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Fate of phosphorus fertilizer in acidic Cambisol assessed using ^{33}P isotope labeling technique

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ABSTRACT

Direct ^{33}P labeling approach is a very powerful technique that has high sensitivity in tracing the fate of added phosphorus (P) fertilizers across various P pools. Nonetheless, only a few studies have used this approach. This study traced the fate of applied P fertilizer in acidic P-limited soil using the ^{33}P labeling approach. The incorporation of ^{33}P-labeled KH_2PO_4 in available P (P_{AEM}), microbial biomass P (P_{mic}) and Fe/Al-bound P (P_{NaOH}) pools was followed in Cambisol as influenced by C and N sources applied as glucose and ammonium sulfate, respectively. Results showed that not all of the added P fertilizer remains in available pool; instead, it was distributed to poorly-available pools. Fast, almost instantaneous P fixation by the Fe and Al oxides and immobilization by microbial uptake were recorded. Applying glucose boosts microbial growth and demand for P, resulting in increased ^{33}P recovery. High ^{33}P recovery in P_{mic} (20% of the applied ^{33}P) and in P_{NaOH} (45% of applied ^{33}P) showed the dominance of P immobilization by microorganisms and adsorption by Fe and Al oxides on the fate of P in an acidic soil. Nevertheless, these can contribute to long-term P availability after the turnover of microbial biomass and desorption of fixed P.

Keywords: ^{33}P isotopic labeling, Phosphorus dynamics, Phosphorus availability, Phosphorus fractions, Microbial biomass P, P-limited soil

INTRODUCTION

Phosphorus (P) as orthophosphate is essential to life as a structural and functional component of all living organisms and cannot be substituted by any other nutrient (Chimdi et al 2014, Frossard et al 2016). Phosphorus is therefore essential for agricultural production. As agricultural systems are open, soil P must be managed at a level that ensures optimal plant growth (Sanchez et al 1997, Follain et al 2009).

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In large areas of the world, P is considered to be a growth limiting nutrient, foremost in areas with old and highly weathered soils, such as in tropical and subtropical regions, and in calcareous soils with high pH. As a consequence, most agricultural land and forest plantations are being heavily fertilized with P to increase crop productivity (Damon et al 2014). However, rock phosphate resources suitable for fertilizer production are decreasing and its reserve is only predicted to last another 50-100 years (Cordell et al 2009). Therefore, a more effective soil management practices to increase the efficiency of P fertilizers is necessary and this can only be achieved with a better understanding of P dynamics in the soil.

Direct labeling of fertilizer sources with P radiotracers (ie, ^{33}P or ^{32}P) can be a powerful technique in determining the fate of applied fertilizer P in the soil (Frossard et al 2011). This technique consists of two approaches: labeling and tracing the fate of a P source, and isotopic dilution. By applying the ^{33}P as a tracer, the fates of fertilizer P to various P pools can be traced. Nonetheless, only a few studies have used this approach. Thus, this study was conducted to trace the fate of applied P fertilizer in an acidic P-limited soil. Incorporation of ^{33}P from potassium dihydrogen phosphate (KH_2PO_4) as a P source into various P pools (ie, microbial P (P_{mic}), available P (P_{AEM}) and Fe/Al-bound P (P_{NaOH})) was followed. Application of ^{33}P tracer added in distilled water was used as a control to determine soil P dynamics in the absence of P additions. It was hypothesized that fertilizer P will be distributed into various P pools and will be distributed quickly to P pools with less availability.

MATERIALS AND METHODS

Soil Collection and Preparation

Soil samples were taken at a site in Unterluess (Luess) located in the district of Celle, Lueneburg Heath (52°50.32'N, 10°16.06'E) at 115m asl, Lower Saxony, Germany. The mean annual rainfall of the area is 780mm, and its mean annual temperature is 8.0°C. The soil type is a Hyperdystric Folic Cambisol developed from Pleistocene sediments. The vegetation is formed by European beech (*Fagus sylvatica* L.). The soil texture is loamy sand with a very low pH (3.72) and low total P (144µg g^{-1} soil) (Bergkemper et al 2016). This site, therefore, well represents acidic soils with very low P availability (Table 1). A bulk sample of the Ah horizon at 0-10cm depth was collected with a shovel after removal of the organic layer. The sample was sieved (2mm) and stored at +4°C.

