BIOAVAILABILITY OF ORGANICALLY-BOUND SOIL PHOSPHORUS[1]

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Introduction

Up to one-half of the total amount of phosphorus in the A horizon of soils from the Midwest and other temperate zones may be present as phosphorus bound to soil humic substances. All current evidence indicates that only inorganic forms of phosphate, orthophosphate in the form of HPO_4^{2-} and $H_2PO_4^-$ ions, are taken up by plants. The phosphate bound to soil humic substances must be mineralized to these inorganic forms before it is available for plant uptake. The process of mineralization is mediated by enzymes, especially phosphatase and phytase.

Soil organic phosphorus occurs in chemically diverse forms. Soil microorganisms play an important role in recycling many of the organic phosphorus compounds in soils. Up to one-half of the organic phosphorus in soils occurs as phytic acid (inositol hexakisphosphate), which is the main compound that plants use to store phosphorus in seeds to support early seedling growth upon germination. The remaining organic phosphorus in soils occurs as mono- and diesters [i.e. $(RO)PO_3H_2$ and $(RO)(R'O)PO_2H$ where R and R' represent aliphatic compounds], phospholipids and nucleotides, sugar phosphates, phosphoproteins, and phosphonates (Tate, 1984). The complexity of this mixture of compounds and the interaction of soil microorganisms with soil organic compounds has prevented the development of an analytical procedure than can measure the quantity and availability of organic phosphorus in soils. The current approach used to estimate the amount of organic phosphorus in soils subtracts the amount of inorganic phosphorus from the total phosphorus. There are no analytical methods to estimate the bioavailability of soil organic phosphorus.

The absence of suitable analytical procedures for soil organic phosphorus coupled with a scarcity of knowledge of the rates and mechanisms for the mineralization of soil organic phosphorus has resulted in fertility management practices that often ignore or minimize the contributions made by this fraction of the total phosphorus in soils. The high loadings of

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inorganic and organic phosphorus resulting from large amounts of manure being applied to soils near large, confined-animal operations and the resulting high concentrations of soil phosphorus has led to renewed emphasis on understanding the role of soil organic phosphorus in plant nutrition and environmental issues.

Current Research

The phosphatase and phytase enzymes are hypothesized to hydrolyze the carbon-oxygen-phosphorous (C-O-P) ester bonds during mineralization of soil organic phosphorus. It is logical to assume that these enzymes might play important roles in the cycling of phosphorus in soils and in the phosphorus nutrition of plants (Sharpley, 1999). However, the experimental evidence is mixed concerning the efficacy of enzymes in mineralizing soil organic phosphorus. Plant roots, mycorrhizal fungi, and soil microorganisms produce these enzymes in soils. The production and secretion of acid phosphatases by some plant species are stimulated by deficient levels of soil phosphorus (Dinkelaker and Marschner, 1992; He, 1998). Roots and mycorrhizal hyphae deplete rhizosphere soil of inorganic phosphorus but do not affect the concentration of organic phosphorus in spite of increased phosphatase activity in soil influenced by roots of cucumber *(Cucumis sativus* L.) (Joner et al., 1995). The results of isotopic exchange experiments with radioactive ^{32}P show that the same pool of soil phosphorus is sampled by plants with or without phosphatase activity (Bolan, 1991). In contrast, Tarafdar and Jungk (1987) report that the concentration of soil organic phosphorus was depleted in soil within 1 mm of clover and wheat roots with high activity levels of phosphatase and microbial populations. These results led to the conclusion that the main role of phosphatases in soil was to recapture organic phosphorus compounds leaked from root cells (Barrett-Lennard et al., 1993).

We have shown that white lupin (*Lupinus albus* L.) samples a much larger pool of soil phosphorus than does soybean (*Glycine max* L.) (Braum and Helmke, 1994; 1995). We hypothesize that white lupin has evolved an especially effective mechanism to mineralize and acquire phosphorus from soil organic phosphorus compounds. This concept is supported by the observation that plants can utilize organic phosphorus supplied as synthetic organo-phosphorus compounds (sodium glycerophosphate, sodium salt of inositol hexaphosphate) in the presence of phosphatases, especially in sand and solution culture (Tarafdar and Marschner, 1994; He, 1998). We have continued our research with white lupin in an attempt to understand how this species might be able to assimilate phosphorus that is bound to soil humic substances.

