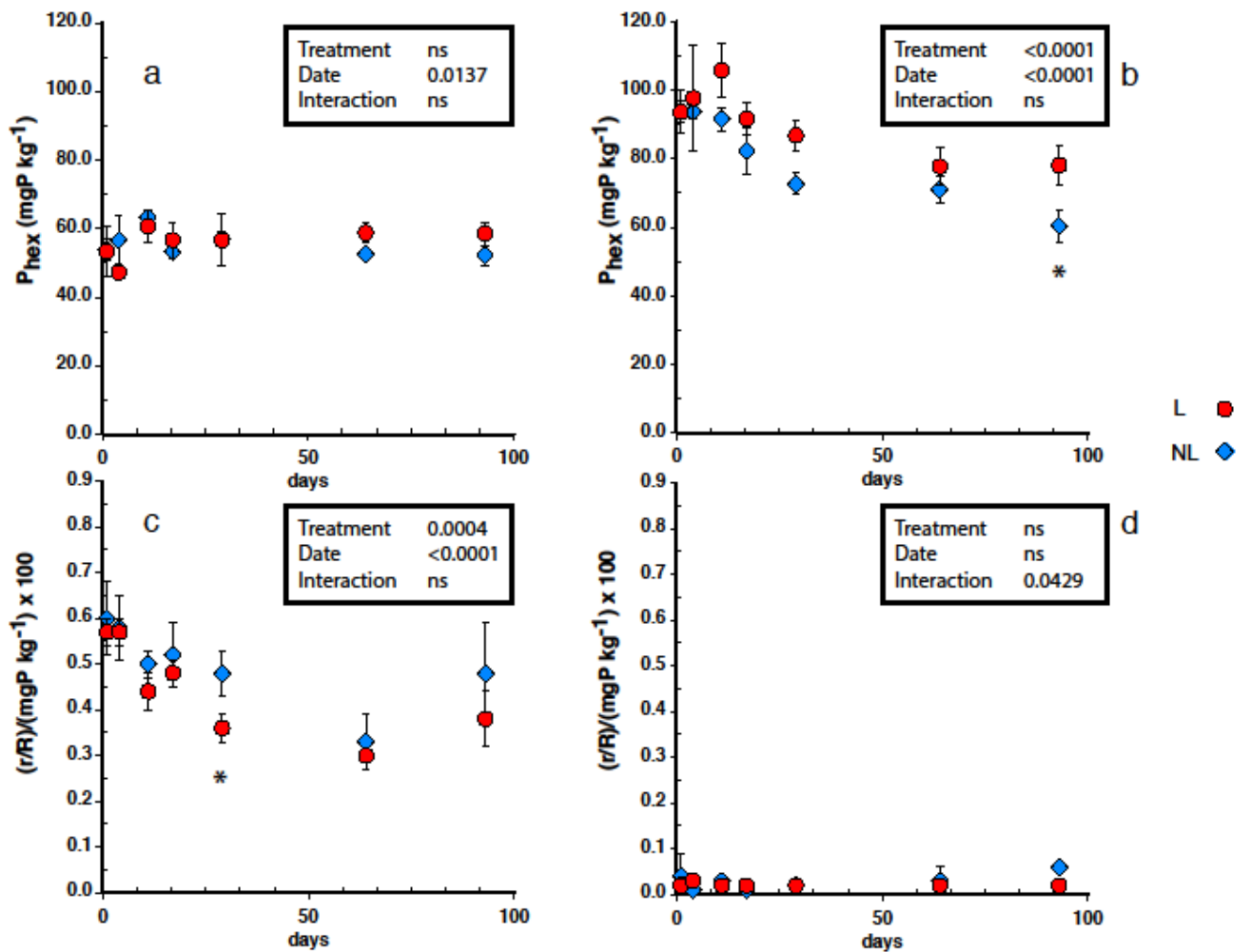

939

940

941   Fig. 4 Mean resin-extractable P (P_{res}) concentration in LUE (a) and BBR (b) and

942   corresponding specific activities in LUE (c) and BBR (d) during incubation, error bars

943   represent the standard deviation (n=4). P-values from the two-factors ANOVA are

