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Measurement of Organic Phosphorus Mineralization in Non-Allophanic Andosols Using Anion Exchange Resin

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Abstract

The objective of this study was to develop a new method to measure organic phosphorus (Po) mineralization in non-allophanic Andosols by incubation of a mixture of soil and an anion-exchange resin (AER, Cl-saturated, strongly basic IRA-900) and to examine the implications of Po mineralization for soil P fertility. Five soil samples with widely different P statuses were collected from forestland, grassland, and cultivated land (corn and garlic fields). The mixing ratio of soil to AER affected the recovery rate of inorganic P (Pi) added to the soil samples along with the AER; a 1:1 ratio seemed to be optimal. Recovery rates of added Pi ranged from 68% to 74% for the five soils, and the amounts of AER-extractable Pi ranged from 2.9 to 27 mg P kg⁻¹. These recovery rates and the amounts of AER-extractable Pi were used for estimating gross Po mineralization. The amounts of Po mineralized after 42 days of incubation ranged from 3.1 to 7.9 mg P kg⁻¹, and the amounts were higher in the soils from the garlic field and the forestland than in the soils from the grassland and the cornfields. The relationship between total Po and mineralized Po suggests that this new method can be used to determine Po mineralization rates in Andosols. Our results indicate that Po mineralization is an important source of plant-available P in unfertilized forest soil.

Introduction

In most soils, organic phosphorus (Po) accounts for 30–65% of total phosphorus (Harrison, 1987). The mineralization of Po plays an important role in the cycling of phosphorus (P) in soils. Oehl et al. (2001) determined a basal daily P mineralization rate of 1.7

mg P kg⁻¹ for a soil that had been managed under a bio-organic cropping system. This rate is equivalent to the equilibrium concentration of inorganic P (Pi) in the soil solution. Lopez-Hernandez et al. (1998) measured daily mineralization rates of 0.22–0.90 mg P kg⁻¹ in Mollisols in the United States. In natural ecosystems and agricultural lands with a low input of P fertilizer, Po mineralization is considered to play an important role in supplying P to plants by replenishing inorganic P (Pi) in the soil solution (Sharpley, 1985). Therefore, methods for measuring Po mineralization are of interest.

Biomass P measurement and ³²P isotope dilution have been proposed as methods for measuring Po mineralization in soil. Biomass P has been measured by means of a chloroform fumigation method using 0.5 M NaHCO₃ as an extractant (Brookes et al., 1982; Hedley and Stewart, 1982). The isotope dilution method involves measuring changes in the amount of ³²P in soil solution (Oehl et al., 2001) or measuring the amount of ³²P extracted by an anion exchange membrane (Lopez-Hernandez et al., 1998). However, these methods cannot differentiate mineralized P from the Pi released from the soil Pi pool. For soils with a high P-retention capacity, such as Andosols, these methods may underestimate Po mineralization, owing to considerable Pi fixation by soils during the fumigation and extraction processes or during the equilibration process after the addition of ³²P.

For more precise measurement of the amounts of mineralized P and biomass P, alternative methods have been proposed. Use of an anion exchange membrane (Kouno et al., 1995) or a Bray extractant instead of an Olsen extractant (Oberson et al., 1997)

has been proposed for improving the recovery rate of P mineralized during biomass P measurements. The use of γ -ray irradiation and autoclave treatments to determine the fractions of gross P mineralization and immobilization has been reported (Zou *et al.*, 1992). However, Po mineralization cannot be directly determined from biomass P measurements, and the γ -ray irradiation method proposed by Zou *et al.* (1992) is not convenient. Anion exchange resins (AER) have been used to assess the amount of plant-available Pi in soils (Amer *et al.*, 1955; Cooke and Hislop, 1963). AER can remove labile Pi from soils without chemical alteration, pH change, or re-sorption or immobilization of Pi produced by mineralization. Parfitt and Tate (1994) developed a method for measuring Po mineralization using an anion exchange membrane with incubation after removal of labile Pi.

Volcanic ash soils (Andosols) commonly contain large amounts of organic matter and Po. Mineralization of Po in Andosols is considered to be an important P source for crops. However, Po mineralization rates for non-allophanic Andosols (Aluandic Andosols) have not been estimated.

The objectives of this study were to develop a simple method, based on the method of Parfitt and Tate (1994), to determine Po mineralization rates in soils using an AER and to determine the Po mineralization rates in non-allophanic Andosols under varied soil management systems.