Table 1. General characteristics of the Cambisol used in the expirement (Bergkemper et al 2016)

Soil	Geographic Location	Soil Type (WRB 2015)	Depth (cm)	Total C (mg g^{-1})	Total N (mg g^{-1})	Total P (µg g^{-1})	pH (H_2O)	Sand (%)	Silt (%)	Clay (%)	Texture (WRB 2015)
Luess (LUE)	52°50′21.77″N 10°16′2.37″E	Hyperdystric Folic Cambisol	0-20	43.0	1.70	144	3.72	77.7	17.3	5.33	Loamy sand

Experimental Treatments and Addition of ^{33}P Labeled Fertilizer

The fate of P fertilization in Cambisol was studied under laboratory incubation. The experiment was composed of three treatments as different rates of P addition.

This includes: 0% (P_0), 10% (P_{10}) and 50% (P_{50}) of the initial extractable P content which is 0.2mg g^{-1}. Each treatment was replicated four times. Fifteen grams of dry weight equivalent soil (sieved at 2mm) were placed into glass jars with caps. Samples were pre-incubated at 25°C and 50% water holding capacity (WHC) in the dark to stabilize microbial activity until a constant CO_2 rate was reached on three consecutive days. After pre-incubation ^{33}P-labeled fertilizer were added.

Potassium dihydrogen phosphate (KH_2PO_4) labeled with ^{33}P was used as P fertilizer. Three P levels were added: i) no P=0.3mL deionized water + ^{33}P tracer only (P_0); ii) 10% P of the initial extractable P (0.2mg g^{-1})=0.3mL solution of 4.4mg KH_2PO_4 dissolved in 1mL of deionized water (P_{10}); iii) 50% P of the initial extractable P=0.3mL solution of 21.95mg KH_2PO_4 dissolved in 1mL of deionized water (P_{50}). An addition of 10% P and 50% P increased the initial extractable P by 20µg g^{-1} and 100µg g^{-1}, respectively. ^{33}P labeling resulted in an addition of 80Bq g^{-1} soil. Each P level addition was combined with substrates: ie, distilled water as control, glucose and ammonium nitrate as C and N source, respectively.

Incubation lasted for 6 days at 70% WHC and 25°C in the dark. Two samplings were done: after 24h and 120h of incubation.

Phosphorus Fractionation and Phosphate Measurement

Microbial P was determined by simultaneous liquid chloroform fumigation and extraction with anion exchange resin membranes (AEM) used (BDH no. 551642S, 1.5x6.25cm with a reactive area of 18.75cm^2 per strip) in bicarbonate form (Kouno et al 1995). The soil samples (fumigated sample) remaining after fumigation-extraction were further extracted using 30mL of 0.1m NaOH to extract the P pool that was adsorbed by Fe/Al oxides (Hedley et al 1982, Maranguit et al 2017). Samples were shaken for 24h in an orbital shaker, centrifuged at 5000rpm for 15min and filtered using Whatman no. 42 filters. Extracts for P measurement were acidified using 0.9m H_2SO_4 to precipitate dissolved organic matter that could interfere in color development and in the measurement.

Phosphate in the fumigated, unfumigated and NaOH-extracts was determined by the malachite green (MG) colorimetric method (D'Angelo et al 2001). Briefly, 150µL of extracts was mixed with 30µL of the first reagent (ammonium molybdate tetrahydrate and sulfuric acid) and 30µL of the second reagent (mixture of MG carbinol hydrochloride and polyvinyl alcohol) in a disposable 96-well polysterene microtiter plate. The plate was shaken for an additional 20min and were exposed to 40°C for 40min. Absorbance was read after 1-1.5h using a spectrophotometer (TECAN; Infinite M200 pro) at 630nm wavelength.
P_{mic}(mg P kg^{-1}) was calculated as:

$$P_{mic} = (P_{fumigated} - P_{unfumigated}) / K_p \qquad (1)$$

where: $P_{fumigated}$=P_{AEM} in fumigated samples in mg P kg^{-1}, $P_{unfumigated}$=P_{AEM} in unfumigated samples in mg P kg^{-1} and K_p is the correction factor to account for the effect of sorption and isotopic exchange and extraction efficiency. R_{sorp} and R_{exch} are equal to 0.9 (Bergkemper et al 2016, Yevdokimov et al 2016). The soil specific correction factor (K_p=0.69) was determined for the soil used in this experiment.