Our earlier results showed that white lupin accesses a pool of soil phosphorus that is unavailable to soybean (Braum and Helmke, 1994:1995). We have since shown that lupin can accumulate all of its phosphorus needs when grown in sterile hydroponic culture where the only source of phosphorus was extracted and purified soil-humic substances or synthetic organic phosphorus compounds. These phosphorus deficient conditions stimulate the lupin roots to develop proteoid root structures that excrete large quantities of citrate ion and phosphatase and phytase (He, 1998).

The combination of citrate and enzymes in the root exudates appears to be the key factor in the ability of lupins to utilize organically bound phosphorus. Our results confirm that enzymes are not very effective in hydrolyzing organically bound soil phosphorus when the compounds in soil humic substances are chemically bonded to metals, especially aluminum and iron (Tate, 1984). Soil humic substances normally bind with many different metal cations, which reduces their solubility and reactivity while increasing their effective size.

Our research results with white lupin suggest that enzymes are much more effective in mineralizing phosphorus if the metals are removed from soil humic substances by plant secreted metal complexing agents, such as the citrate anion. White lupin develops proteoid roots that secrete large quantities of citrate or citric acid as an adaptive response to phosphorus deficiency. Citrate ion forms complexes with many metals and it removes the metals from soil humic substances. This greatly increases the solubility and reactivity of the soil organic phosphorus compounds and leaves them susceptible to hydrolysis by enzymes, similar to the results found with the synthetic organic phosphorus compounds in nonsoil culture cited above. Microorganisms can also secrete citric and oxalic acids, which can explain the utilization of soil organic phosphorus when phosphatases are associated with high microbial populations as reported by Tarafdar and Jungk (1987) cited above.

Our current research is attempting to utilize this new information to develop methods to assess the potential bioavailability of organically bound soil phosphorus. The approach is to treat soils with and without citric acid with the enzymes phosphatase and phytase and measure the amount of orthophosphate released. Examples of our current results for soils are shown in Figures 1 through 3. In these experiments, five grams of dry soil was incubated at 37^{o} for one hour in 12 mL of solution containing varying concentrations of citrate in a 0.1 molar sodium acetate buffer at pH 6. The concentration of orthophosphate was then determined by the molybdenum blue colorimetric method.

These and other preliminary data indicate that most of the mineralizable organic phosphorus in soils is in the form of phytic acid. In general, only small amounts of orthophosphate are released by phosphates. It is very apparent that increasing concentrations of citrate greatly enhance the effectiveness of the enzymes in mineralizing soil organic phosphorus compounds. These results are consistent with our hypothesis of how white lupin acquires phosphorus from soil humic substances. Our current research is examining the effects of dry versus field-moist soils on the mineralization of organic phosphorus. Concentrations of citrate above 5 mM interfere with the determination of phosphorus by the molybdenum blue colorimetric method, and we are trying to resolve this problem so that the experiments can be extended to higher citrate concentrations.

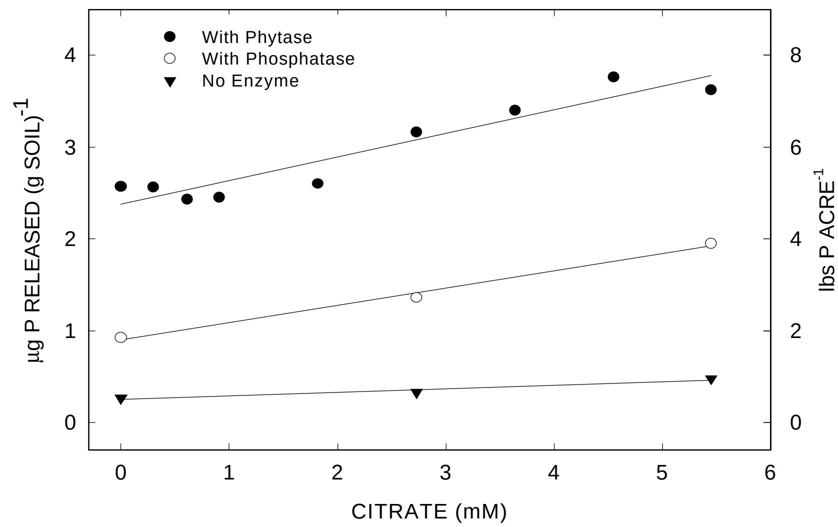

Figure 1. The concentration of inorganic phosphate released from the Houghton muck soil by phytase and phosphatase as a function of the concentration of citrate.