Materials and methods

1) Soil samples

Soils used in this study were collected at Field Science Center of Graduate School of Agricultural Science, Tohoku University, in northeastern Japan (Table 1). Five soil samples, which had different Po and labile Pi contents, were collected from forestland, grassland, and cultivated land (two corn fields and a

garlic field). The fields from which the soil samples were collected were adjacent to one another. The soils showed high P retention and large amounts of active Al and Fe and were classified as Melanic Aluandic Andosols according to IUSS Working Group (2006). Forest soil was collected from a depth of 0–10 cm, and the other 4 samples were collected from a depth of 0–15 cm. The grassland had been used for forage production for 9 years, and P-containing fertilizer had been applied at the rate of 42 kg P ha⁻¹ year⁻¹. Phosphorus fertilizer and cattle manure compost had been applied to the garlic field at rates of 340 kg P ha⁻¹ year⁻¹ and 27 t ha⁻¹ year⁻¹ for 5 years, respectively. Phosphorus fertilizer had been applied to the cornfields at the rate of 87 kg P ha⁻¹ year⁻¹ for 5 years. The Po and Pi contents of each soil differed substantially and were in the ranges of 164–391 mg P kg⁻¹ and 808–3870 mg P kg⁻¹, respectively.

Soils were air-dried and sieved (<0.5 mm) after removal of plant roots. Before incubation with the AER, the soil pH was adjusted to 6.0 using CaCO₃, so that Po mineralization rates for the various soils could be compared under the same pH conditions.

2) Soil analysis

Soil pH was measured in aqueous solution with a glass electrode at a soil to water ratio of 1:2.5. Organic C was determined by the Tyurin method (Tyurin, 1931). Total P was determined by the colorimetric method of Murphy and Riley (1962) after digestion by HNO₃–HClO₃. Po was measured by the ignition method (550 °C, 1 h) described by Saunders and Williams (1955). Available P was measured by the method of Truog (1930). Phosphate retention and the amounts of acid oxalate-extractable Al and Fe were determined by the method of Blakemore *et al.* (1981).

3) Preparation of anion exchange resin and extrac-

Table 1. Chemical properties of non-allophanic Andosols used in this study

	pH (H ₂ O)	Organic carbon (g/kg)	P retention (%)	Acid oxalate extractable Al and Fe Al+ 1/2 Fe (g/kg)	Total P (mg P/kg)	Organic P (mg P/kg)	Inorganic P (mg P/kg)	Truog P (mg P/kg)
Forest soil	4.8	144	94	19.0	1078	270	808	3.49
Grassland soil	4.8	81	95	27.2	1759	213	1546	37.3
Corn field soil 1	6.1	79	96	24.7	2257	205	2052	72.0
Corn field soil 2	6.0	85	95	22.1	2483	164	2319	91.6
Garlic field soil	5.9	96	91	21.3	4261	391	3870	453

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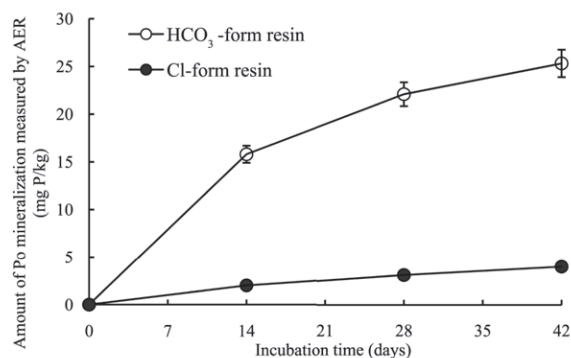


Fig. 1. Effect of saturated anion type of AER added to forest soil on soil Po mineralization (Bars show standard deviations (n=2))

tion of P recovered by resin

Preliminary studies were conducted to determine the most suitable anion for the AER. Figure 1 shows the amounts of P mineralized from the Po pool of the forest soil during incubation with a Cl-form resin and a HCO₃-form resin. The amounts of P mineralized from the Po pool were obtained by subtracting the amount of Pi extracted by the AER during a 24-h period prior to incubation (labile Pi) from the amount of Pi extracted by the AER after incubation (details described below). The HCO₃-form resin extracted much more Pi than the Cl-form resin because the soil pH changed from 6 to 7 upon the addition of the HCO₃-form resin; addition of the Cl-form resin did not affect the soil pH. Because the HCO₃-form resin artificially increased mineralization of soil Po, the Cl-form resin was more suitable for estimating Po mineralization.