^{33}P Activity Measurement and Calculations

One mL each of the fumigated, unfumigated and NaOH-extract were transferred into 6mL vials and mixed with 3mL of scintillation cocktail Rotiszint EcoPlus (Carl Roth Company, Germany) and were measured using a HIDEX 300 SL Liquid Scintillation Counter (Hidex Oy, Finland). The recovery of ^{33}P (%) in a specific P pool was calculated as:

$$^{33}\text{P recovery (\%)} = (r/R) \times 100, \qquad (2)$$

where: r and R is the radioactivity (Bq g^{-1} soil) in the extracted pool and the total amount of added ^{33}P activity, respectively (Bünemann et al 2004).

The recovery of ^{33}P in P_{mic} was corrected for the effect of sorption, isotopic exchange and extraction efficiency. ^{33}P recovery in P_{mic} was calculated as:

$$^{33}P_{mic} = (^{33}P_{fumigated} - {}^{33}P_{unfumigated}) / K_p \qquad (3)$$

where: Kp=0.69 is the correction factor to account for the effect of sorption, isotopic exchange and extraction efficiency as mentioned above.

Data Analysis

The results given are arithmetic means of four replicates in each treatment and expressed on an oven-dry weight basis. Normality and homogeneity of variance were checked using Shapiro-Wilk's *W* test and Levene tests, respectively. For each sampling time (24h & 120h) of the incubation experiment, data were tested by one-way analysis of variance (ANOVA). Multiple comparisons (all-pairwise comparisons) using Tukey's test were performed whenever the ANOVA indicated significant differences at $p \leq 0.05$. All statistical analyses were carried out using STATISTICA 12 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Fates of Applied P Fertilizer

Inorganic P fertilization increased the size of soil P pools and also revealed a distinct temporal pattern in the various P pools over the 120h incubation period. Basically, the high rate of P application yielded the greatest available P content (P_{AEM}) in the soil compared to low P (P_{10}) and no P addition (P_0) (Figure 2a). Importantly, not all the applied P remained in the available pool for plant uptake. The P_{AEM} content decreased ($p<0.05$) by about 27%, 52% and 40% in P_0, P_{10} and P_{50}, respectively, after 120h. Therefore available P was rapidly distributed to less available pools: both high and low P application resulted in strong P immobilization by microorganisms and fixation by Fe and Al oxides, as is evident in the elevated P_{mic} (Figure 1a) and P_{NaOH} (Figure 3a) content, respectively. Nonetheless, these pools are very important P reserves, buffering available P.

