

A Comparison of Four Methods of Measuring Gross Phosphorus Mineralization

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ABSTRACT

This study compared three previously published methods of estimating gross P mineralization: (i) an isotope dilution method that relies on specific activity, (ii) a later isotope dilution method that uses isotopically exchangeable P as the response variable, and (iii) a differential extraction method. We adapted the isotope dilution method (KB) commonly used for gross N mineralization for gross P mineralization. We evaluated two methods used to correct for adsorption of ^{32}P : sterilized soil incubations and a simulation model. Finally, we examined the necessity of including microbial biomass P as a component of labile P for the isotope dilution methods. The three previously published methods gave highly variable estimates of gross P mineralization, and our data suggest that critical assumptions of each method were violated. We recommend the KB method because its assumptions were generally met and it requires no sterilized samples. The KB method represents net mineralization because there is no correction for adsorption/desorption, which we have shown to be complex and difficult to interpret in nonsterilized samples. Modeled and estimated adsorption were often different, and relative differences varied among soil types. We also recommend combining the extractable inorganic P and microbial biomass P fractions into a single “labile” pool for isotopic-dilution studies and that the incubations are run over no more than 3 to 5 d. Although the KB method represents a conservative estimate of P mineralization as net P mineralization, it corresponds to a useful indicator in comparing potentially any soil type.

PHOSPHORUS is the primary limiting nutrient in most freshwater systems (Schindler, 1977; Chapin, 1998; Bedford et al., 1999) and in highly weathered terrestrial soils, such as in Oxisols in the tropics and Ultisols of the southeastern USA (Stevenson, 1986). Soil organic matter represents a large reservoir of potentially available P in natural and managed ecosystems, with up to 80% of total P found as organic P in some areas (Tiessen et al., 1994; Kellogg and Bridgham 2003). Despite the obvious importance of measuring gross P mineralization rates, the lack of comparisons among techniques and a critical evaluation of their assumptions has hampered acceptance of any standard method.

Studies that have attempted to determine gross P mineralization rates have used a number of different methods, none of which have been determined to be comparable or effective for multiple soil types (Tran et al., 1988; Walbridge, 1991; Zou et al., 1992; Di et al.,

1995; Frossard et al., 1996; López-Hernández et al., 1998; Oehl et al., 2001). Many are based on interpreting isotopic dilution of an introduced rare isotope (^{32}P or ^{33}P) in the soil solution to estimate gross P mineralization rates (e.g., Walbridge and Vitousek, 1987; Frossard et al., 1996; López-Hernández et al., 1998; Oehl et al., 2001, 2004). Kirkham and Bartholomew (1954) defined and Di et al. (2000) revised the major assumptions that must be met for the use of isotopes to be viable for determining gross P mineralization rates: (i) isotopic equilibrium, or the available pool and all exchangeable pools, have equivalent specific activities after a time of equilibration; (ii) the soil organic pool is the only pool not labeled by the isotope; (iii) all mineralized ions equilibrate in the soil solution before moving into other pools; and (iv) there is no discrimination between the rare and the more common form of the ion. Oehl et al. (2001) have suggested a fifth prerequisite from Shepard's work (1961): The pool sampled must be the pool in which the isotope was introduced. However, the dilution method has long been used in estimating gross nitrogen (N) mineralization rates, but although ^{15}N is usually added in water, the available pool is sampled with a mild extractant, such as 2 M KCl (Davidson et al., 1991). Whether or not this last stipulation is necessary remains questionable.

The purpose of this article is to compare the three published methods of estimating gross P mineralization rates: (i) an early isotope dilution method (Walbridge and Vitousek, 1987) (WV), (ii) an isotopically exchangeable P method (López-Hernández et al., 1998) (LH), and (iii) an extraction method (Zou et al., 1992) (Z). Other studies have used minor variations of these methods. Additionally, we adapted the Kirkham and Bartholomew (1954) (KB) method used for gross N mineralization for gross P mineralization, as suggested by Di et al. (2000). For two of the soil types used, we examined the difference between using a water extraction and a mild sodium bicarbonate extraction to examine the potential usefulness of each extract. Furthermore,

Abbreviations: AC, sodium azide plus autoclaving treatment incubated for 1 h in the Z method, correcting for lysing of microbial cells; E, isotopically exchangeable P; I, immobilization rate; KB, isotope-dilution method of Kirkham and Bartholomew adapted for gross P mineralization; LH, isotope-dilution method for gross P mineralization of López-Hernández et al.; M, mineralization rate; MA, sodium azide plus autoclaving treatment incubated for 24 h in the Z method, representing the solubilization of inorganic P; N , slope of $\ln(^{32}\text{P}_i/t^{32}\text{P}_a)$ versus $\ln t$ in the model of ^{32}P adsorption in the LH method; PM, sodium azide treatment in the Z method, representing solubilization of inorganic P and the mineralization of organic P by exoenzymes but no immobilization by the microbial community; SA, specific activity; slope, slope of \ln of SA of P_{i+m} pool versus t in WV method; t , time; WV, isotope dilution method for gross P mineralization method of Walbridge and Vitousek (1987); X , correction term for adsorption of $^{32}\text{P}_i$ in WV method; Z, differential extraction method for gross P mineralization of Zou et al.

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we tested the validity of using (i) a sterilized incubation and (ii) a simulation model to correct for adsorption. We also examined the utility of including microbial biomass P (P_m) in the labile P pool rather than just the extractable inorganic P (P_i) based on the findings of Walbridge and Vitousek (1987) that the P_i and P_m pools did not reach isotopic equilibrium. Equations from each method have been standardized for clarity and are outlined in the next section.

Four Models of Gross Phosphorus Mineralization

The WV method uses the change in specific activity of the combined extractable + microbial pool (P_{i+m}) to determine the mineralization rate. An incubation of non-sterile soils amended with ^{32}P is required, as well as an incubation of sterile soils amended with ^{32}P . Included in this model is a formula to correct for adsorption of $^{32}\text{P}_i$ using the nonsterile and sterile soil incubations:

$$X = {}^{32}\text{P}_{i+m,0} - [{}^{31}\text{P}_{i+m,0} (\text{SA}_{i+m,0} + \text{SA}_{i,f,s} - \text{SA}_{i,0,s})] \quad [1]$$

where X is the correction term, ${}^{32}\text{P}_{i+m,0}$ is the ${}^{32}\text{P}_{i+m}$ concentration in the initial time period in nonsterile samples, ${}^{31}\text{P}_{i+m,0}$ is the ${}^{31}\text{P}_{i+m}$ concentration in the initial time period in nonsterile samples, $\text{SA}_{i+m,0}$ is the specific activity of the P_{i+m} pool in the initial time period in nonsterile samples, $\text{SA}_{i,f,s}$ is the specific activity of the P_{i+m} pool in the final time period in sterile samples, and $\text{SA}_{i,0,s}$ is the specific activity of the P_{i+m} pool in the initial time period in sterile samples. The correction term is added back into the final activity of the P_{i+m} pool. After correction, the mineralization rate (M) is computed as:

$$M = \text{slope} \times ({}^{31}\text{P}_{i+m,0} - {}^{31}\text{P}_{i+m,f}) \quad [2]$$

where slope is the slope of the natural log of the specific activity of the P_{i+m} pool ($\text{SA}_{i+m,t}$) versus time, and ${}^{31}\text{P}_{i+m,f}$ is the final concentration of the ${}^{31}\text{P}_{i+m}$ pool.