The strongly basic AER resin (IRA-900 Amberlite) used in this study was sieved to less than 0.5 mm and saturated with Cl ion using 1 M HCl and 1 M NaCl. The Cl-saturated AER was washed with distilled water until the filtrate pH reached 4.5. The air-dried AER contained 20% moisture. Nearly 100% of P absorbed by the AER (10 g) was recovered after 3 extractions with 50 ml of 1 M NaCl (data not shown).

4) Measurement of Po mineralization

Incubation method

Distilled water was added to the air-dried and sieved soil to attain a soil moisture condition of 60% water-filled pore space, a value that is equivalent to the field moisture capacity. Moistened soil equivalent to 10 g of oven-dry soil was mixed with 10 g of AER in a polyethylene bag with a small hole. Three such

bags were prepared and incubated at 30 °C under aerobic conditions. The soil moisture content was adjusted to the initial value every 7 days.

Measurement of Po mineralization in soils

Po mineralization in soils was measured as follows. Mixtures of soil and AER, both before and after incubation, were transferred to 100-ml plastic bottles containing 50 ml of distilled water, and the slurries were shaken for 24 h at 25 °C. Soil particles were removed by sieving (mesh size, <0.5 mm) and supersonication (5 min) after the shaking. The collected clean AER (10 g) was shaken with 50 ml of 1 M NaCl solution for 1 h to elute Pi from the AER. The resin was extracted 3 times, and the recovered Pi was measured by the colorimetric method of Murphy and Riley (1962).

The amount of P mineralized during incubation was calculated with the following equation: amount of P mineralized from the soil Po pool = (amount of Pi extracted by AER after incubation – amount of Pi extracted by AER before incubation)/recovery ratio.

Determination of the optimal soil:AER ratio for efficient capture of Pi from soils

It is possible that not all of the Pi produced by mineralization of the Po pool was recovered by the AER, because some of the mineralized P could have been fixed by soil colloids during incubation. Therefore, we examined the effect of the soil:AER mixing ratio on the Pi recovery rate. KH₂PO₄ solution (50 ml, 8.7 µg P ml⁻¹) was added to the moistened forest soil (10 g oven-dry basis) mixed with 5, 10, 15, or 20 g of AER in 100-ml plastic bottles, and the mixtures were shaken for 1 h. Two sets were prepared for this experiment. We determined the best mixing ratio on the basis of the amounts of Pi taken up by the AER.

Method for distinguishing between P mineralized from the soil Po pool and released from the soil Pi pool

Because labile Pi released from the soil Pi pool can be taken up by the AER during incubation, the Pi produced by mineralization of the Po pool had to be distinguished from the Pi released from the Pi pool in the soils. We tried to estimate net Po mineralization rates in soils by subtracting the amount of AER-extractable Pi before incubation from the amount of Pi recovered by the AER after incubation. We de-

terminated the shaking time required for effective extraction of labile Pi released from the soil Pi pool. A mixture of each of the soils (10 g oven-dry basis) and AER (10 g) was shaken with 50 ml of distilled water for 3, 6, 12, 24, or 48 hours. Two sets were prepared for each shaking time.

Determination of the efficiency of P recovery with the AER

Because mineralized P can be competitively absorbed by soil and AER during incubation, Po mineralization rates obtained by means of the above method were net values. To evaluate gross Po mineralization, we determined the recovery rate of Pi with the AER. A mixture of soil (10 g oven-dry basis) and AER (10 g) was shaken with 50 ml of KH_2PO_4 solution ($8.7 \mu\text{g P ml}^{-1}$). After shaking for 1 h, the amount of P recovered from the AER was determined for each soil.

Results and Discussion

1) Effect of soil:AER mixing ratio on recovery rate of added Pi

The recovery rate of added Pi was examined at different soil:AER mixing ratios for the forest soil. The recovery rate of Pi was significantly lower at a soil:AER ratio of 1:0.5, whereas there were no significant differences between the Pi recovery rates for mixing ratios of 1:1, 1:1.5, and 1:2, which were in the range of 73.5–75.3% (Table 2). Sibbesen (1978) reported that the amount of P extracted from soils by AER varied only slightly even when the AER:soil ratio was varied. In addition, Amer *et al.* (1955) and Olsen and Sommers (1982) used a resin:soil ratio of 1:1 for assessing labile P. Taken together with the results from previous studies, our results indicated that a soil:AER ratio of 1:1 was suitable for effective recovery of mineralized P.