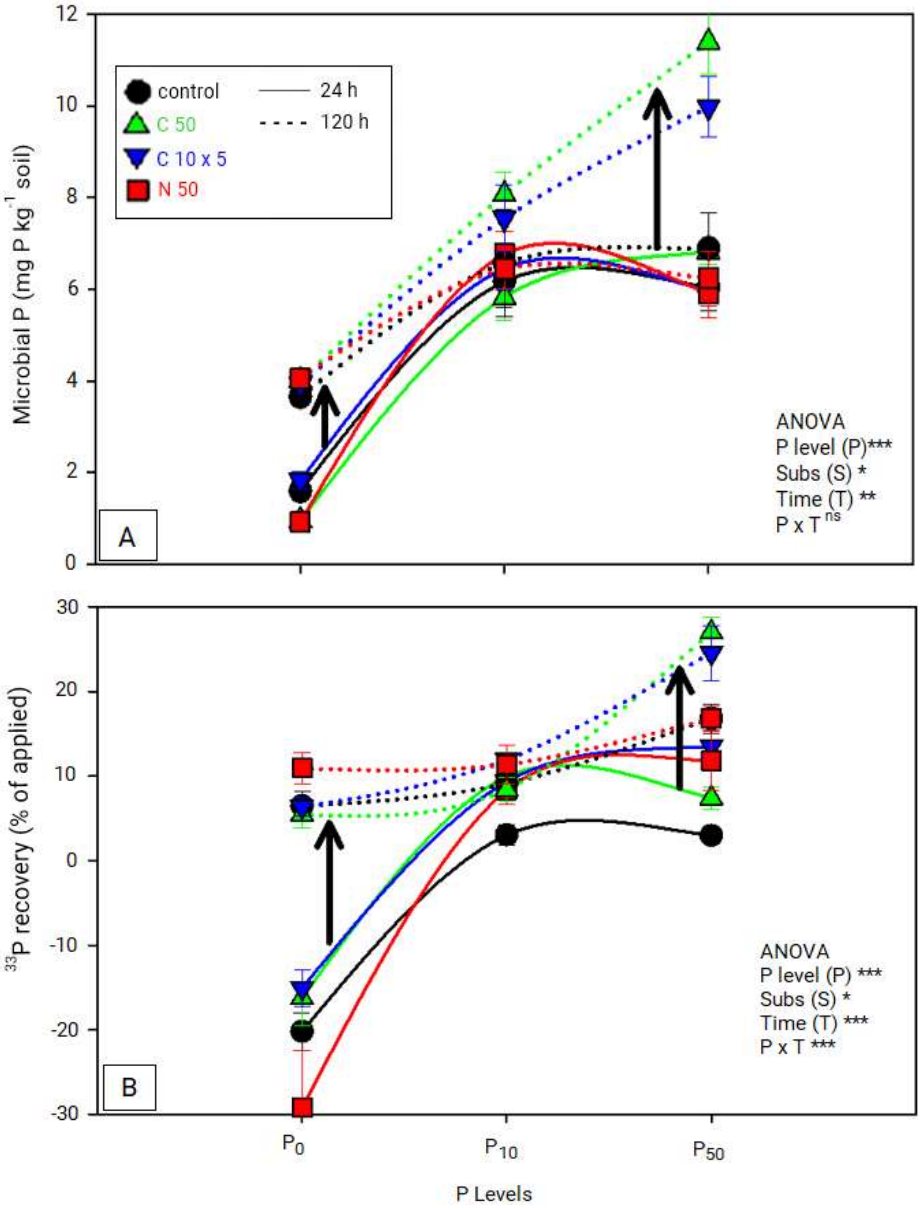

Figure 1. (a) Phosphorus content and (b) ^{33}P recovery in microbial P (P_{mic}) after the addition of ^{33}P tracer alone (P_0), ^{33}P-labeled fertilizer as KH_2PO_4 applied to soil as 10% (P_{10}) and 50% (P_{50}) of the initial P content and combined with substrates: ie, distilled water as control, glucose and ammonium nitrate as C and N source, respectively. Bars indicate standard error of four replicates. Arrows indicate significant increase or decrease between 24 and 120h.