The LH method uses isotopically exchangeable phosphorus as the available pool and estimates P mineralization as the change in that pool over time. This is also determined from an incubation of nonsterile soils amended with ^{32}P . To correct for adsorption of $^{32}\text{P}_i$, a short-term (100-min) batch experiment is performed under nonsterile conditions. The decrease in ^{32}P in soil solution over time (${}^{\text{mod}}{}^{32}\text{P}_{i,t}$) is modeled with the following equation:

$$\frac{{}^{\text{mod}}{}^{32}\text{P}_{i,t}}{{}^{32}\text{P}_{i,a}} = \left(\frac{{}^{32}\text{P}_{i,t=1}}{{}^{32}\text{P}_{i,a}} \right) \left[t + \left(\frac{{}^{32}\text{P}_{i,t=1}}{{}^{32}\text{P}_{i,a}} \right)^{1/N} \right]^{-N} + \frac{{}^{32}\text{P}_{i,\infty}}{{}^{32}\text{P}_{i,a}} \quad [3]$$

where ${}^{32}\text{P}_{i,a}$ is the amount of ${}^{32}\text{PO}_4^{-3}$ added, and ${}^{32}\text{P}_{i,t=1}$ and ${}^{32}\text{P}_{i,\infty}$ are the amounts of ${}^{32}\text{P}_i$ remaining in solution after 1 min and infinity, respectively. The ratio ${}^{32}\text{P}_{i,\infty}/{}^{32}\text{P}_{i,a}$ is the maximum possible dilution of ${}^{32}\text{P}_i$ and is estimated by the ratio of soil solution P_i to total soil P. The parameter N is estimated from the batch experiment using a linear regression of $\log({}^{32}\text{P}_{i,t=1}/{}^{32}\text{P}_{i,t})$ versus $\log(t)$. This equation for N is different from the published equation from Frossard et al. (1996), which

included a slight mathematical error. Frossard et al. (1996) derived N to be the slope for $\log({}^{32}\text{P}_{i,t}/{}^{32}\text{P}_{i,a})$ versus $\log(t)$. For our derivation of N , see the appendix.

Adsorbed isotopically exchangeable P (${}^{\text{mod}}E_t$) at time t is estimated as:

$${}^{\text{mod}}E_t = {}^{32}\text{P}_{i,t} \left(\frac{{}^{32}\text{P}_{i,a}}{{}^{\text{mod}}{}^{32}\text{P}_{i,t}} \right) \quad [4]$$

The assumptions in this adsorption correction are that no microbial uptake of ${}^{32}\text{P}_i$ occurs over the 100-min incubation and that adsorption over this period can be extrapolated out to days and weeks. Thus, we extrapolated this over the period of our original incubation of nonsterile soil (López-Hernández et al., 1998).

The gross mineralization rate of P is computed as the difference between measured isotopically exchangeable ${}^{32}\text{P}$ at time t [${}^{\text{mes}}E_t$] and ${}^{\text{mod}}E_t$ (Eq. [4]), where

$${}^{\text{mes}}E_t = {}^{31}\text{P}_{i,t} \left(\frac{{}^{32}\text{P}_{i,a}}{{}^{\text{mes}}{}^{32}\text{P}_{i,t}} \right) \quad [5]$$

We compared the modeled value with the sterile soil incubations. We also compared the simulation and the sterilized adsorption with an estimation of adsorption based on the recovery within the nonsterile soils using a mass balance approach. Within the nonsterile samples, we determined all ${}^{32}\text{P}$ pools with the exception of adsorbed ${}^{32}\text{P}$; therefore, any ${}^{32}\text{P}$ not recovered could be assumed to be adsorbed.

Kirkham and Bartholomew (1954) developed a number of formulas to be used for mineralization based on whether the immobilization rate (I) is higher than, equal to, or less than the mineralization rate (M). As in the previous two isotope models, the nonsterile soil incubations are used to determine the variables in the following formulas. We determined the equation to use by using a t test to see if the initial and final concentrations of ${}^{31}\text{P}_i$ were significantly different and, if they were, whether net mineralization or immobilization occurred. We used P_i and the combined P_i and P_m pools (P_{i+m}) as an estimate of available P, as in the WV method, to examine potential isotopic disequilibria between these two pools. The following are the formulas used:

$$I > M: M = \frac{{}^{31}\text{P}_{i+m,0} - {}^{31}\text{P}_{i+m,t}}{t} \times \frac{\ln(\text{SA}_{i+m,0}/\text{SA}_{i+m,t})}{\ln({}^{31}\text{P}_{i+m,0}/{}^{31}\text{P}_{i+m,t})} \quad [6]$$

$$I < M: M = \frac{{}^{31}\text{P}_{i+m,t} - {}^{31}\text{P}_{i+m,0}}{t} \times \frac{\ln(\text{SA}_{i+m,0}/\text{SA}_{i+m,t})}{\ln({}^{31}\text{P}_{i+m,t}/{}^{31}\text{P}_{i+m,0})} \quad [7]$$

$$I = M: M = \left(\frac{{}^{31}\text{P}_{i+m,0}}{t} \right) \times \ln \left(\frac{{}^{32}\text{P}_{i+m,0}}{{}^{32}\text{P}_{i+m,t}} \right) \quad [8]$$

The Z method uses a mass balance approach to estimate the gross P mineralization rate, with sterilization and autoclaving treatments to correct net estimations of P mineralization for adsorption and mineralization by phosphate exoenzymes present in soil. Anion-exchange

resins are used as a measure of P availability. The original method calls for γ radiation sterilization; however, we did not have access to a γ source, and therefore we used a 10% sodium azide solution. The first treatment receives a sodium azide addition (PM), representing solubilization of inorganic P and the mineralization of organic P by exoenzymes but no immobilization by the microbial community. The second treatment receives sodium azide and is also autoclaved (MA) to inactivate phosphatase exoenzymes and thus represents only the solubilization of inorganic P. The PM and MA treatments are incubated for 24 h. The addition of sodium azide and autoclaving soils cause a large flush of P_i from the lysing of microbial cells (Zou et al., 1992). To correct for this release, the sodium azide plus autoclaving treatment is applied to another subsample of the soil, but is only incubated for an hour (AC). Gross P mineralization (M) is then calculated as:

$$M = PM - (MA - AC) \quad [9]$$

MATERIALS AND METHODS

Soils

We used four soils (Table 1) that represent a range of physical properties and expected P mineralization rates: an Ultisol (Aquic Paleustults) and Entisol (Quartzipsamments) from Venezuela, a Histosol (Houghton muck) from northern Indiana, USA, and an Alfisol (Aquic Arenic Hapludalf) from southern Michigan, USA. We chose these soils to represent a range of adsorption rates because previously published methods have specified that isotopic dilution is restricted in utility to less adsorptive soils. However, for a method to be used as a standard method, it should be able to overcome these difficulties. The Venezuelan soils were collected, air-dried, sieved to pass through a 2-mm diameter mesh, and shipped via overnight express to the University of Notre Dame (South Bend, IN). Fifteen cores (10-cm depth) were taken from each site, composited, and roots and large debris were removed by hand. We did not collect the soil samples from Venezuela and relied instead on them being collected and sent by students in the López-Hernández lab. Because of this, it would have been difficult to obtain fresh soils when shipping time and USDA regulations were factored in; therefore, the tropical soils arrived air-dried. Because we were comparing the four methods within a soil type and not the soil types themselves, differences between soil-preservation techniques are not a problem.