2) Measurement of labile Pi extracted by the AER

The amounts of Pi extracted by the AER before incubation increased with the shaking time and reached their maximum values after 24 h for all 5 soils (Fig. 2). This result indicates that a shaking time of 24 h was sufficient to extract the labile Pi from the soils. The amounts of Pi extracted by the AER at 24 h were 2.9, 5.2, 8.3, 14, and 27 mg P kg^{-1} in the forest, grassland, cornfield-1, cornfield-2, and garlic field soils, respectively. The AER-extractable Pi correlated positively with the total Pi ($R^2 = 0.956^{**}$, Fig. 5) and Truog P ($R^2 = 0.921^{**}$). These relationships indicate that AER-extractable Pi was released from the soil Pi pool and that subtraction of AER-Pi removed before incubation from the AER-Pi removed after incubation was an effective method for determining Po mineralization.

3) The recovery rates of added Pi with AER

The recovery rates of added Pi with AER are shown in Table 3. The AER took up 68–74% of Pi added to the mixture of AER and soil. The result suggests that AER effectively absorbed Pi from soil solution, even for Andosols with a high P-fixation capacity. We used the values as correction factors to determine gross Po mineralization in this study.

4) Po mineralization during incubation

Po mineralization profiles for the 5 soils are shown in Fig. 3. These values were corrected with the amounts of labile Pi and the Pi recovery rates. Po mineralization increased with incubation time for all 5 soils. Nitrogen mineralization also occurred during the incubation experiment (Fig. 4). This result suggests that Po mineralization proceeded along with decomposition of organic matter. The amounts of Po mineralized after 42 days, which depended on the total Po contents in soils, were 4.6, 3.4, 3.1, 3.1, and

Table 2. Effect of mixing ratio of soil to AER on recovery rate of added Pi in the forest soil

Mixing ratio of soil to AER	Recovery rate of added Pi (%)	SD
1 : 0.5	69.9 a	0.95
1 : 1	74.2 b	0.55
1 : 1.5	75.3 b	0.90
1 : 2	73.5 b	1.05

There are significant difference ($P < 0.05$) between the values with different letters by Tukey's HSD method. SD means standard deviation ($n=2$)

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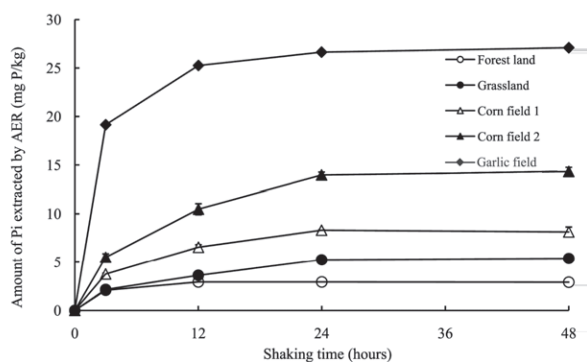


Fig. 2. Effect of shaking time on the amounts of labile Pi extracted by AER (Bars show the standard deviations (n=2))

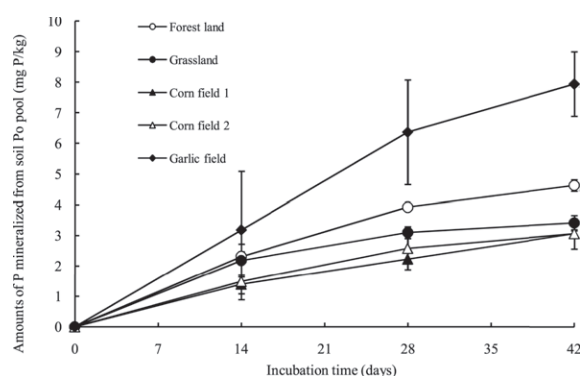


Fig. 3. Amounts of P mineralized from Po pools in non-allophanic Andosols with different land use (Bars show standard deviations (n=3))

Table 3. Percentage of Pi recovered by AER

Soil samples	Recovery rates of added Pi (%)	SD
Forest soil	74	0.55
Grassland soil	71	0.35
Corn field soil 1	71	0.95
Corn field soil 2	72	1.00
Garlic field soil	68	1.35

SD means standard deviation (n=2)

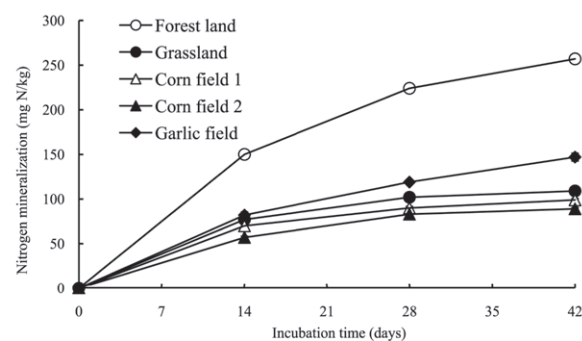


Fig. 4. Nitrogen mineralization in AER added soils during incubation (Bars show standard deviation (n=3))

