Phosphorus Dynamics in the Environment[☆]

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Glossary

Adsorption The physical adherence or bonding of organic and inorganic phosphate ions onto the surfaces of soil or mineral particles.
Assimilation (transitory immobilization) The process of incorporating nutrients into cellular biomass.
Authigenic Formed *in situ* rather than by having been transported or deposited in a location through secondary processes.
Chelation The reversible binding (complexation) of a ligand to a metal ion.
Decomposition The conversion of organic matter into simple inorganic compounds.
Diagenesis The process by which sediment undergoes chemical and physical changes during its lithification.
Eolian transport Advection of material via the atmosphere or wind.
Eutrophication Excessive nutrients in a body of water that causes a dense algal growth, followed by algal decomposition, anoxia, and impairment of the aquatic community.
Flux (phosphorus) Rate of flow of phosphorus per unit area, which has the dimensions [quantify] · $[\text{time}]^{-1} \cdot [\text{area}]^{-1}$ (used for describing phosphorus turnover within a reservoir of flow from one reservoir to another.
Immobilization The process by which labile phosphorus is sequestered and removed from the environmental reservoir of bioavailable phosphorus for a period of time.
Inorganic phosphorus A class of chemical compounds that comprises those not existing in or derived immediately from living organisms (e.g., phosphate).
Mineral formation Processes in which anions react with cations in the environment to form insoluble precipitates.
Mineralization The conversion of organic matter into simple inorganic compounds by enzymes.
Mycorrhiza A symbiotic relationship between fungi and higher plants.
Nutrient limitation The cessation of photosynthetic biomass production in response to low availability of a physiologically important nutrient or nutrients.
Organic phosphorus A class of chemical compounds that comprises those existing in or derived from living organisms.
Orthophosphate Form of orthophosphoric acid (H_3PO_4) in which three protons have been lost (i.e., PO_4^{3-}).
Oxidation state The total number of electrons that an atom either gains or losses in order to form a chemical bond with another atom.
Phosphate Form of orthophosphoric acid (H_3PO_4); will be found as $H_2PO_4^-$ or HPO_4^- at pH 4–9, the pH range most common in nature.
Primary production The conversion of radiant or chemical energy into organic matter.
Productivity The rate at which radiant energy is used by producers to form organic substances as food for consumers.
Redox cycling Chemical reactions whereby the oxidation state of a molecule is either increased (oxidation) or decreased (reduction).
Soil solution The liquid phase of soil, surrounding mineral particles, from which plants and microbes draw nutrients.
Solubilization The conversion of insoluble compounds into soluble compounds.
Weathering Processes by which rocks, minerals, and soils are transformed through physical or chemical processes.

Defining Statement

This review includes a discussion of the global phosphorus cycle, including sources, sinks, and transport pathways of phosphorus in the environment, microbially-mediated transformations of phosphorus and their genetic regulation, and a discussion of microbial responses to anthropogenic changes to the phosphorus cycle.

Introduction

Phosphorus is an essential nutrient for all living organisms, and the phosphorus cycle is an important link between Earth's living and non-living entities. The availability of phosphorus strongly influences primary production, the process by which

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photosynthetic organisms fix inorganic carbon into cellular biomass. Therefore, knowledge of the phosphorus cycle is critically important for understanding the global carbon budget and hence how biogeochemical cycles impact and are influenced by global climate.

The Global Significance of the Phosphorus Cycle

Natural assemblages of microbes have a critical role in the phosphorus cycle because they forge a link between phosphorus reservoirs in the living and non-living environment. Microbes facilitate the weathering, mineralization, and solubilization of non-bioavailable phosphorus sources, making phosphate available to microbial and plant communities and hence to higher trophic levels within the food web. However, microbes also contribute to immobilization of phosphorus, a process that diminishes bioavailable phosphorus by converting soluble forms to insoluble forms. The mechanisms of microbial involvement in these processes vary from passive (e.g., resulting from microbial metabolic byproducts) to highly regulated active contributions (e.g., the regulation of gene expression in response to environmental cues).

Microbially-mediated processes also link the phosphorus cycle to the carbon cycle and hence to global climate. During photosynthesis, photoautotrophs incorporate phosphorus and carbon at predictable ratios, with approximately 106 carbon atoms assimilated for every phosphorus atom for marine photoautotrophs. The phosphorus cycle therefore plays an important role in regulating primary productivity, the process in which radiant energy is used by primary producers to form organic substances as food for consumers, as in photosynthesis.

Because phosphorus is required for the synthesis of numerous biological compounds, its availability in the environment can limit the productivity of producers even when other nutrients are readily available. This fact carries important implications for the global carbon budget because the rate of incorporation (fixation) of carbon dioxide into photosynthetic biomass can be directly controlled by the availability of phosphorus; if phosphorus is not available, carbon dioxide fixation is halted. The intersection of the phosphorus and carbon cycles is of particular significance for global climate, which is affected by atmospheric carbon dioxide levels. Hence, through the growth of producers the phosphorus cycle contributes to the regulation of global climate.

In aquatic environments, photosynthetic microbes (i.e., phytoplankton) may comprise a substantial portion of the photosynthetic biomass and hence primary production. Marine and freshwater phytoplankton are characterized by extensive biodiversity and, as a group, inhabit an incredible number of different niches within aquatic environments. Phytoplankton, like other microbes, have strategies that enable them to adapt to changes in the amounts and forms of phosphorus available within their environment. For example, the production of phosphatase enzymes, which hydrolyze complex phosphorus compounds to simple, bioavailable inorganic phosphate, helps to mediate the mineralization of organic phosphorus compounds in surface waters, and allows cells to adapt when phosphate levels are low.

Recent estimates suggest that phytoplankton are responsible for as much as half of global carbon fixation, thereby contributing significantly to the regulation of Earth's climate. Phytoplankton productivity is strongly influenced by nutrient availability, and the nutrient that ultimately limits production depends on (1) the relative abundance of nutrients and (2) the nutritional requirements of the phytoplankton. With some exceptions, production in most lakes is limited by the availability of phosphorus, whereas limitation of primary production in the ocean has traditionally been attributed to nitrogen, another nutrient required by cells in large quantities. However, phosphorus differs from nitrogen in that the major source of phosphorus to natural aquatic environments is the weathering of minerals on land that are subsequently introduced into water bodies by fluvial and eolian sources. In contrast, microbially-mediated nitrogen fixation, in which bioavailable forms of nitrogen are generated from nitrogen gas in the atmosphere, is a major pathway by which phytoplankton gain access to nitrogen (in addition to continental weathering). Because there is no phosphorus input process analogous to nitrogen fixation, both marine and terrestrial productivity over geological time scales are considered to be a function of the supply rate of phosphorus from weathering and the rate at which phosphorus is recycled in soil or aquatic systems. Accordingly, the phosphorus cycle influences phytoplankton ecology, productivity, and carbon cycling in both marine and freshwater ecosystems as well as those of natural and agriculture vegetation systems on land.

Characterizing and Measuring Environmental Phosphorus Pools

Occurrence of elemental phosphorus in the environment is rare, given that it reacts readily with oxygen and combusts when exposed to oxygen; thus, phosphorus is typically found in nature bound to oxygen. Phosphorus has the chemical ability to transfer a 3s or p orbital electron to a d orbital, permitting a relatively large number of potential configurations of electrons around the nucleus of the atom. This renders the structures of phosphorus-containing molecules to be quite variable. These properties are likely responsible for the ubiquity and versatility of phosphorus-containing compounds in biological systems.

Because phosphorus exists in many different physical and chemical states in the environment, specific definitions are needed to clarify different parts of the phosphorus pool. Chemical names reflect the chemical composition of the phosphorus substance in question, whereas other classifications are based on methodological aspects of how the substance is measured. For example, "orthophosphate" is a chemical term that refers specifically to a phosphorus atom bound to four oxygen atoms, forming the orthophosphate molecule (PO_4^{3-}); at pH 4–9, the range typically found in nature, this would exist as $H_2PO_4^-$ or HPO_4^{2-} (which are referred to as phosphate). In contrast the aquatic term "soluble reactive phosphorus," (SRP), is a methodological term referring to everything that gets measured when a phosphate assay is performed (such as the molybdate blue or ascorbic acid method,

described below). The majority of measured SRP comprises phosphate but other forms of phosphorus may be included as well due to experimental inaccuracy. Therefore, while SRP tends to closely reflect the amount of phosphate in a sample, the values may not be identical.

The most common assay for measuring SRP is the ascorbic acid method, which is approved by the US Environmental Protection Agency for monitoring phosphate in environmental samples. In this method, ascorbic acid and ammonium molybdate react with dissolved phosphate in the sample, forming a blue compound that can be observed visually or determined spectrophotometrically. Assays for measuring total phosphorus are also based on the ascorbic acid method but begin with a step to transform all phosphorus in the sample to phosphate (typically through digestion by heating the sample in the presence of acid). Following digestion, the sample is analyzed by the ascorbic acid method.