Isotope Dilution

To obtain the variables for each of the models based on isotope dilution, we used nonsterile soils amended with ^{32}P .

Table 1. Soil properties of the four soil types. P, N, and C are based on total dry mass of soil. Soil texture is based on ashed dry mass of soil. Analyses were done for composited samples with <1% difference of replicate samples.

Soil type	pH	Soil texture			Total P $\mu\text{g g}^{-1}$ soil	Total N mg g^{-1} soil	Total C
		Sand	Clay	Silt			
Alfisol	7.1	60	20	20	223.1	3.19	44.97
Histosol	6.9	—	—	—	231.2	4.36	110.0
Entisol	5.4	90	5	5	59.8	0.21	7.86
Ultisol	5.4	50	20	30	93.0	0.32	18.54

However, gross P mineralization is difficult to ascertain due to geochemical P_i interactions. Therefore, we tested the possibility of using a sterile soil control (i.e., without microbial mineralization or immobilization of P) to correct for adsorption in determining gross P mineralization rates. Approximately 100 g (dry wt) of each soil type was weighed into two sets of Mason jars (nonsterile and sterile incubation), with three replicates of each soil type-treatment combination. The previously air-dried soils were brought to 80% field capacity, and the field-moist soils were kept at their original moisture levels. Jars were covered with lids containing septa holes, which were plugged with glass wool to allow air exchange but reduce moisture loss. For the sterile soils, we autoclaved them (120°C and 2.12 MPa for 45 min) and then added a 10% sodium azide solution. The autoclaving provided an initial sterilization that also denatured phosphatase enzymes within the soil matrix, and the addition of sodium azide discouraged recolonization of the soil by bacteria. Aseptic techniques were used with the sterile samples over the course of the incubation. Nonsterile and sterile soils were kept at 15°C and incubated in the dark.

We labeled each sterile and nonsterile soil with 5.0 kBq g^{-1} soil of $\text{KH}_2^{32}\text{PO}_4$. At 4 h (0 d), 1 d, 4 d, 8 d, and 15 d, the nonsterile and sterile soils were extracted for P_i and the nonsterile soils for P_m . Previous isotope-dilution studies have used soil solution (Oehl et al., 2001), soil extracts (Walbridge and Vitousek, 1987), or anion-exchange resins (Frossard et al., 1996; López-Hernández et al., 1998) to estimate available P_i . To maintain a consistent comparison among models, we determined P_i by extracting 2-g dry-wt soil with 40 mL of 0.5 M sodium bicarbonate (Kuo, 1996). For the two tropical soils, we also determine P_i by extracting 4-g dry-wt soil with 40 mL of distilled water to ascertain the possibility of using water extracts to compute gross P mineralization.

Microbial biomass P was determined as the increase in P_i after chloroform fumigation of subsamples (Walbridge 1991). The subsample of soil (2-g dry-wt) was placed in a 50-mL centrifuge tube, and 10 μL of chloroform was added. The tubes were capped and incubated for 24 h in the dark before bicarbonate extraction. At 1, 8, and 15 d, to verify that our sterile soils were inactive, we determined microbial biomass P and CO_2 respiration for the sterile soils. The recovery of P_m from chloroform-fumigated soils is an underestimate because of (i) incomplete lysis of P_m to P_i , which is often corrected for with a standard efficiency factor (Brookes et al., 1984), and (ii) differential recovery of the lysed P_m , which may vary substantially among different soil types. We use the standard correction in this article ($k_p = 0.4$). However, if one makes the reasonable assumption that this correction is constant within a soil type, then relative dynamics of $^{32}P_m$ and $^{31}P_m$ over time within a soil type should be qualitatively correct. Estimates of gross P mineralization in the WV and KB methods are under- or overestimated to the extent that the $^{32}P_{i+m}$ and $^{31}P_{i+m}$ pools and their SA are under- or overestimated (Eq. [2], [6], [7], [8]). There was no measurable microbial biomass P or CO_2 respiration in sterile samples (data not shown).

An estimate of adsorbed ^{32}P in the soils was determined in the soils through a mass-balance approach to compare to the estimations from the modeled method. To do this, any ^{32}P not recovered in the P_i or P_{i+m} pool was considered "adsorbed."

Before determining ^{32}P content of each pool, we used a molybdate-butanol separation (Jayachandran et al., 1992) to separate the inorganic ^{32}P from any possible organic ^{32}P . Although dissolved organic P may be a significant pool of soil solution P, because of attempts to replicate each method and the assumption that organic P is not labeled, we did not analyze the dissolved organic P fraction for ^{32}P . We counted the inorganic ^{32}P with a Beckman scintillation counter using

Cerenkov counting. The ^{31}P concentrations in each pool were determined using the Murphy-Riley colorimetric method (Kuo, 1996). Finally, we calculated gross mineralization rates of P based on the equations presented in the introduction.

Simulation of Adsorption

The simulation model was parameterized with a subsample from each soil type. This took place within days of starting the above incubations. Soil (10 g) was shaken for 24 h with 99 mL of deionized water. After equilibration, 1 mL of carrier-free $\text{KH}_2^{32}\text{PO}_4$ (150 MBq) was added to each of the soil slurries and shaken for 100 min. At five time periods over 100 min, water was removed from each sample using a syringe with a Millipore filter (0.2 μm). We analyzed the water sample for $^{32}\text{P}_i$ activity and $^{31}\text{P}_i$ concentrations as previously and calculated adsorption with Eq. [4].

Nonisotopic Method

Using two of the four soil types (Alfisol and Histosol), we also tested a nonisotopic method for gross P mineralization (Zou et al., 1992). These incubations took place within days of beginning the isotope-amended incubations. We weighed out six sets of three 2.5-g dry-wt equivalent samples into 250-mL flasks for a total of 18 flasks for each soil type ($n = 3$). Treatments were (i) control (no addition or autoclaving), (ii) sodium azide addition, and (iii) sodium azide + autoclaving. Two anion-exchange resin bags (0.75 g of Dowex 1-X8 Cl^- resin in nylon mesh bags) were added to the soil slurries after treatment.

Three of the six sets of samples from each soil type were used to correct for the initial flush of P_i from lysing of microbial cells from the treatments. These correction soils were shaken for 1 h, after which the resin bags were removed. The other three sets of each soil were shaken for 24 h before removal of the resin bags. All resin bags were rinsed thoroughly with deionized water and allowed to air dry to dampness before extraction. The bags were put in 50-mL centrifuge tubes with 30 mL of 0.5 M HCl to extract for P. We analyzed the extractions as described previously. We calculated the gross mineralization rate using Eq. [9].

Statistics

Within each soil type, we used a repeated measures analysis of variance to test for interactions between the method used and time of sampling if the methods differed. If there were no interactions, pairwise comparisons were determined with a Tukey post-hoc test.

