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Phosphorus cycle in agricultural soils
Phosphorus forms and P-sorption properties after long-term mineral and manure applications

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

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Summary

Food production requires the application of phosphorus (P) containing-fertilizers but phosphate is a finite reserve that has been estimated to last about 90 yr. In agricultural lands, where the excessive use of fertilizers occurs, significant diffuse P losses to surface and groundwater have been found. Thus, the high P concentration in waters is a major cause of eutrophication with detrimental impacts on the water quality for both of the ecosystem and human health. The knowledge of the different forms of P in the soil and the reactions that happens between P and the soil components is important to better understand the phosphorus behaviour in soils. The effects of a long-term mineral and organic fertilization in three different alkaline soils was considered and the occurrence of different forms of P were investigated. The high fertilisation strictly increased the P saturation of soils, especially in the superficial layers, thus evidencing the resulting movement of P throughout the soil profiles. The movement of the soluble and available P forms from the upper to lower layers has been confirmed in a study on soil profiles and down to 100 cm depth. Relationship between soil test P (STP) and the water soluble P evidenced the necessity of estimating the losses of P via subsurface when P exceed both agronomic and environmental thresholds. Keeping agronomic STP below the environmental threshold is important in developing sustainable agricultural systems, as well as many other areas where human activity impacts on the environment.

Chapter 1 - Soil phosphorus in agriculture and environment

Introduction

Food production requires application of fertilizers containing phosphorus (P) on agricultural fields in order to sustain crop yields. More than 90% of phosphate rock mined serves to make chemical fertilizers, animal feed, elemental P and other industrial phosphates. Today the world production of phosphate is about 133 Mt and is in the hands of four countries: the USA, former Soviet Union, Marocco, and China collectively produce about 75% of the world's phosphate rock (Stewart et al., 2005). Phosphate rock is a finite non-renewable resource and the depletion of phosphate rock reserves is difficult to predict. However, if future consumption equalled the 5-yr average mine production between 1997 and 2001, the global peak in phosphorus production is predicted to occur around 2030 (Cordell et al., 2009). The estimated world reserves would last about 90 yr, while USA reserves would last only 25 yr. Yet, future access to phosphorus receives little or no international attention.

In agriculture the excessive use of fertilizers and manures may result in diffuse P losses to surface and groundwater by erosion, surface runoff, and subsurface leaching (Buczko and Kuchenbuch 2007). High P concentrations in waters are a major cause of eutrophication, with detrimental impacts on water quality, since P usually is the nutrient that limits algae growth in freshwater bodies. Both in the USA and Europe, there has been a large-scale trend of increasing P concentrations in freshwaters during the last decades (European Environment Agency, 2003). P emissions from point sources have decreased during the past decades, mainly due to improved sewage treatment and reduction of industrial P emissions. On the other hand, P loss from diffuse sources, mainly agriculture, has not been reduced (Behrendt et al., 2003), ranging from less than 50 kg P km^{-2} y^{-1} to more than 200 kg P km^{-2} y^{-1}. Thus, there is a need to assess the risk of P loss from fields into surface waters at the field scale in order to decide, which fields are prone to P loss and therefore should not receive further phosphorus-fertilizer additions or at which fields other measures against P loss should be implemented.

The phosphorus cycle

The phosphorus (P) cycle in soil is a dynamic system involving soils, plants and microorganisms. Major processes include uptake of soil P by plants, biological turnover through mineralization-immobilization, fixation reactions at clay and oxides surfaces, and solubilisation of mineral phosphates through the activities of microorganisms (Stevenson, 1986). In the natural state, essentially all the P consumed by plants is returned to the soil in plant and animal residues; under cultivation, some P is removed in the harvest and only part is returned. Losses of soil P occur through leaching and erosion. In many respect the P cycle in soil is analogous to the N cycle. However, the former is less spectacular in that no valency changes occur during assimilation of inorganic phosphate by living organisms or during breakdown of organic P compounds by microorganisms. Next to N, P is the most abundant nutrient contained in microbial tissue, making up as much as 2% of the dry weight. Partly for this reason, P is the second most abundant nutrient in soil organic matter. Unlike the C, N and S, the P cycle does not have an important gaseous component; accordingly, movement of P to and from the atmosphere is of minor importance because of the small amounts that are circulated as atmospheric particulates. More than 90% of the total P in the soil-plant-animal system is in soils and less than 10% is in the remaining biological systems. The P content of the lithosphere is ~1200 ppm while it is 200-5000 ppm in soils. In the hydrosphere, typical concentration of total P in domestic wastewater, agricultural drainage, and lake surface waters are 3-15, 0.05-1, and 0.01-0.04 ppm, respectively (Arai and Sparks, 2007).

