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Soil Phosphorus

Characterization and Total Element Analysis

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Phosphorus is a major element in soil organic matter, and in natural terrestrial ecosystems is derived from the weathering of minerals in parent rock material. It is usually the second most limiting nutrient for terrestrial primary production (after nitrogen) and is often the primary limiting nutrient in freshwater ecosystems. Due to its critical role in controlling aquatic primary production, there is an increased concern over runoff of P from terrestrial to aquatic ecosystems and the role of P in affecting water quality.

Studies of P cycling and availability pose a challenge to agronomists and ecologists, in part because P occurs in soils in many different physicochemical forms that are operationally difficult to define, and in part because P is involved in a myriad of biological and chemical processes. The cycling of P can be controlled by inorganic chemical reactions, but in many systems the turnover of organic P controls the availability of P to plants through organic matter decomposition or the release of P from microbial biomass. Phosphate sorption by various soil constituents is a dominant process in maintaining very low concentrations of P in soil solutions, particularly in mineral soils. Tight control of P concentrations by adsorption creates difficulty for the measurement of the amount of P available for plant uptake or P release upon organic matter mineralization. Whereas important pools and fluxes of N are readily measured in soils, the different physicochemical forms of soil P are more difficult to categorize or define operationally. As a result, it is challenging to define or measure bioavailable P.

Understanding the cycling of P through soils has also proven challenging to ecologists. In contrast to measurements of N mineralization, the determination of changes in extractable P pools over time yields little information, in part due to the high background of sorbed mineral P and in part due to the lack of a single extract

that can measure the newly released P. Although researchers have used various techniques, including the use of ^{32}P, to measure the mineralization of organic P in soils, these approaches either have not been widely tested or have met with some skepticism by other researchers, and they are not yet standard enough to be recommended here (for examples and comments see Walbridge and Vitousek 1987; Zou et al. 1992, 1995). Sorption/desorption also plagues measurements of solution or available P as well as measurements of organic P.

Due to these difficulties, it is important to realize that many pools of P such as "available" P are functional concepts rather than measurable quantities. Many measurements of P are simple to conduct (e.g., extractable P, sorbed P) but are more difficult to interpret. The measurement of total P in soils is one of the few measures of this element that is both operationally and theoretically well defined.

In this chapter we will discuss (1) the measurement of labile, or readily available, P in soils; (2) a current recommended method to estimate soil organic P; (3) total P and total element analysis by fusion; (4) an index of P sorption; and (5) a discussion of a current method to estimate operationally defined fractions of P in soils for use in specific research questions. Finally, procedures for measuring inorganic and organic P in solution are given.

General procedures used for sample collection, processing, and determining soil pools on an areal basis are provided in detail in Chapter 1, this volume. However, the sampling and handling protocols associated with the measurement of pools of P deserve special consideration. Due to the strong geochemical reactions of P in soils, certain measures of P, such as the estimation of total P pools, might best be analyzed by soil profile horizon rather than by depth, since distinct horizons will differ strongly in strength of geochemical reactions. Additionally, the preservation of soils prior to analysis, if samples cannot be analyzed or handled immediately while still field-moist, is an issue that is still debated by soil scientists. The drying of soils may present problems for the analysis of specific P fractions such as available or microbial P, as well as sorbed P. In many cases, particularly in heavy clays, soils may aggregate irreversibly upon even air drying, making them difficult to handle. Although grinding soils solves the aggregation problem, grinding exposes previously unexposed surfaces and may lyse microbial P, resulting in an apparent increase in measured labile P (Potter et al. 1991). In such cases soils should be analyzed for available P while still field-moist if at all possible; researchers have kept soils in plastic bags, refrigerated or cooled, without measurable changes in properties such as P sorption potential (Parfitt et al. 1989). However, other studies have shown that even short-term moist storage at 4 °C decreased labile P pools (Chapman et al. 1997), just as sieving soil has been shown to increase labile P.

Drying may also result in the conversion of P into physicochemical fractions that are fairly resistant to redissolution. Changes in field moisture and air drying have been shown to have pronounced effects on microbial biomass (Powlson and Jenkinson 1976; Potter et al. 1991), although adjustment of moisture content to approximately 60% of field capacity, followed by incubation, reduces this drying effect on microbial P (Potter et al. 1991). Of course, drying is less of a problem for soils that dry naturally under field situations. If analysis of field-moist soils is not possible, soils should be air-dried rather than oven-dried. However, since even air

drying of specific soils, such as many tropical soils, can result in unalterable changes in soil chemistry, individual researchers should be aware of the properties of their soils before assuming that air drying will not cause problems. In all cases, final values of available P concentrations should be converted to an oven-dry (105 °C) basis.

Available P by Laboratory Resin Extraction

Problems with the definition of a "bioavailable" pool of P go beyond the measurement problems caused by the complexity of P chemistry in soils. Research has shown that different plants can extract different quantities of P from the same soils, and can derive this P from different physicochemical pools (Lajtha and Harrison 1995). Thus pools of available P differ among plant species. However, researchers have found useful indices of P availability and thus fertilizer requirements for many crop species by developing different chemical extractants for use in specific soil types. Unfortunately, these different extractants do not necessarily yield specific soil P fractions that are useful in understanding P cycling in an ecosystem or allow for comparisons across ecosystems and soil types. Because the purpose of this volume is to suggest methods that can be used across ecosystems and soil types, these extracts, commonly used in agronomic applications, will not be discussed here.

Anion-exchange resins, enclosed in permeable nylon or polyethylene mesh bags, have been used in both field and laboratory situations, and across a wide variety of soil types, as an index of labile P. The resins are assumed to simulate the ion uptake action of plant roots and, like active roots, provide a strong sink for P released into solution. In the bicarbonate form, anion-exchange resins will most closely mimic the chemical conditions of the rhizosphere, where bicarbonate accumulates and buffers soil pH (Sibbesen 1978). The relative affinity of anion-exchange resins for bicarbonate is low, thus favoring exchange with ortho-P, and resins in the bicarbonate form extract more ortho-P than resins in either the chloride form or the hydroxyl form (Lajtha 1988).

Researchers have used both Teflon-based anion-exchange membranes (Saggar et al. 1990; Schoenau and Huang 1991; Abrams and Jarrell 1992) and iron oxide strips (Lin et al. 1991; Menon et al. 1989; Sharpley 1991) in laboratory assessments of P availability. The anion-exchange membranes can certainly be used in place of the resin bags, but it is essential that a sufficient number of membranes of a sufficient size be used to ensure an excess of exchange sites. Resin strips and iron oxide strips give nearly the same result for labile P in poorly weathered soils; however, the iron oxide strip removes 5 to 10 times more P than do resins in highly weathered soils. Clearly the iron oxide strips are better competitors for P in these highly weathered soils, but they do not necessarily better mimic the action of plant roots. Myers et al. (1995) discuss some of the problems with the preparation and use of the iron oxide strips. These problems include abrasion and removal of the FeO coating during extractions, problems with different pore sizes of the papers, and adherence of soil particles to the strips. Thus here we recommend the use of resin bags and/or resin membrane filters over the use of iron oxide filters.