RESULTS AND DISCUSSION

Change in Specific Activity over Time

We begin by examining the specific activities of various P fractions to determine if the first assumption of isotope-dilution theory holds: Do the available pool and all exchangeable pools have equivalent specific activities after equilibration (i.e., is the system in isotopic equilibrium)? The absolute magnitude and temporal dynamics of SA differed in P_i extracted with water or bicarbonate (SA_i , Fig. 1a, b) and in microbial biomass (SA_m , Fig. 1c). Isotopic dilution theory predicts that because all pools equilibrate with the soil solution pool, the only pool directly altered is the soil solution pool; mineralization of

the unlabeled organic pool causes the soil solution pool to be “diluted” by the inputs of unlabeled P. This would be seen as a decrease in specific activity of the soil solution pool as specific activity is the ratio of labeled P (^{32}P) to unlabeled P (^{31}P). In all soil types, the bicarbonate SA_i significantly decreased over time as would be predicted from isotopic dilution theory (Fig. 1a; $p < 0.05$). However, the water SA_i did not act as would be predicted by theory. There was no change in this pool for the Entisol, whereas there was a sharp decline between 4 h and Day 1 for the Ultisol ($p < 0.001$, Fig. 1b). There were no clear trends for SA_m . The Entisol SA_m did not significantly differ over time, whereas the Alfisol SA_m increased between Day 4 and 8 (Fig. 1c). In the Histosol, SA_m was not significantly different between 4 h and Day 1 but sharply declined between Day 1 and Day 4 ($p = 0.002$) and remained relatively constant through Day 15 (Fig. 1c). Finally, SA_m in the Ultisol increased from 4 h

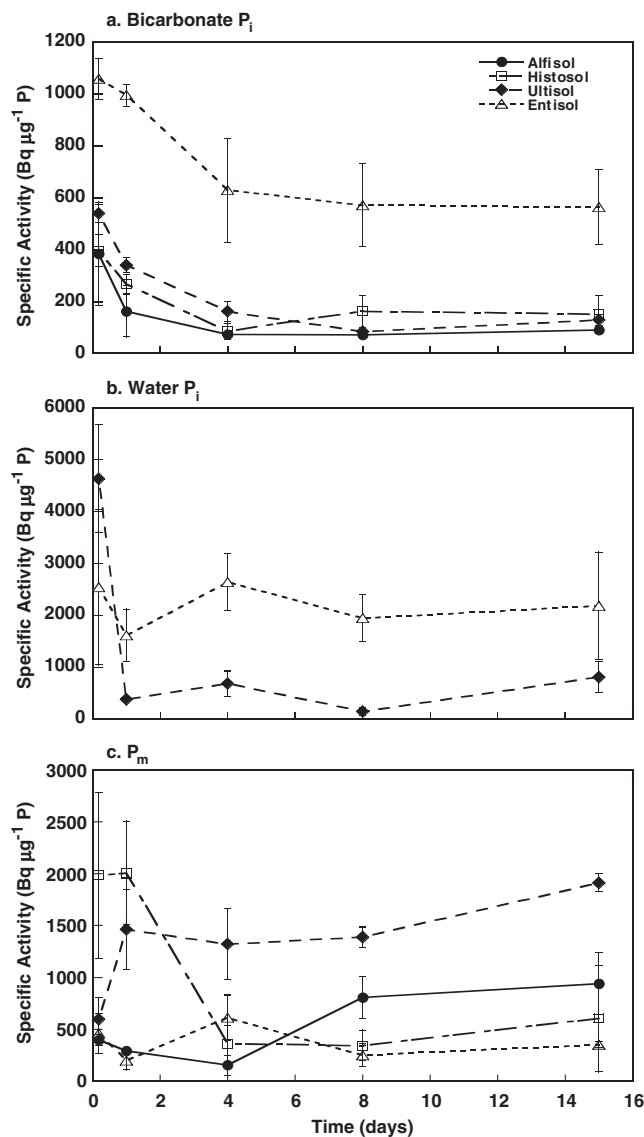


Fig. 1. Specific activity for (a) bicarbonate-extracted P_i , (b) water-extracted P_i , and (c) P_m pool for all four soil types. Values are mean \pm SD.

to Day 1 ($p = 0.02$) and increased between Day 8 and Day 15 (Fig. 1c; $p = 0.045$).

Our results indicate that the SA of the P_m pool does not equilibrate with the SA of the P_i pool even after 15 d. Therefore, the first assumption of isotopic equilibrium was violated if the labile P pool is only operationally defined as “soil solution” P (see Four Methods of Gross P Mineralization). Walbridge and Vitousek (1987) also found that the turnover of bacterial biomass with high SA caused an increase in soil solution SA leading to lower estimates of isotope dilution in the soil solution. Based on our results and the findings from previous work, we concur with Walbridge and Vitousek (1987) that mineralization studies should combine the P_i and P_m pools into a single “labile” P pool that undergoes isotopic dilution. However, because others have not done this, for the purpose of illustrating the difference between the two methods we calculated P mineraliza-

tion rates for P_i and the combined the P_i and P_m pools as “labile” P below.

Correction for Adsorption

Both $^{31}P_m$ and $^{32}P_m$ were dynamic through time, and turnover of P_m generally did not result in an increase in the P_i pool (Fig. 2, 3). Thus the third assumption of isotope dilution theory, namely that all mineralized ions equilibrate in the soil solution before moving into other pools (see Four Methods of Gross P Mineralization), seems to have been violated. It is likely that ^{32}P released from the microbial pool was adsorbed without equilibrating with the soil solution and, similarly, that mineralized P is being taken up directly by microorganisms without equilibrating with the soil solution. Therefore, adsorption needs to be corrected for to compute gross P mineralization accurately, whereas microbial biomass

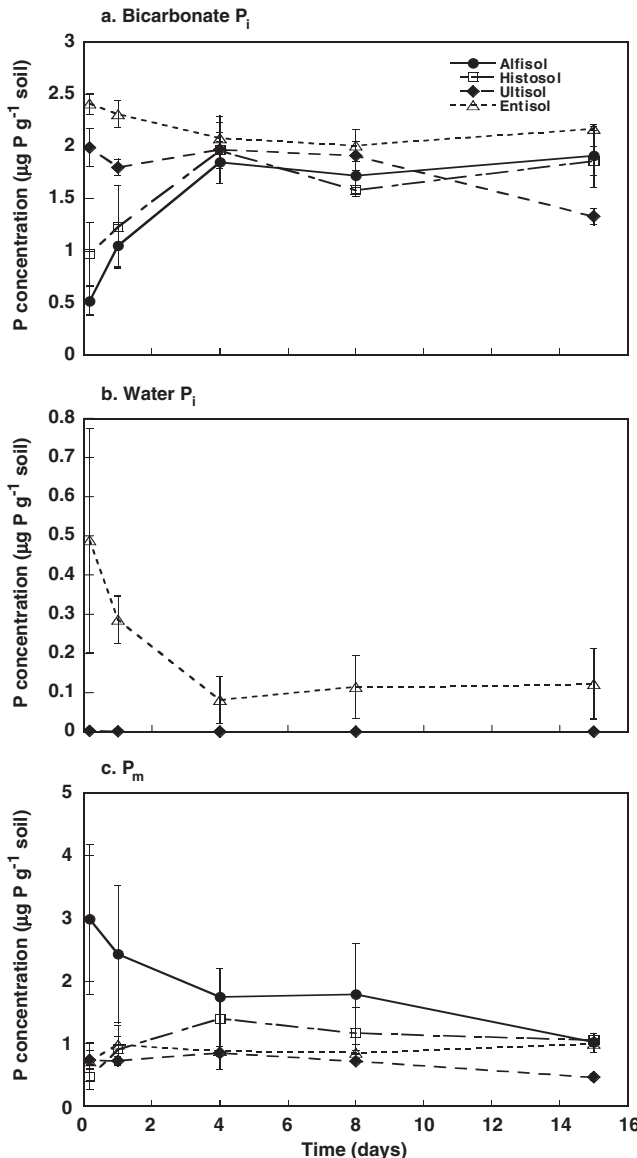


Fig. 2. Concentrations for (a) bicarbonate- and (b) water-extracted P_i and (c) microbial P (P_m). Values are mean \pm SD.