When phosphorus is measured in water samples, distinguishing forms that are part of particulate matter from those that are in solution is often useful. Phosphorus is therefore classified as "soluble" or "particulate". Soluble phosphorus includes all forms of phosphorus that are distributed in solution and that pass through a filter with a given pore size (typically 0.45 μm), while the particulate fraction is the amount retained on the filter. Measurements of soluble phosphorus include both the "dissolved" and "colloidal" fractions. Dissolved phosphorus includes all forms that have entered a solute to form a homogenous solution. By contrast, colloidal forms include any tiny particles that are distributed evenly throughout the solution but that are not dissolved in solution. Soluble phosphorus is commonly reported because colloidal phosphorus particles are very small and differentiating between colloidal and dissolved phosphorus is methodologically difficult.

In soils, soil test phosphorus (STP) is a similar measurement to SRP, in that it measures labile or easily accessible phosphorus in the soil solution surrounding soil particles. There are several different methods to measure STP, each of which involves extracting a soil sample with an extractant, then measuring phosphate in solution with a colorimetric method such as the ascorbic acid method. The extractant will depend on soil pH, among other factors, and includes Bray P1 (dilute hydrochloric acid and ammonium fluoride), for low pH soils, and Olsen P (dilute sodium bicarbonate) for higher pH soils. Although STP is sometimes referred to as "available phosphorus", this is not correct; these tests are designed to predict a crop response to phosphate fertilizer. Other methods to estimate soil bioavailable phosphate include placing anion exchange resins into soil for set periods of time. Total soil phosphorus is determined by digestion, as previously described for water, as is total plant phosphorus. The phosphorus contained in living soil microbial biomass is determined in extracts before and after soil fumigation with chloroform; the chloroform will kill any living organisms, causing them to release phosphorus into the extract.

When living organisms assimilate phosphorus into their cells, the resulting phosphorus-containing compounds are collectively called "organic phosphorus." It must be stressed that this definition of the biologically-associated phosphorus as "organic phosphorus" is not identical to the chemical definition of organic compounds (e.g., containing carbon). For example, some intracellular biologically-synthesized compounds such as polyphosphate may not contain carbon bonding. The term organic phosphorus encompasses molecules within living cells as well as molecules that are liberated into the environment following decay of organisms. The major source of terrestrial organic phosphorus is plant material, which is released as vegetation decomposes, but microbial and animal sources also contribute significantly. In the marine environment, organic phosphorus comes from a variety of sources (e.g., plankton, fish excrement, advection from land, etc.), the relative contributions of which differ widely depending on location in the ocean. In contrast to organic forms, inorganic phosphorus compounds are not always directly of biogenic origin. Rather, they also include phosphate derived from the weathering of phosphorus-containing minerals, including soluble and particulate phosphate.

Although phosphate can be identified and quantified by simple colorimetric techniques, identifying and quantifying more complex phosphorus forms requires the use of advanced techniques and instruments, such as mass spectrometry or ^{31}P nuclear magnetic resonance (NMR) spectroscopy. These more complex phosphorus forms are discussed in detail in the section on Immobilization, below.

Phosphorus Sources, Sinks, and Transport Pathways

The phosphorus cycle encompasses numerous living and non-living environmental reservoirs and various transport pathways. In tracing the movement of phosphorus in the environment, the interplay between physical and biological processes becomes apparent. In addition to acting as reservoirs of phosphorus in the environment (as discussed in this section), microbes contribute to the transformation of phosphorus within other reservoirs such as in soil or aquatic environments (discussed below in Section "Microbially-Mediated Processes").

Within the earth's crust, the abundance of phosphorus is 0.10%–0.12% (on a weight basis), with the majority of phosphorus existing as inorganic phosphate minerals and phosphorus-containing organic compounds. A phosphate mineral is any mineral in which phosphate anion groups form tetrahedral complexes in association with cations, although arsenate (AsO_4^{3-}) and vanadanate (VO_4^{3-}) may also substitute into the crystalline structure. Apatite is the most abundant group of phosphate minerals, comprising hydroxyapatite, fluorapatite, and chlorapatite (**Table 1**). These three forms of apatite share nearly identical crystalline structures, but differ in their relative proportions of hydroxide, fluoride, and chloride, each being named for the anion that is most abundant in the mineral. Phosphate minerals generally form in the environment in magmatic processes or through precipitation from solution (which may be microbially-mediated), and the chemical composition of the minerals depends on the ion or ions present in solution at the time of precipitation. For this reason, it is not uncommon for natural deposits of phosphate minerals to

Table 1 Phosphate minerals and their chemical compositions. Apatite is the general term for the three minerals hydroxylapatite, fluorapatite, and chlorapatite

Apatite	*$Ca_5(PO_4)_3(F,Cl,OH)$*
Hydroxylapatite	$Ca_5(PO_4)_3OH$
Fluorapatite	$Ca_5(PO_4)_3F$
Chlorapatite	$Ca_5(PO_4)_3Cl$
Frankolite	$Ca_{10-a-b}Na_aMg_b(PO_4)_{6-x}(CO_3)_{x-y-z}(CO_3F)_y(SO4)_zF_2$
Lazulite	$(Mg,Fe)Al_2(PO_4)_2(OH)_2$
Monazite	$(Ce,La,Y,Th)PO_4$
Pyromorphite	$Pb_5(PO_4)_3Cl$
Strengite	$FePO_4 \cdot 2H_2O$
Triphylite	$Li(Fe,Mn)PO_4$
Turquoise	$CuAl_6(PO_4)_4(OH)_8 \cdot 5H_2O$
Variscite	$AlPO_4 \cdot 2H_2O$
Vauxite	$FeAl_2(PO_4)_2(OH)_2 \cdot 6H_2O$
Vivianite	$Fe_3(PO_4)_2 \cdot 8H_2O$
Wavellite	$Al_3(PO_4)_2(OH)_3 \cdot 5H_2O$

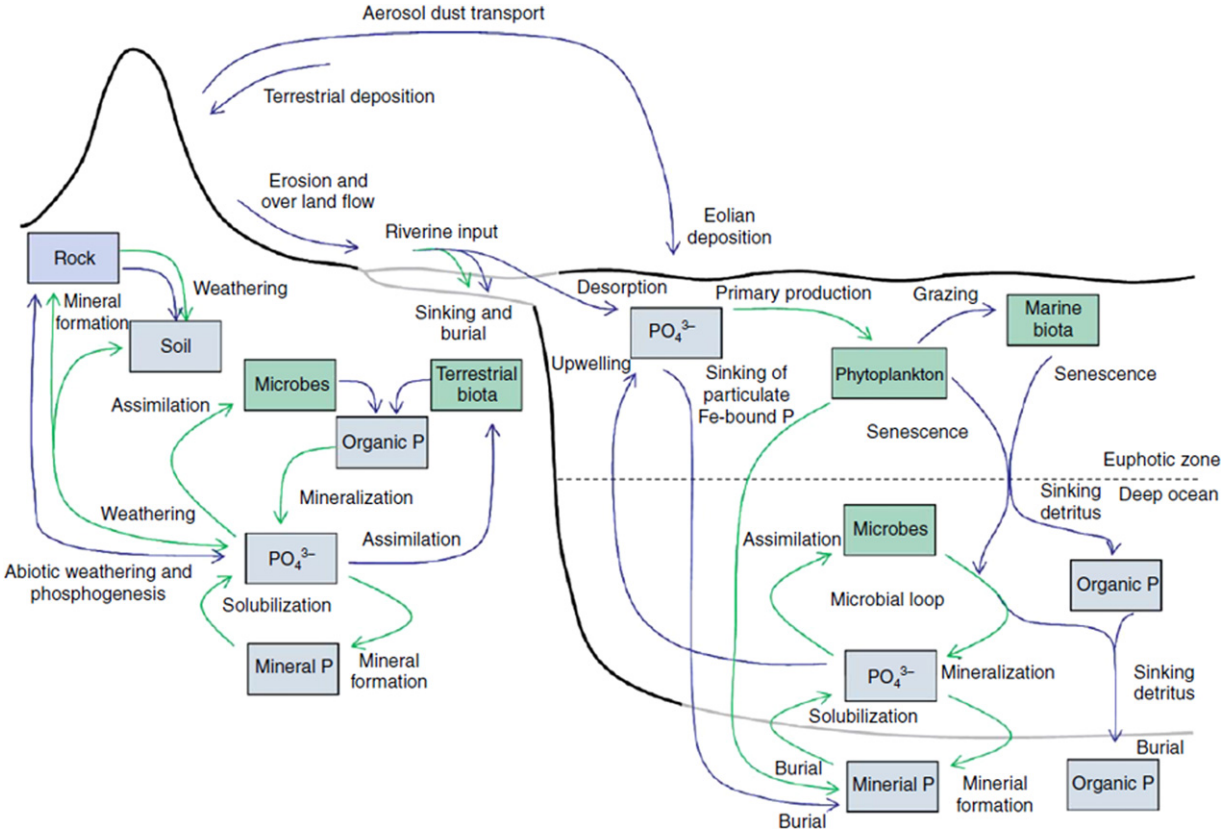

Fig. 1 Schematic diagram of the phosphorus cycle showing phosphorus reservoirs (living in green boxes; non-living in blue boxes), physical transport pathways (blue arrows), and microbially-mediated transformations (green arrows).

be heterogeneous, rather than composed of a single type of phosphate mineral. These natural deposits of phosphate minerals are collectively called "phosphorites" to reflect variations in their chemical compositions.