Materials

1. Dowex 1-X8 anion-exchange resin beads (or any type I resin), 20–50 mesh (or the largest available bead size); OR anionic resin strips, 2 × 6 cm (available commercially, e.g., BDH, marketed through VWR or through Soil, Plant, Water Quality Inc., 12505 NW Cornell Rd., Portland OR 97229)
2. Nylon or polyethylene small-mesh fabric (if using resin beads)
3. Salad spinner or open-basket hand centrifuge (if using resin beads)
4. 0.5 mol/L $NaHCO_3$
5. 0.5 mol/L HCl
6. 250 mL polyethylene bottles
7. Mechanical shaker
8. 50 μg P/mL stock solution: 0.2195 g oven-dried primary standard-grade KH_2PO_4 dissolved in 1000 mL deionized water. Stored in a polyethylene bottle, refrigerated, with a few drops of chloroform.
9. P solution standards. 0–4 μg P/mL : 0.01 μg P/mL, 0.1 μg P/mL, etc. Up to two times the expected maximum concentration of samples is diluted from the primary stock daily.

Procedures

Resin Bag Preparation

1. Sew resin bags (with a surface area approximately 50 cm^2, or 4–5 × 8–10 cm) from undyed stocking material or small-mesh rigid polyester screen. Any porous nylon or polyethylene material with a mesh size smaller than the resin bead may be used, but it is critical that dyed stocking material not be used because dye leached from the stockings may interfere with P analysis.
2. Weigh 4 g of dry-weight equivalent (wet/dry conversions are usually given on the bottle) resin, or 10 meq total anion exchange capacity (if total anion exchange capacity equivalents are given) into each bag.
3. Convert the anion resin to the bicarbonate form by shaking bags for 10 minutes in three successive 100 mL 0.5 mol/L $NaHCO_3$ solutions, rinsing with deionized water between each $NaHCO_3$ equilibration. Alternatively, resins may be converted to the bicarbonate form in large batches before placing in bags, but bicarbonate-form resins do not store as long as the hydroxyl-form resins, even when refrigerated.
4. Rinse bags thoroughly in deionized water and spin dry in a hand centrifuge or a salad spinner (do not air- or oven-dry). If a salad spinner is used, mesh should be placed around the open edges of the spinner so that the bags do not protrude through the slots and tear.

Resin Membrane Preparation

1. Cut membranes into strips of a standard dimension, usually 2 × 6 cm, and convert to the bicarbonate form as for resin beads. Membranes are often eas-

ier to use because they can be rinsed directly under deionized water and may be shaken rather than spun dry.
2. Membranes may be stored after rinsing in weak HCl, and then refrigerated in Ziplock bags. Because they are not completely abrasion resistant they need to be checked before each reuse.

Use of Bags and Membranes

1. Place 100 mL deionized water and 10 g of soil in a polyethylene bottle along with two resin strips or a resin bag, cap bottle lightly, and shake gently for 18 hours.
2. Remove bags, rinse thoroughly in deionized water, and spin (bags) or shake (membranes) dry.
3. Place bags or membranes in clean 250 mL bottles with 100 mL of 0.5 mol/L HCl and extract for 1 hour. Because bicarbonate still associated with the resin will be protonated by HCl, CO_2 will outgas, and extracting bottles will need to be uncapped periodically to release trapped CO_2, especially for the first 15 minutes.
4. Prepare standards by shaking bags or resin strips in 100 mL of the solution standards and extracting using the same procedure as described for soil solutions. Extracts and standards are then analyzed for ortho-P using methods described in the "Inorganic P in Solution" section, below.

Calculations

$$\mu g\ P/g\ soil = C \times F \times 1/f_{dry\ soil} \times 1/f_P$$

where

C = concentration of P in the extract solution as μg P/mL extract
F = mL extract/g soil at field moisture content (e.g., 100 mL/10g soil)
$f_{dry\ soil}$ = the fraction of field-moist soil that is dry soil, or (oven-dry mass / fresh mass);
f_p = the fractional recovery efficiency of the ion exchange material as determined from the extracted standards, or (recovered P/standard P).

Special Considerations

If resin bags are to be recharged and reused, the extraction of ortho-P needs to take place with intact bags. The extraction of intact bags is time-consuming, and CO_2 is not readily released from nylon bags. If resins are to be recycled but the nylon or polyethylene bags disposed, bags can be cut and the resins placed into bottles before the addition of acid. After acid extraction, resins can be reconverted to the bicarbonate form and reused. Resins will degrade if completely dried; they should be refrigerated in weak (0.5 mol/L) HCl for storage. The efficiency of recycled resins

should be monitored periodically using standard solutions as part of the standard protocol, described earlier.

Many researchers have used resin bags, prepared as described earlier, to monitor P availability in the field (Lajtha 1988; Giblin et al. 1994). For these applications, care should be taken to have all resin bags of uniform size because the extent of sorption depends more on total surface area than on total volume or weight of resin. Bags are flattened and placed horizontally in soil. It is generally assumed that water percolates vertically through the soil and the bags, yet it has been shown that if the bag material is of a hydrophobic fabric or if the mesh size is very small the movement of water will be impeded. Bags have been left in the field for periods of 2 weeks to 4 months before retrieval, although Giblin et al. (1994) found that phosphate was desorbed under field conditions if bags were left for long periods, and thus deployment times on the order of a few weeks are recommended. It is not recommended that field-placed resins be reused because some decomposition and fouling of the organic resin material occurs.

Anion-exchange resin–impregnated membranes have been used under field situations as well, and they appear to be simpler and easier to use and maintain than resin bags (Abrams and Jarrell 1992; Cooperband and Logan 1994). Ion-exchange membranes are flat, and thus they enhance the interaction of the exchange surface with soil, although they may inhibit percolation through exchange sites. This technique is described in detail in Chapter 9, this volume.

Soil Organic P

Even though plants assimilate inorganic P, organic P is an important source of inorganic P in most soils. This organic pool is affected by weathering and soil age, parent geochemistry, management practices and cultivation, and organic matter dynamics (Walker and Syers 1976; Tiessen et al. 1983; Stewart and Tiessen 1987). Organic P is potentially available to plants or microbes, or soil sorption sites, after mineralization. The turnover of organic P is, in part, dependent on the mineralization of organic carbon pools, although soluble pools of organic P can be mineralized by soil enzymes, thus disconnecting soil C and P cycles (McGill and Cole 1981). Due to the difficulties of measuring organic P mineralization, an estimate of organic P pools serves as an index of potentially available P over a longer time scale than that obtained from a resin extract, although the lability of this pool is highly variable (Tiessen and Stewart 1985). Because of this variability, "potentially mineralizable" organic P in soils is not analogous to the mineralizable N pools that are easily measured in field or laboratory incubations (see Chapter 13, this volume). Phosphorus fractionation techniques, such as the Hedley fractionation described below, may serve to better differentiate specific pools of organic P with different turnover times.

There are many extraction methods for the determination of organic P. These methods vary in complexity and time required. Perhaps the most common current method for measuring organic P is the dry combustion method of Saunders and Williams (1955) as modified by Walker and Adams (1958). This method is simple,

requires few steps, and requires no special apparatus. The basic technique uses a strong acid extraction of ignited and nonignited soil samples. However, recoveries using this method are erratic and vary from Ca-dominated to Fe/Al-dominated soils and with weathering intensity. In particular, the ignition method may be highly inaccurate for weathered soils rich in Fe and Al oxides. Several researchers have observed negative values in tropical soils, perhaps because ignition changes the chemistry of oxides by driving off water of hydration, changing water and hydroxide balance at mineral surfaces, and thus affecting sorption. A more detailed discussion of potential errors with this method can be found in Olsen and Sommers (1982).