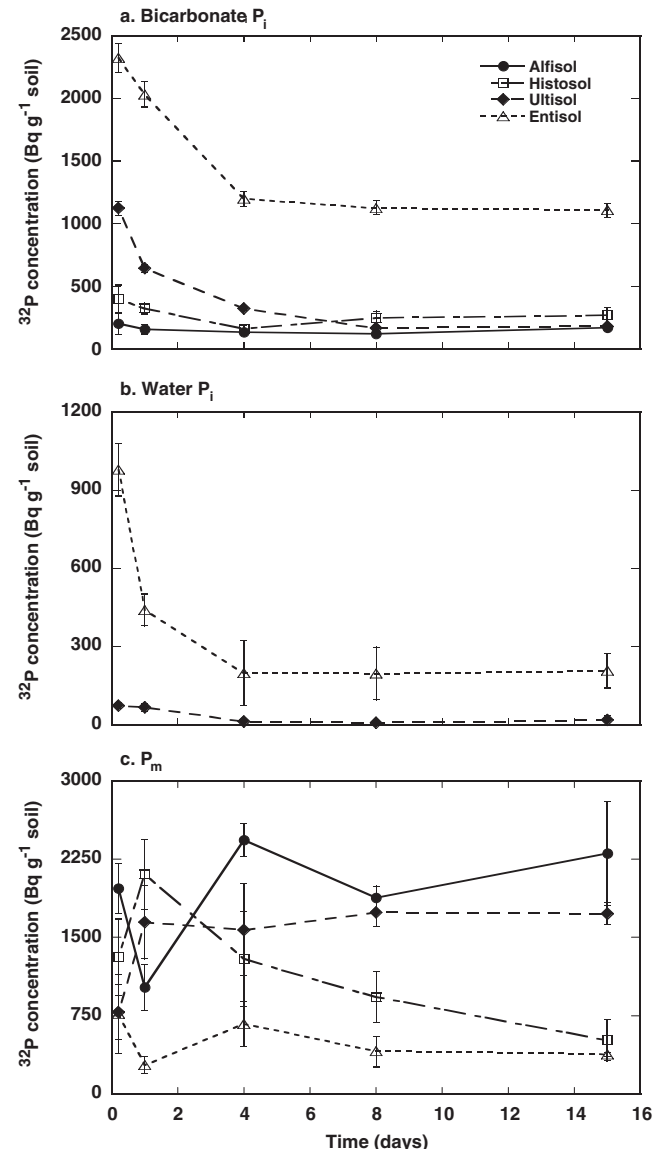


Fig. 3. Concentrations of ^{32}P for (a) bicarbonate- and (b) water-extracted P_i and (c) microbial (P_m). Values are mean \pm SD.

should be included in the estimation of “labile” P. We looked at two methods that attempt to correct for adsorption, WV and LH.

The WV method corrects for adsorption using sterilized soils according to Eq. [1]. When we applied this formula to our four soil types, the correction amount (data not shown) varied and at times was very large or a negative number. It is unclear as to why we did not obtain a correction factor that worked with our data; however, the correction formula is not viable for all soil types. The calculated gross mineralization rates using WV presented in this article includes the correction factor, but because of its high variability even within a soil type, we do not recommend its use.

We compared sorption obtained with sterilized soils to that calculated with the adsorption model using $E(t)$ (Eq. [4]). In most cases, adsorption in the model and in sterilized incubations behaved differently (Fig. 4). In the Entisol, adsorption calculated using water extractions from the sterile incubations remained less than the model ($p < 0.03$), except at 4 h (no difference). Adsorption in the bicarbonate extraction from the sterile incubation was much lower than the other two estimates of adsorption (Fig. 4; $p < 0.05$), except for Day 8, when water and bicarbonate extractions were similar. In the Ultisol, adsorption calculated from the water extractions from the sterile soil was orders of magnitude greater than bicarbonate extractions ($p < 0.001$), with the exception of 4 h, when all three adsorption calculations were similar and Day 1 when the water and bicarbonate extractions in the sterile incubations were similar. Adsorption calculated from the modeled and water extractions from the sterile incubations were similar across time with the exception of Day 1, when the water-extracted sterile incubation decreased. Adsorption calculated from the bicarbonate extractions, similar to the other soil types, rose to a plateau by Day 4 and remained stable through Day 15. In the Histosol, adsorption measured in the model and sterilized incubations were similar, although this could be due to high variation in the sterilized incubations. The discrepancies among the different methods of calculating adsorption rates demonstrate the difficulties behind assessing adsorption.

Two major assumptions of calculating adsorption of P from the simulation model are that (i) microbial uptake of P during the 100 min incubation is negligible, and (ii) adsorption during 100 min can be extrapolated over days to weeks. The model makes no attempt to correct for microbial uptake of P, even though there exists an abundance of evidence in this study (see Fig. 3) and others that microbial uptake is often rapid and a large percentage of the added ^{32}P (Richardson and Marshall, 1986; Walbridge and Vitousek, 1987). Because of microbial uptake, the model is a poor estimation of adsorption in many soil types, as seen when compared with the sterile incubations in this study. Additionally, different adsorption sites are accessed by P over time (Froelich, 1988), making extrapolation of adsorption from 100 min to days or weeks inappropriate. Oehl et al. (2001) repeated a 1 min batch experiment at several time points over 28-d incubations for a number of soils and found the adsorp-

tion model's parameters to be relatively constant over time, suggesting that this validated its use for soils of low to medium adsorption capacity. However, we suggest that their results demonstrate that very short-term adsorption dynamics remain constant when repeated over time, not that adsorption dynamics over minutes can be extrapolated over order-of-magnitude longer time periods.

Finally, we wanted to test the ability of a sterilized-soil incubation to mimic potential adsorption dynamics in nonsterilized soils, which is an inherent assumption of the WV and Z methods. To do this, we assumed that any ^{32}P not recovered in the P_i (or P_{i+m} within the nonsterile soil) pool was adsorbed and compared the fraction of the added ^{32}P found in the adsorbed pool in sterile and nonsterile incubations. In the Entisol, adsorbed ^{32}P in the water-extracted sterile soil was higher than the bi-

