**PHOSPHORUS IN THE COARSE SOIL FRACTION IS RELATED TO SOIL ORGANIC PHOSPHORUS
MINERALIZATION MEASURED BY ISOTOPIC DILUTION**

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Abstract

A sound prediction of soil organic phosphorus (P_o) mineralization would be helpful to improve fertilizer recommendations. However, in most mineral soils expensive and time consuming isotopic dilution experiments are required to assess P_o mineralization. A proposed method to estimate P_o mineralization is the quantification of P in the coarse fraction (CF, $>53 \mu m$) of the soil. The aim of this study was to evaluate if P in the CF can effectively predict P_o mineralization. Soil samples from three North American long-term arable field experiments (>10 years) with different textures (sand content 15 to 82%) and different fertilization treatments (non-P-fertilized, mineral P fertilizer, poultry litter) were analyzed. Soil samples were physically fractioned into CF and fine fraction (FF, $<53 \mu m$). Total P (P_t), P_o , and total carbon (C_t) were determined in each fraction. Gross

and net P_o mineralization rates as well as soil respiration were determined in a 13-day-incubation experiment. The cumulative gross P_o mineralization over 13 days ranged from 8.3 to 38.6 mg P kg⁻¹, while P_t in CF varied between 9.7 and 90.7 mg P kg⁻¹ and P_o in CF between 6.1 and 17.7 mg kg⁻¹. No association between P_t in CF and gross or net mineralized P_o was observed. However, a significant linear relationship between P_o in CF and net (R^2 : 0.68, $p < 0.01$) and gross mineralized P_o (R^2 : 0.72, $p < 0.01$) was observed after 6 days and 13 days of incubation. Additionally, gross and net mineralized P_o could also be predicted from C respiration, and these models were significantly improved by incorporating the $C_t:P_o$ ratio of the CF. Our results suggest that the quantification of P_o in the CF as well as the measurement of respiration corrected by the $C_t:P_o$ ratio of the CF are promising non-isotopic indicators of P_o mineralization rates.

Keywords

Organic phosphorus, mineralization, isotopic dilution, poultry litter, mineral fertilizer, physical fractionation

1. Introduction

Phosphorus (P) is a macronutrient involved in the energy metabolism and in the synthesis of cellular structures of plants (Raghothama et al., 2005). Consequently, the availability of P controls the growth and development of all crops. It is estimated that 5,700 million hectares of soils worldwide do not have the adequate available P content required to achieve optimum crop production (Batjes, 1997). To alleviate this deficiency, usually mineral P fertilizers are used at rates based on extraction methods calibrated by field trials, such as those developed by Bray and Kurtz (1945), Mehlich (1984) and Olsen et al. (1954). However, all of these soil P tests consider only the available inorganic P (P_i) pool, without taking into account organic P (P_o) which can represent up to 80% of total P (P_t) (Condon et al.; 2005; Harrison, 1987) and can become plant available through

mineralization. Therefore, the measurement or estimation of P_o mineralization rates is important to better estimate P plant availability and P fertilizer recommendations.

This conversion from P_o to P_i can be expressed as gross or net P_o mineralization. Since P_i released by mineralization to the soil solution can be taken up again by microorganisms, i.e. immobilized, gross P_o mineralization is the sum of net P_o mineralization and microbial P immobilization (Bünemann, 2015). Consequently, net P_o mineralization can be derived as gross P_o mineralization minus the amount of immobilized P_i . The quantification of gross and net P_o mineralization is complicated by the high reactivity of phosphate ions, which are rapidly adsorbed by the soil mineral matrix after being released by mineralization from the organic pool to the soil solution. This reactivity makes it generally impossible to quantify net P_o mineralization by incubation-extraction methods as the ones used to measure net mineralization rates of other nutrients such as nitrogen (N) (Stanford and Smith, 1972) and sulfur (S) (Tabatabai and Al-Khafaji, 1980). An exception are soils with low reactivity, for which extraction methods could be appropriate (Achat et al., 2010; Grierson et al., 1998). Currently, the only method to quantify P_o mineralization on most mineral soils is based on isotopic dilution experiments under steady-state conditions, rendering gross and net P_o mineralization rates in the absence of flush effects (caused by, for example, recent inputs of labile C substrates or climatic variations) (Bünemann, 2015; Oehl et al., 2001). The isotopic dilution method has been used and improved in several studies (Oehl et al., 2001; Bünemann et al., 2007, 2012; Achat et al., 2009a; Radriamanantsoa et al., 2015) as recently reviewed by Bünemann (2015). However, such experiments are expensive and time consuming. Thus, there is a need to develop and evaluate non-isotopic methods to estimate the P_o mineralization potential of soils, based for instance on C mineralization (Achat et al., 2009a, 2010) or soil properties (Achat et al., 2013).

The P_t concentration in the coarse fraction (CF) (soil fraction $> 53 \mu m$) has been suggested as a potential estimator of P_o mineralization (Ciampitti et al., 2011). These authors observed that the concentration of P_t in the CF ($mg P_t\text{-CF kg}^{-1}$ soil) was positively related to the P accumulation in corn (*Zea mays* L.) plants growing in unfertilized plots on a Mollisol in Argentina, and suggested that this association is based on the rapid turnover rate of the P_o in the CF. However, there are no studies analyzing the association between P_o mineralization and P_t or P_o content in the CF to support this hypothesis. The CF has been previously named “particulate organic matter” (Cambardella and Elliott, 1992) or “particulate fraction” (Wyngaard et al., 2013). Here, we will refer to it as “coarse fraction” (CF), since this fraction is composed of both organic matter and mineral particles. As the determination of P_t or P_o in the CF is simple and economical, it could be a promising indicator to estimate potential P_o mineralization.

Other labile soil P_o fractions that vary depending on the climate, vegetation, mineralogy, and fertilization history can be characterized using enzyme addition assays (Annaheim et al., 2015; Requejo and Eichler-Löbermann, 2014; Keller et al., 2012; He et al., 2008; Koopmans et al., 2007). Bünemann et al. (2012) observed that an enzyme-labile P fraction (P hydrolyzed by phytase) was not associated with the measured gross and net P_o mineralization rates in a grassland soil from Switzerland. It should be evaluated for other land-uses and contrasting management practices if there is a relationship between enzyme-labile P_o fractions and P_o mineralization rates.

Others have suggested to use soil respiration ($C\text{-CO}_2$ released during soil incubations) as an estimator of gross (Achat et al. 2010) or net (Bünemann et al. 2012) mineralization of non-living P_o when microbial P remains constant, assuming that mineralization of non-living P_o is driven by the microbial need for energy. The empirical evidence that P_o in CF as well as soil respiration are associated with P_o mineralization should be validated by comparison with results from isotopic dilution experiments.

Our objective was to evaluate if P_t and P_o in the CF, enzyme-labile P_o fractions, and / or respiration can be used as indicators of gross and net P_o mineralization measured by isotopic dilution. To this end, we analyzed soils which were not fertilized with P or were amended with inorganic fertilizer (IF) or poultry litter (PL) for more than 10 years.

2. Materials and methods

2.1. Field experiments and soil sampling

Soil samples were collected from three long-term agricultural field experiments in the United States in August 2014 at 0 to 20 cm depth. These sites were located in Delaware (39°40'13"N 75°45'10"W, on a Hapludult, Woodstown series, according to the Natural Resources Conservation Service (1999)), Maryland (39°10'42"N 76°10'58"W, Hapludult, Mattapex series) and Iowa (42°01'22"N 93°46'35"W, Hapludoll, Nicollet/Webster series). These soils were classified by their particle size distribution as sandy loam (14.5 % clay and 82.0 % sand), silt loam (21.8 % clay and 16.1 % sand) and clay loam (29.2 % clay and 42.0 % sand), respectively. At each site, plots fertilized with inorganic fertilizers or PL (a mixture of excreta, organic bedding material, feathers and wasted feed) were sampled to obtain a wide range of values for P_o in CF and enzyme-labile P_o fractions.

The experimental design in these long-term experiments was a complete randomized plot arrangement with six replications in Delaware and Maryland (55 m² per plot), and four replications in Iowa (400 m² per plot). In Delaware and Maryland, three different treatments have been compared since 2000: 1) no P fertilizer application (Control), 2) IF (30 kg P ha⁻¹ year⁻¹, as triple super phosphate) and 3) PL (30 kg P ha⁻¹ year⁻¹, as PL). In Iowa, the evaluated treatments were: 1) Control, 2) inorganic N fertilization without P (Control+N) (90 kg N ha⁻¹, as 32-0-0), and 3) PL (90 kg P ha⁻¹ year⁻¹), all maintained since 1998. Since their establishment, Delaware and Maryland field

116 trials were managed under corn monoculture while the Iowa study was managed under a
117 corn/soybean (*Glycine max* L.) rotation.

118 All soil samples were taken eleven months after the last fertilizer application. Three out of the
119 four-six field replications were sampled (one composite soil sample per field replicate), resulting in
120 three samples per treatment and site. After sampling, soils were sieved (4 mm), mixed, and kept at
121 4°C for up to 3 months until the laboratory experiments were performed. A portion of the soil was
122 air-dried right after sampling, sieved through a 2-mm sieve and used for soil analysis (Section 2.2).
123 Replicates from each soil and treatment combination were analyzed separately, except for the
124 isotopic dilution experiment (Section 2.4), for which equal portions of all replicates of a given soil
125 and treatment combination were mixed into a composite soil sample.