Mehta et al. (1954) described a sequential extraction procedure using HCl and NaOH. Bowman (1989) described a much simpler and faster procedure involving sequential extractions of H_2SO_4 and NaOH.

The alkaline EDTA extraction procedure of Bowman and Moir (1993) is simple, should work equally well in soils across a broad range of pH and clay content, and compares well with the Bowman (1989) procedure. It is recommended that this procedure be used to calibrate any other method; it is quite likely, in calcareous or low P sorbing soils, that the ignition method and the EDTA method will yield similar results. The principle behind the Bowman and Moir (1993) method is that an alkaline solution solubilizes soil organic matter (SOM) and associated organic P. EDTA chelates metal cations (and thus reduces cationic bridges with SOM) to increase the efficiency of SOM extraction. Persulfate is used to oxidize the extracted solution from dissolved organic P into ortho-P, which can then be measured colorimetrically. Organic P is calculated as the difference between persulfate (total) P and extracted inorganic P.

Materials

1. Electric ball mill (e.g., Spex mixer-mill)
2. Mechanical mixer
3. Incubator, heating water bath, or oven
4. 50 mL polypropylene screw-cap centrifuge tubes
5. Centrifuge plus adapters
6. Extracting solution: 0.25 mol/L NaOH in 0.05 mol/L Na_2EDTA

Procedure

1. Dry soils at 105 °C until no additional weight is lost, and store in a warm oven or dessicator.
2. Dried soils should be finely ground (<1 mm mesh) with an electric ball mill.
3. Weigh 0.5 g soil into a 50 mL polypropylene screw-top centrifuge tube (less mass should be used for highly organic or calcareous soils), add 25 mL of extracting solution, cap the vessel, and mix about 15 seconds with a touch mixer.
4. Loosen caps (to minimize gas buildup inside tubes) and place for 10 minutes in an oven, incubator, or water bath that has been preheated to 85 °C.
5. After 10 minutes, tighten caps and continue heating for a total heating time of 2 hours.

6. Remove tubes, cool until comfortable to handle, remix, and centrifuge at 10,000 rpm for 10 minutes. (Tabletop centrifuges often operate at lesser speeds; any speed is acceptable, but at lower speeds the time required for filtration increases.)
7. Filter the supernatant through no. 40 filter paper. Reserve one aliquot for inorganic P (ortho-P) determination (see "Inorganic P in Solution" section, below) and one for total P (persulfate) digestion (see "Analysis of Dissolved Organic P . . . " section, below).

Calculations

Convert both inorganic ortho-P and total P extract concentrations to soil quantities as:

$$\mu g\ P/g\ soil = C \times F$$
$$\text{organic P} = \text{total P} - \text{inorganic P}$$

where

C = concentration of P in extract solution as μg P/mL extract
F = mL extract/g dry soil (e.g., 25 mL extract/0.5 g dry soil)

Microbial P

In many soils, the cycling of P is highly dependent on microbial population dynamics, with labile P released only during episodic population crashes (Chapin et al. 1978). Microbial P may constitute a significant fraction of total P in highly organic soils (Walbridge 1991), yet might be insignificant in less organic soils (Lajtha and Schlesinger 1988). Problems with the measurement of microbial P include the calculation of a K_p, or extraction efficiency coefficient, which accounts for P not extracted after fumigation due to incomplete lysis and to soil sorption of released P. K_p values will vary with different soils and will be most problematic in high P fixing soils.

The method proposed here was developed by Hedley and Stewart (1982), following the chloroform fumigation technique of Anderson and Domsch (1978) first adapted by Cole et al. (1978) to estimate microbial P. In the Hedley and Stewart (1982) procedure, removal of labile P by resin extraction before the $NaHCO_3$ extraction step was found to increase the accuracy of the measurement of microbial P.

In the Hedley and Stewart (1982) procedure, dried and ground soil was used. However, microbial populations can change significantly with either storage or drying, and thus immediate processing of field-moist soils is strongly preferred. Fresh samples of field soils that are not ground require sample sizes of 2–5 g to allow for soil heterogeneity and for correction to oven-dry weight. Since soil:solution ratios need to be between 1:30 and 1:60, larger centrifuge tubes or bottles may be required. If immediate processing is not possible, then refrigeration is still preferable to air

drying soils; air-dried soils should be rewetted and incubated before use (Powlson and Jenkinson 1976).

Materials

1. Resin strips (prepared as described in the section "Available P by Laboratory Resin Extraction," above).
2. 50 mL screw-cap polypropylene centrifuge tubes (250 mL centrifuge bottles if field-moist soils are used)
3. Chloroform ($CHCl_3$)
4. 0.5 mol/L $NaHCO_3$ adjusted to pH 8.5
5. 0.5 mol/L HCl
6. Centrifuge
7. Mechanical shaker with basket for holding tubes horizontally
8. 0.45 μm filters with syringe filter holders (e.g., Swinnex or Gelman filter holders) and 50 mL syringes
9. Acid-washed vials with polyethylene or polypropylene screw caps, 20–50 mL capacity

Procedure

1. Filters must be cleaned before use. This is accomplished by either filtering approximately 150 mL deionized water through the filter just prior to use or, preferably, soaking all filters in 0.5 mol/L HCl and then rinsing in deionized water several times before use. In the latter case, deionized water should still be passed through the filter before use to remove any remaining acid. If filters are not dried before use, about 5 mL of the extract that is being filtered should be passed through the filter and discarded before the filtered extract is saved for analysis.
2. Place duplicate 0.5 g finely ground, oven-dried soil subsamples in separate centrifuge tubes and add 30 mL deionized water and two resin strips in the bicarbonate form to each. If field-moist soils are used, 2–3 g soil and 150 mL deionized water should be used.
3. Cap the tubes and shake gently for 16 hours.
4. Remove resin strips, gently rinsing soil particles back into the tube with 1–2 mL deionized water. Swirl tubes gently while vertical before placing in a centrifuge in order to dislodge soil particles lodged in the cap or the tops of tubes. Centrifuge tubes for 10 minutes at 10,000 rpm, or at lower speeds for longer times. Decant the supernatant carefully so as not to lose soil and discard. If there is interest in measuring the resin P pool, the resin strips should be rinsed thoroughly in deionized water, placed in clean 50 mL tubes with 30 mL of 0.5 mol/L HCl, and shaken gently for 2 hours. The resulting solution is saved for analysis.
5. Add 1 mL $CHCl_3$, in a hood, to one tube (the "fumigated" tube), cap, and shake for 1 hour. Remove the cap and allow the chloroform to evaporate overnight under the hood.

6. Allow the other, nonfumigated tube to sit overnight, uncapped; refrigeration is suggested to avoid microbial P release, although this was not mentioned in the original procedure.
7. Add 30 mL of 0.5 mol/L $NaHCO_3$ (or 150 mL to bottles containing field-moist samples) to the fumigated and unfumigated tubes, cap, and shake for 16 hours. After 16 hours centrifuge the tubes and filter and save the supernatants.
8. Analyze both supernatants ("fumigated" and "unfumigated") for total (persulfate) P (described later in this chapter).