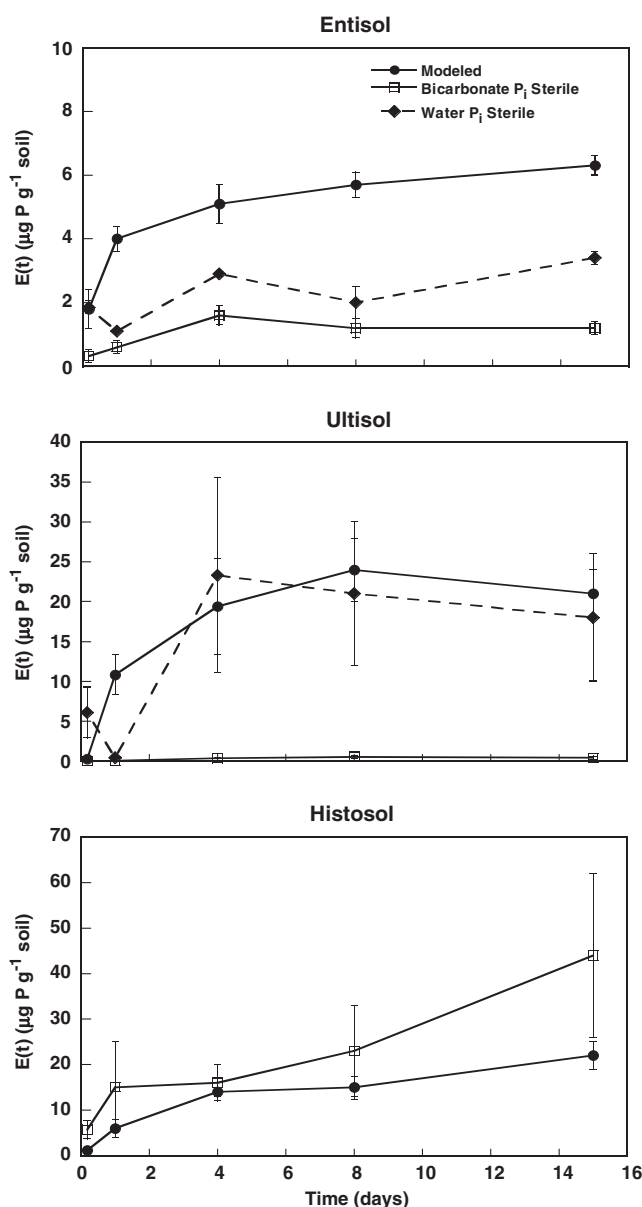


Fig. 4. $E(t)$ for the sterilized incubations and the simulation model for three of the four soil types: (a) Histosol, (b) Entisol, and (c) Ultisol. Values are mean \pm SD.

carbonate-extracted sterile and nonsterile soils ($p < 0.01$; Fig. 5). The bicarbonate-extracted sterile soils and the nonsterile soils were different only at Days 1 and 4 ($p < 0.04$; Fig. 5). In the Alfisol, adsorbed ^{32}P was higher in sterile than in the nonsterile soil after Day 1 ($p < 0.001$). In the Histosol, adsorption was higher in the sterile than the nonsterile soil at Days 1 and 8 ($p < 0.04$; Fig. 5). In the Ultisol, adsorbed ^{32}P was higher with water extractions than bicarbonate extractions, and the sterile water-extracted adsorption was higher than in the nonsterile soil ($p < 0.04$), with the exception of Day 1. Adsorption in the bicarbonate extractions was generally highest in the sterile incubation ($p < 0.04$; Fig. 5).

A major assumption with the use of a sterilized sample to correct for adsorption is that microbial activity does not affect the rate of ^{32}P adsorption. However, because of microbial competition for P in nonsterile soils, we often observed that adsorption of ^{32}P was lower in control than in sterilized soils. He and Zhu (1998) dem-

onstrate microbial uptake of ^{32}P associated with minerals such as kalonite and aluminum oxide. This shows the potential of microorganisms to access P that is not considered readily available. Therefore, using sterile soils as a correction for adsorption leads to underestimates of gross P mineralization.

Computation of Mineralization Rates

We computed gross P mineralization rates using the four methods previously described (Table 2). The water extraction generally gave much lower values than the bicarbonate extraction ($p < 0.02$; Table 2). The extreme case was the Entisol for the LH method, where use of a water extraction to represent the labile P pool resulted in negative mineralization rates. Oehl et al. (2001) argue that the pool in which isotope dilution is measured must be the same pool to which the ^{32}P is added, but use of a water-extractable pool in our soils often gave nonsen-

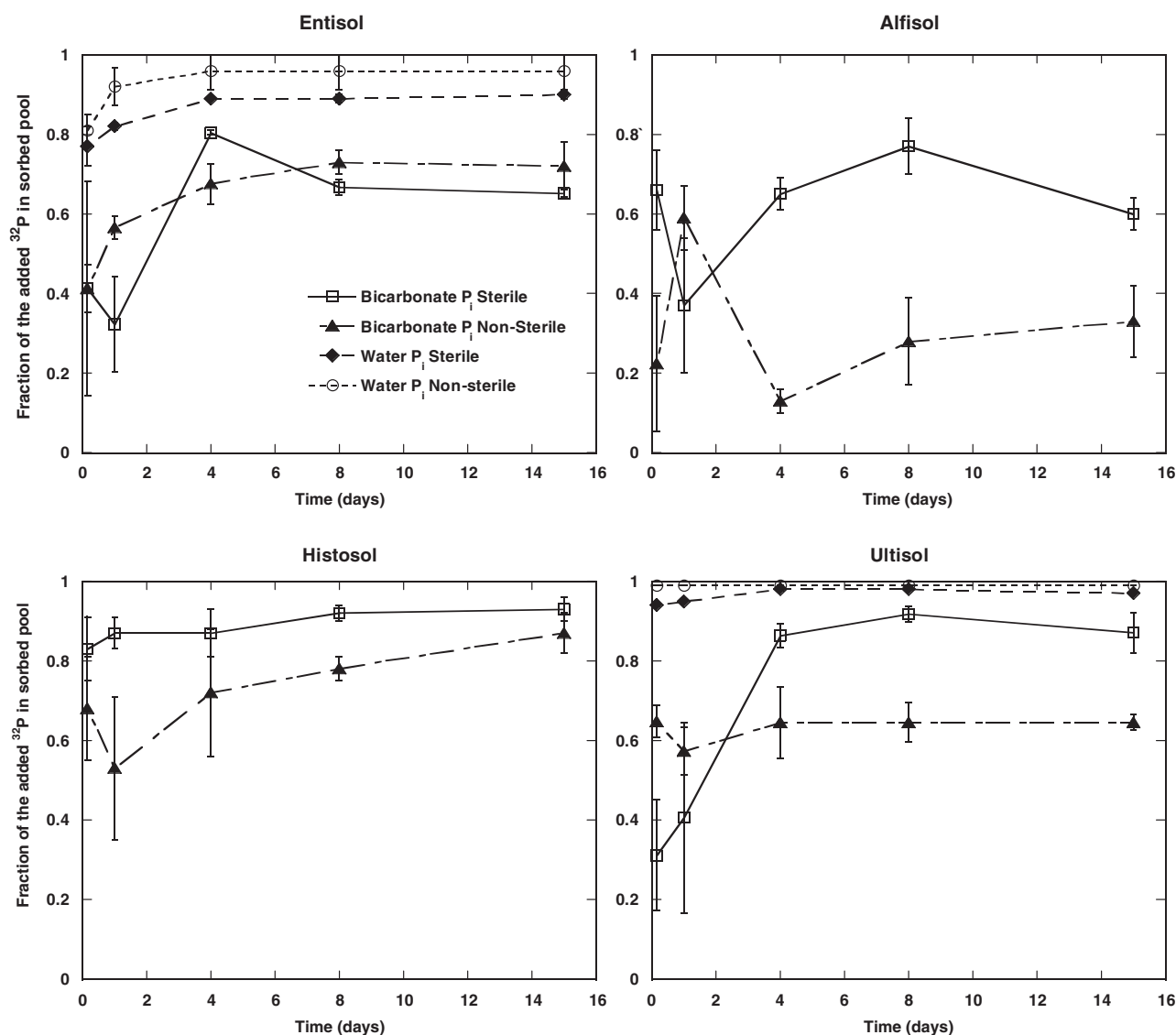


Fig. 5. Fraction of added ^{32}P found in the adsorbed pool in the sterile versus the nonsterile soil incubations with bicarbonate or water extractants in the four soil types: (a) Alfisol, (b) Histosol, (c) Entisol, and (d) Ultisol. Values are mean \pm SD.