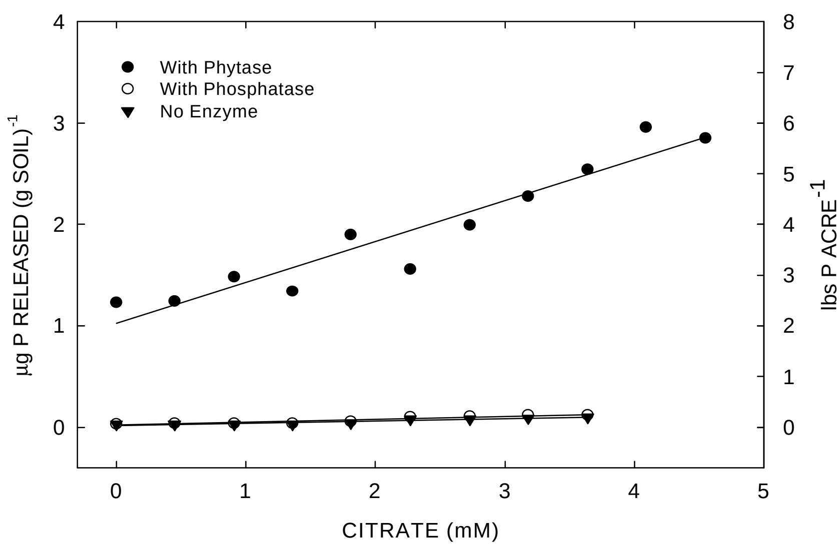

Figure 2. The concentration of inorganic phosphate released from the Plano silt loam soil by phytase and phosphatase as a function of the concentration of citrate.

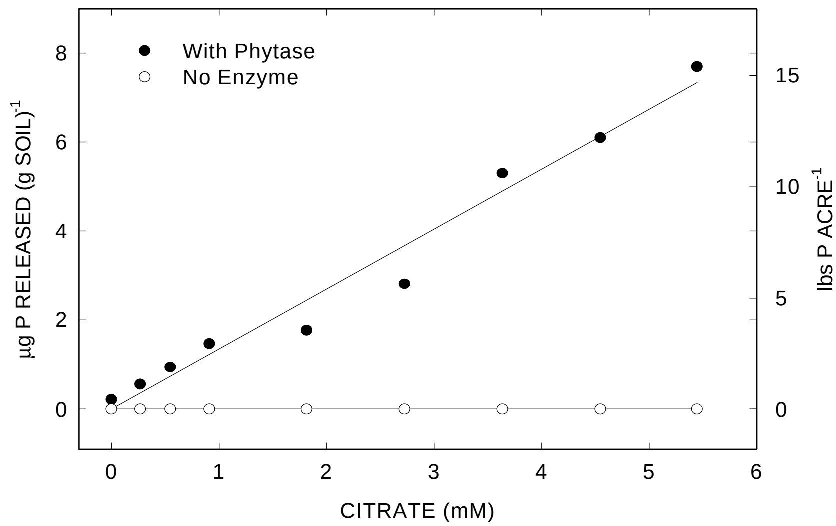

Figure 3. The concentration of inorganic phosphate released from the Antigo silt loam soil by phytase as a function of the concentration of citrate.

Future Directions

The removal of metals from soil humic substances by plant secreted complexing agents, which mobilizes part of the soil organic matter and increase its reactivity with phytase and phosphatases, appears to be an important but under recognized part of the phosphorus cycle in natural and agricultural soils. Knowledge and an understanding of the chemical and biological reactions in the natural cycle of phosphorus in the soil-water-plant system will pro-vide critical support for the sustainability of both high- and low-input agricultural practices and for the protection of the environment.

This new knowledge has the potential for reducing the fertilizer needs for phosphorus and thus extending the lifetime of the nation’s reserves of phosphate. It should also provide the knowledge base for better soil tests for phosphorus that include the organic fraction of soil phosphorus. This research also has the potential for developing knowledge that will lead to improved phosphorus utilization by plants through different soil management or fertilizer practices. Greater plant utilization of the soil organic phosphorus pool will allow soils to be managed with a lower total concentration of phosphorus, thereby reducing the impact of phosphorus pollution of surface waters by runoff from agricultural lands.

For the longer term, additional information about our hypothesis of how white lupin acquires soil phosphorus from soil humic substances will encourage plant geneticists to identify the genetic control of this characteristic. Hokkadio University, Japan, has an active program with this objective. They have already isolated a cDNA clone for an acid phosphatase from the roots of white lupin grown in phosphorus deficient conditions (Wasaki, et al., 1999). Genetic transfer of this phosphorus acquisition strategy to mainstream agronomic crops has the potential to transform phosphorus fertilization and soil management practices.

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