Calculations

$$\mu g\ P/g \text{ in microbial biomass} = [(\textit{Total P} \text{ in fumigated soil}) - (\textit{Total P} \text{ in unfumigated soil})]/k_p$$

where

Total P = μg P/g dry soil as determined by acid persulfate digestion (see later)
k_p = extraction efficiency = 0.4 (see the section "Special Considerations," below)

Special Considerations

This extraction removes only part of the microbial P that is lysed by the chloroform fumigation, and thus the k_p, or extraction efficiency factor, differs from 1.0. Ideally, a k_p factor is measured for each soil type. However, because this procedure involves culturing appropriate fungi and bacteria that are radiolabeled (Hedley and Stewart 1982), it cannot be casually undertaken for each site or study. Over a wide variety of soils, k_p factors have been found to range from 0.32 to 0.47, and thus an approximate k_p factor of 0.4 may be used with some degree of accuracy. While k_p factors are generally lower in highly weathered soils, the relationship between k_p and sorption capacity is not quantitative and thus cannot be modeled. However, some authors have used sorption capacity in place of a measured k_p factor.

P Sorption

Sorption is considered to be the most important process controlling P availability in soils. The dominant sorption reactions are those with Fe-Al oxides and hydroxides and Ca-carbonates, but the strength of sorption can vary considerably among soils. Since sorption is to some degree reversible, sorbed P may be a source of plant-available P either immediately or over a longer term. However, the degree of reversibility is not well understood, and there is no single easy measurement of P sorption.

The approach taken by most researchers to measure sorption has been to run a series of sorption studies at constant temperatures, or sorption isotherms, for a set period of time and then fit a model to the data in order to get a single number, or index, that can be compared across sites. Models include the simple Langmuir equa-

tion (Olsen and Watanabe 1957), from which a sorption maximum can be calculated, but this model does not fit empirical curves of sorption very well. Several authors have modified this simple equation to include distinct sorption surfaces or reactions (e.g., Holford and Mattingly 1975). The Freundlich equation, which describes a model of adsorption in which the affinity for adsorption decreases exponentially as adsorbed P increases (Barrow 1978), seems to fit many highly P-deficient soils. However, no one model is currently the accepted norm in all soils. Sorption maxima are often calculated, but soils rarely show a distinct sorption maximum; an initial, rapid period of sorption is generally followed by slower rates of P uptake. Studies have shown a long-term migration of P into aggregate or particle surface sorption sites that are of decreasing accessibility to solution (Willett et al. 1988; Fardeau and Jappe 1980; Tiessen et al. 1991), and this makes modeling the dynamics of P sorption complex. For the simple indices described here, however, we use a standard reaction time of 24 hours.

All of these models require that P sorption be measured over a range of P concentrations in solution so that curves can be generated. Bache and Williams (1971) suggested that valid comparisons could be made among different soils with an index that measured sorption at only one single high P addition, making the measurements faster and easier. Here we recommend that simple P sorption curves be generated and that the Bache and Williams (1971) index be calculated so that soils may be compared. Once the curves are made, models may be fitted if desired, depending on the questions being addressed.

Most of the original procedures recommend that sieved, oven-dried soils be used. However, oven drying soils with high clay contents could cause irreversible aggregation, making their handling nearly impossible. Drying may result in the conversion of fairly lightly sorbed P into physicochemical fractions that are fairly resistant to redissolution, thus causing major errors in estimates of already sorbed P. In such cases soils should be analyzed for available P while still field moist. Grinding soils is not recommended, since new mineral surfaces are thus exposed that would not be exposed under field conditions. Authors working in relatively young or unweathered soils have not reported significant problems in the use of air-dried or oven-dried soils, although comparisons to results with field-moist soils are rarely reported.

Materials

1. 50 mL screw-cap polypropylene centrifuge tubes
2. Centrifuge with adapters
3. Chloroform ($CHCl_3$)
4. Acid-washed vials with polyethylene or polypropylene screw caps, 20–50 mL capacity
5. 0.01 mol/L KCl
6. 1000 μg P/mL stock solution: 4.39 g oven-dried primary standard-grade KH_2PO_4 dissolved in 1000 mL deionized water. Stored in a polyethylene bottle, refrigerated, with a few drops of chloroform.
7. P working solutions: 0–200 μg P/mL solutions should be diluted daily from the stock solution and made up in 0.01 mol/L KCl.

Procedure

1. Add 30 mL of working solution to 3.0 g of oven-dry equivalent, unground soil in a 50 mL centrifuge tube with one drop of chloroform or toluene. Each soil at each working solution level should be run in duplicate.
2. Shake tubes for 24 hours at a constant temperature (approx 25 °C), centrifuge at 10,000 rpm for 30 minutes, and decant the supernatant, filtering if necessary.
3. Analyze solutions for ortho-P (described later in this chapter).

Each soil at each working solution level should be run in duplicate, and at least three working solutions, plus blank solution, should be used to generate the sorption isotherm curve. The range of working solution concentrations used does not affect intersite comparisons even if the range differs among studies, since curves of soil sorption *vs.* resulting equilibrium solution are plotted.

Calculations

Sorbed P

$$X_s = (s - c) \times F$$

where

X_s = sorbed P at working solution concentrations (μg P/g soil)
s = μg P/mL of original working solution
c = μg P/mL in equilibrium solution
F = mL working solution/g dry soil (e.g., 30 mL/3 g dry soil)

Note that if isotopically exchangeable P can be measured (see Bache and Williams 1971 and "Special Considerations," below) or if resin-extractable P is measured, then the correct final equation is

$$X_s = [(s - c) \times F] - E$$

where

E = isotopically exchangeable or resin-extractable P, as μg P/g soil

Sorption Curves

Sorption curves are plotted as X versus c. At this point, curves may be fitted by eye or by using the models discussed earlier. A somewhat artificial absorption maximum (X_m) may be calculated using the Langmuir equation, which can be expressed as

$$c/X = c/X_m = 1/kX_m$$

A plot of c/X against c should give a straight line of slope $1/X_m$; k is a constant that relates to the bonding energy. The Freundlich equation may be written as

$$X = a \times c^{1/n} \text{ or } \log(X) = \log(a) + (1/n) \times \log(c)$$

where a and n are constants, and a plot of log (X) versus log (c) would yield a straight line, and the slope can be calculated as an index for comparison.

Phosphorus Adsorption Index

The phosphorus adsorption index (*PAI*) of Bache and Williams (1971) is calculated from sorption with a working solution of 150 μg P/mL, and is calculated as

$$PAI = X/\log(c)$$

This index is used rather than a simple measure of X at 150 μg P/mL to correct for the different P equilibrium concentrations (c) that will result from differential sorption capacities of soils that are being compared.

Special Considerations

Many researchers have used background electrolyte solutions such as $CaCl_2$ for pH stabilization and to maintain electrochemical neutrality, although Carreira and Lajtha (1997) have shown that this leads to erroneous results in neutral to high pH soils and recommend KCl as a background solution. Strength of the background electrolyte has been shown to affect sorption, and although researchers have used KCl at concentrations that can range from 0.02 mol/L (Bache and Williams 1971) to 0.1 mol/L (Barron et al. 1988) in sorption studies, we here recommend a standard concentration of 0.01 mol/L KCl.