Table 2. P mineralization rates (P $\mu\text{g g}^{-1}$ soil d^{-1}) in four different soils at 1, 4, and 15 d after the addition of the radiotracer.

P mineralization	Alfisol			Histosol			Entisol			Ultisol		
	1 d	4 d	15 d	1 d	4 d	15 d	1 d	4 d	15 d	1 d	4 d	15 d
Kirkham and Bartholomew (1954)												
P_i	0.710 (0.47)‡	0.399 (0.07)	0.105 (0.03)	0.455 (0.17)	0.574 (0.39)	0.043 (0.02)	0.223 (0.06)	0.288 (0.09)	0.090 (0.02)	0.871 (0.09)	0.616 (0.09)	0.157 (0.01)
P_{i+m}	2.918 (0.84)	0.525 (0.09)	0.161 (0.06)	0.634 (0.40)	0.762 (0.03)	0.359 (0.20)	0.645 (0.30)	0.329 (0.03)	0.085 (0.006)	0.926 (0.42)	0.111 (0.08)	0.068 (0.02)
P_w	nd§	nd	nd	nd	nd	nd	0.248 (0.07)	0.026 (0.02)	0.009 (0.003)	0.031 (0.006)	0.006 (0.001)	0.003 (0.001)
López-Hernández et al. (1998)												
P_i	nd	nd	nd	14.86 (1.31)	13.67 (4.6)	1.289 (1.01)	0.833 (0.44)	0.336 (0.12)	0.168 (0.11)	2.915 (0.97)	4.181 (0.51)	1.001 (0.20)
P_{i+m}	nd	nd	nd	2.863 (1.59)	23.75 (9.90)	14.36 (3.35)	1.127 (0.53)	0.568 (0.440)	0.223 (0.125)	10.07 (3.14)	5.12 (1.95)	1.82 (0.34)
P_w	nd	nd	nd	nd	nd	nd	17.842 (3.42)	12.101 (2.34)	1.579 (1.52)	3.741 (1.99)	1.817 (1.13)	0.889 (0.53)
Walbridge and Vitousek (corrected) (1986)												
P_i	0.238 (0.03)	0.091 (0.04)	0.009 (0.003)	0.191 (0.07)	0.067 (0.05)	0.002 (0.001)	0.067 (0.02)	0.017 (0.006)	0.004 (0.002)	0.098 (0.03)	0.006 (0.002)	0.016 (0.009)
P_{i+m}	0.429 (0.15)	0.044 (0.01)	0.026 (0.01)	0.355 (0.13)	0.036 (0.008)	0.028 (0.019)	0.209 (0.13)	0.028 (0.007)	0.004 (0.003)	0.135 (0.07)	0.025 (0.02)	0.023 (0.01)
P_w	nd	nd	nd	nd	nd	nd	0.009 (0.009)	0.002 (0.001)	0.001 (<0.001)	0.061 (0.01)	0.015 (0.003)	0.002 (0.001)
Walbridge and Vitousek (uncorrected) (1987)												
P_i	0.055 (0.02)	0.030 (0.004)	0.009 (0.001)	0.024 (0.006)	0.012 (0.003)	0.003 (0.001)	0.006 (0.002)	0.001 (0.001)	0.0004 (0.0002)	0.031 (0.006)	0.006 (0.002)	0.005 (0.001)
P_{i+m}	0.038 (0.01)	0.004 (0.001)	0.002 (0.001)	0.081 (0.02)	0.021 (0.01)	0.003 (0.002)	0.019 (0.01)	0.002 (0.001)	0.001 (<0.001)	0.039 (0.003)	0.007 (0.004)	0.006 (0.001)
P_w	5.95 (1.7)			13.8 (5.2)			0.002 (0.001)	0.001 (0.0002)	0.0003 (0.0001)	0.013 (0.003)	0.0002 (0.0001)	0.0003 (0.0002)
Zou et al. (1992)†							nd	nd	nd	nd	nd	nd

† The Zou method was for 24 h only.

‡ Numbers in parentheses are standard errors.

§ nd = not done.

sical P mineralization rates, probably because of low P_i concentrations (Fig. 2) and rapid adsorption of ^{32}P (Fig. 5) in this pool. P_i in soil solution is strongly buffered by and undergoes rapid equilibration with easily adsorbed P_i , which is why weak extractants such as bicarbonate have routinely been used to quantify the labile P_i pool. Moreover, a water extractant is not equivalent to soil solution in terms of osmotic chemistry or quantity. Last, the gross N mineralization method has long used a salt solution to measure the dilution of the added ^{15}N for reasons to those described previously (Davidson et al., 1991). Thus, our results and the results of others (Davidson et al., 1991) indicate that an appropriate extractant, such as bicarbonate, must be used to quantify isotopic dilution of ^{32}P in any P mineralization method.

When we calculated P mineralization rates using P_i or P_{i+m} as the labile pool, we found little agreement within a soil type and method. The P mineralization rate was generally greater with the inclusion of the P_m pool in the calculations than when using only P_i ($p < 0.03$; Table 2). Most importantly, we have shown that the SA of the P_i and P_m pools does not equilibrate (Fig. 1); therefore, the two pools should be combined into a common (P_{i+m}) labile pool to estimate isotopic dilution.

In comparing the four methods, P mineralization rates from the Z method (Zou et al., 1992) were orders of magnitude greater than the other three. This method depends on the difficult-to-evaluate assumption that the various treatments act specifically on solubilization, microbial uptake, and mineralization by exoenzymes. At the very least, we have shown that the use of sterilized soils to estimate sorption dynamics should be interpreted guardedly (Fig. 5). Among the methods using isotopic dilution, rates calculated from the KB method were consistently higher than those determined from the WV method. This difference is likely due to the correction for adsorption in the WV method that is lacking in the KB method and problems with units in the WV method (see below). The mineralization rate computed from the LH method in relation to the KB and WV method depended on the time sampled because of the variability of the LH method. The LH method also gave negative results in some soil/extractant combinations.

Although the WV model is conceptually similar to the KB method, when analyzing the original model used for these calculations, we discovered a problem with the final units. Phosphorus mineralization rates are normally presented in $\mu\text{g P g}^{-1}$ soil d^{-1} . Walbridge and Vitousek (1987) adapted a litter P mineralization model of Correll and Miklas (1975) to estimate gross P mineralization in soils. In this model, P mineralization rates are calculated based on the change of specific activity over time, which gives units of Bq g^{-1} soil d^{-1} . We could duplicate the final results in Walbridge and Vitousek (1987) if the units are Bq g^{-1} soil d^{-1} but not if units are in $\mu\text{g P g}^{-1}$ soil d^{-1} . For the purposes of this article, we have computed the rates exactly as noted in Walbridge and Vitousek (1987).

Equation [5] for the LH method is similar to the KB method when immobilization equals mineralization (Eq. [8]). The differences between the LH method and $I = M$

case for the KB method are (i) the use of a modeled $^{32}\text{P}_i$ adsorption term in the LH method but not the KB method, (ii) the log term in Eq. [8] but not in Eq. [5], (iii) the time at which an initial $^{32}\text{P}_i$ or $^{32}\text{P}_{i+m}$ value is taken, and (iv) the typical length of the incubations.