P chemistry

P belongs to the Group VA in the periodic table with an electronic configuration of ([Ne] $4s^2$ $4p^3$). It is stable in the pentavalent state to form an orthophosphate anion (i.e., phosphate) that retains a near tetrahedral complex surrounded by four oxygen atoms. In most soil/water environments, $H_2PO_4^-$ and HPO_4^{2-} are the thermodynamically favourable species (pK_{a1} = 2.1, pK_{a2} = 7.2, and pK_{a3} = 12.3). There are several other forms of P-

containing compounds (polyphosphates, metaphosphates, and organic P). The condensed forms of inorganic P (i.e., polyphosphates and metaphosphates) are formed with two or more orthophosphate groups. Whereas polyphosphates are linear O-P-O linkages, the mataphosphates are cyclic.

The organic P percentage of total soil P can range from 20 to 80% (Arai and Sparks, 2007). Several forms of organic P have been identified in soils. There are inositol phosphate, nucleic acids, and phospholipids. Inositol phosphate (phytic acid) makes up more than 50% of the total organic P due to its high stability in soils whereas the phospholipid content comprises as little as 0.5-7% of total organic P. Nucleic acid, which originates from decomposition of microbes, plants, and animal remains, is the smallest (less than 3%) fraction of the total organic P (Dalal, 1977).

P forms complex minerals with a wide variety of elements. About 150 P minerals are known (Cathcart, 1980). According to Povarennykh's structural classification, P minerals can be placed into four groups: framework, insular, chain, and layer minerals. A majority of the P minerals belong to insular minerals, including the apatite group $Ca_{10}(X)(PO_4)_6$ (where X represents F^-, Cl^-, OH^-, or CO_3^{2-}), and wavellite ($Al_3(PO_4)_2(OH)_3\ 5H_2O$). Some secondary minerals of phosphate are vivianite [$Fe_3(PO_4)_2\ 8H_2O$], dufrenite [$FePO_4\ \ Fe(OH)_3$], strengite [$Fe(PO_4)\ H_2O$] and variscite [$Al(PO_4)\ 2H_2O$].

Origin of P in soils

Phosphorus in most soils originates from the weathering of apatite. As soil weather, aluminium (Al) and iron (Fe) phosphates and organic forms of P become more prominent. Soil solution P concentrations typically range from <0.01 m P L^{-1} in very infertile soils to 1 mg P L^{-1} in well-fertilized soils and can be as high as 7 to 8 mg P L^{-1} in soils recently amended with fertilizers or organic wastes (Pierzynski et al., 2005). Within the soil solution, orthophosphate acts as a hard Lewis base and therefore has a tendency to form soluble inner- or outher-sphere complexes with hard Lewis acids such as H^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}. A Lewis base has valence electrons that are not being shared in a covalent bond and can form complexes with Lewis acids which have vacant valence orbitals that can accept electrons. Soluble complexes (ion pairs or complex ions) at high pH values can also represent a significant amount of the P in solution, but in most cases chemical

disassociation of these soluble complexes rapidly converts these to orthophosphate. Soluble organic P species and their contribution to soil P bioavailability are not well understood although it is believed the microbial hydrolysis to orthophosphate occurs rapidly (Stevenson, 1986). A soil solution P concentration of 0.003 to 0.3 mg P L^{-1} is considered optimum for plant growth, depending on the plant species, while P concentrations in freshwater associated with eutrophication are of 0.01-0.03 mg dissolved P and 0.0035-0.10 mg total P L^{-1}.

Phosphate fixation

Sorption and desorption reactions equilibrate with the soil solution. Orthophosphates can adsorb to the surfaces and edges of hydrous oxides, clay minerals, and carbonates by replacing H_2O or OH^-. Bonds can be monodentate or bidentate with P adsorbed by monodentate bonds considered to be more labile than that with bidentate bonds. The sorption process is believed to involve two steps: adsorption, the accumulation of P on the surfaces of solid soil constituents; and absorption, the diffusion of P into solid soil constituents (Corey, 1981; Sposito, 1986).