Tiessen et al. (1991) added an interesting twist to this method by determining not only P sorbed from a water solution but also the amount of P that is sorbed strongly enough so as not to be extractable by anion-exchange resin. After pouring off the supernatant following the centrifugation step, resin-extractable P is determined using 30 mL deionized water and two resin strips in the bicarbonate form (*as above*). This resin-extractable P (in μg P/g soil) is subtracted from the calculation of sorbed P as described in "Available P by Laboratory Resin Extraction," above. This is not a standard method, but curves of sorbed P (X) and sorbed P that is not resin-extractable vs. c on the same graphs can be informative.

A problem may arise for soils with high amounts of already sorbed P, such as highly fertilized agricultural soils. Because P that is adsorbed in a soil sample includes both the P sorbed from the equilibration solution and this initially sorbed P, the measurement of initially sorbed P is important if accurate adsorption capacities are to be calculated. However, because the measurement of the initially sorbed P is both difficult and controversial (i.e., there is no general agreement on whether this term should be measured with a resin extract or with stronger extractants such as a weak acid extract), many authors have ignored the initially sorbed P because it is a small percentage of total sorption capacity, or have produced nonlinear sorption curves when initially sorbed P is significant. Bache and Williams (1971) found that a 24 hour measure of isotopically exchangeable P (referred to as E) could accurately

correct for this initially sorbed P in fertilized soils. However, the use of ^{32}P in lab sorption studies will never be routine and thus cannot be recommended as a standard measurement here. This problem with initially sorbed P will be most serious for plotted sorption curves (see the calculation for sorption curves, above), but will not be as significant for the Bache and Williams (1971) single-addition method (see the calculation for phorphorus adsorption index, above), which was created in part to swamp out the effect of initially sorbed P. Thus the Bache and Williams index may prove to be the most useful index for comparing a large number of soils. However, for plotted sorption curves we recommend the analysis of resin P and using this value as E in the calculation for μg P sorbed g^{-1} soil, above.

Total P and Total Element Analysis by Fusion

Various methods for the analysis of total P have been proposed, including both wet and dry digestion techniques. Unfortunately, many of these methods are plagued with poor recoveries for certain types of soils, and thus they are not useful for cross-system comparisons. One fairly common and accurate procedure for total P analysis is fusion with Na_2CO_3 (Brenner et al. 1980; Olsen and Sommers 1982). The lithium metaborate fusion procedure has the advantage of being simpler, and it allows for the simultaneous total analysis of most major and minor ions (Thompson and Walsh 1983). In addition, less expensive graphite crucibles may be used instead of the platinum or nickel crucibles that are used for the Na_2CO_3 fusion, and fusions can be conducted on multiple samples in a muffle furnace rather than by hand, individually, over a flame. Although the Na_2CO_3 fusion technique certainly yields accurate results, we recommend the lithium metaborate fusion procedure here.

Materials

1. Ultrapure graphite crucibles (e.g., from Ultra Carbon, Bay City, MI; 517-894-2911)
2. Soil or rock mill (e.g., Spex mixer-mill) for powdering rock
3. Reagent-grade $LiBO_2$
4. Muffle furnace (small is better than large due to the high heat used in this procedure)
5. Long furnace tongs, furnace gloves, protective eyewear, etc.
6. 10% HNO_3 solution by volume (200 mL conc. HNO_3 in approx. 1 L deionized water, bring to 2 L final volume). Note: if trace elements are to be determined simultaneously, Ultrapure or trace metal grade HNO_3 must be used.
7. 150 mL acid-washed beakers with watch glass lids
8. Stir plates and Teflon-coated stir bars
9. 100 mL or 250 mL acid-washed volumetrics

Procedure

1. All soils must be very finely powdered prior to fusion. Using 10 g soil, 1–2 minutes are required for grinding in most rock mills. Consistency should be that of very fine talcum powder, without contamination by larger particles.

2. Place powdered soil (0.25 g) and 0.75 g lithium metaborate in a prefired graphite crucible. Mix the powders thoroughly with a thin spatula until the color is uniform and independent bands of soil or lithium metaborate cannot be observed.
3. Using extreme caution and heat-resistant gloves, eye protection, and long tongs, place crucibles in a muffle furnace that has been preheated to 1000 °C. Fusion is usually completed in 10–15 minutes and is observed as a glowing white (not red) bead. Fusion time is furnace-specific, and trial runs should be conducted to determine the minimum time required to achieve complete fusion in order to extend crucible life.
4. While fusion is proceeding, place beakers with 50 mL of 10% HNO_3 on individual stir plates with stir bars, and start stirring just before opening the furnace. As each crucible is removed from the furnace, the molten bead is immediately poured into the acid and a watch glass placed over the beaker to reduce evaporation and contamination. The molten bead dissolves within 1–2 hours.
5. Transfer solutions to volumetric flasks and make up to either 100 mL or 250 mL with deionized water, depending on the estimated total P in the soil or the concentrations of the other elements that are being analyzed.
6. Analyze solutions for ortho-P. Standards are made in the same normality of HNO_3 as final sample solutions. If 250 mL volumetrics are used, standards should be made over the range of 0–1000 μg P/mL. Note that these levels are very high, and dilution of solutions will be necessary before ortho-P analysis in most cases.

Calculations

$$\mu g\ P/g\ soil = C \times F$$

where

C = concentration of P in the extract solution as μg P/mL extract
F = mL extract/g dry soil (e.g., 250 mL/0.25 g dry soil)

Special Considerations

For best results, graphite crucibles should be briefly ashed two or three times before use and wiped clean with lint-free Kimwipes. New crucibles with unconditioned walls and older crucibles (those with a reduction in wall thickness of 50% after repeat use) may have a tendency to retain small beads of flux. In most cases these beads can be scraped and added to the 10% HNO_3 solution, but they may not dissolve. For soils rich in Fe, pouring may be difficult and the amount of $LiBO_2$ flux may need to be increased to 1 g.

Platinum crucibles may be used as well, although they are significantly more expensive. In this case, crucibles with sample and flux are heated as described earlier, allowed to cool, and placed whole in the beaker with 10% HNO_3.

Soil P Fractionation

Soil P fractionation is too complicated to recommend as a routine soil procedure; instead, it is a focused research tool that provides insight on the biogeochemistry of P in terrestrial ecosystems. Researchers may wish to examine organic versus inorganic fractions of P in soils, or available versus recalcitrant P fractions, and how soil P fractions change with cultivation (e.g., Hedley et al. 1982; Tiessen et al. 1983) or in different landscape positions (e.g., Lajtha and Schlesinger 1988). Cross-site comparisons might also be useful; extensive soil survey comparisons have already been made (e.g., Tiessen et al. 1984; Cross and Schlesinger 1995).

There is a long history of procedures that have been developed to fractionate phosphorus pools in soils and in sediments. The Chang and Jackson (1957) procedure was used widely for many years and was subsequently modified by Williams et al. (1967). However, there are problems associated with several extractants used in these procedures (for a discussion see Tiessen and Moir 1993), and they are not recommended.