Soils and lake sediments are another terrestrial reservoir of phosphorus (Fig. 1). The phosphorus in recently formed soils will resemble the parent material on which the soil was formed, namely apatite and other phosphate minerals. Over time, the soil forming factors of climate (rainfall and temperature), topography and biota will transform phosphorus into other forms in conjunction with soil development. In warm, high-rainfall environments such as the tropics, cations will be lost with leaching and the soil pH will drop. Phosphorus forms in these soils will shift from associations with calcium and magnesium to associations with iron and aluminum. Phosphorus in soil is chemically controlled by precipitation with cations such as calcium and

magnesium (here termed 'mineral formation'), by adsorption (the physical adherence or bonding of organic and inorganic phosphate ions onto the surfaces of soil or mineral particles) onto iron or aluminum oxyhydroxides, and by microbially-mediated mineralization of organic phosphorus. Mineralization is discussed in detail in the Section "Microbially-Mediated Processes".

Plants and microbes remove phosphate from the soil solution surrounding mineral particles. This creates a gradient, and the soil solution phosphate is replenished by desorption from mineral surfaces, by dissolving precipitated phosphorus forms or by mineralizing organic phosphorus. Plants draw inorganic phosphorus from lower soil depths and convert it to organic forms. These will be deposited on the soil surface and mixed into the soil by organisms such as earthworms. Over time, phosphorus will become stratified in soil in undisturbed natural ecosystems, with higher concentration and more organic forms at the soil surface and lower concentrations and more inorganic forms with depth. Land use can alter the forms and distributions of phosphorus in soils. Soils managed as croplands may have phosphorus added as fertilizers and pesticides, and will have phosphorus removed from crops, while animals grazing in pastures add phosphorus as urine and feces and remove phosphorus as they eat plant material. Management practices such as tillage will redistribute phosphorus forms within the profile, while fire can convert organic phosphorus to inorganic phosphate.

Marine and lake sediments also represent an important phosphorus reservoir, but because the physical and chemical factors affecting marine sediment differ considerably from those on land, some processes controlling phosphorus dynamics in aquatic sediments are different than for soils, although many are the same. In sediments, phosphate can be present in insoluble inorganic phosphates minerals (such as phosphorites), which are relatively immobile. Phosphate can also be adsorbed onto iron or manganese oxyhydroxides. The adsorbed phosphate can regain mobility in response to changes in the redox potential at the sediment-water interface and thus is considered more mobile. As in soil, phosphorus in aquatic detrital organic matter can also become remobilized as decomposition progresses through microbially-mediated processes.

Biota (i.e., microbes, plants, and animals) serve as another reservoir of phosphorus in the environment, as they assimilate phosphorus within their cellular biomass. Biota can contribute significantly to environmental phosphorus levels; for example, microbial communities contribute 0.5%–7.5% of total phosphorus in grassland and pasture topsoil, and up to 26% in indigenous forests. Microbes are also responsible for generating the myriad of organic phosphorus compounds found throughout the environment. In particular, microbes and primary producers play an important role in providing nutrition, including phosphorus, to higher trophic levels by making it biologically available (bioavailable). Phosphorus assimilation is a microbially-mediated process discussed in the "Transitory immobilization" section below.

Phosphorus is transported within the environment through various mass transfer pathways. For example, rivers are important in the phosphorus cycle as both reservoirs and transport pathways. Phosphorus that has weathered from minerals and leached or eroded from soils enters rivers through a variety of vectors, including dissolved and particulate forms in water from overland flow and in groundwater, and particulates brought by wind. Approximately 95% of phosphorus in rivers is particulate, and approximately 40% of that is bound within organic compounds. Rivers influence the distribution of phosphorus in soils and lakes by contributing or removing phosphorus, and riverine input is the single largest source of phosphorus to the oceans.

A number of outcomes are possible for phosphorus entering the ocean. Much of the phosphorus entering from rivers is trapped in near-shore areas of the ocean, such as continental margins and estuaries, through immediate sedimentation and biological assimilation. The remaining phosphorus enters the dynamic surface ocean, also called the euphotic zone, in which nearly all bioavailable phosphorus is sequestered within biota through primary production. Upon death of the organisms, a fraction of the biologically-sequestered phosphorus sinks below the euphotic zone and most of it is regenerated into bioavailable forms like phosphate by heterotrophic organisms. This recycling is part of the so-called "microbial loop". Physical processes such as upwelling and deep convective mixing draw the deep water, which in most parts of the ocean is nutrient rich compared to the surface waters, back into the euphotic zone where up to 95% of it is re-used in primary production. The remainder is removed from the ocean reservoir through particulate sedimentation, mineral formation (which may be microbially-mediated), and scavenging by iron and manganese oxyhydroxides, all of which deposit phosphorus as a component of ocean sediment.

The phosphorus cycle differs from the cycles of other biologically-important elements, such as carbon, nitrogen, and sulfur, in that it lacks a significant gaseous component; nearly all phosphorus in the environment resides either in solid or aqueous forms. The one exception to this rule is the volatile compound phosphine (PH_3, also called phosphane), a colorless, poisonous gas formed in the environment from the breakdown of alkali metal or alkali earth metal phosphides with water. This process is poorly characterized and likely comprises various multistage chemical reactions. Microbially-mediated phosphine production can be a major source of the gas in engineered systems (e.g., sewage treatment facilities and constructed wastewater treatment wetlands) where organic phosphorus is abundant and reducing conditions are common, suggesting that microbes could also play a roll in phosphine formation in natural systems (although direct enzymatic production of phosphine has not yet been identified). While phosphorus can exist as phosphine, the gas does not persist in the environment due to rapid autoxidation, preventing significant accumulation of phosphine in the atmosphere. Phosphine is therefore a minor component of the environmental phosphorus pool.

The absence of a significant gaseous phase does not eliminate the atmosphere as an important reservoir in the phosphorus cycle. When weathering and erosion of soil generate inorganic and organic particulate phosphorus, wind transports some of the particles from their source to a new location. These particles can include mineral dust, pollen and plant debris, insect fragments, and organic phosphorus bound to larger particles. This distribution of terrestrial particulate phosphorus, termed eolian deposition, plays an important role in delivering nutrients to the oceans. In ocean waters where nutrient levels are naturally low, such as in the open ocean gyres where riverine inputs do not extend and significant upwelling does not occur, eolian deposition may

comprise a large portion of the nutrient flux that is available for primary production. The eolian phosphorus flux to the oceans is approximately 1×10^{12} g yr^{-1}, of which approximately half is organic and half is inorganic. The solubility, and therefore bioavailability, of the phosphorus in eolian particulate matter differs significantly depending on its source; however, estimates suggest that approximately 15%–50% is typically soluble.

Microbially-Mediated Processes

Immobilization

Immobilization refers to the process by which phosphorus is sequestered and removed from the environmental reservoir of labile phosphate for some time. Immobilization processes can generally be grouped into two categories. The first category, transitory immobilization or cellular assimilation, includes all processes that sequester phosphorus within living microbial cells and is rapidly reversible upon cell death, although some phosphorus may be incorporated into non-labile organic compounds. The second category, mineral formation, encompasses processes that generate phosphorus-containing minerals.

Transitory immobilization

Transitory immobilization, or assimilation, is an important mechanism of phosphorus sequestration in soil and aquatic environments. Within cells, phosphorus is incorporated in numerous essential biological-molecules and is required in larger quantities than many other elements. However, unlike other biologically important nutrients such as nitrogen and sulfur that must first undergo reduction prior to being incorporated into the cell, phosphorus remains oxidized before and after assimilation. Because mineralization of cellular material occurs rapidly following cell death, cellular assimilation of phosphorus into biological macromolecules leads to relatively short-term phosphorus retention in living cells where the duration is related to the characteristics of the microbial community in question, and to the nature of the compounds produced. Within the cellular reservoir, phosphorus is present as different compounds that serve various functions.