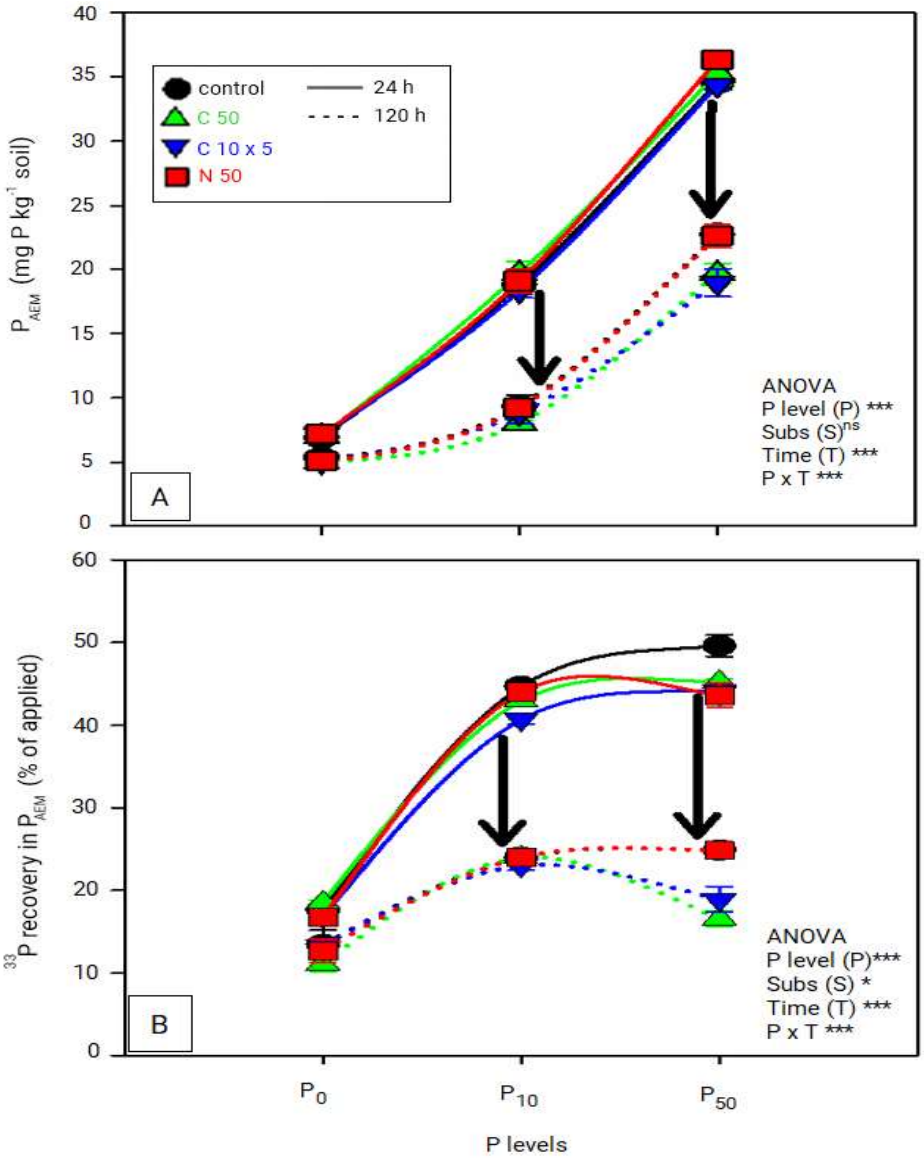

Figure 2. Phosphorus content and ^{33}P recovery in available P ($_{PAEM}$) after the addition of ^{33}P tracer alone (P_0), ^{33}P-labeled fertilizer as KH_2PO_4 applied to soil as 10% (P_{10}) and 50% (P_{50}) of the initial P content and combined with substrates: ie, distilled water as control, glucose and ammonium nitrate as C and N source, respectively. Bars indicate standard error of four replicates. Arrows indicate significant increase or decrease between 24 and 120h.

Microbial P increased (p<0.05) exponentially after P addition by about 3-fold compared to soil with no P after 24h. The average difference recorded between P_0 and P_{10} was 5.1mg P kg^{-1} soil and remained constant for P_0 and P_{50}. After 120h, however, P_{mic} in P_0 increased (p<0.05), which resulted in a smaller difference (3.9mg P kg^{-1} soil) compared to P_{10} and P_{50}. On the other hand, the P content in P_{NaOH} increased (p<0.05) after P_{10} and P_{50} addition compared to P_0, with an average