Perhaps the most widely used method in recent years for soil P fractionation is the Hedley fractionation (Hedley et al. 1982), outlined in modified form in Figure 7.1. The Hedley procedure was designed to examine pools of P that have fairly clearly defined chemical properties. Note, however, that this fractionation procedure is evolving, and many modifications have been proposed. For example, many researchers do not include the sonification step, although this pool ranges from 10% to 40% of P extracted by the previous step. The determination of microbial P is often deleted from this procedure in part due to erratic results in acid, highly weathered soils (Potter et al. 1991). Tiessen and Moir (1993) recently suggested a hot concentrated HCl step to follow the dilute HCl step to extract some of the organic P that remains and that is otherwise counted as part of the residual P pool in the original procedure. Perrott (1992) found that where exchangeable Ca was present in soils, precipitation of calcium phosphate and calcium-organic matter complexes occurred during the NaOH extraction; dissolution of this precipitated calcium phosphate during the subsequent acid extraction resulted in an overestimation of Ca-bound P. Perrott (1992) suggested prewashing soils with a buffered NaCl-EDTA solution. Certainly this step should be considered for soils with high concentrations of exchangeable Ca. It is likely that more modifications and caveats to this procedure will appear in the future. Unfortunately, as the fractionation procedure is modified, it becomes difficult to compare results among studies. Moreover, it is virtually impossible to compare results obtained using different fractionation procedures that have used different extractants altogether.

The principles behind the extraction steps in the Hedley et al. (1982) procedure are straightforward. The pool measured by the resin strips is fairly labile inorganic P that is directly exchangeable with the soil solution (see section "Available P by Laboratory Resin Extraction," above). The alkaline bicarbonate extract (Olsen et al. 1954) provides a measure of relatively labile and plant-available P sorbed onto soil surfaces, as the bicarbonate mimics the respiration activity of plant roots and will depress the activity of Ca^{2+} in high Ca soils. This step is more effective in relatively unweathered soils than in tropical soils, however. NaOH extracts amorphous and

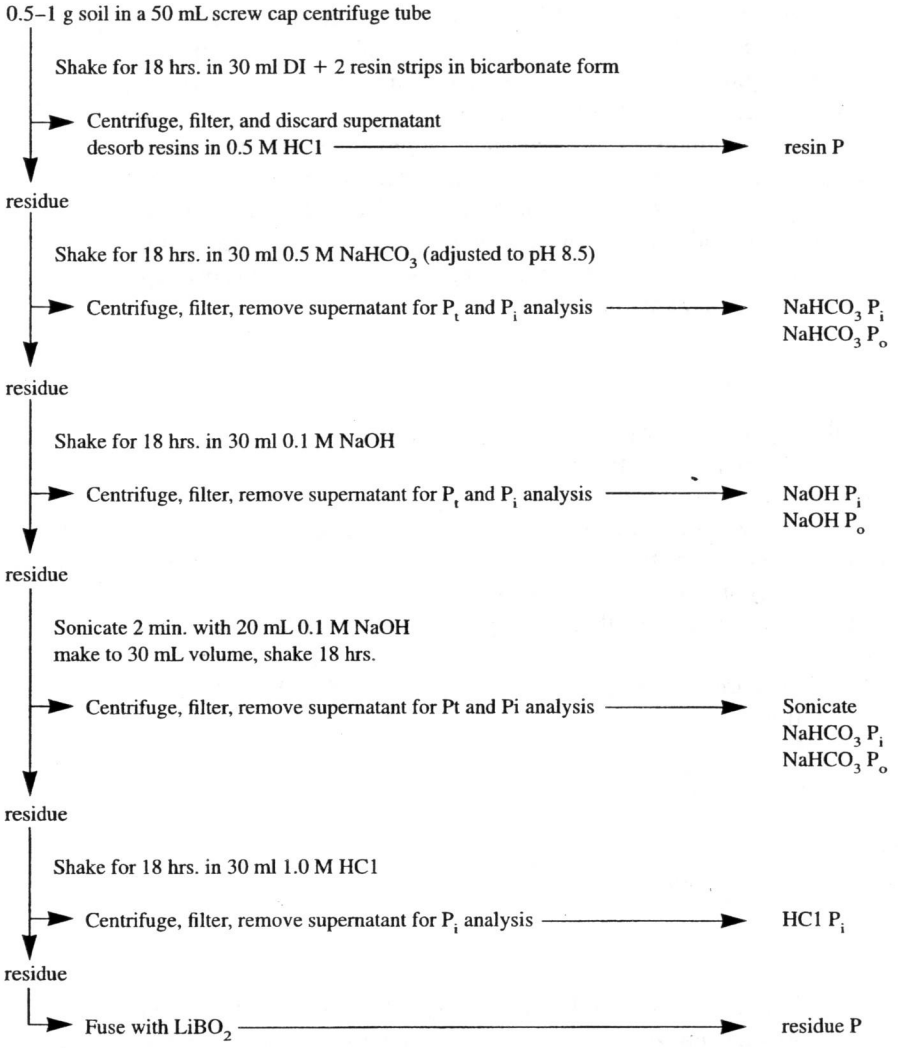

Figure 7.1. Hedley fractionation procedure for soil phosphorus (Hedley et al. 1982).

some crystalline Fe and Al phosphates, as well as P strongly bound by chemisorption to Fe and Al compounds. There is also a large organic pool of P in this fraction in some soils. Ultrasonification with fresh NaOH enables extraction of P held at internal surfaces of soil aggregates. Relatively insoluble Ca-P minerals, including apatite, are extracted with HCl. Residual P is a combination of organic and inorganic forms, and is determined by fusion (see earlier discussion) or as the difference between total P and the sum of the fractions determined.

The question of drying, and grinding, of soils emerges in this procedure as well (see earlier discussion). The original procedure suggested using dried and ground soils, although Potter et al. (1991) noted problems with dried versus field-moist

soils. Lajtha and Schlesinger (1988) found few significant differences between using ground and unground soils for fairly unweathered arid soils. However, most researchers recommend using dried and ground soils to reduce sample heterogeneity, realizing that such pretreatment will influence results. High-clay soils either must be analyzed while still field-moist or, if dried, must be ground. Certainly grinding will release new mineral surfaces, which will be important for poorly weathered soils, and drying will affect microbial pools and estimates of sorbed P in many cases. Because using field-moist soils may be impossible in many cases, we recommend using dried and ground soils for ecosystem intercomparisons—with full knowledge of the associated problems and assumptions.

Materials

1. 50 mL polypropylene centrifuge tubes with screw-cap lids
2. Centrifuge with adapters for the tubes
3. Sonicator, probe type rather than bath
4. Mechanical shaker with basket for holding tubes horizontally
5. 0.45 μ filters with syringe filter holders (e.g., Swinnex or Gelman filter holders) and 50 mL syringes
6. Acid-washed vials with polyethylene or polypropylene screw caps, 20–50 mL capacity
7. Resin strips (prepared as described the section "Available P by Laboratory Resin Extraction," above)
8. 0.5 mol/L HCl
9. 0.5 mol/L $NaHCO_3$ adjusted to pH 8.5
10. 0.1 mol/L NaOH
11. 1.0 mol/L HCl
12. Concentrated HCl (optional)