944   shown on the figure.
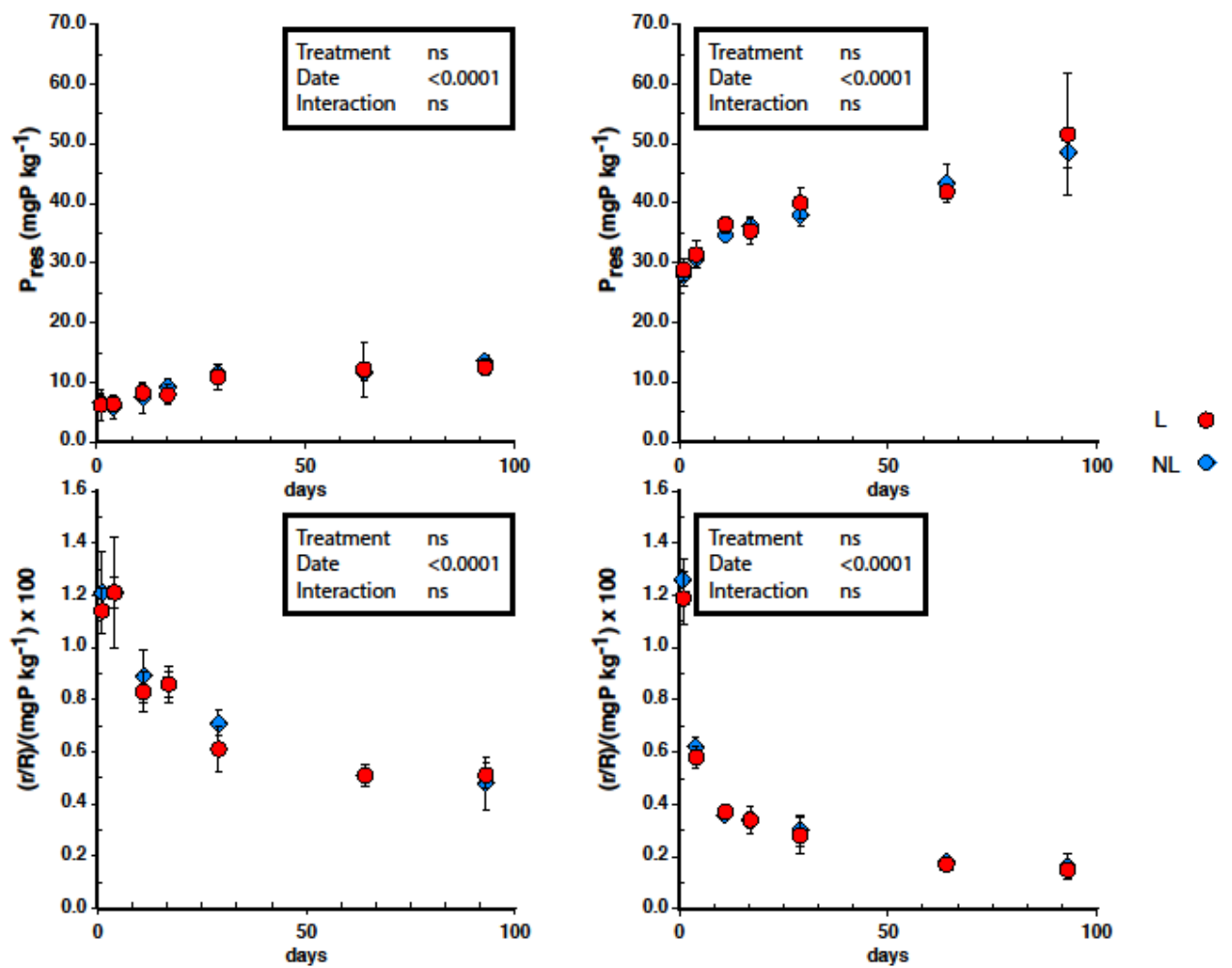
945

946

947   Fig. 5 Mean hexanol-labile P (P_{mic}) concentration in LUE (a) and BBR (b) and

948   corresponding specific activities in LUE (c) and BBR (d) during incubation, error bars

949   represent the standard deviation (n=4), * below a single time point indicates

950   significant differences between non-amended (NL) and litter-amended (L) treatments

951   according to t-test. P-values from the two-factors ANOVA are shown in the figure.

952

953