126 **2.2. Soil analysis**

127 Total C (C_t) and N (N_t) concentrations in soil were determined by dry-combustion. Soil P_o was
128 determined by the ignition method (Saunders and Williams, 1955), P_t by wet digestion with H_2O_2
129 and H_2SO_4 (Anderson and Ingram, 1989), and P_i was derived from P_t minus P_o . Soil pH was
130 measured in a 1:2.5 soil-to-water suspension, and particle size distribution was determined by the
131 pipette method (Robinson, 1922). All soil analyses were performed with three analytical replicates
132 per plot.

133 Additionally, the soil was physically separated into two fractions: the CF and the fine fraction (FF),
134 following a modification of the procedure described by Cambardella and Elliott (1992). Briefly, 10 g
135 of dry soil sample was shaken overnight with 30 ml of 1 M NaCl instead of 0.05 M $(NaPO_3)_6$ to
136 avoid the addition of P (Salas et al., 2003). After this, the suspension was wet-sieved through a 53-
137 μm sieve. The fraction that remained on top of the sieve was designated the CF, while the one that
138 passed through the sieve was called the FF. After drying and milling both fractions, the

concentrations of P_i , P_o , P_t and C_t were measured in CF and FF as detailed above. These concentrations were always expressed on a whole-soil basis ((mg P or C)/(kg CF or FF) x (kg CF or FF)/kg soil).

2.3. Enzyme addition assay

To quantify enzyme-labile P_o fractions in NaOH-EDTA extracts, the protocol described by Keller et al. (2012) was performed. To this end, soil samples were shaken for 16 h with a 0.25 M NaOH-0.05 M EDTA solution in a 1:10 ratio. After this, filtered aliquots of the extract were transferred to a microplate, buffered with a MES buffer (final molarity 0.2, pH 5.2) and submitted to four separate enzyme treatments: control (no enzyme addition), acid phosphatase (from *Solanum tuberosum*, Sigma chemicals, product No. P1146), phytase (from *Peniophora lycii*, RONOZYME® NP, DSM Nutritional Products, Denmark) and acid phosphatase + nuclease (from *Penicillium citrinum*, Sigma chemicals, product N8630). The samples were agitated and incubated for 24 h in the dark at 37°C. Then, the P_i concentration in the incubated extracts was quantified colorimetrically in a microplate reader (EL 800, Biotek) using malachite green (Ohno and Zibilske, 1991). The P_i released by each enzyme was calculated as the difference in P concentration in each enzyme treatment minus the control, accounting for any background phosphate in the enzyme preparations.

On another plate handled in parallel, the activity of the enzymes was evaluated without the NaOH-EDTA soil extracts by their reaction with *myo*-inositol phosphate (Sigma chemicals, product P8810), glycerol phosphate (Sigma chemicals, product G7879) and deoxyribonucleic acid (DNA) (Sigma chemicals, product D3159). The P release by the enzymes from the model substrates in water averaged 100%, 94% and 98% for acid phosphatase, acid phosphatase + nuclease and phytase, respectively. The model substrates together with the respective enzymes were also added to the NaOH-EDTA soil extracts in MES buffer to verify that the extracts did not affect the

enzyme activity. The recovery of a $10 \mu\text{g ml}^{-1}$ orthophosphate solution was used as a correction factor for P sorption or precipitation. This recovery averaged 96%, 99% and 100% in the presence of acid phosphatase, acid phosphatase + nuclease and phytase, respectively.

Three enzyme-labile P_o pools were calculated: simple monoester-like P (P released by phosphatase), DNA-like P (P released by nuclease + phosphatase minus P released by phosphatase alone) and phytate-like P (P released by phytase minus P released by phosphatase). Also, the non-hydrolysable P_o pool was calculated as the difference between P_o in the NaOH-EDTA extract and the sum of all three enzyme-labile pools. The P_o concentration was estimated as the difference between P_t (determined by digestion with ammonium persulfate and sulfuric acid in an autoclave) and P_i in the extract, both measured colorimetrically (Ohno and Zibilske, 1991).

2.4. Isotopic dilution experiment

This experiment was performed as described by Oehl et al. (2001), incorporating the estimation of microbial P immobilization suggested by Bünemann et al. (2007). The protocol consists of two separate and complementary studies: 1) a batch experiment to determine the baseline of isotopic dilution caused by physicochemical processes (Section 2.4.1) and 2) an incubation experiment with ^{33}P -labeled soils to determine the isotopic dilution caused by both biological and physicochemical processes (Section 2.4.2). This second part of the experiment was accompanied by the measurement of soil respiration.

2.4.1. Batch experiment: isotopic exchange kinetics (IEK)

The aim of this part of the experiment was to assess changes in the specific activity (SA, i.e. $^{33}\text{P}/^{31}\text{P}$) of water-extractable phosphate caused by physicochemical processes (e.g. adsorption/desorption, precipitation/solubilization) (see Figure S1 in supplementary material) (Fardeau et al., 1991). Soil samples were pre-incubated at 30°C for two weeks at 40% water-

holding capacity. This procedure has been described as sufficient to reach a constant soil respiration rate and constant parameters of isotopic exchange after the disturbance generated by sieving and mixing the soil (Oehl et al., 2001). After this, the water content was elevated to 55% and an amount of pre-incubated soil equivalent to 10 g dry weight was shaken for 16 hours in a 250-ml Nalgene bottle with 99 ml of water (including the water contained in the soil sample). At time zero, 1 ml of carrier-free $^{33}\text{PO}_4^{3-}$ tracer was added to the soil-water suspension while stirring. The introduced activity (R) ranged between 24-206 kBq, depending on the soil. The suspension was sampled with a syringe after 1, 4, 10, 30, 60 and 90 min and immediately filtered through a membrane filter (0.2 μm pore size). At each time point, the radioactivity in the filtrate (r) was quantified by liquid scintillation counting in a betacounter (TRI-CARB, 2500 TR). Additionally, at the end of the experiment, the suspension was sampled and filtered as above to determine the concentration of water-extractable phosphate (C_p in mg l^{-1}) using malachite green (Ohno and Zibilske, 1991).

The temporal development of radioactivity remaining in the soil solution ($r_{(t)}/R$) is described by the following formula (Fardeau et al., 1985):

$$r_{(t)}/R = m \cdot (t + (m)^{1/n})^{-n} + r_{\infty}/R \quad (\text{Equation 1})$$

where t is time, m and n are model fitting parameters, and r_{∞}/R is the maximum possible dilution of the isotope. This last value was estimated by the ratio between the water-extractable phosphate ($10 \cdot C_p$, in mg kg^{-1}), and total P_i (estimated by the difference between P_t determined by digestion and P_o measured by ignition). Equation 1 was used to extrapolate $r_{(t)}/R$ to time-points corresponding to those used in the incubation experiment (1, 6 and 13 days, section 2.4.2).

Finally, the quantity of isotopically exchangeable P at each time point, the so-called E-value ($E_{(t)}$) (mg P kg^{-1}), was estimated as:

208 $E_{(t)est} = 10 * C_p / (r_{(t)} / R)$ (Equation 2)

209 using the $r_{(t)} / R$ calculated according to equation 1, for time-points of 1, 6 and 13 days.

210 The approach of using IEK experiments to estimate changes in the SA of the water-extractable
211 phosphate (SA_{H_2O}) caused only by physicochemical processes is based on the assumption that
212 during the short time of the experiment there is no incorporation of ^{33}P into microorganisms (Oehl
213 et al., 2001). In order to confirm this assumption, the experiment was repeated in the presence of
214 a microbial inhibitor (10 mg l^{-1} $HgCl_2$ solution added 10 min before addition of ^{33}P). Since C_p , r_1 / R
215 and n were unaffected by the presence of the inhibitor (data not shown), it was concluded that no
216 inhibitor was required during the short-term IEK experiments.

217 Six analytical replicates of the IEK experiment were performed for each soil and treatment. Three
218 of these replicates were performed at the beginning and three at the end of the incubation
219 experiment (2.4.2) to verify that IEK parameters were constant over time. As no change over time
220 was observed (data not shown), the six replicates were analyzed jointly.

221 **2.4.2. Incubation experiment**

222 The aim of this part of the experiment was to assess changes in the SA of water-extractable
223 phosphate caused by both physicochemical and biological processes (see Figure S1 in
224 supplementary material). Additionally, soil respiration and microbial ^{31}P and ^{33}P were quantified.
225 The incubation experiment was performed in three separate sets, one for each soil. In detail, soil
226 samples were pre-incubated at 30°C for two weeks at 40% water-holding capacity. After this, a
227 fraction of the pre-incubated soil equivalent to 220 g dry soil was labeled with 10 ml of a ^{33}P
228 solution (from 468 to 894 kBq ml^{-1} , depending on the soil) and additional water was added to
229 achieve a moisture level corresponding to 55% water-holding capacity. The resulting label was
230 21.7, 27.1 and 41.4 kBq g^{-1} for Delaware, Maryland and Iowa, respectively. The labeled soil was

mixed thoroughly for 10 min and then divided into three sub-samples per soil and treatment, which were placed in plastic bags and incubated at 30°C in the dark, keeping a constant water content. We did not divide the soil into further sub-samples because the relative water loss during the incubation increases with decreasing sub-sample weight.

Three different time-points were considered: 1, 6 and 13 days. These time-points represent the moment at which the samples were filtered, even though the extraction procedure started 16 hours before. This is because the isotopic exchange continues during the extraction. For each time-point, one of the incubated sub-samples was used to measure the SA of water-extractable phosphate and microbial P, each with four analytical replicates.