Procedure

1. Filters must be cleaned before use. This is accomplished either by filtering approximately 150 mL deionized water through the filter just prior to use or, better yet, by soaking all filters in 0.5 mol/L HCl and then rinsing in deionized water several times before use. In the latter case, deionized water should still be passed through the filter prior to use to remove any acid residue. If filters are not dried before use, then about 5 mL of the extract that is being filtered should be passed through the filter and discarded before filtered extract is saved for analysis.
2. Each step outlined in Figure 7.1 is conducted on a separate day. Place 1 g finely ground, oven-dried soil in a centrifuge tube and add 30 mL deionized water and two resin strips converted to the bicarbonate form. Cap tubes and shake gently for 18 hours.
3. Remove resin strips, making sure that soil particles are gently rinsed back into the tube with 1–2 mL deionized water. Swirl tubes gently while vertical to dislodge soil particles lodged in the cap or the tops of tubes, then centrifuge for 10 minutes at 10,000 rpm, or at lower speeds for longer times. Discard the

supernatant, pouring out as much solution as possible by hand without losing soil. Carefully aspirate the remaining supernatant into the syringe, which is then fitted with the filter holder containing a cleaned filter. Soil remaining on the filter is scraped back into the tube or rinsed off with some of the 30 mL of the next extractant, and the tubes are ready for the next extractant. Resins are rinsed thoroughly in deionized water, placed in clean 50 mL tubes with 30 mL 0.5 mol/L HCl, and shaken gently for 2 hours. The resulting solution is saved for analysis.

4. For the rest of the extracts, the supernatant is saved after centrifugation for both dissolved inorganic and dissolved total P analysis (see later discussion). As much suspension-free supernatant as possible is decanted into acid-washed vials, but all cloudy supernatant should be filtered. Remaining supernatant is filtered as described earlier, with soil remaining on the filter returned to the tube.

Special Considerations

The fractions will consist of solutions with different pH values and levels of extracted organic matter. Therefore, solutions should be neutralized before P analysis (see below) or else analysis reagents should be made up to account for the various levels of acidity. In many soils the $NaHCO_3$ and the NaOH fractions will be highly colored. These solutions should be acidified to <pH 2 with a few milliliters of 1 mol/L H_2SO_4 before analysis of inorganic P or the dissolved organic matter will precipitate in the acid conditions of the P reagents. The exact number of milliliters needed should be determined either stoichiometrically or else separately using blank extracting solution, since pH electrodes should never be used in solutions that are to be analyzed. Solutions are refrigerated for 30 minutes and then brought back to room temperature, and aliquots are removed for analysis once organic matter has precipitated and solutions are clear. Alternatively, solutions may be centrifuged.

Analysis of Dissolved Organic P and Total Dissolved P by Acid Persulfate Digestion

Several persulfate digestion procedures can be used for the analysis of total dissolved phosphorus in soil solutions or other aquatic samples, and by difference, dissolved organic P. The principle behind persulfate digestion is to use potassium (or ammonium) persulfate to oxidize dissolved organic P to ortho-P, and total P is measured as soluble reactive phosphorus (see earlier discussion). Organic P is then calculated as total P less inorganic P in the solution. The procedure that will be described here is an acid persulfate digest (Bowman 1989) that should be used only for the oxidation of P (not N) in solutions or extracts. A common alternative when both dissolved organic N and dissolved organic P are to be determined in soil solutions or extracts is the alkaline persulfate digest (Chapter 5, this volume; Ameel et al. 1993; D'Elia et al. 1976).

Johnes and Heathwaite (1992) describe a very fast and simple procedure for the simultaneous digestion of total N and total P in aqueous samples using persulfate

microwave digestion. Because this procedure requires an automated microwave that is adjustable in 1% power increments and specialized Teflon vessels, it will not be described here. However, if the user has access to an automated microwave system (e.g., CEM Digestion Systems), the microwave technique is the easiest and fastest procedure for total dissolved N and P analysis.

Materials

1. 25 mL volumetric flasks (or double all volumes and use 50 mL volumetrics), or block-digestion tubes
2. Potassium persulfate, low-N ($K_2S_2O_8$)
3. 5.5 mol/L H_2SO_4
4. Hot plate or block digestor
5. Organic standards (may include glycerophosphate, ATP, p-nitrophenyl phosphate, sodium inositol hexaphosphate, etc.)

Procedure

1. Pipette an appropriate aliquot or standard (e.g., 1 mL) into a 25 mL volumetric flask or block-digestion tube, then add 0.5 g $K_2S_2O_8$ and 1 mL of 5.5 mol/L H_2SO_4 to each flask. Start with an aliquot of 1 mL sample; increase volume if concentrations are too low, and adjust the normality of the acid reagent accordingly.
2. Digest samples on a hot plate or in a block digestor (approx. 150 °C) for 20–30 minutes. Digestion is complete once vigorous boiling subsides. After solution cools, deionized water is added to volume.
3. Analyze total P as ortho-P after neutralization (see "Inorganic P in Solution," below). The malachite green method is recommended.
4. Two organic P standards, two replicate analyses, two spikes, and two blanks (deionized water) should be brought through the entire procedure for each batch of 25 sample unknowns to control for contamination of reagents and contamination during the digestion process, as well as to check for digestion completion. Spikes are 0.05 μg P/mL (15 μL of the 50 μg P/mL stock, for low-P solutions) or 0.1 μg P/mL (30 μL of the 50 μg P/mL stock, higher P solutions) added to samples to check for recoveries and matrix interferences. When analyzing for ortho-P, regular standards made up in blank solution are run to determine oxidant contamination and volume loss.

Calculations

$$\mu g\ P/mL \text{ in solution} = C \times F$$

where

C = concentration of P in the digestion solution as μg P/mL digest
F = mL digestion solution/mL sample (e.g., 25 mL/1 mL).

Special Considerations

An alternative approach for large numbers of samples or low-P solutions is to use an autoclave as for the alkaline persulfate digestion. In this case, 5 mL sample are added to a 50 mL volumetric or a 40 mL glass screw-top vial. 10 mL of 0.9 mol/L H_2SO_4 and approximately 0.8 g $K_2S_2O_8$ are added. Vials are capped tightly, or volumetrics are covered with tin foil, and are autoclaved at 121 °C and 17 psi for 50 minutes. After cooling, vials are brought to volume with deionized water, and soluble reactive phosphorus (see later) is measured after neutralization. For very low levels of P in solution, more sample solution can be added. The normality of the acid reagent should be adjusted accordingly.

Inorganic P in Solution—Murphy and Riley Procedure

The level of inorganic P in solutions measured by the colorimetric techniques described later is often referred to as soluble reactive phosphorus (SRP) to distinguish it from orthophosphate, or "free" phosphate, in solution. Phosphate sorbed onto Al- or Fe-colloidal complexes may not be directly available to bacteria or to phytoplankton, and perhaps not to roots in soils except over the long term, but this form of P is partially measured by both the Murphy and Riley (1962) procedure and the malachite green procedure due to the high acidity of the reagents. Acid hydrolyzable organic P is also measured with these colorimetric procedures. While this consideration does not represent a problem for ecosystem-level studies of element budgets or fluxes, it has been a major impediment to studies of P cycling using ^{32}P in soils and aquatic ecosystems. Unfortunately, measures of SRP do not provide a good estimate of the size of the unlabeled P compartment because SRP can be 2–10 times the true ortho-P pool.

Several techniques have been developed to try to estimate true "free" ortho-P in solutions, often using plant bioassays or algal cultures (Rigler 1966). Such measures are beyond the scope of this chapter. SRP here is used as an estimate for free ortho-P, with an understanding of the limitations of the results.