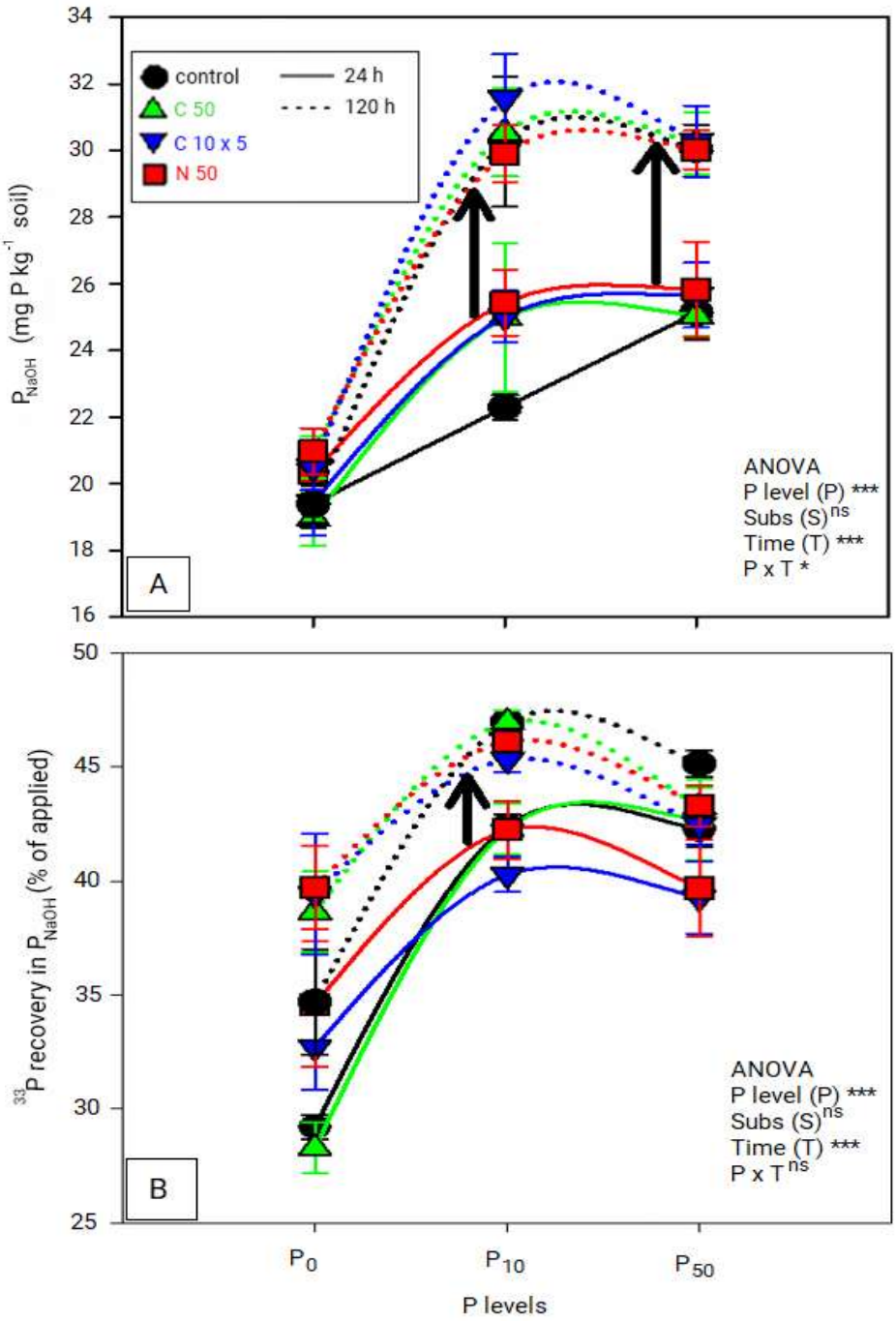

Figure 3. Phosphorus content and ^{33}P recovery in Fe/Al-bound P (P_{NaOH}) after the addition of ^{33}P tracer alone (P_0), ^{33}P-labeled fertilizer as KH_2PO_4 applied to soil as 10% (P_{10}) and 50% (P_{50}) of the initial P content and combined with substrates: ie, distilled water as control, glucose and ammonium nitrate as C and N source, respectively. Bars indicate standard error of four replicates. Arrows indicate significant increase or decrease between 24 and 120h.