Complex phosphorus compounds are generally classified based on the bonding of phosphate (Fig. 2). Phosphomonoesters are compounds in which one phosphate group is linked to one carbon atom through an ester bond (through oxygen), phosphodiesters have one phosphate group linked to two separate carbon atoms, each through an ester bond, and phosphonates have one

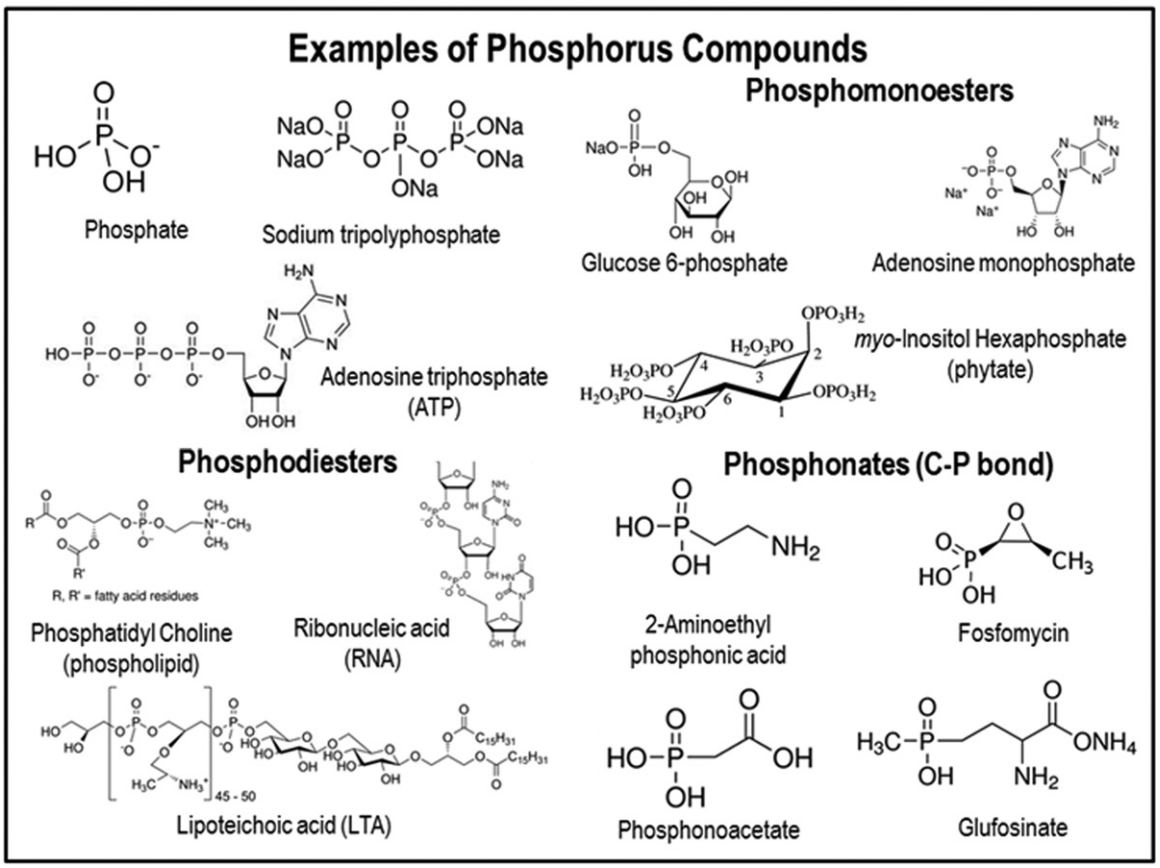

Fig. 2 Examples of phosphorus containing compounds associated with microbes.

carbon-oxygen-phosphorus bond replaced by a direct carbon-phosphorus bond. Phosphoramidates contain a nitrogen-phosphorus bond in place of a carbon-oxygen-phosphorus bond; however, these are rare compared to other phosphorus compounds, and are poorly studied for microbes, and will not be discussed further in this article. Polyphosphates have phosphate groups linked by energy-rich phosphoanhydride bonds.

Phospholipids, lipids in which a phosphate group has replaced one of the fatty acid groups, are phosphodiesters. Lipids are generally hydrophobic; however, phospholipids are amphipathic, meaning that each molecule has a hydrophilic portion and a hydrophobic portion. The phosphate group is responsible for giving phospholipids their partially hydrophilic character, hence imparting a wide range of biochemical properties. In the cell, phospholipids are important in the formation of biological membranes and in some signal transduction pathways. The cell walls of gram-positive bacteria such as *Bacillus subtillis* and *Staphylococcus aureus* contain lipoteichoic acid (LTA), which contain long chains of ribitol phosphate or glycerol phosphate. Gram-positive bacteria cause many human infections (e.g., staph infections), which is linked to LTA. Phosphonolipids have phosphonates attached to lipids instead of phosphates. Phosphonolipids are wide-spread in nature; one example is 2-acyloxyethylphosphonate, which has been isolated from blooms of the aquatic cyanobacterium *Aphanizomenon flos-aquae*.

Nucleotides are biological compounds consisting of a pentose sugar, a purine or pyrimidine base, and one or more phosphate groups. Nucleotides are the structural subunits (monomers) of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), and alternating bonds between the sugar and phosphate groups form the backbones of these nucleic acids. The phosphate groups form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings, imparting directionality to the molecule. In addition, nucleotides are present as several major cofactors in the cell (e.g., flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide phosphate (NADP), etc.) that have important functions in cell signaling and metabolism.

Adenosine triphosphate (ATP) is a nucleotide that performs multiple functions of considerable importance to the cell and contains polyphosphate and phosphomonoester bonds. The primary function of ATP is to aid in intracellular energy transfer by storing energy that is generated during photosynthesis and respiration so that it can be used in other cellular processes that require energy (e.g., cell division and biosynthetic reactions). In the cell, phosphate can be assimilated in ATP through substrate-level phosphorylation, oxidative phosphorylation, photo-phosphorylation, and via the adenylate kinase reaction. ATP is also active in signal transduction pathways, where it can be used as a substrate for kinase enzymes in reactions that transfer phosphate groups to proteins and lipids, forming phosphoproteins and phospholipids respectively. Phosphorylation is an important and ubiquitous form of signal transduction in many organisms.

Inositol phosphates are phosphomonoesters and are complex molecules in which one to six phosphates are bonded with carbons on the inositol ring. Inositol phosphates with six phosphate groups, inositol hexaphosphates (IHP) are more common in nature than those with fewer phosphate groups, and the best-known and studied of these is the *myo*-IHP stereoisomer phytate. This compound is widely distributed in nature because it is a storage compound produced by higher plants, especially in seeds. Phytate is a highly reactive compound that forms stable complexes with a variety of mineral cations, and so can accumulate in soils. There is no evidence that phytate is synthesized by microbes. However, other IHP stereoisomers have been detected in soils and lake sediments, including *chiro*-, *scyllo*- and *neo*-IHP, and are thought to be produced by microbes by direct synthesis or by microbial transformation of phytate.

Other phosphomonoesters include intermediaries in many biochemical pathways, such as phosphoproteins and sugar phosphate such as phosphoenol pyruvate and glucose 6-phosphate, as well as mononucleotides such as adenosine monophosphate.

Phosphonates are widespread in terrestrial and aquatic environments. However, their role is poorly understood, but the C-P bond is thought to be more resistant to degradation than the more common ester bonds. In addition, some microbially-synthesized phosphonates have anti-microbial properties, providing protection. Some of these have been developed for commercial use. For example, some soil *Streptomyces* species produce glufosinate (phosphinothricin), which is used as a herbicide, while other soil *Streptomyces* species produce fosfomycin, which is used as a broad-spectrum antibiotic. In oceans, dissolved phosphonates have been shown to comprise up to a quarter of all high molecular weight dissolved organic phosphorus. Although the microbial sources of phosphonates are not fully understood, two genera of cyanobacteria and one species of *Thaumarchaeon* have been definitively implicated.

In addition to biological molecules like nucleic acids and phospholipids, which by weight consist primarily of carbon along with smaller portions of phosphorus, the intracellular accumulation of polyphosphate granules in microbial cells is an important type of transitory phosphorus immobilization. Polyphosphates form through the activity of polyphosphate kinase enzymes, and therefore represent a highly concentrated reservoir of phosphorus in the cell. The energy stored in the anhydride bonds can be used by the microbial cell during periods of starvation. Polyphosphates may also serve as ligands for metal cations such as calcium, aluminum, and manganese in some microbes; however, the extent and elemental stoichiometry of intracellular polyphosphate cation complexation varies among organisms. Short-chain polyphosphates, with only two phosphate groups, are called pyrophosphate.

Phosphorus immobilized within cells is considered transitory because many phosphorus compounds can rapidly reenter the reactive phosphate pool following cell death, through microbially-mediated mineralization processes. The immobilization of phosphorus via cellular assimilation is therefore relatively brief (i.e., within the lifetime of a cell) compared to other processes in the phosphorus cycle, some of which occur over geological time scales. However, the strong adsorption to mineral surfaces in soils and sediments by some phosphorus compounds such as IHP stereoisomers and DNA will increase their immobilization.