Water-extractable phosphate was extracted by shaking moist soil equivalent to 10 g dry soil with 100 ml water (including the water in the sample) for 16 h. After this, the soil-water suspension was stirred for 10 min to generate conditions similar to those in the IEK experiment (2.4.1), sampled with a plastic syringe and filtered through a 0.2-μm membrane. The r and C_p values were quantified as described in 2.4.1, with four analytical replicates. Using the r and C_p obtained at each incubation time, the measured E-value (E_{meas}) was calculated according to equation 2. At a given time point, the difference between E_{meas} and E_{est} (determined as described in 2.4.1) represents the cumulative gross P_o mineralization (ΔE).

The fumigation-extraction procedure described by Kouno et al. (1995) was used to quantify P and radioactivity in the microbial biomass, but using hexanol as fumigant instead of chloroform as suggested by Bünemann et al. (2004). The fumigation-extraction method is based on shaking a soil sample with water and an anion exchange resin membrane (BDH #55164, 6 cm x 2 cm) saturated with CO_3^{2-} for 16 h, with and without hexanol. The membranes are then eluted with a 0.1 M

NaCl/HCl solution, and the radioactivity and phosphate content in the eluates are quantified as described in 2.4.1.

The ^{31}P content in the microbial biomass ($^{31}\text{P}_{\text{mic}}$) was determined as the difference in phosphate concentration (mg kg^{-1}) between fumigated ($^{31}\text{P}_{\text{hex}}$) and non-fumigated ($^{31}\text{P}_{\text{resin}}$) samples after correction for adsorption of the P released during fumigation (Equation 3).

$$^{31}\text{P}_{\text{mic}} = (^{31}\text{P}_{\text{hex}} - ^{31}\text{P}_{\text{resin}}) / ^{31}\text{P}_{\text{rec}} \quad (\text{Equation 3})$$

The ^{31}P sorption correction ($^{31}\text{P}_{\text{rec}}$) was obtained from the recovery of a P spike, corresponding to the average $^{31}\text{P}_{\text{mic}}$ determined in a preliminary analysis of all soils (7.5 mg P kg^{-1}). This spike was added to additional non-fumigated samples, and evaluated for each soil and treatment at all sampling dates. The recovered fraction of added P averaged 0.89 ± 0.13 for Delaware, 0.92 ± 0.10 for Maryland and 0.88 ± 0.23 for Iowa.

No conversion factor to take into account the fumigation efficiency (K_p) was measured in our study. This factor varies depending on the soil (Oberson and Joner, 2005). Therefore, we present the results of P in the microbial biomass using or not a K_p factor, as performed by Achat et al. (2009b), in order to assess the effect of K_p on the calculated results. The selected K_p factor was 0.4, which is the one given by Brookes et al. (1984) and Kouno et al. (1995), and is very similar to the 0.37 value used by Hedley and Stewart (1982).

In principle, the radioactivity recovered in the microbial biomass ($^{33}\text{P}_{\text{mic}}$) was derived from the difference in radioactivity between fumigated ($^{33}\text{P}_{\text{hex}}$) and non-fumigated samples ($^{33}\text{P}_{\text{resin}}$), with additional corrections to account for physicochemical processes that affect the recovery of $^{33}\text{P}_{\text{mic}}$:

1) Upon release of ^{31}P from the lysed microbial cells, some ^{33}P is liberated from the mineral matrix. A simple subtraction of ^{33}P in non-fumigated subsamples would therefore overestimate ^{33}P in

microbial P. At each sampling time, the linear relationship between ^{31}P added ($^{31}\text{P}_{\text{spike}}$) (0 or 7.5 mg P kg^{-1}) and ^{33}P released and recovered ($^{33}\text{P}_{\text{released}}$) (Bq g^{-1}) was established (equation 4), with one P spike considered sufficient since a pretest with three P spikes had shown the relationship to be linear.

$$^{33}\text{P}_{\text{released}} = a * ^{31}\text{P}_{\text{spike}} + b \quad (\text{Equation 4})$$

The measured concentration of $^{31}\text{P}_{\text{hex}}$ was then inserted into this equation as the concentration of $^{31}\text{P}_{\text{spike}}$, and the resulting value was subtracted from $^{33}\text{P}_{\text{hex}}$.

2) Consecutively, the potential sorption of ^{33}P released during fumigation was corrected for based on the recovery of a ^{33}P spike (4.4 kBq g^{-1}) added to non-fumigated samples together with ^{31}P (7.5 mg P kg^{-1}). This procedure is analogous to the one used in Equation 3, but for ^{33}P in this case instead of ^{31}P . The recovery of the ^{33}P spike averaged 0.88 ± 0.17 for Delaware, 0.88 ± 0.09 for Maryland and 0.97 ± 0.22 for Iowa. No significant difference was found between the recovery of ^{31}P and ^{33}P spikes.

3) As for $^{31}\text{P}_{\text{mic}}$, $^{33}\text{P}_{\text{mic}}$ was calculated using K_p values of 1 or 0.4.

Immobilized P was calculated as suggested by Bünemann et al. (2007):

$$\text{P immobilized (mg P kg}^{-1}\text{)} = ((^{33}\text{P}_{\text{mic}}/^{31}\text{P}_{\text{mic}})/(^{33}\text{P}_{\text{H}_2\text{O}}/^{31}\text{P}_{\text{H}_2\text{O}})) * ^{31}\text{P}_{\text{mic}} \quad (5)$$

where the term $^{33}\text{P}_{\text{mic}}/^{31}\text{P}_{\text{mic}}$ represents the SA of microbial phosphate and $^{33}\text{P}_{\text{H}_2\text{O}}/^{31}\text{P}_{\text{H}_2\text{O}}$ represents the SA of water-extractable phosphate. Finally, the immobilized P was subtracted from the cumulative gross P_o mineralization to derive the cumulative net P_o mineralization. The gross and net P_o mineralization rates were calculated by dividing the cumulative gross and net P_o mineralization, respectively, by the number of incubation days.

In parallel to the incubation experiment, soil respiration was measured according to Alef (1995). To this end, pre-incubated soil samples were thoroughly mixed with water instead of ^{33}P labeling solution for 10 min to emulate the physical disturbance generated during soil labeling. After this, soil at 55% water-holding capacity equivalent to 20 g dry soil was placed in a plastic vial inside a 1-l Mason jar together with a 50-ml Erlenmeyer beaker containing 10 ml of 0.5 M NaOH. Blanks without soil were also included. At day 5, traps were exchanged and the NaOH was back-titrated with HCl after the addition of 4 ml of 1.5 M BaCl_2 and phenolphthalein. The respiration measurement was finished with a second titration at day 12. Linear regression between cumulated soil respiration and incubation period in days was used to estimate the respiration rates at days 6 and 13. We did not use this linear function to estimate respiration values for day 1, considering that a flush in soil respiration is commonly observed after the soil labeling (Achat et al., 2009a; Bünemann et al., 2007; Bünemann et al., 2012), which cannot be predicted by the linear function of respiration measured after 5 or 12 days of incubation.

The C:P ratio of net mineralization was calculated as the ratio between measured C respiration rate and net P_o mineralization rate. On the other hand, the C:P ratio of gross mineralization was calculated as the ratio between gross C respiration rate and gross P mineralization. The gross C respiration rate was calculated from the measured C respiration rate, assuming that 60% of the C mineralized is released as CO_2 while 40% is assimilated into the microbial biomass (Murphy et al., 2003).

2.5. Statistical analysis

Due to the different fertilizer treatments and rates, each experimental site was analyzed separately. The normality of distribution was confirmed using the Shapiro and Wilk (1965) procedure, while the homogeneity of variances was confirmed using the Levene (1960) test.

Differences between treatments were tested with a one-way ANOVA using the SAS PROC MIXED procedure (SAS Institute Inc, 2013). Effects were considered significant at $p < 0.05$, and the Tukey-Kramer test was used to compare fertilizer treatment means if analysis of variance indicated significant treatment effects.

The full model in the IEK experiment (Equation 1) was fitted using R in order to determine the parameters m and n (R Core Team, 2013). As the field replications were combined for the incubation experiment (Section 2.4.2), no statistical comparison between treatments was performed for the results of this part, and only the means and standard deviations are shown. The measured means for net and gross P_o mineralization determined in the incubation experiment were associated by fitting linear models with P_t and P_o in the whole soil, CF and FF, $^{31}P_{mic}$, simple-monoester-like P, phytate-like P and DNA-like P, using PROC REG (SAS Institute Inc, 2013). Multiple linear regression (PROC REG) was used to estimate P_o mineralization rates using respiration (estimated at days 6 and 13) and C:P ratios of the soil and soil physical fractions as regressors.

3. Results

3.1. Soil analysis

As expected, fertilization treatments affected P_t and P_o in most of the measured fractions (Tables 1 and 2), resulting in a large range of values for these soil properties, which allowed testing their relationships with gross and net P_o mineralization (Section 3.3.4). Concentrations of P_i in the whole soils ranged between 91 and 481 mg kg⁻¹, while P_o ranged from 119 to 370 mg kg⁻¹ (Table 1). The P_t in the CF varied from 10 to 91 mg kg⁻¹, with P_o representing 20 to 88% of it (Table 2). The recovery of P_t and P_o in both soil fractions (Table 2) represented 90 ± 7 and $109 \pm 9\%$ of the P_t and P_o measured in the non-fractioned samples, respectively (Table 1).

3.2. Enzyme addition assay

The concentration of P_i in NaOH-EDTA extracts of all soils was greater in P fertilized treatments than in the Control, irrespective of the P source (Table 3). Enzyme-labile P_o fractions showed a high variability between replications, and no differences between fertilizer treatments were observed for simple monoester-like P, DNA-like P and non-hydrolysable P_o . However, the concentration of phytate-like P was increased by PL application in Maryland and Iowa and additionally by IF fertilization in Maryland.