The significant microbial P pool in our study (Figure 1a), coupled with the rapid turnover time of soil microbial P, suggests that it is an increasingly important source of plant-available P as soils age and become P-limited (Brookes et al 1984, Kouno et al 2002). Likewise, the significant amount of ^{33}P recovered in the P_{NaOH} pool (Figure 2a & b) reflects the importance of P sorption on sesquioxides (Bünemann et al 2004). This buffers the available P supply (Hedley et al 1982, Maranguit et al 2017). Although the transformation of this pool to the labile pool requires a long time, the long-term release of the P_{NaOH} reserve is very important. It protects P from leaching and surface run-off and potentially buffers available P for plant-uptake.

^{33}P Recovery and Dynamics Over time

^{33}P recovery in P_{AEM} (Figure 2b) was in accordance with the principles of isotopic exchange (Fardeau 1996, Bünemann et al 2004): it diminished steadily, with the greatest decrease at 120h. These findings agree with the trends observed during the incubation of temperate soils amended with ^{33}P-labeled soybean residues (Daroub et al 2000), in highly weathered Oxisols from Colombia (Bühler et al 2002) and in kaolinitic Oxisols from Kenya (Bünemann et al 2004). Furthermore, a significant fraction of the added labeled P was irreversibly fixed in soils that sorb very high P amounts, thereby reducing the ^{33}P fraction actually participating in the isotopic exchange (Wolf et al 1986). In this study, the lower ^{33}P recovery in P_{AEM} (Figure 2b) was accompanied by a simultaneous increase in the P_{mic} (Figure 1b) and P_{NaOH} (Figure 3b) after 120h of incubation. This reverse trend of ^{33}P recovered in different P pools after 120h suggests that ^{33}P-labeled phosphate was distributed from the labile pool (P_{AEM}) to the immobilized pool by microorganisms (P_{mic}) and by mineral sorption (P_{NaOH}).

The ^{33}P recovered in immobilized pool (P_{mic}) after 120h–with a strong increase at P_0 compared to no increase at P_{10} and less increase in P_{50} (Figure 1b)–can be explained by the microbial response to labile P. It was earlier hypothesized that microorganisms fully trap the limiting resource (P) until P becomes totally restricted. During the first 24h at P_0, microorganisms were actually P-limited and not fully activated even though the addition of C and N led to less consumption and saturation of ^{33}P in the microbial biomass. After the longer incubation period (120h), the increasing microbial activity and growth (reflected in the microbial biomass C; Figure 4) resulted in greater (p<0.05) ^{33}P recovery. In P_{10}, although labile P was increased only minimally (eg, 10% of initial P content (0.2mg g^{-1}), it was apparently enough for microorganisms to become activated and saturated with P during the first 24h. These results suggest that, when new labile P is applied in the system, P can be rapidly immobilized by soil microorganisms under limiting P conditions (Bünemann et al 2012). Accordingly, the P concentration in the soil solution is strongly influenced by microbial P immobilization (Frossard et al 2000). The results further support our hypothesis that, after being saturated with P and initial stimulation, microorganisms return to dormancy, explaining why the ^{33}P recovered in the microbial biomass has been found to be rather constant over time (Oberson et al 2001, Bünemann et al 2004, 2012). This increases the chance for the remaining labeled fertilizer to be adsorbed/fixed by Fe/Al oxides, which is reflected in the slight increase of ^{33}P recovered (Figure 3b) and of the P content (Figure 3a) in the P_{NaOH} pool after 120h. A longer incubation study (34 days) showed a subsequent movement of the labeled P from the labile pool to the P_{NaOH} pool (Daroub et al 2000).