Phosphate mineral formation

Phosphate mineral formation represents another phosphorus sink that is influenced by microbial activity; however, it encompasses processes other than cellular assimilation and short-term storage of phosphorus as biological molecules. In mineral formation (also called phosphogenesis), phosphate anions react with cations in the environment to form insoluble precipitates. Sediments that comprise significant amounts of phosphorus-containing minerals are called "phosphorites," and may contain apatite, francolite, and other phosphorus minerals. Mineral formation is generally an important mechanism of phosphorus sequestration in marine environments, where the high seawater calcium levels facilitate microbially-mediated formation of insoluble phosphorites. The sequestration of phosphorus by this process retains (immobilizes) phosphorus for longer periods of time than does transitory immobilization.

In order for an insoluble mineral to form, the concentration of the ions forming the mineral should be high enough such that super saturation is reached, and equilibrium of the precipitation reaction is shifted toward the product. In addition, the physical and chemical characteristics of the environment must be conducive to precipitation of that mineral based on its solubility characteristics, and factors such as pH, redox state, and concentrations of co-occurring ions all influence mineral precipitation. The mineral should also be stable in the environment for mineral formation to constitute a long-term sink for phosphorus.

Mineral formation occurs both authigenically and diagenetically in the environment. Authigenic mineral formation is the formation of insoluble precipitates (minerals) *in situ* rather than by having been transported or deposited in a location through secondary processes. In contrast, diagenetic mineral formation is the alteration of existing minerals by chemical changes occurring after the initial deposition of a mineral (i.e., during or after burial and lithification). The primary type of diagenetic phosphorus mineral formation in marine environments is the substitution of phosphate into calcium carbonate minerals such as calcite or aragonite. Microbes contribute to this process by mineralizing organic phosphorus to reactive phosphate, which then substitutes diagenetically into calcium carbonate.

In authigenic mineral formation, microbes may generate reactive phosphate from the mineralization of organic phosphorus sources, and the resulting localized high phosphate concentrations favor precipitation of phosphate minerals. In soils, microbes convert organic phosphorus to phosphate, increasing its concentration in the soil and promoting formation of stable minerals such as apatite. In productive areas of the ocean, microbial mineralization of detrital matter at the sediment-water interface generates reactive phosphate, some of which reacts with seawater calcium to form phosphorite. (Reactive phosphate that does not contribute to mineral formation is available for biological assimilation by benthic microbes or may be reintroduced to the euphotic zone by diffusion and upwelling for use by phytoplankton).

The accumulation of phosphorus in microbial cells during transitory immobilization also contributes to mineral formation by increasing the pool of phosphate that could react with cations to form minerals. This is particularly important in anoxic areas of the ocean and soils where phosphate levels are low. Under oxic conditions where phosphate is more abundant, luxury uptake and storage of phosphate as polyphosphate molecules occurs in some microbes (e.g., *Pseudomonas spp.*, *Actinobacter spp.*), as discussed above. Cells use the energy stored in polyphosphates to activate an alternative organic electron acceptor when conditions shift toward anoxia, freeing substantial levels of phosphate in the process. The sequestration and release of phosphate by the cell under oxic and anoxic conditions respectively represents a mechanism by which microbes contribute to mineral formation because it generates locally-elevated phosphate concentrations near the cells; these concentrations could be high enough to induce precipitation of minerals. Accumulation of phosphate within cells may also lead to phosphorus immobilization via mineral formation if phosphorus minerals are generated and stored within the cell. Intracellular formation of mineral apatite is an example of phosphorus immobilization that has been observed in some microbes (e.g., *Escherichia coli, Bacterionema matruchotii*) following incubation with calcium phosphate at a slightly basic pH. This process occurs in living and dead cells, suggesting that the locally-elevated phosphate concentrations within the cell help initiate apatite formation. Similarly, the formation of carbonate fluorapatite following cell death has been observed in Gram-negative rods, possibly pseudomonads, in coastal marine sediment, and is thought to be an important phosphorite formation process in locations where sedimentation rates are low.

Weathering

Rock material exposed to the atmosphere breaks down, or weathers, as the result of numerous environmental processes. Weathering processes are classified into two categories. In mechanical weathering, physical processes (including thermal expansion, pressure release, hydraulic action, salt crystal formation, freeze-thaw, and frost wedge events) cause deterioration of rock material without changing the chemical composition of the parent material. In contrast, chemical weathering causes deterioration by altering the chemical structure of the minerals from which the rock is made. Chemical weathering processes include dissolution, hydrolysis, hydration, and oxidation-reduction (redox) reactions. Living organisms can contribute to mechanical weathering by altering the microenvironments at the surface of the parent material (e.g., by increasing local humidity or forming bio-films on surfaces); however, most biological weathering processes are classified as chemical weathering because they chemically alter the composition of the parent rock material directly or indirectly. These biological weathering processes are also referred to as solubilization.

Solubilization

Inorganic phosphorus can occur in nature in soluble and insoluble forms. The solubility of the most abundant form of inorganic phosphorus, phosphate, is determined by the ambient pH and the cation to which it is bound as a mineral (e.g., K^+, Ca^{2+}, Mg^{2+},

Fe^{2+}, Fe^{3+}, and Al^{3+}). Microbially-mediated phosphorus solubilization plays an important role in the conversion of insoluble phosphorus minerals to soluble forms of phosphorus. Solubilization directly benefits the microbes that perform it by providing the bioavailable phosphorus needed for growth. Similarly, the process benefits other organisms (including other cells and higher plants) from the surplus of solubilized phosphorus.

Production of organic and inorganic acids is the primary mechanism of microbial phosphorus solubilization. In this process, biogenic acids interact with phosphorus minerals to form phosphates, thereby bringing phosphorus into solution. Chemoautotrophic bacteria (e.g., nitrifying bacteria and *Thiobacillus spp.*) generate inorganic acids (e.g., nitric and sulfuric acid) by oxidizing ammonium and sulfur respectively, and these acids can liberate soluble phosphorus from apatite, the most abundant phosphorus mineral. Production of organic acids occurs in numerous microbial taxa and can also contribute to the solubilization of phosphorus minerals. Many higher plants can also produce organic acids in their rhizosphere (the area adjacent to plant roots) to solubilize phosphorus. Many organic acids, such as citrate, oxalate, lactate and 2-ketogluconate, are chelating compounds. Chelation is the reversible binding (complexation) of a ligand to a metal ion. Chelators increase the solubility of insoluble phosphate mineral salts by complexing the metal cations, thereby making dissolution of the salt more energetically favorable.In addition to acid production, microbially-mediated redox reactions contribute to phosphorus solubilization through the reduction of iron oxyhydroxides and associated ferric (Fe^{+3}) phosphate (strengite). In this process, dissimilatory iron reduction of ferric phosphates liberates soluble ferrous (Fe^{+2}) iron as well as the phosphate associated with it. This occurs under reducing conditions, such as in flooded, anoxic soils and in some benthic (e.g., the sediment surface and some sub-surface layers) aquatic environments. In another redox process, hydrogen sulfide (H_2S) produced by sulfur-reducing bacteria reduces the ferric iron in iron phosphate ($FePO_4$) to ferrous iron. In this reaction iron sulfide and elemental sulfur are precipitated, and phosphate is generated.

Mineralization

Plant and animal detritus in the soil and in particulate and dissolved forms in aquatic environments comprise a large reservoir of organic phosphorus. However, because complex phosphorus forms such as those bound to carbon are generally unable to cross cell membranes, most of the organic phosphorus from the detrital pool is not directly available to many living organisms. To become bioavailable, phosphorus bound to organic material must be converted to phosphate by mineralization, which is accomplished through the activity of a number of enzymes, many of which are produced by microbes. Because this process makes nutrients available that would otherwise be sequestered in unavailable forms, mineralization provides a vital link between the detrital pool and living organisms. It is estimated that approximately 70%–80% of microbes can participate in phosphorus mineralization. As in many enzyme-catalyzed systems, mineralization is encouraged by higher levels of available substrate; however, high levels of inorganic phosphate (the product) do not necessarily impede the reaction, and mineralization will often occur even if an abundance of phosphate is present. Ambient conditions favoring phosphorus mineralization include those that also favor mineralization of other elements, such as optimal temperatures and pH values for the specific enzyme; in soil, adequate but not excessive soil moisture is also required.