3.3. Isotopic dilution experiment

3.3.1. Water-extractable phosphate and microbial P

The concentration of water-extractable phosphate during the soil incubation ranged between 0.2 and 10.7 mg kg⁻¹ and was not always constant between sampling times (Figures 1 a-c). Water-extractable ³³P decreased over time in all soils and treatments (Figures 1 d-f). Therefore, also the SA in water-extractable phosphate decreased over time (Figure 1 g-i). The analytical variability between replications was low, with an average coefficient of variation of 4.4% for ³¹P, 4.0% for ³³P and 4.1% for SA.

Microbial P ($K_p = 1$) ranged between 1.2 and 11.9 mg P kg⁻¹ (Figures 2 a-c). Temporal trends were significant mainly in Delaware (all treatments), with an increase on the last sampling date. The recovery of ³³P and the SA in the microbial P pool showed a high analytical variability (average variation coefficients of 29% and 27%, respectively), and for most soils and treatments did not change significantly over time (Figures 2 d-i).

3.3.2. Batch experiment: IEK

The fitting parameter m of the IEK experiment ranged between 0.11 and 0.64, while the parameter n ranged between 0.18 and 0.37 (Table 4). Water-extractable phosphate measured in

the IEK experiment ranged between 0.2 and 10.4 mg P kg⁻¹ (Table 4) and was similar to the concentrations of water-extractable phosphate observed during the incubation experiment (Figure 1).

3.3.3. Gross and net P_o mineralization

The difference between extrapolated E-values (E_{est}) and measured E-values (E_{meas}) was statistically significant in most cases, except in the soils from Iowa under Control or PL treatments at day 1 (see Table S1 in supplementary material). The resulting cumulative gross P_o mineralization values ranged between 0.5 and 12.6 mg kg⁻¹ at day 1, between 7.3 to 26.9 mg kg⁻¹ at day 6 and between 9 to 38.6 mg kg⁻¹ at day 13 (Table 5). The resulting gross P_o mineralization rates ranged from 0.5 to 12.6 mg kg⁻¹ day⁻¹.

The net P_o mineralization rate was on average (between sites, treatments and incubation times) 3.0 and 2.1 mg kg⁻¹ d⁻¹ when using a K_p of 1 or 0.4 respectively (Table 5). The difference in net P_o mineralization when using different K_p values derived from the increase in the calculated P_{imm} when moving from a K_p of 1 to a K_p of 0.4 (see Table S1 in supplementary material).

3.3.4. Association between P pools and P_o mineralization

Neither gross nor net P_o mineralization (K_p = 1 and K_p = 0.4) were significantly associated with simple monoester-like P, phytate-like P, DNA-like P, non-hydrolysable P_o, P_o and P_t in the whole soil and in the FF, P_t in CF, nor hexanol-labile P at any sampling date (see Table S2 in supplementary material). In contrast, a highly significant association between P_o in the CF with gross and net (K_p = 1 and K_p = 0.4) P_o mineralization was found for Day 6 and Day 13 (see Table S2 in supplementary material and Figure 3), but not for Day 1.

In the Iowa soil amended with PL, less P_o was mineralized than estimated by the P_o in the CF. For example, on day 1 the linear regression between these two variables would have been highly significant without this soil (e.g. $R^2=0.87$; $p=0.0007$ for gross P_o mineralization). However, on day 1 the Iowa soil amended with PL which was supposed to mineralize $16 \text{ mg } P_o \text{ kg}^{-1}$, according to the estimation based on the P_o content in the CF, actually immobilized P_o .

3.3.5 . Respiration

Soil respiration rates (Table 5) during the first 6 days varied between 5.8 and $13.7 \text{ } \mu\text{g C g}^{-1} \text{ d}^{-1}$. Averaged across soils and treatments, rates were 37% lower for the second measurement period (day 6 - 13). The C:P ratios of gross mineralization ranged between 5 and 22, while for net mineralization they ranged between 3 and 16 when using a $K_p = 1$ and between 3 and 52 when using a $K_p = 0.4$ for the calculations (Table 5).

The association between respiration rates and gross and net P_o ($K_p=0.4$ and 1) mineralization rates was statistically significant (Table 6) but presented a low coefficient of determination ($R^2=0.48$, 0.39 and 0.45, respectively). It was observed that this low association between C and P_o mineralization was caused by the relatively small P_o mineralization rate in some treatments such as Delaware PL, as compared to other treatments with similar respiration rates. This trend coincided with a greater $C_t:P_o$ ratio in the soil and in the CF of these treatments (Tables 1 and 2). Therefore, the $C_t:P_o$ ratio was incorporated as a regressor to account not only for the organic matter decomposition rate, but also for its composition. The incorporation of the $C_t:P_o$ ratio of the soil or of the FF did not improve the model significantly. However, the incorporation of the $C_t:P_o$ ratio of the CF to the model significantly improved the coefficient of determination to 0.72 for gross mineralization and to 0.55 and 0.67 for net P_o mineralization using a K_p of 0.4 and 1,

respectively. The estimation of net P_o mineralization based on respiration and P_o concentration in the CF was significant, irrespective of the K_p factor used.

The $C_t:P_o$ ratios of the whole soil and the FF were not associated with the gross and net mineralization rate (see Table S2 in supplementary material). However, a significant linear association between the $C_t:P_o$ ratio of the CF and the C:P ratio of gross and net mineralization (for both $K_p=1$ and $K_p=0.4$) was observed at Day 13 ($R^2 = 0.73, 0.72$ and 0.43 , respectively).

4. Discussion

4.1. Soil P pools

The determined concentration of P_o in the studied samples (Table 1) was in the range of that commonly observed in soils (Havlin et al., 2005). The observed values of P_t and P_o in the CF were also in a similar range as in previous studies (Ciampitti et al., 2011; Wyngaard et al., 2013), except for the PL treatment in Iowa, which exceeded all other values. The change in P_t and P_o caused by PL was significant in the CF but not in the FF (Table 2), which agrees with the findings of Ciampitti et al. (2011) that P_t in the CF is more sensitive to the long-term application of fertilizers than P_t in whole soil samples.

The concentrations of DNA-like P, phytate-like P, simple monoester-like P and non-hydrolysable P_o (Table 3) were generally lower than in other studies (Annaheim et al., 2015; Requejo and Eichler-Löbermann, 2014; Keller et al., 2012; Koopmans et al., 2007). This difference can be a consequence of an incomplete extraction of the total P_o by the NaOH-EDTA extract, which is supported by the fact that the sum of all organic fractions extracted by NaOH-EDTA (Table 3) represented on average 19% of the P_o measured by calcination (Table 1). The observed high concentration of phytate-like P in PL amended soils (Table 3) can be a consequence of the

accumulation of the phytate-like P present in bird feces, where this form of P is the major component of the hydrolysable P_o (He et al., 2008).

The range of values for water-extractable phosphate was broader in this study (Figure 1 a-c) than in other studies (Bünemann et al., 2012; Achat et al., 2009b; Bünemann, 2008; Oehl et al., 2004). This is a consequence of the high concentrations of water-extractable phosphate in soils amended with PL. Both water-extractable and microbial P changed over time (Figures 1 and 2). This demonstrates that steady state conditions were not fully met throughout the experiment.

4.2. Gross and net P_o mineralization

The rates of gross P_o mineralization at days 6 and 13 were in a similar range as those determined by Oehl et al. (2004) for arable soils, and were similar at days 1, 6 and 13 to those determined by Bünemann et al. (2012) in permanent grassland soils in Switzerland. The decrease of these rates over time can be explained by the disturbance generated during the soil labeling and also by the recycling of ^{33}P labelled microbial P compounds during the incubation (remineralization *sensu strictu*) (Bünemann, 2015).

The calculated net P_o mineralization has been described to be biased by the used K_p value (Achat et al., 2009b). Analyzing a forest soil from France, Achat et al. (2009b) observed a 35% decrease in the calculated net P_o mineralization when using a K_p value of 0.5 instead of 1. In our study, this difference was 22%, 33% and 70% for the soils of Delaware, Maryland and Iowa, respectively, when using a K_p value of 0.4 (Table 5).

4.3. Association between P pools and P_o mineralization

The fact that P_o mineralization was not related to the concentrations of any form of P_o in the bulk soil but to its concentrations in a soil physical fraction (CF) (Figure 3) reinforces the importance of

physical protection for the stabilization of organic compounds (Six et al., 2002). This stabilization is a consequence of processes of adsorption of organic molecules to the FF (Six et al., 2002) and of the formation of micro-aggregates (Fonte et al., 2014; Six et al., 2002). Consequently, even though 89% to 98% of the P_o was located in the FF, the unprotected P_o in the CF mostly determined the mineralization rate of the soil during the incubation. However, we cannot prove that P_o in the CF was indeed mineralized and we have to consider it as an indicator of P_o mineralization rather than as the mineralized substrate. If microbial activity was ongoing during storage of soils at 4°C before starting the experiment, P_o in the CF may have been mineralized before the start of the incubation experiment. We determined P_o in the CF on soils that were dried just after sampling. Therefore, we are indeed discussing a correlation and not necessarily a causal relationship. In future experiments, soil storage should be avoided and net changes in P_o in the CF during incubation should be assessed.

The association between gross or net P_o mineralization and P_o in the CF was above the 1:1 regression line on days 6 and 13. This indicates that the cumulative P_o mineralized is greater than the amount of P_o in the CF of the soil, suggesting that other sources of P_o (either living or non-living) are actively mineralized during the incubation. Previous studies conceptualized two-pool models to describe P_o mineralization, dividing the mineralizable pool between living and non-living soil organic matter (Müller and Bünemann, 2014; Achat et al., 2010). In future studies, it would be important to analyze if the dynamics of non-living organic P can also be divided into two pools of different turnover, as it was proposed for nitrogen (Deans et al., 1986).