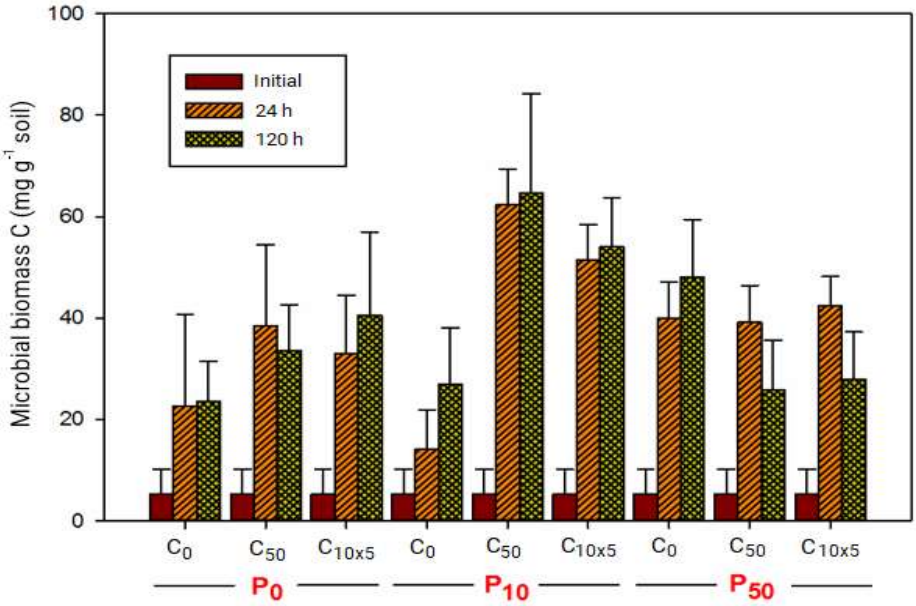

Figure 4. Microbial biomass C (MBC) after addition of ^{33}P tracer alone (P_0), ^{33}P-labeled fertilizer as KH_2PO_4 applied to soil as 10% (P_{10}) and 50% (P_{50}) of the initial P content and combined with substrates: ie, distilled water as control and glucose as C source. Bars indicate standard error of four replicates.

In contrast to P_0 and P_{10}, P_{50} almost tripled the labile P content of the soil available for microorganisms and for plant-uptake. This explains the higher microbial activity even during the first 24h, which led to the greatest ^{33}P recovery in microbial biomass (Figure 1b). Nonetheless, the ^{33}P recovered in P_{10} and P_{50} was almost the same in all soils throughout the incubation time, except for soils amended with glucose as a source of C. Therefore, microorganisms will be easily saturated at a certain P level regardless of how high the available P content is in the soil. Consequently, in P_{50}, part of the ^{33}P-labeled fertilizer not taken-up by microorganisms went to the P_{NaOH} pool. Likewise, free Fe and Al for P binding also had a saturation point. This explains the more or less identical P content and ^{33}P recovery in the P_{NaOH} pool in soil with P_{10} and P_{50} (Figure 3b). In the experiment without plants, the excess of label fertilizer in soils with P_{50} remains in the labile pool, free to be accessed again by microorganisms. Under natural conditions, however, competition between plant and microorganisms will influence the availability and depletion of available P. At any rate, the activation of microorganisms is stimulated by adding any substrate as a C source (Ayaga et al 2006, Spohn & Kuzyakov 2013). In fact, very small amounts of labile C substrate (5-15µg g^{-1}) can activate soil microorganisms (De Nobili et al 2001, Mason-Jones & Kuzyakov 2017). This increases the demand for P, boosting the recovery of label in the ^{33}P microbial pool (Bünemann et al 2004). Therefore, microorganisms took up the remaining labile P from solution, and ^{33}P recovery increased in the P_{mic} pool in soils amended with C in P_{50} after 120h (Figure 1b). This was in accordance to the ^{33}P recovery of 66% recorded in chloroform-labile P after 2 d when soils were amended with glucose and ammonium nitrate, compared with 8% in the absence of easily available sources of C and N (Oehl et al 2001).

CONCLUSIONS

Tracing the fate and distribution of added P fertilizer to various P pools was made possible and faster using ^{33}P labeling technique. Results of the study confirmed our hypothesis that the extent to which P remains in the soil solution depends on the degree to which it is adsorbed, desorbed and mineralized. Fertilizer P was distributed into various P pools and was distributed quickly to P pools with less availability. High P application enhanced the available content for plant-uptake, but only for a short time. Applied P, if not directly taken-up by plants, will be distributed quickly to less available pools. Applying glucose as a C source boosts the microbial activity, growth and demand for P, which increases the microbial biomass P pool. Nonetheless, the turnover of microbial biomass P and desorption of fixed P sustains long-term P fertility.

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