Although regularly used to determine gross N mineralization (e.g., Davidson et al., 1991), the equations in the KB method were originally presented in a generalized form appropriate for mineralization of any element (Kirkham and Bartholomew, 1954; see also Di et al., 2000). These equations are derived from the parameterization of a simple model, and the log terms in each equation come from the integration of the appropriate mass balance equations. In contrast, the LH model is empirically based; thus, the log term in Eq. [8] is more theoretically justified.

Our results indicate that the time at which the initial value for $^{32}\text{P}_i$ or $^{32}\text{P}_{i+m}$ is taken is important. The LH method uses the amount of $^{32}\text{P}_i$ added ($^{32}\text{P}_{i,a}$) as the initial value, whereas the KB method for gross N mineralization typically includes a short equilibration period of a few minutes to 1 d (Davidson et al., 1991; Di et al., 2000) before taking the initial measurement of the ^{15}N atom % enrichment (the equivalent of SA for a stable isotope) of the available pool. We recovered 33 to 76% of ^{32}P in nonsterile samples after 4 h, with typically much smaller changes in recovery afterward (data not shown). Thus, it seems important to provide an initial equilibration period before measuring $^{32}\text{P}_{i,0}$ or $^{32}\text{P}_{i+m,0}$. Oehl et al. (2001) attempted to overcome problems with equilibration by incubating the soils until respiration had stabilized, taking up to 30 d or more. However, because these are incubations and, therefore, closed systems, this stabilization is the result of exhausting the labile carbon pool, which is associated with a decrease in nutrient mineralization rates (Nicolardot et al., 1994; Bridgman et al., 1998; McLauchlan and Hobbie 2004). The standard Stanford and Smith (1972) incubations to determine labile nutrient pools are based on this phenomenon. Therefore, pre-incubating soils for such long periods of time gives artificially depressed nutrient mineralization rates.

The LH method typically has incorporated long incubation times (weeks to months). We incubated our soils to 15 d because of the probability of organic matter contamination with ^{32}P over long incubations, as seen by others with ^{15}N (Bjarnason, 1988). Although one would expect P mineralization to decrease over time due to exhaustion of the labile organic pool, the plateau often seen in cumulative $_{\text{mes}}E_t$ (e.g., López-Hernández et al., 1998) may be an artifact of ^{32}P being remineralized. Therefore, to compute a mineralization rate using ^{32}P , a time period that is least likely to result in mineralization of ^{32}P labeled organic matter is an important consideration. Also, short incubation times are more likely to give mineralization rates that are more representative of in situ conditions.

CONCLUSIONS

Because of the inherent problems in the WV, LH, and Z methods discussed previously, we recommend the use of the KB method. The KB method has a strong theo-

retical basis for its equations based on the appropriate mass balance equations, whereas the equations in the other methods are largely empirical in nature. Di et al. (2000) suggested that the KB method could be used for gross P mineralization, but they did not test it or its assumptions against other previously published methods.

Our results show that adsorption is difficult to correct for in computing P mineralization rates. The WV and Z methods require sterilized samples, which we have shown to give varying results in estimating adsorption for nonsterile soils when compared with mass balance estimates of adsorption in the nonsterile soil. Although the LH method does not use sterile soils to estimate adsorption, it assumes that adsorption over 100 min can be extrapolated over much longer time periods and that microbial uptake of ^{32}P is insignificant over the 100-min incubation. Our results indicate that both assumptions are violated.

Because of the erratic uptake and release of P by the microbial community, the isotopic disequilibria of the P_i and P_m pools, and the apparent lack of equilibration of mineralized P_m with the P_i pool, we recommend, as did Walbridge and Vitousek (1987), combining the P_m and the P_i pools to determine isotopic dilution. Further, in disagreement with Oehl et al. (2001), we recommend the use of a mild extractant, such as sodium bicarbonate, for sample extraction, as is done with the isotopic dilution method to measure gross N mineralization (Davidson et al., 1991).

Using the KB method with no sterilization and a mild extractant represents a conservative estimate of P mineralization that can be used in potentially any soil type. The rapid decrease in recovery of $^{32}\text{P}_{i+m}$ over several hours suggests that an equilibration period of several hours to 1 d be used before determining the initial SA. Furthermore, this method could easily be developed to include intact cores as discussed in Di et al. (2000) to give in situ rates. Finally, because we recommend combining the inorganic and microbial pool, the estimation for the microbial pool requires higher precision than we have demonstrated here. An appropriate estimation requires that estimations of a k_p value be determined for each soil type as well as the recovery efficiency of ^{32}P from chloroform fumigated soils.

APPENDIX

Derivation of N

To solve for N , we begin with the original equation:

$$\frac{^{32}\text{P}_{i,t}}{^{32}\text{P}_{i,a}} = \left(\frac{^{32}\text{P}_{i,1}}{^{32}\text{P}_{i,a}} \right) \left[t + \left(\frac{^{32}\text{P}_{i,1}}{^{32}\text{P}_{i,a}} \right)^{1/N} \right]^{-N} + \frac{^{32}\text{P}_{i,\infty}}{^{32}\text{P}_{i,a}}$$

As pointed out by Frossard et al. (1996), $\frac{^{32}\text{P}_{i,\infty}}{^{32}\text{P}_{i,a}}$ approaches zero and therefore is negligible compared with $\left(\frac{^{32}\text{P}_{i,1}}{^{32}\text{P}_{i,a}} \right) \left[t + \left(\frac{^{32}\text{P}_{i,1}}{^{32}\text{P}_{i,a}} \right)^{1/N} \right]^{-N}$, so it is removed from the equation. Also, $\left(\frac{^{32}\text{P}_{i,t=1}}{^{32}\text{P}_{i,a}} \right)^{1/N}$ is negligible compared with t .

Therefore, the equation to solve N is as follows:

$$\frac{{}^{32}\text{P}_{i,t}}{{}^{32}\text{P}_{i,a}} = \left(\frac{{}^{32}\text{P}_{i,1}}{{}^{32}\text{P}_{i,a}} \right) [t]^{-N}$$

$$\log\left(\frac{{}^{32}\text{P}_{i,t}}{{}^{32}\text{P}_{i,a}}\right) = \log\left[\left(\frac{{}^{32}\text{P}_{i,1}}{{}^{32}\text{P}_{i,a}}\right)[t]^{-N}\right]$$

$$\log({}^{32}\text{P}_{i,t}) - \log({}^{32}\text{P}_{i,a}) = \log\left(\frac{{}^{32}\text{P}_{i,1}}{{}^{32}\text{P}_{i,a}}\right) + \log(t^{-N})$$

$$\log({}^{32}\text{P}_{i,t}) - \log({}^{32}\text{P}_{i,a}) = \log({}^{32}\text{P}_{i,1}) - \log({}^{32}\text{P}_{i,a}) - N\log(t)$$

$$N\log(t) = \log({}^{32}\text{P}_{i,1}) - \log({}^{32}\text{P}_{i,t})$$

$$N = \log({}^{32}\text{P}_{i,1}/{}^{32}\text{P}_{i,t})/(\log t)^{-1}$$

N is the slope of $\log({}^{32}\text{P}_{i,1}/{}^{32}\text{P}_{i,t})$ versus $\log(t)$, rather than the slope of $\ln({}^{32}\text{P}_{i,t}/{}^{32}\text{P}_{i,a})$ versus $\ln(t)$.

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