The K_p value was shown to affect the calculation of net P_o mineralization. However, we demonstrated that the association between P_o concentration in the CF and net P_o mineralization is strong in the evaluated soils, irrespective of the K_p value used (Figure 3). Further research is required to verify the association between P_o in the coarse fraction and P_o mineralization in a

broader range of land-uses and soil types. For example, given the high sorption capacity of Al and Fe oxides for P_o and its effect on microbial processes (Achat et al., 2013), it would be important to analyze if the association between P_o mineralization and P_o content in the CF is significant also in weathered soils with a high concentration of these minerals.

4.4. Prediction of P_o mineralization from soil respiration

Soil respiration rates (Table 5) were similar to those observed by Oehl et al. (2004) in arable fields. Also, the observed trends in soil respiration between soils and fertilization treatments coincided, in general, with those observed for soil C_t accumulation (Table 1).

The observed C:P ratios of gross mineralization (Table 5) were similar to those observed in other arable soils (Randriamanantsoa et al., 2015; Oehl et al., 2004). Kirkby et al. (2011) suggested to compare the $C_t:P_o$ ratio of stable organic matter versus the C:P ratio of mineralization. The fact that in our study the $C_t:P_o$ ratio of the CF was associated with the P_o mineralization rate (Table 6), while the $C_t:P_o$ of the soil and the FF were not, supports the hypothesis of the CF having a relative greater contribution to P_o mineralization than the FF. It would be important to confirm these findings in future studies by measuring C_t and P_o concentrations in CF and FF at the beginning and at the end of the incubation period, to determine the depletion of these pools.

Even though there is a relationship between P_o in the CF and P_o mineralization, the $C_t:P_o$ ratio of the CF averaged 253 (Table 2), which is much greater than the C:P ratio of gross and net mineralization (average 8 for gross P_o mineralization and 6 and 11 for net P_o mineralization using a K_p of 1 or 0.4, respectively). These last values are most similar to those described for the C:P ratio of the microbial biomass in arable soils, which ranges between 8 and 26 (Brookes et al., 1984; Oehl et al., 2004; Bünemann et al., 2012). Unless gross P_o mineralization is overestimated, for reasons unknown, this would suggest that gross and net mineralization rates originated entirely from

immobilization and remineralization (*sensu latu*) (Bünemann, 2015), and that mineralization of non-living P_o was negligible. The net P_o mineralization rate given in Table 5 would thus resemble net remineralization. Further experiments of longer duration which allow assessing the mineralization of non-living P_o (Achat et al. 2010), possibly in combination with modelling approaches (Müller & Bünemann, 2014) are needed to better understand the processes underlying gross P_o mineralization.

The comparison of C:P ratios of potential substrates and rates suggests that the contribution of P_o in the CF to P_o mineralization is indirect: P_o in CF positively influences the microbial biomass, which in turn controls the gross and net P_o mineralization rates. Bünemann (2015) likewise suggested that microbial immobilization and remineralization represent the majority of the gross P_o mineralization flux, rather than the mineralization of non-living P_o . A link between P in the CF and microorganisms has been indicated before: Salas et al. (2003) showed that plant residues added to soil are quickly colonized by fungi, which become part of the CF. Consequently, over time, P in particulate organic matter may originate mainly from fungal P immobilization rather than from plant residues.

5. Conclusions

The concentration of P_o in the CF of the soil ($>53 \mu\text{m}$) was significantly related to P_o mineralization measured by isotopic dilution. Thus, the concentration of P_o in the CF surges as a promising indicator to differentiate soils according to their P_o mineralization potential, without focusing on the actual rate of mineralization which will be affected by other factors such as temperature and water content. In order to estimate P_o mineralization rates, we recommend to measure soil respiration and correct it by the $C_t:P_o$ ratio of the CF. Further research is required to verify the

association between P_o in the coarse fraction and P_o mineralization in a broader range of land-uses and soil types.

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References

- Achat, D.L., Bakker, M.R., Augusto, L., Saur, E., Dousseron, L., Morel, C., 2009a. Evaluation of the phosphorus status of P-deficient podzols in temperate pine stands: combining isotopic dilution and extraction methods. *Biogeochemistry* 92, 183-200.
- Achat, D.L., Bakker, M.R., Morel, C., 2009b. Process-based assessment of phosphorus availability in a low phosphorus sorbing forest soil using isotopic dilution methods. *Soil Science Society of America Journal* 73, 2131-2142.
- Achat, D.L., Bakker, M.R., Zeller, B., Pellerin, S., Bienaimé, S., Morel, C., 2010. Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biology and Biochemistry* 42, 1479-1490.
- Achat, D.L., Bakker, M.R., Augusto, L., Morel, C., 2013. Contributions of microbial and physical-chemical processes to phosphorus availability in Podzols and Arenosols under a temperate forest. *Geoderma* 211, 18e27.

542 Alef, K., 1995. Soil respiration, In: Nannipieri, P. (Ed.), Methods in applied soil microbiology and
 543 biochemistry. Academic press, London, pp. 214-219.

544 Anderson, J.M., Ingram, J., 1989. Tropical soil biology and fertility. A handbook of methods. CAB
 545 international Wallingford, Oxon, UK, 221 pp.

546 Annaheim, K., Doolette, A., Smernik, R., Mayer, J., Oberson, A., Frossard, E., Bünemann, E., 2015.
 547 Long-term addition of organic fertilizers has little effect on soil organic phosphorus as
 548 characterized by ³¹P NMR spectroscopy and enzyme additions. *Geoderma* 257-258, 67-
 549 77.

550 Batjes, N., 1997. A world dataset of derived soil properties by FAO–UNESCO soil unit for global
 551 modelling. *Soil Use and Management* 13, 9-16.

552 Bray, R.H., Kurtz, L., 1945. Determination of total, organic, and available forms of phosphorus in
 553 soils. *Soil Science* 59, 39-46.

554 Brookes, P., Powlson, D., Jenkinson, D., 1984. Phosphorus in the soil microbial biomass. *Soil*
 555 *Biology and Biochemistry* 16, 169-175.

556 Bünemann, E.K., 2008. Enzyme additions as a tool to assess the potential bioavailability of
 557 organically bound nutrients. *Soil Biology and Biochemistry* 40, 2116-2129.

558 Bünemann, E., 2015. Assessment of gross and net mineralization rates of soil organic phosphorus -
 559 a review. *Soil Biology & Biochemistry*. [dx.doi.org/10.1016/j.soilbio.2015.06.026](https://doi.org/10.1016/j.soilbio.2015.06.026).

560 Bünemann, E.K., Smithson, P.C., Jama, B., Frossard, E., Oberson, A., 2004. Maize productivity and
 561 nutrient dynamics in maize-fallow rotations in western Kenya. *Plant and Soil* 264, 195-208.

562 Bünemann, E., Marschner, P., McNeill, A.M., McLaughlin, M.J., 2007. Measuring rates of gross and
 563 net mineralisation of organic phosphorus in soils. *Soil Biology and Biochemistry* 39, 900-
 564 913.

565 Bünemann, E., Oberson, A., Liebisch, F., Keller, F., Annaheim, K., Huguenin-Elie, O., Frossard, E.,
 566 2012. Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a
 567 grassland soil with low inorganic phosphorus availability. *Soil Biology and Biochemistry* 51,
 568 84-95.

569 Cambardella, C., Elliott, E., 1992. Particulate soil organic-matter changes across a grassland
 570 cultivation sequence. *Soil Science Society of America Journal* 56, 777-783.

571 Ciampitti, I.A., García, F.O., Picone, L.I., Rubio, G., 2011. Soil carbon and phosphorus pools in field
 572 crop rotations in Pampean soils of Argentina. *Soil Science Society of America Journal* 75,
 573 616-625.

574 Condon, L.M., Turner, B.L., Cade-Menun, B.J. 2005. Chemistry and dynamics of soil organic
 575 phosphorus. In: Sims, J.T., Sharpley, A.N. (Eds) *Phosphorus: Agriculture and the*
 576 *Environment*. ASA, CSSA and SSSA, Madison, WI, pp 87-122

577 Deans, J., Molina, J., Clapp, C., 1986. Models for predicting potentially mineralizable nitrogen and
 578 decomposition rate constants. *Soil Science Society of America Journal* 50, 323-326.

579 Fardeau, J.-C., Morel, C., Jappe, J., 1985. Cinétique d'échange des ions phosphate dans les
 580 systèmes sol-solution: vérification expérimentale de l'équation théorique. *Comptes rendus*
 581 *des séances de l'Académie des sciences. Série 3, Sciences de la vie* 300, 371-376.

582 Fardeau, J., Morel, C., Boniface, R., 1991. Cinétiques de transfert des ions phosphate du sol vers la
 583 solution du sol: paramètres caractéristiques. *Agronomie* 11, 787-797.

584 Fonte, S.J., Nesper, M., Hegglin, D., Velásquez, J.E., Ramirez, B., Rao, I.M., Bernasconi, S.M.,
 585 Bünemann, E.K., Frossard, E., Oberson, A., 2014. Pasture degradation impacts soil
 586 phosphorus storage via changes to aggregate-associated soil organic matter in highly
 587 weathered tropical soils. *Soil Biology and Biochemistry* 68, 150-157

588 Grierson, P., Comerford, N., Jokela, E., 1998. Phosphorus mineralization kinetics and response of
589 microbial phosphorus to drying and rewetting in a Florida Spodosol. *Soil Biology and*
590 *Biochemistry* 30, 1323-1331.