Enzymes involved in mineralization comprise a diverse group of proteins, called phosphatases, with a broad range of substrates and substrate affinities, and varying conditions for optimal activity. In addition, phosphatases can either be constitutively expressed by an organism (produced all the time), or facultatively expressed under conditions of low phosphate (and in some cases, low carbon). The microbial phosphatases can be classified into general broad categories such as phosphonatases, phosphomonoesterases and phosphodiesterases, or into more specific categories such as nucleases or phytases, depending on the compounds they will hydrolyze. Phosphomonoesterases catalyze reactions with phosphomonoesters. The reaction involves the hydrolysis of the phosphorus-carbon bond, generating a free phosphate molecule and an alcohol as products. Phosphomonoesterases are further classified as "acid" or "alkaline" based on their optimal pH ranges for maximum catalytic activity. Because of their ubiquity and importance in phosphorus mineralization, phosphomonoesterases are often referred to simply as phosphatases in the scientific literature, rather than by their full name, and context is often necessary to determine which group is being referenced. Therefore, a phosphomonoesterase with a pH optimum near 8 might be referred to as an "alkaline phosphatase" rather than an "alkaline phosphomonoesterase". In soils, both acid and alkaline phosphomonoesterase activities are usually measured, although the pH optima for these enzyme assays will vary with soil pH in some but not all soils.

Phosphodiesterases hydrolyze phosphodiesters. Cleaving of a diester proceeds similarly to the monoester reaction, with water added across the phosphorus-carbon bond yielding phosphate and an alcohol. Once a phosphodiester has undergone hydrolysis, the resulting alcohol phosphomonoester may undergo another hydrolysis step, catalyzed by a phosphomonoesterase. Specific examples of phosphodiesterases include depolymerizing nuclease enzymes such as deoxyribonuclease (DNAse) for DNA and ribonuclease (RNAse) for RNA.

Inositol hexaphosphates such as phytate are specifically hydrolyzed by phytases, of which there are four subclasses differing in pH optima, cofactor requirements, or mechanisms of action. Because it is found in seeds, phytate is a common part of human and animal diets. However, humans and many animal species do not produce phytases. The six phosphate groups of phytate allow it to sorb tightly to soils, and it can accumulate, especial when manure is applied to soils. Below pH 5, DNA can also sorb to soil minerals and accumulate because enzymatic mineralization will be restricted. A combination of organic acids to desorb phytate or DNA, followed by enzyme hydrolysis, will be required to release phosphate from these tightly sorbed molecules.

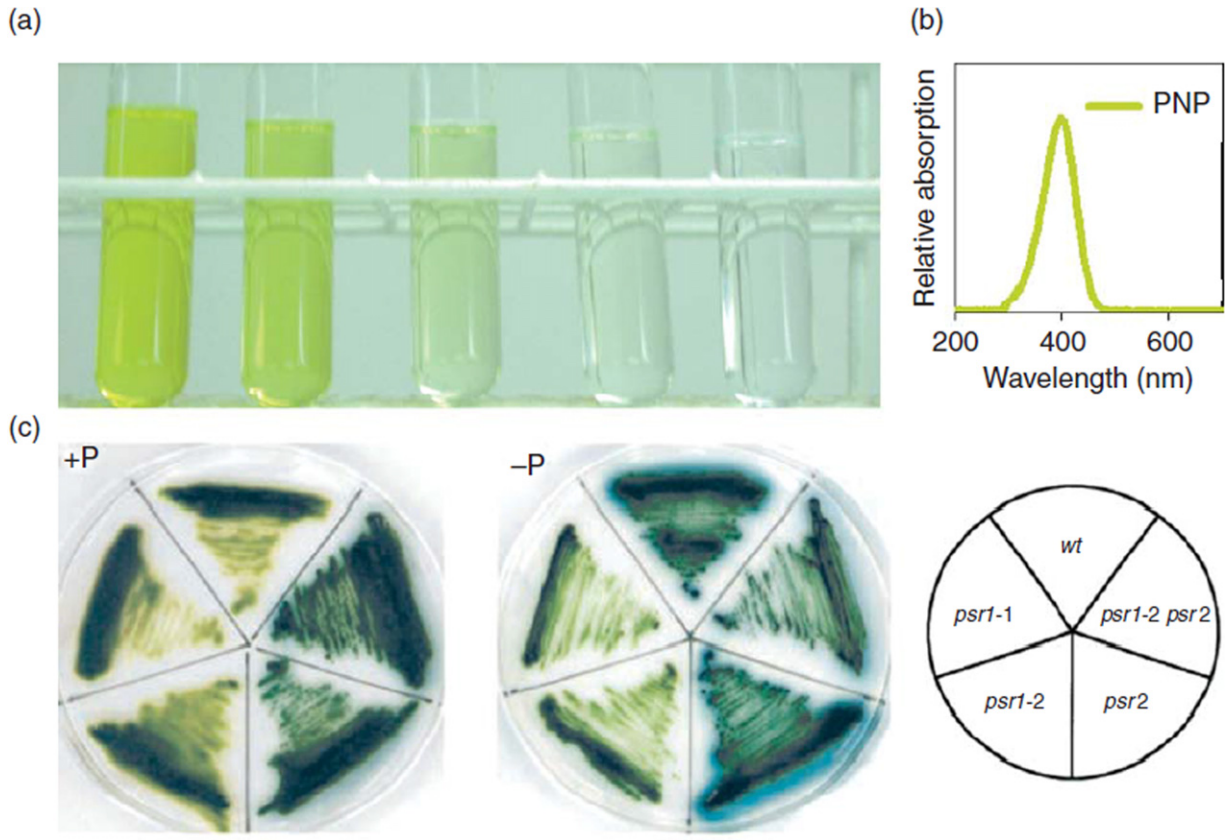

Fig. 3 (a) The *para*-nitrophenyl phosphate (PNP) assay for alkaline phosphatase activity produces a yellow color in the presence of the enzyme. (b) The PNP assay quantifies alkaline phosphatase activity based on the absorption of light at 380 nm. (c) The 5-bromo-4-chloro-3-indolyl-phosphate assay with *Chlamydomonas* algae under phosphate replete (left) and phosphate limited (middle) conditions shows phosphatase activity using the blue coloration formed around the cells when they express the enzyme. Key (right) identifies mutants used in the study (wt is wild type). The phosphatase of the wild type cells is induced in phosphate-free medium. Reproduced with permission from Shimogawara, K., Wykoff, D.D., Usuda, H., Grossman, A.R., 1999. *Chlamydomonas reinhardtii* mutants abnormal in their responses to phosphorus deprivation. Plant Physiology 120, 685–693. Copyright 1999 by the American Society of Plant Biologists.

Because many phosphatase enzymes are synthesized in response to low environmental phosphate levels (i.e., when the cells experience phosphate limitation), phosphatase activity has been used extensively as a metric for determining the nutrient status of microbial communities in aquatic ecosystems. This is less common in soils, where microbes are generally more limited by carbon or nitrogen than phosphorus. The activity of phosphatase enzymes has been measured in many terrestrial, lake, and marine environments using a variety of methods, and numerous laboratory studies have also been conducted. In the environment, the bulk phosphatase activity of an entire microbial community is commonly measured by incubating soil, sediment, or water samples with a phosphate-bound substrate in which the hydrolytic product undergoes a color change that can be observed visually or spectrophotometrically, such as *para*-nitrophenyl phosphate (PNP) (**Fig. 3(a)** and **(b)**), phenolphthalein phosphate (PPP), glycerophosphate, and 5-bromo-4-chloro-3-indolyl-phosphate (**Fig. 3(c)**). In addition, measurements can be made fluorometrically for the substrates 3-o-methylfluoresein phosphate (MFP) and 4-methylumbellyferyl phosphate (MUP). Radiometric analyses can similarly be made using ^{32}P labeled glycerol phosphate (or an equivalent molecule). Chemical analysis of the hydrolytic products of glycerol phosphate and other bioenergetically important molecules has also been used to estimate phosphatase activity in bulk microbial populations.