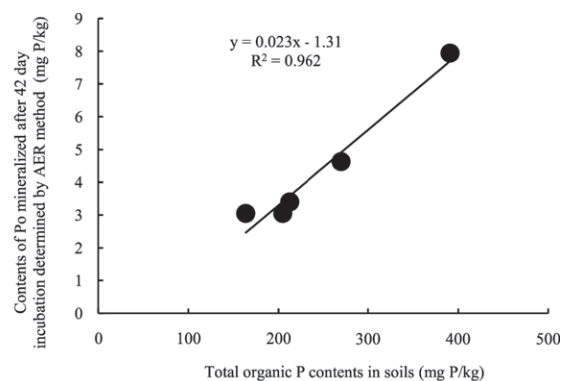
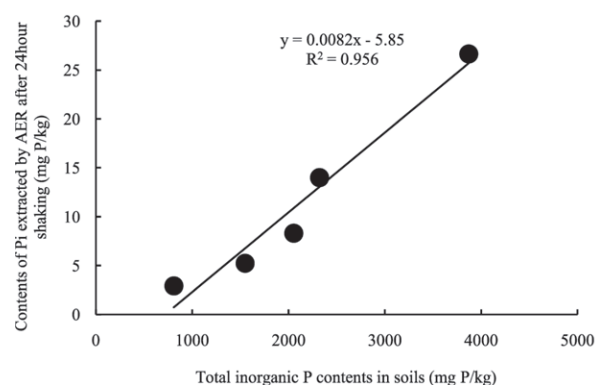


Fig. 5. Relationships between AER-Pi and inorganic P contents, and mineralized Po and organic P contents

7.9 mg P kg⁻¹ in the forest, grassland, cornfield-1, cornfield-2, and garlic field soils, respectively. The amount of P mineralized after incubation for 42 days was positively correlated with total Po content (Fig. 5). Stewart and Sharpley (1987) also observed positive correlation between total Po and mineralized P

in Texas soils. However, mineralized P was not significantly correlated with total Pi and labile Pi such as AER-extractable and Truog-extractable Pi in the soils. These results suggest that increased AER-P after incubation was released not from the Pi pools but from the Po pools in the soils. The method described

in this study is considered to be useful for measurement of Po mineralization in soils such as Andosols that have high P-fixation capacity.

5) Implications of Po mineralization for soil P fertility

The ratio of Po mineralized over a period of 42 days to bioavailable Pi (Truog-Pi) was 1.3 in the forest soil and <0.1 in the other soils. This result indicates that Po mineralization is an important source of P for plants in unfertilized forest soil. The percentage of Po mineralized over a period of 42 days ranged from 1.5% to 2.0% of the total Po in each soil. The amount of mineralized Po was highest in the garlic field soil, which had the highest Po content, followed by the forest soil. Po content greatly influenced the Po mineralization rates through microbial activity. Parfitt and Tate (1994) showed that the amounts of Pi released from Po in Andosols under forest vegetation ranged from 0.5 to 10.7 mg P kg⁻¹ after incubation for 7 days at 39 °C. Assuming that the Q₁₀ for Po mineralization is 2, the mineralization values at 14 days in this study correspond to the values obtained by Parfitt and Tate. The amounts of P mineralized after 14 days (1.3 to 3.1 mg P kg⁻¹) in our research were within the range obtained by Parfitt and Tate (1994).

In this study, air-drying and pH adjustment were carried out before incubation. These treatments were conducted to prepare uniform soil samples and measure Po mineralization under the same pH conditions. Air-drying of soil is known to reduce the biomass P and increase inorganic P extracted by NaHCO₃ (Brookes *et al.*, 1982; Sparling *et al.*, 1985). Organic P mineralization may decrease in an air-dried soil compared with a moist soil because the Pi derived from soil microorganisms killed by desiccation may be estimated as labile Pi by our method. Marumoto *et al.* (1982) reported that increasing the soil pH increased nitrogen mineralization. Increasing the soil pH may increase Po mineralization. Therefore, in future studies it will be necessary to measure Po mineralization using a moist soil without pH adjustment for estimating Po mineralization under in situ conditions.

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