591 Harrison, A.F., 1987. *Soil organic phosphorus: a review of world literature*. CAB International
592 Wallingford.

593 Havlin, J., Beaton, J.D., Tisdale, S.L., Nelson, W.L., 2005. *Soil fertility and fertilizers: An introduction*
594 *to nutrient management*. Pearson Prentice Hall Upper Saddle River, New Jersey, USA, 515
595 pp.

596 Hedley, M.J., Stewart, J.W.B., 1982. Method to measure microbial phosphate in soils. *Soil Biology &*
597 *Biochemistry* 14, 377-385.

598 He, Z., Honeycutt, C.W., Cade-Menun, B.J., Senwo, Z.N., Tazisong, I.A., 2008. Phosphorus in poultry
599 litter and soil: Enzymatic and nuclear magnetic resonance characterization. *Soil Science*
600 *Society of America Journal* 72, 1425-1433.

601 Keller, M., Oberson, A., Annaheim, K.E., Tamburini, F., Mäder, P., Mayer, J., Frossard, E.,
602 Bünemann, E.K., 2012. Phosphorus forms and enzymatic hydrolyzability of organic
603 phosphorus in soils after 30 years of organic and conventional farming. *Journal of Plant*
604 *Nutrition and Soil Science* 175, 385-393.

605 Kirkby, C., Kirkegaard, J., Richardson, A., Wade, L., Blanchard, C., Batten, G., 2011. Stable soil
606 organic matter: a comparison of C: N: P: S ratios in Australian and other world soils.
607 *Geoderma* 163, 197-208.

608 Koopmans, G., Chardon, W., McDowell, R., 2007. Phosphorus movement and speciation in a sandy
609 soil profile after long-term animal manure applications. *Journal of Environmental Quality*
610 36, 305-315.

611 Kouno, K., Tuchiya, Y., Ando, T., 1995. Measurement of soil microbial biomass phosphorus by an
 612 anion exchange membrane method. *Soil Biology and Biochemistry* 27, 1353-1357.

613 Levene, H., 1960. Robust tests for equality of variances. *Contributions to probability and statistics:*
 614 *Essays in honor of Harold Hotelling* 2, 278-292.

615 Mehlich, A., 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant.
 616 *Communications in Soil Science & Plant Analysis* 15, 1409-1416.

617 Müller, C., Bünemann, E.K., 2014. A ^{33}P tracing model for quantifying gross P transformation rates
 618 in soil. *Soil Biology and Biochemistry* 76, 218-226.

619 Murphy, D., Recous, S., Stockdale, E., Fillery, I., Jensen, L., Hatch, D., Goulding, K., 2003. Gross
 620 nitrogen fluxes in soil: theory, measurement and application of ^{15}N pool dilution
 621 techniques. *Advances in Agronomy* 79, 69-118.

622 Natural Resources Conservation Service, 1999. *Soil Taxonomy: A basic system of soil classification*
 623 *for making and interpreting soil surveys.* US Department of Agriculture.

624 Oberson, A., Joner, E.J., 2005. Microbial turnover of phosphorus in soil. In: Turner, B.L., Frossard,
 625 E., Baldwin, D. (Eds.), *Organic phosphorus in the environment.* CABI, Wallingford, Oxon,
 626 UK, pp. 133-164.

627 Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., Oberson, A., 2004. Basal organic phosphorus
 628 mineralization in soils under different farming systems. *Soil Biology and Biochemistry* 36,
 629 667-675.

630 Oehl, F., Oberson, A., Sinaj, S., Frossard, E., 2001. Organic phosphorus mineralization studies using
 631 isotopic dilution techniques. *Soil Science Society of America Journal* 65, 780-787.

632 Ohno, T., Zibilske, L.M., 1991. Determination of low concentrations of phosphorus in soil extracts
 633 using malachite green. *Soil Science Society of America Journal* 55, 892-895.

634 Olsen, S.R., Cole, C., Watanabe, F.S., Dean, L., 1954. Estimation of available phosphorus in soils by
635 extraction with sodium bicarbonate. US Department of Agriculture Washington, DC.

636 R Core Team, 2013. A language and environment for statistical computing. Vienna, Austria: R
637 Foundation for Statistical Computing. URL <http://www.R-project.org>.

638 Raghothama, K.G., Sims, J., Sharpley, A., 2005. Phosphorus and plant nutrition: an overview.
639 Phosphorus: agriculture and the environment, 355-378.

640 Randriamanantsoa, L., Frossard, E., Oberson, A., Bünemann, E.K., 2015. Gross organic phosphorus
641 mineralization rates can be assessed in a Ferralsol using an isotopic dilution method.
642 Geoderma 257-258, 86-93.

643 Requejo, M.I., Eichler-Löbermann, B., 2014. Organic and inorganic phosphorus forms in soil as
644 affected by long-term application of organic amendments. Nutrient Cycling in
645 Agroecosystems 100, 245-255.

646 Robinson, G.W., 1922. A new method for the mechanical analysis of soils and other dispersions.
647 The Journal of Agricultural Science 12, 306-321.

648 Salas, A., Elliott, E., Westfall, D., Cole, C., Six, J., 2003. The role of particulate organic matter in
649 phosphorus cycling. Soil Science Society of America Journal 67, 181-189.

650 SAS Institute Inc, 2013. SAS® 9.4 In-Database Products: User's Guide, Third Edition. Cary, NC: SAS
651 Institute Inc.

652 Saunders, W., Williams, E., 1955. Observations on the determination of total organic phosphorus
653 in soils. Journal of Soil Science 6, 254-267.

654 Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples).
655 Biometrika, 591-611.

656 Six, J., Conant, R., Paul, E., Paustian, K., 2002. Stabilization mechanisms of soil organic matter:
657 implications for C-saturation of soils. Plant and Soil 241, 155-176.

Stanford, G., Smith, S.J., 1972. Nitrogen Mineralization Potentials of Soils. Soil Science Society of America Journal 36, 465-472.

Tabatabai, M.A., Al-Khafaji, A.A., 1980. Comparison of Nitrogen and Sulfur Mineralization in Soils. Soil Science Society of America Journal 44, 1000-1006.

Wyngaard, N., Vidaurreta, A., Echeverría, H.E., Picone, L.I., 2013. Dynamics of Phosphorus and Carbon in the Soil Particulate Fraction Under Different Management Practices. Soil Science Society of America Journal 77, 1584-1590.

FIGURE CAPTIONS

Figure 1. Concentration of phosphate (a, b and c), recovery of ^{33}P (d, e and f) and specific activity (g, h and i) in water-extractable phosphate during an incubation experiment in three different soils subject to different fertilizers treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Time-points: 1, 6 and 13 days after labeling. Significance values in the legend indicate the effect of time for each treatment separately in a one-way ANOVA. Error bars represent the standard deviation (n=4).

Figure 2. Concentration of phosphate (a, b and c), recovery of ^{33}P (d, e and f) and specific activity (g, h and i) in microbial P ($K_p=1$) during an incubation experiment with three different soils subject to different long-term fertilizers treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Time-points: 1, 6 and 13 days after labeling. Significance values in the legend indicate the effect of time for each treatment separately in a one-way ANOVA. Error bars represent the standard deviation ($n=4$).

Figure 3. Association between organic phosphorus in the coarse fraction (CF) of the soil and the cumulative gross and net P_o mineralization (P_{min}) at 1, 6 and 13 days of incubation. Net mineralization calculated using two different conversion factors to account for incomplete recovery of microbial P during fumigation-extraction ($K_p = 0.4$ and $K_p = 1$). Different shapes represent different soils: circle (Delaware), square (Maryland) and triangle (Iowa). Different colors represent different fertilizer treatments: white (control), grey (inorganic fertilizer) and black (poultry litter). The 1:1 line is shown to increase the readability of the graph.

Figure S1. Conceptual models to describe inorganic and organic P (P_i and P_o , respectively) transformations in the soil during our study (a) and the principles of the isotopic dilution method (b) (Adapted from Bünemann, 2015). In the latter, gross P_o mineralization is determined as the difference in the E-value extrapolated from a batch isotopic exchange kinetics experiment (IEK) (Section 2.4.1) and the E-value measured during a 13-days incubation experiment (Section 2.4.2). The net P_o mineralization is calculated by subtracting P immobilization from gross P_o mineralization.

Table 1

Table 1. General soil properties (pH, total carbon (C_t), total nitrogen (N_t), inorganic P (P_i) and organic P (P_o) in three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Means ± standard deviation of three field replicates. Different letters indicate significant (p < 0.05) differences between treatments in a given soil as indicated by the Tukey-Kramer test.