A major drawback to measuring bulk phosphatase activity is that it provides limited information, if any, about which members of the microbial community experience phosphate limitation at the time of sampling. For aquatic samples, this can be addressed to some extent by size fractionating cells (as on a filter) prior to incubation with the substrate, thereby allowing phosphatase activity to be assigned to gross taxonomic classes of organisms. However, size fractionation introduces other obstacles for data interpretation, and results must be interpreted with care. For example, activity in different size fractions can be skewed by groups of bacteria that coalesce to form larger particles, although the individual cells are small and would otherwise be grouped with smaller size fractions. Studies with mixed populations of bacteria and green algae showed that 44% of the measured phosphatase activity was attributable to aggregated groups of cells. Moreover, in the marine environment substantial phosphatase activity has been shown to persist for 3–6 weeks at 50% of initial levels in water samples filtered to remove particles. These observations suggest that phosphatases free in solution or bound to soluble organic material can contribute a significant amount of phosphatase activity,

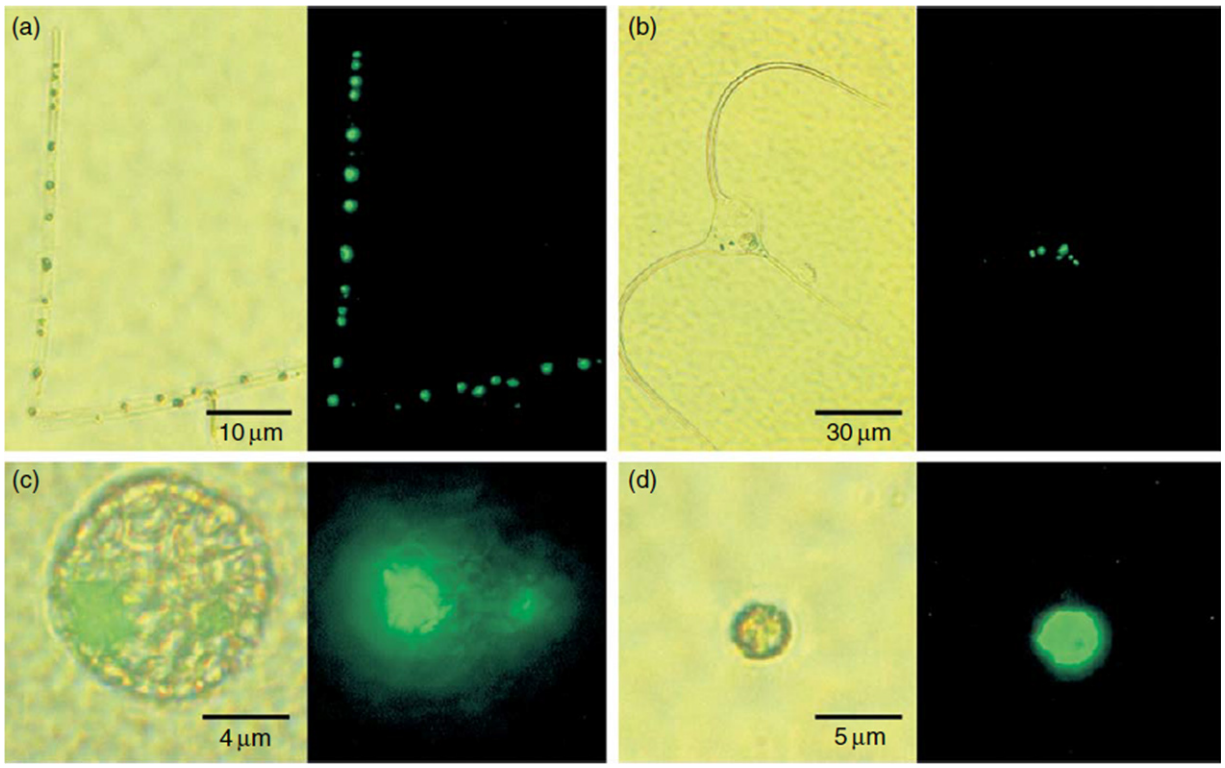

Fig. 4 Micrographs of ELF-97 labeled phytoplankton from the euphotic zone in the Gulf of Aqaba (a) *Trichodesmium sp.*, (b) *Ceratium sp.*, (c) coccolithophore, and (d) *Cyanothece sp.* For each pair, the left panel is a view under visible light and the right panel under UV illumination. ELF-97 labeled areas appear as bright areas under UV illumination and show the location of phosphatase enzymes on the cells. Reproduced with permission from Mackey, K.R.M., Labiosa, R.G., Calhoun, M., *et al.*, 2007. Phosphorus availability, phytoplankton community dynamics, and taxon-specific phosphorus status in the Gulf of Aqaba, Red Sea. Limnology and Oceanography 52, 873–885. Copyright 2007 by the American Society of Limnology and Oceanography, Inc.

potentially leading to overestimates of cell-bound phosphatase activity in the small-cell size fraction. A difficulty common to both bulk and size fractionated samples is that phosphatases can persist for long periods of time without being bound to a living cell. It is not uncommon for microbial cells to retain phosphatase activity for months or years after being dried or preserved, including in dried soil samples, indicating that cell viability is not critical for maintaining phosphatase enzymes over these time periods, and dead cells may contribute to the overall phosphatase activity in a sample.

Several methods have been developed to overcome the limitations of bulk enzyme activity measurements by directly labeling cells when phosphatases are present. These methods allow phosphatase activity to be attributed to individual cells or taxa, allowing greater resolution of the phosphate status of organisms within a mixed community. In aquatic samples, direct cell staining with azo-dyes or precipitation of lead phosphate at the site of enzyme-mediated phosphate release have been used together with light microscopy to visualize phosphatase activity on individual cells. Similarly, enzyme-labeled fluorescence (ELF) labels individual cells with a fluorescent precipitate (ELF-97) following hydrolysis of the non-fluorescent substrate molecule (2-(5′-chloro-2′phosphoryloxyphenyl)-6-chloro-4-(3H)-quinazolinone) at the site of the enzyme (**Fig. 4**).

Oxidation-Reduction

Phosphorus can be found in a broad range of oxidation states. Like nitrogen, which occurs just above phosphorus in the periodic table of elements, the valence electrons of phosphorus allow -3, 0, $+1$, $+3$ and $+5$ oxidation states. However only the $+5$ oxidation state, i.e., phosphate, is thermodynamically predicted to remain stable in aqueous solutions (including soil solution). Consequently, inorganic phosphate is nearly always the dominant form of phosphorus in the ocean, lakes, sediments, and soils. The phosphorus-containing organic compounds that generally account for the vast majority of cellular phosphorus, including DNA, RNA, and phospholipids, also contain phosphorus in the $+5$ oxidation state. While the geochemistry and biochemistry of phosphorus generally reflects the chemical characteristics of phosphorus in the $+5$ oxidation state, there are pools of phosphorus at reduced oxidation states (-3, 0, $+1$ and $+3$) whose roles on Earth remain to be fully elucidated. The contribution of inorganic reduced phosphorus species from geochemical sources, which include primarily metal phosphides produced via lightning strikes or meteorite strikes, is quite small. Thus, microbes are regarded as the primary sources of reduced phosphorus species. Phosphine (PH_3), which contains phosphorus in the -3 oxidation state, is a gas produced in environments where organic

matter is actively degraded, such as sewage treatment plants or rice paddies. Atmospheric phosphine concentrations are much higher on land than over the open ocean. Phosphite (HPO_3^{2-}), which contains phosphorus in the $+3$ oxidation state, has been observed in low concentrations in soils, lakes, wetlands and rivers. Recent laboratory innovations are making phosphite and hypophosphite (H_2PO^{2-}; $+1$ oxidation state) analyses more accessible to investigators, and a growing body of observations suggests that these two reduced inorganic phosphorus species may be more prevalent than once thought. The biochemical mechanisms by which microorganisms produce reduced phosphorus species are poorly understood. Thermodynamic calculations suggest that the direct reduction of phosphate to phosphite is prohibitively endergonic in the presence of water. Thus, if this process is indeed mediated by microbes, then it would require highly specialized enzyme systems that have yet to be identified. By contrast, the direct oxidation of phosphite is highly exergonic, and dissimilatory phosphate oxidation has been tied to the microbial sulfate and nitrate reduction and possibly carbon dioxide fixation.

Plant-Microbe Interactions

Like all organisms, phosphorus is an essential nutrient for terrestrial plants. However, unlike aquatic environments where both phosphorus and organisms are mobile, terrestrial plants are fixed in place and can easily deplete the labile phosphate in the rhizosphere (the zone surrounding plant roots). To ensure a continued supply of labile phosphate, relationships between higher plants and rhizosphere microbes have developed. Carbon is the nutrient most limiting to many soil microbes; plants release carbon directly into the rhizosphere by exuding simple sugars or high molecular weight compounds, or indirectly by root turnover. Rhizosphere microbes in turn can produce organic acids to release adsorbed or chelated phosphate, phosphatases to mineralize organic phosphorus, or hormones to stimulate the growth of roots and root hairs. The majority of higher plants also form symbiotic relationships with fungi called mycorrhizas. These vary depending on the plant and fungal species. Arbuscular mycorrhizas are endomycorrhizas, where fungi colonize root cells and extend hyphae beyond the rhizosphere to increase phosphate uptake. In ectomycorrhizas, fungal hyphae form sheaths around plant roots and do not enter plant cells. The fungi involved in mycorrhizas extend hyphae into the soil to access phosphate beyond the reach of plant roots and root hairs and may also transform phosphorus by producing enzymes and organic acids.

Genetic Regulation of Microbially-Mediated Processes

Microbially-mediated processes, including those involved in the phosphorus cycle, are the outcome of numerous biological pathways occurring in concert across diverse microbial communities. Even cursory observations of natural microbial communities demonstrate that while microbially-mediated processes influence and change the environment, the environment likewise shapes the activity of microbes, in many cases by providing feedback that either inhibits or enhances the processes. (An example of this type of feedback is the synthesis of phosphomonoesterase enzymes, many of which are only present during periods of phosphate deprivation but not when phosphate is abundant in the environment). Likewise, microbially-mediated processes can also be controlled indirectly by secondary factors (other than phosphorus) that influence growth and metabolism, such as the availability of oxidized nitrogen or sulfur in some chemoautotrophic microbes.