Two methods are commonly used to measure SRP in solution, although several others exist. The Murphy and Riley (1962) procedure, as modified by Watanabe and Olsen (1965), is commonly adapted for automated analysis. The manual procedure is given here; most autoanalyzers come with instructions for automated procedures. The malachite green procedure (described next) is significantly more sensitive than the Murphy and Riley (1962) procedure but has the disadvantage that malachite green stains plastic ware, countertops, and skin.

Materials

1. Ammonium molybdate
2. Antimony potassium tartrate
3. Ascorbic acid
4. 50 mL volumetrics for samples

5. 500 mL (2), 1000 mL (1), and 2000 mL (1) volumetrics for reagents
6. Spectrophotometer
7. 50 μg P/mL stock solution: 0.2195 g oven-dried primary standard-grade KH_2PO_4 dissolved in 1000 mL deionized water. Stored in a polyethylene bottle, refrigerated, with a few drops of chloroform.
8. P solution standards. Up to two times the expected maximum concentration of samples is diluted from the primary stock daily.

Procedure

1. The following solutions should be made:
   A. H_2SO_4 (2.5 mol/L). Add 278 mL concentrated H_2SO_4 to approximately 1000 mL deionized water in a volumetric that is partly submerged in cold water. When cool, bring to 2000 mL. This solution is extremely stable.
   B. Ammonium molybdate (40.0 g in 1000 mL deionized water). If kept refrigerated it will precipitate, but the mixture can be shaken and used without problem. This solution is stable for several months.
   C. Antimony potassium tartrate (1.454 g in 500 mL deionized water). This solution is stable.
2. In a 500 mL volumetric, add 250 mL solution A, 75 mL solution B, 2.64 g ascorbic acid, and 25 mL solution C. Solution C reduces interferences from silica and thus is critical for total P analyses, but adding solution C is less critical for freshwater samples. Bring to 500 mL with deionized water. This mixed reagent should be kept in a dark bottle and needs to be made fresh daily; sensitivity is noticeably lower after 8 hours.
3. Many solutions will not be neutral (e.g., resin extracts, total P fusions, most solutions from the fractionation procedure). Neutralization can be accomplished in two ways. In the first method, after 40 mL of the solution is added to the 50 mL volumetric, a few drops of paranitrophenol indicator are added. If the solution is acid, then add 4 mol/L NaOH dropwise until the solution turns yellowish, then add 0.25 mol/L H_2SO_4 dropwise until the solution just turns clear, and make to volume. If the solution is alkaline, just add acid until the solution plus indicator turns clear, and then make to volume. The second method involves adjusting the acidity of the mixed reagent to compensate for the acidity or alkalinity of solutions to be analyzed. Because the final pH of the solution plus reagent is critical for complete color development, the amount of acid added to the final mixture from acidic solutions can be subtracted from the amount of acid added to the final solution by the mixed reagent.
4. To 40 mL of a neutral (pH 5–7.5) solution (see later) in a 50 mL volumetric, add 8 mL of mixed reagent, bring to volume with deionized water, wait exactly 10 minutes, and read at 880 nm (most sensitive) or 660 nm (less sensitive) with a spectrophotometer. For high-P samples, less sample solution and more diluent can be used as long as the final volume remains constant across samples. In many spectrophotometers, path lengths can be increased for greater sensitivity. Note that all volumes can be reduced to save solution and

reagents, but proportions should be maintained. The standard curve should span the range of measured samples.

Special Considerations

It is very easy to contaminate collecting bottles or laboratory equipment with phosphates. Thus any material that will come in contact with solutions that will be analyzed for P, such as collection bottles, filters, and bottles containing reagents, should not be washed with laboratory detergents. In addition, some materials such as Parafilm have been found to contain high concentrations of P; even Parafilm placed over acidic solutions has been shown to contaminate solutions. Bottles and other equipment should be acid-washed before use and rinsed thoroughly with deionized water, and plastic gloves should be worn whenever handling solutions or glassware that will be used for P analyses.

If solutions are highly stained with dissolved organic matter (e.g., alkaline soil extracts), this organic matter needs to be precipitated before analysis or else it will precipitate in the acidic reagents used for analysis. These solutions should be acidified to <pH 2 and refrigerated for 30 minutes; after the cleared sample returns to room temperature, an aliquot is removed for analysis with a pipette from the surface. The exact number of milliliters needed should be determined on a separate blank solution using a pH meter; never use a pH electrode in solution that is to be analyzed. If solutions are only lightly colored, reading at 880 nm rather than 660 will reduce interferences. A nonreagent blank may be run to account for any remaining color in the solution.

Inorganic P in Solution—The Malachite Green Procedure

The malachite green procedure for inorganic P is more sensitive than the Murphy and Riley (1962) procedure and should be used where warranted because of low concentrations. The manual procedure of Ohno and Zibilske (1991) is offered here; an automated procedure can be found in Fernandez et al. (1985) that is fast and simple. Note, however, that there is a misprint in the Fernandez et al. (1985) paper: reagent B should be in 8 mol/L acid, not 4 mol/L as printed. Thus the volume of acid used should be doubled from their directions.

Materials

1. Concentrated H_2SO_4
2. 1000 mL volumetrics
3. 20 mL vials
4. Ammonium para-molybdate
5. Polyvinyl alcohol
6. Malachite green
7. Hotplate
8. 50 μg P/mL stock solution: 0.2195 g oven-dried primary standard-grade

KH_2PO_4 dissolved in 1000 mL deionized water. Store in a polyethylene bottle, refrigerated, with a few drops of chloroform.

9. P solution standards. Up to two times the expected maximum concentration of samples is diluted from the primary stock daily.

Procedure

1. Prepare reagent 1: Add 106 mL concentrated H_2SO_4 to 500 mL deionized water—never the reverse—in a 1000 mL volumetric flask. Dissolve 17.55 g ammonium para-molybdate in the acid and bring to volume.
2. Prepare reagent 2: Heat approximately 800 mL deionized water to 80°C, and add 3.5 g polyvinyl alcohol and stir until dissolved. Add 0.35 g malachite green and stir until dissolved. Cool to room temperature and dilute to 1000 mL with deionized water.
3. Pipette 10 mL of sample or standard into a 20 mL vial. Add 2 mL of reagent 1 and mix. After 10 minutes add 2 mL reagent 2 and mix. After 30 minutes read absorbance at 630 nm in a 20 mm cuvette and compare with standard curve.

Special Considerations

See the description of the Murphy and Riley (1962) procedure in the section "Inorganic P in Solution" (above) for procedures to use for samples that are highly colored with organic matter.

Summary

The study of P cycling and availability in soils has been hampered by the difficulty

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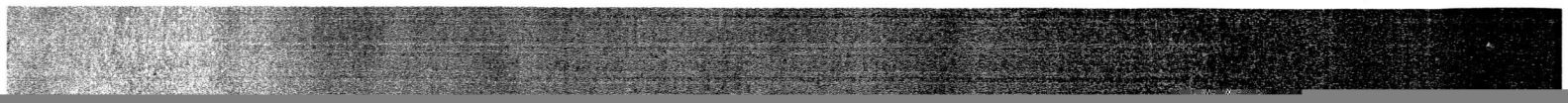

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STANDARD SOIL METHODS

FOR

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