Soil	Treatment	pH	C _t g kg ⁻¹	N _t g kg ⁻¹	P _i -----mg kg ⁻¹ -----	P _o	C _t :P _o
Delaware	Control	5.1 ± 0.0 b	12.6 ± 0.2 b	0.68 ± 0.02 b	90.9 ± 0.9 b	118.5 ± 0.9 b	106.1 ± 2.0 a
	IF	5.2 ± 0.1 b	13.1 ± 0.3 b	0.81 ± 0.02 a	145.2 ± 3.8 a	140.7 ± 3.8 a	93.3 ± 0.3 b
	PL	5.5 ± 0.0 a	14.2 ± 0.3 a	0.86 ± 0.01 a	155.6 ± 7.8 a	141.8 ± 7.8 a	100.0 ± 4.6 ab
Maryland	Control	5.5 ± 0.3	10.2 ± 0.1 c	0.90 ± 0.02 b	349.7 ± 3.4 c	345.5 ± 3.4 b	29.6 ± 0.2
	IF	6.1 ± 0.4	10.5 ± 0.1 b	0.95 ± 0.02 ab	413.4 ± 8.8 b	352.3 ± 8.8 b	29.7 ± 0.7
	PL	5.9 ± 0.2	11.0 ± 0.1 a	1.01 ± 0.03 a	443.3 ± 2.3 a	369.6 ± 2.3 a	29.7 ± 0.3
Iowa	Control	6.6 ± 0.1 b	17.2 ± 0.3 b	1.27 ± 0.04 b	137.0 ± 4.7 b	245.5 ± 4.7 b	69.9 ± 1.2 ab
	Control + N	5.8 ± 0.1 c	18.7 ± 0.2 a	1.41 ± 0.02 a	119.5 ± 4.0 c	281.3 ± 4.0 a	66.4 ± 1.5 b
	PL	7.2 ± 0.1a	17.0 ± 0.1 b	1.34 ± 0.01 ab	481.4 ± 3.8 a	230.6 ± 3.8 c	73.6 ± 1.0 a
Significance level (p)	Delaware	< 0.001	0.003	< 0.001	< 0.001	0.006	0.013
	Maryland	0.274	< 0.001	0.012	< 0.001	0.013	0.933
	Iowa	< 0.001	< 0.001	0.003	< 0.001	< 0.001	0.003

Table 2

Table 2. Concentrations of total C (C_t), total P (P_t), organic P (P_o) and C_t:P_o ratio in two physical fractions (coarse and fine) of three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). All concentrations are expressed on a whole soil basis. Means ± standard deviation of three field replicates. Different letters within columns indicate significant (p < 0.05) differences between treatments in a given soil as indicated by the Tukey-Kramer test.

Soil	Treatment	Coarse fraction (>53µm)				Fine fraction (<53 µm)			
		C _t g kg ⁻¹	P _t [†] ----- mg kg ⁻¹ -----	P _o [†] ----- mg kg ⁻¹ -----	C _t :P _o	C _t g kg ⁻¹	P _t ----- mg kg ⁻¹ -----	P _o	C _t :P _o
Delaware	Control	3.8 ± 0.4	9.7 ± 1.2 b	8.5 ± 0.4 b	478.0 ± 25.5 a	8.8 ± 0.3 b	161.6 ± 23.1	110.2 ± 15.5	81.1 ± 10.5
	IF	3.3 ± 0.5	19.9 ± 5.0 ab	11.3 ± 0.7 b	293.7 ± 30.7 b	9.6 ± 0.3 ab	208.5 ± 36.4	120.9 ± 22.4	90.1 ± 21.0
	PL	4.0 ± 0.6	25.9 ± 6.7 a	14.9 ± 1.0 a	302.2 ± 18.7 b	10.3 ± 0.3 a	219.4 ± 38.7	138.6 ± 27.4	86.2 ± 6.6
Maryland	Control	1.9 ± 0.2	19.9 ± 4.2 b	11.3 ± 0.4 b	156.7 ± 13.8	8.3 ± 0.2 b	613.4 ± 31.0	379.6 ± 50.1	22.2 ± 2.7
	IF	1.8 ± 0.2	31.0 ± 0.5 ab	12.2 ± 0.7 ab	132.3 ± 7.4	8.6 ± 0.2 ab	664.9 ± 30.1	366.7 ± 26.1	23.7 ± 2.1
	PL	1.9 ± 0.1	42.5 ± 1.0 a	14.7 ± 0.7 a	133.5 ± 9.8	9.0 ± 0.1 a	697.5 ± 51.0	379.3 ± 33.6	24.0 ± 2.3
Iowa	Control	1.7 ± 0.4 b	25.0 ± 1.5 b	6.1 ± 0.9 b	285.4 ± 91.0	15.5 ± 0.6 a	354.9 ± 21.8 a	261.2 ± 7.4	59.3 ± 3.7
	Control + N	2.2 ± 0.3 ab	25.2 ± 1.5 b	7.2 ± 0.4 b	335.8 ± 122.5	16.4 ± 0.3 a	375.8 ± 31.5 a	294.8 ± 24.2	56.2 ± 5.0
	PL	2.9 ± 0.3a	90.7 ± 8.3 a	17.7 ± 2.3 a	164.1 ± 13.8	14.1 ± 0.2 b	592.4 ± 35.5 b	278.0 ± 17.9	50.8 ± 2.6
Significance level (p)	Delaware	0.364	0.041	0.003	0.003	0.020	0.262	0.481	0.864
	Maryland	0.897	0.010	0.008	0.107	0.020	0.170	0.929	0.745
	Iowa	0.025	0.000	0.001	0.070	0.004	0.000	0.251	0.171

†P_t and P_o determined by the ignition method (Saunders and Williams, 1955)

Table 3

Table 3. Concentrations of different phosphorus forms in NaOH-EDTA-extracts determined by enzyme additions in three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Means $\pm$ standard deviation of three field replicates. Within columns, different letters indicate significant ($p < 0.05$) differences between treatments in a given soil as indicated by the Tukey-Kramer test.

Soil	Treatment	Inorganic P	Organic P			
			Simple monoester-like P	Phytate-like P	DNA-like P	Non-hydrolysable P _o
			----- mg P kg ⁻¹ -----			
Delaware	Control	34.0 ± 3.0 b	2.5 ± 1.8	9.9 ± 0.6	0.3 ± 0.5	3.4 ± 3.7
	IF	79.9 ± 3.8 a	2.8 ± 1.0	12.6 ± 1.3	0.9 ± 0.6	10.7 ± 5.4
	PL	86.2 ± 6.4 a	0.6 ± 1.0	11.3 ± 2.9	0.5 ± 0.6	5.8 ± 1.6
Maryland	Control	52.7 ± 4.9 b	6.2 ± 3.5	2.9 ± 0.9 c	1.5 ± 1.8	40.0 ± 4.2 ab
	IF	97.7 ± 0.2 a	5.3 ± 4.0	7.2 ± 0.5 b	0.5 ± 0.0	59.9 ± 16.3 a
	PL	106.0 ± 6.6 a	1.9 ± 2.3	12.6 ± 1.5 a	0.8 ± 1.2	29.1 ± 7.8 b
Iowa	Control	57.3 ± 2.5 b	2.6 ± 0.3	14.9 ± 1.2 b	0.0 ± 0.0	38.1 ± 2.3 ab
	Control + N	43.2 ± 5.4 b	3.9 ± 2.5	14.1 ± 1.0 b	0.5 ± 0.5	62.2 ± 15.7 a
	PL	265.9 ± 5.6 a	13.7 ± 18.7	19.8 ± 0.8 a	1.3 ± 2.2	29.4 ± 4.3 b
Significance level (<i>p</i>)	Delaware	< 0.001	0.177	0.326	0.656	0.302
	Maryland	< 0.001	0.293	< 0.001	0.679	0.024
	Iowa	< 0.001	0.495	0.002	0.635	0.016

Table 4

Table 4. Parameters of isotopic exchange in three different soils subjected to different long-term fertilizer treatments: Control, inorganic P fertilization (IF), Control + N and poultry litter (PL). Means $\pm$ standard deviation of six analytical replicates.

Soil	Treatment	10*Cp (mg P kg ⁻¹)	<i>m</i>	<i>n</i>
Delaware	Control	0.20 $\pm$ 0.01	0.39 $\pm$ 0.04	0.32 $\pm$ 0.02
	IF	1.22 $\pm$ 0.08	0.60 $\pm$ 0.03	0.25 $\pm$ 0.00
	PL	1.75 $\pm$ 0.12	0.64 $\pm$ 0.03	0.22 $\pm$ 0.01
Maryland	Control	0.20 $\pm$ 0.01	0.11 $\pm$ 0.01	0.37 $\pm$ 0.01
	IF	0.95 $\pm$ 0.08	0.23 $\pm$ 0.01	0.28 $\pm$ 0.01
	PL	1.18 $\pm$ 0.05	0.27 $\pm$ 0.01	0.27 $\pm$ 0.00
Iowa	Control	0.45 $\pm$ 0.04	0.20 $\pm$ 0.01	0.30 $\pm$ 0.01
	Control + N	0.27 $\pm$ 0.04	0.21 $\pm$ 0.03	0.35 $\pm$ 0.01
	PL	10.35 $\pm$ 0.27	0.37 $\pm$ 0.00	0.18 $\pm$ 0.00

Table 5. Soil respiration rate at 6 and 13 days of incubation, and gross and net mineralization (calculated using two different conversion factors to account for incomplete recovery of microbial P during fumigation-extraction: $K_p = 0.4$ and $K_p = 1$) at 1, 6 and 13 days of incubation in an experiment with three soils subjected to long-term fertilizer treatments (Control, inorganic P fertilization (IF), Control + N and poultry litter (PL)). Values of organic P mineralization are expressed as cumulative at each sampling date and also as daily rate, and the C:P ratio of each flux is also included. Means $\pm$ standard deviation of four analytical replicates.