These processes, which are manifest in the environment as the combined outcome of activities from a diverse microbial community, are in fact a result of genetic mediation within single cells. The regulation of genes in response to environmental stimuli determines how a cell will respond to its environment, including if and how it will contribute to microbially-mediated processes in the phosphorus cycle. To understand gene regulation in greater detail, highly sensitive genetic and molecular methods have been developed. Under laboratory conditions, these methods have elucidated pathways important in the immobilization (i.e., phosphorus assimilation into cell biomass) and mineralization (i.e., phosphatase production) of phosphorus, as well as countless other pathways and processes.

In *E. coli*, two major phosphate assimilation pathways have been identified. The phosphate transport system (Pit), which comprises a hydrogen-phosphate symport powered by proton motive force, is expressed constitutively and provides the cell with sufficient phosphorus for growth when phosphate concentrations in the media are not limiting. When media phosphate concentrations drop below a threshold concentration, the high affinity phosphate specific transport (Pst) system becomes engaged. This system has a 100-fold greater affinity for phosphate than Pit, enabling the cell to acquire phosphate from a limited reservoir. Uptake of phosphate through Pst is an ATP dependent process (e.g., requires energy input from the cell).

Pst is activated as part of the Pho regulon, a group of operons that is expressed when phosphate levels are low. Activation of the Pho regulon is initiated through phosphorylation of the PhoB cytoplasmic protein which, in its phosphorylated state, is a transcriptional activator of the operons within the Pho regulon. In addition to Pst, the Pho regulon also includes genes encoding alkaline phosphomonoesterase (PhoA), outer-membrane porin proteins that facilitate diffusion of phosphate into the periplasm (PhoE), and proteins for the uptake and processing of glycerol-3-phosphate (*ugp* operon) and phosphonates (*phn* operon).

Metabolism of glycerol 3-phosphate is an interesting strategy for heterotrophic microbes, such as *E. coli*, because it is a potential source of both phosphate and carbon for the cell. However, when grown under phosphate-deplete conditions and expressing *ugp* genes, cells are only able to use glycerol 3-phosphate as a phosphate source, not as a carbon source. For cells to

grow with glycerol 3-phosphate as the only phosphate source, another carbon source must also be provided. The *ugp* system is less efficient when internal cell phosphate levels are high and is no longer expressed if external phosphate levels increase above a threshold level. Another system that is not part of the Pho regulon, the *glp* transport system, is regulated by external and internal glycerol-3-phosphate levels rather than phosphate concentrations. Unlike in the *ugp* system, glycerol-3-phosphate acquired by the *glp* system can serve as the sole source of carbon and phosphate for the cell. Both *ugp* and *glp* systems facilitate direct cellular uptake of glycerol-3-phosphate; however, each is regulated by different internal and external cues (i.e., phosphate or glycerol-3-phosphate levels) and has a different nutritional strategy (i.e., supplying phosphate alone versus phosphate and carbon together).

These two systems are an example of how microbes, by developing multiple inter-related pathways, can contribute to microbially-mediated processes in the phosphorus cycle under a range of environmental and physiological conditions.

Experimental evidence shows that the phosphate assimilation pathways in other heterotrophic bacteria are similar to those of E. coli, and many microbes are known to have portions of the Pho regulon. In particular, the alkaline phosphomonoesterase gene (*phoA*) and homologues have been identified in numerous microbial taxa, and although the primary function of the protein remains the same, factors that influence its expression and activity vary from organism to organism. The diversity of organisms and environmental conditions in which this gene exist allow microbially-mediated mineralization of phosphorus to occur in nearly every environment where microbes are found. For example, photosynthetic cyanobacteria in the genus *Synechococcus*, which populate freshwater environments, coastal waters, and vast areas of the open ocean, have the *phoA* gene along with many of the other genes encoded in the Pho regulon, highlighting the global ubiquity of microbially-mediated processes in the phosphorus cycle.

Metagenomic techniques have the potential to significantly increase our understanding of phosphorus mineralization, as well as other aspects of phosphorus cycling, in terrestrial and aquatic ecosystems. These molecular-based techniques sequence extracted RNA and DNA to accurately identify and quantify a large number of microbial genes. In addition to better identification of the microbial community in a sample, metagenomics can identify the microbial genes coding for various phosphorus cycling processes, such as phytase or phosphonatase production. Metagenomics will only identify the presence and abundance of these genes, which can only give the potential gene activity; it does not indicate if these genes are actually active in a sample or ecosystem. However, these metagenomic results can be linked to measures of ecosystem processes, such as the enzyme activity measurements previously described, or to the presence of substrates such as phytate, identified and quantified by techniques such as ^{31}P-NMR spectroscopy.

Anthropogenic Alteration of the P Cycle: Eutrophication in Aquatic Ecosystems

As discussed above, microbes have an important role in nearly every aspect of the phosphorus cycle, and their activities help control the relative rates at which phosphorus is mobilized and immobilized within the environment. However, humans also influence the phosphorus cycle and alter the structure of microbial communities, sometimes causing devastating ecological consequences.

Post-industrial human activities, including deforestation, phosphorus mining, and agricultural practices, affect the phosphorus cycle by increasing the mobility of phosphorus in the environment and causing it to accumulate in soils and aquatic environments. Several factors contribute to the mobilization of phosphorus by these activities. Deforestation and mining expose phosphate (and other) minerals in rock and soil to the atmosphere, leading to increased rates of weathering and erosion. Some agricultural practices such as tillage can also increase soil erosion, while the localized elevation of phosphorus levels from application of fertilizers can be a large source of the anthropogenic phosphorus input into receiving water. Elevated soil phosphorus levels increase the amount of phosphorus in runoff and ultimately lead to the accumulation of phosphorus in lakes and estuaries. Livestock production for meat or dairy will produce manures containing high concentrations of phosphorus. When applied to soil as fertilizer at rates to meet plant nitrogen requirements, this will apply excess phosphorus that may be lost in runoff. Increasing human populations, both in cities and in rural areas, will increase phosphorus in human sewage that can enter water systems if not properly treated and disposed.

As a result of these practices, recent estimates suggest that the net storage of phosphorus in terrestrial and freshwater habitats has increased 75% over pre-industrial levels, and the total phosphorus flux to the ocean is 2-fold higher than pre-human levels. When phosphorus enters water bodies where phosphorus limits production, substantial changes in the microbial community occur. Reversal of phosphorus limitation leads to eutrophication, the rapid growth of bloom-forming phytoplankton, some of which are toxic or nuisance species (like *Pfiesteria sp.*) that are harmful to aquatic organisms and humans. As the bloom exhausts the supply of phosphorus, the phytoplankton senesce, sink to the bottom of the water body, and are decomposed by the heterotrophic microbial community. At depth, where light levels are low, photosynthetic phytoplankton are not able to balance the metabolic oxygen demands of the heterotrophs, and anoxia occurs in the bottom waters. Anoxia damages the benthic environment, leading to fish kills and harming benthic invertebrate communities. Loss of submerged aquatic vegetation, coral reef death, human shellfish poisoning, and a reduction in biodiversity are among the possible outcomes caused by microbial responses to the anthropogenic introduction of excess phosphorus to sensitive aquatic ecosystems. Eutrophication has been observed in many ecosystems, including fresh water lakes like Lake Erie, large estuaries like the Chesapeake Bay, and coastal areas like the hypoxic "dead zone" of the Gulf of Mexico.

Conclusion

Microbially-mediated processes in the phosphorus cycle forge a critical link between the geosphere and biosphere by assimilating phosphorus within biological molecules and contributing to chemical transformations of phosphorus in the environment. In addition to acting as living reservoirs of phosphorus, microbes also contribute to the transformation of phosphorus within other non-living reservoirs, such as rock, soils, rivers, lakes, and oceans. Microbially-mediated phosphorus transformation includes processes that increase the bioavailability of phosphorus in the environment, such as weathering, solubilization, and mineralization, as well as those that decrease its bioavailability, such as assimilation and mineral formation. These large-scale environmental processes are the outcome of numerous biological pathways occurring in concert across diverse environmental settings and microbial communities. Genetic diversity and finely-tuned regulation of gene expression allow microbes to adapt to harsh environments, and to contribute to the phosphorus cycle under numerous and diverse environmental conditions. Human alteration of the natural phosphorus cycle causes unintended consequences in microbial communities, and serious environmental, economic, esthetic, and human health problems are caused by microbial responses to the anthropogenic introduction of excess phosphorus to sensitive aquatic ecosystems.

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