Soil	Treatment	Days	Respiration	----- Gross P _o mineralization -----			----- Net P _o mineralization (K _p = 1) -----			----- Net P _o mineralization (K _p = 0.4) -----		
			Rate	Cumulative	Rate	C:P	Cumulative	Rate	C:P	Cumulative	Rate	C:P
			mg C kg ⁻¹ d ⁻¹	mg P kg ⁻¹	mg P kg ⁻¹ d ⁻¹		mg P kg ⁻¹	mg P kg ⁻¹ d ⁻¹		mg P kg ⁻¹	mg P kg ⁻¹ d ⁻¹	
Delaware	Control	1	-	3.6 ± 0.3	3.6 ± 0.3	-	2.6 ± 0.3	2.6 ± 0.3	-	1.1 ± 0.3	1.1 ± 0.3	-
		6	11.7 ± 0.2	7.3 ± 0.5	1.2 ± 0.1	16.7 ± 1.2	6.2 ± 0.5	1.0 ± 0.1	11.7 ± 0.2	4.6 ± 0.5	0.8 ± 0.1	15.7 ± 0.2
		13	8.7 ± 0.4	9.0 ± 0.4	0.7 ± 0.0	22.1 ± 1.7	7.4 ± 0.4	0.6 ± 0.0	16.1 ± 0.7	5.0 ± 0.4	0.4 ± 0.0	23.7 ± 1.1
	IF	1	-	7.7 ± 0.5	7.7 ± 0.5	-	7.2 ± 0.5	7.2 ± 0.5	-	6.4 ± 0.5	6.4 ± 0.5	-
		6	11.8 ± 0.1	20.9 ± 1.7	3.5 ± 0.3	5.9 ± 0.6	19.0 ± 1.7	3.2 ± 0.3	3.9 ± 0.0	16.2 ± 1.7	2.7 ± 0.3	4.6 ± 0.1
		13	8.6 ± 0.1	28.0 ± 1.0	2.2 ± 0.1	7.0 ± 0.3	26.5 ± 1.0	2.0 ± 0.1	4.4 ± 0.0	24.4 ± 1.0	1.9 ± 0.1	4.8 ± 0.1
	PL	1	-	11.1 ± 1.8	11.1 ± 1.8	-	9.7 ± 1.8	9.7 ± 1.8	-	7.6 ± 1.8	7.6 ± 1.8	-
		6	12.8 ± 0.1	26.9 ± 0.8	4.5 ± 0.1	5.0 ± 0.2	24.4 ± 0.8	4.1 ± 0.1	3.4 ± 0.0	20.6 ± 0.8	3.4 ± 0.1	4.0 ± 0.0
		13	7.5 ± 0.2	34.5 ± 2.3	2.7 ± 0.2	5.2 ± 0.4	31.2 ± 2.3	2.4 ± 0.2	3.4 ± 0.1	26.4 ± 2.3	2.0 ± 0.2	4.1 ± 0.1
Maryland	Control	1	-	9.0 ± 0.9	9.0 ± 0.9	-	5.6 ± 0.9	5.6 ± 0.9	-	0.5 ± 0.9	0.5 ± 0.9	-
		6	10.9 ± 0.2	13.9 ± 3.6	2.3 ± 0.6	9.0 ± 2.7	10.9 ± 3.6	1.8 ± 0.6	6.3 ± 0.1	6.5 ± 3.6	1.1 ± 0.6	10.7 ± 0.2
		13	6.4 ± 0.4	28.1 ± 2.1	2.2 ± 0.2	5.1 ± 0.2	26.5 ± 2.1	2.0 ± 0.2	3.5 ± 0.2	20.8 ± 2.1	1.6 ± 0.2	4.4 ± 0.2
	IF	1	-	12.1 ± 0.5	12.1 ± 0.5	-	10.1 ± 0.5	10.1 ± 0.5	-	7.2 ± 0.5	7.2 ± 0.5	-
		6	12.7 ± 0.0	21.0 ± 2.7	3.5 ± 0.5	6.5 ± 0.9	18.2 ± 2.7	3.0 ± 0.5	4.5 ± 0.0	14.0 ± 2.7	2.3 ± 0.5	5.8 ± 0.0
		13	7.5 ± 0.1	37.4 ± 2.0	2.9 ± 0.2	4.8 ± 0.3	35.0 ± 2.0	2.7 ± 0.2	3.1 ± 0.0	31.4 ± 2.0	2.4 ± 0.2	3.4 ± 0.0
	PL	1	-	12.6 ± 5.3	12.6 ± 5.3	-	9.6 ± 5.3	9.6 ± 5.3	-	5.2 ± 5.3	5.5 ± 5.3	-
		6	13.7 ± 0.3	21.1 ± 0.9	3.5 ± 0.2	6.5 ± 0.3	18.4 ± 0.9	3.1 ± 0.2	4.4 ± 0.1	14.4 ± 0.9	2.4 ± 0.2	5.5 ± 0.1
		13	7.4 ± 0.4	38.6 ± 2.9	3.0 ± 0.2	4.7 ± 0.2	35.6 ± 2.9	2.7 ± 0.2	3.0 ± 0.1	31.0 ± 2.9	2.4 ± 0.2	3.5 ± 0.1
Iowa	Control	1	-	1.0 ± 0.6	1.0 ± 0.6	-	-0.5 ± 0.6	-0.5 ± 0.6	-	-2.6 ± 0.6	-2.6 ± 0.6	-
		6	6.2 ± 0.0	8.6 ± 0.5	1.4 ± 0.1	7.2 ± 0.6	4.2 ± 3.7	0.7 ± 0.6	9.3 ± 0.4	0.8 ± 3.7	0.1 ± 0.6	11.9 ± 0.6
		13	3.3 ± 0.6	8.3 ± 1.0	0.6 ± 0.1	10.0 ± 1.8	5.3 ± 1.0	0.4 ± 0.1	8.9 ± 1.2	0.7 ± 1.0	0.1 ± 0.1	52.1 ± 7.4
	Control + N	1	-	5.0 ± 0.8	5.0 ± 0.8	-	3.6 ± 0.8	3.6 ± 0.8	-	1.5 ± 0.8	1.5 ± 0.8	-
		6	5.8 ± 0.1	10.5 ± 3.4	1.7 ± 0.6	6.3 ± 1.8	8.0 ± 3.4	1.3 ± 0.6	4.5 ± 0.1	4.2 ± 3.4	0.7 ± 0.6	8.7 ± 0.2
		13	3.8 ± 0.1	10.5 ± 1.5	0.8 ± 0.1	8.6 ± 1.3	7.8 ± 1.5	0.6 ± 0.1	6.8 ± 0.1	3.7 ± 1.5	0.3 ± 0.1	14.3 ± 0.3
	PL	1	-	0.5 ± 0.2	0.5 ± 0.2	-	-3.6 ± 0.2	-3.6 ± 0.2	-	-9.8 ± 0.2	-9.8 ± 0.2	-
		6	12.0 ± 0.4	21.6 ± 2.3	3.6 ± 0.4	5.8 ± 0.7	17.4 ± 2.3	2.9 ± 0.4	4.2 ± 0.1	11.1 ± 2.3	1.9 ± 0.4	6.4 ± 0.2
		13	6.3 ± 0.2	31.5 ± 1.7	2.4 ± 0.1	4.9 ± 0.3	27.0 ± 1.7	2.1 ± 0.1	3.4 ± 0.1	20.2 ± 1.7	1.6 ± 0.1	4.6 ± 0.1

Table 6

Table 6. Linear models to estimate gross or net P_o mineralization rate (P_{min}) using respiration rate (Resp) and the $C_t:P_o$ ratio in the non-fractionated soil, in the coarse fraction and in the fine fraction ($C:P_{Total}$, $C:P_{CF}$ and $C:P_{FF}$, respectively) as regressors. Net P_o mineralization was calculated using two different conversion factors to account for incomplete recovery of microbial P during fumigation-extraction ($K_p = 0.4$ and $K_p = 1$).

Variables	Model	Significance level (p)	R^2
Resp	Gross $P_{min} = 0.26 + 0.24 \text{ Resp}^{\dagger}$	0.0015	0.48
Resp + $C:P_{Total}$	Gross $P_{min} = 0.82 + 0.24 \text{ Resp}^{\dagger} - 0.01 \text{ C:P}_{Total}$	0.0036	0.53
Resp + $C:P_{CF}$	Gross $P_{min} = 1.7 + 0.20 \text{ Resp}^{\dagger} - 0.0049 \text{ C:P}_{CF}^{\dagger}$	0.0001	0.72
Resp + $C:P_{FF}$	Gross $P_{min} = 0.65 + 0.24 \text{ Resp}^{\dagger} - 0.01 \text{ C:P}_{FF}$	0.0051	0.51
Resp	Net $P_{min} (K_p=0.4) = -0.10 + 0.19 \text{ Resp}^{\dagger}$	0.0060	0.39
Resp + $C:P_{Total}$	Net $P_{min} (K_p=0.4) = 0.24 + 0.19 \text{ Resp}^{\dagger} - 0.01 \text{ C:P}_{Total}$	0.0189	0.41
Resp + $C:P_{CF}$	Net $P_{min} (K_p=0.4) = 0.95 + 0.17 \text{ Resp}^{\dagger} - 0.0036 \text{ C:P}_{CF}^{\dagger}$	0.0025	0.55
Resp + $C:P_{FF}$	Net $P_{min} (K_p=0.4) = 0.07 + 0.19 \text{ Resp}^{\dagger} - 0.0031 \text{ C:P}_{FF}$	0.0239	0.39
Resp	Net $P_{min} (K_p=1) = 0.06 + 0.23 \text{ Resp}^{\dagger}$	0.0022	0.45
Resp + $C:P_{Total}$	Net $P_{min} (K_p=1) = 0.54 + 0.23 \text{ Resp}^{\dagger} - 0.01 \text{ C:P}_{Total}$	0.0058	0.50
Resp + $C:P_{CF}$	Net $P_{min} (K_p=1) = 1.36 + 0.21 \text{ Resp}^{\dagger} - 0.0044 \text{ C:P}_{CF}^{\dagger}$	0.0003	0.67
Resp + $C:P_{FF}$	Net $P_{min} (K_p=1) = 0.38 + 0.23 \text{ Resp}^{\dagger} - 0.01 \text{ C:P}_{FF}$	0.0079	0.48

[†]Indicates a significant contribution ($p < 0.05$) of the variable to the model.

Figure 1

Figure 2

Figure 3