Numerous studies have shown that much of the P applied to soil in water-soluble forms e.g., monocalcium phosphate $Ca(H_2PO_4)_2$, does not remain as such for long but is converted to one of many insoluble or complex forms. Both biological and chemical processes are involved in fixation, with the latter being of greatest importance from the standpoint of retention of fertilizer P.

It is known the importance of pH is governing fixation reactions and thereby the availability of phosphates to plants. For most soils, maximum availability would be expected in the slightly acid to neutral pH range. Phosphate fixation is known to be influenced by clay mineralogy and decreases in the following order: amorphous hydrous oxides > goethite-gibbsite > kaolinite > montmorillonite (Stevenson, 1986). In highly acid soils, phosphate is readily precipitated as the highly insoluble Fe- or Al-phosphates or adsorbed to oxide surfaces (Stevenson, 1986). Both forms are poor sources of P for higher plants. This is particularly true for soils rich in Fe, such as lateritic soils (Oxisols) of the tropics and subtropics. On such soils, plants often show severe P deficiency symptoms and relatively large amounts of fertilizer P must be applied to meet plan requirements. In

calcareous soils the less soluble di- and tricalcium phosphates $Ca_2(HPO_4)_2$ and $Ca_3(PO_4)_2$ are formed, with the latter being gradually converted to carbonate apatite $3[Ca_3(PO_4)_2]CaCO_3$. This highly insoluble compound is not a good source of P for plants but availability may be enhanced in the presence of growing plants through the action of organic acids secreted from plant roots or synthesized by microorganisms in the rhizosphere.

Phosphorus is adsorbed by plants largely as the negatively charged primary and secondary orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}), which are present in the soil solution. Thus the water-soluble pool is of particular interest because this P has a direct effect on plant growth, and hence yields. Plants have the ability to absorb certain soluble organic phosphates but it is not known whether they serve as direct sources of P to plants. From the standpoint of plant nutrition, the P in soil can be considered in terms of pools. Only a small fraction of the P occurs in water-soluble forms at any one time. A portion of the insoluble P appears to be somewhat more available to plants than the bulk of the soil reserves. This fraction, designated as the labile pool is believed to consist of easily mineralized organic P and phosphates weakly adsorbed to clay colloids. The bulk of the soil P (>90%) occurs in insoluble of fixed forms, namely, as primary phosphate minerals, humus P, insoluble phosphates of Ca, Fe and Al, and phosphates fixed by colloidal oxides and silicate minerals. The ability of the soil to provide P to higher plants is determined by a variety of factors (Stevenson, 1986) that include: 1) the quantity of $H_2PO_4^-$ and HPO_4^{2-} in the soil solution, 2) the solubility of Fe- and Al-phosphates and phosphate complexes with hydrous oxides and clay minerals in acid soils, 3) the solubility of Ca phosphate and P minerals in calcareous soils, 4) amount and stage of decomposition of organic residues, and 5) activities of microorganisms.

Soil tests for available P

Numerous soil tests have been developed over the years for measurement of available P in soils (STP). Some procedures vary only in minor detail; others represent extremes in extraction conditions. No single test will apply for all soils and all methods have limitations that must be taken into account when making fertilizer P recommendations. A limitation common to most methods is that little, if any, information is provided on the rate at which

the P in insoluble complexes is converted to plant available (soluble) forms during the course of the growing season.

Dalal and Hallsworth pointed out that a description of available soil P must include an intensity factor I, a quantity factor Q, and a capacity factor $\Delta Q/\Delta I$, as well as rate and diffusion factors. The immediate source of available P for plants is that contained in the soil solution-the intensity factor I. The labile portion of the solid phase is the quantity factor Q, which is approximated by most soil test extraction techniques. The capacity of the soil system to maintain P concentration in the solid phase as P is removed by plants $\Delta Q/\Delta I$ is not directly determined by soil tests, nor is the rate of replenishment of soil solution P from solid phase forms.

P soluble in water

The P soluble in water determine the P concentration in the soil solution that is required for the optimum plant growth, the intensity factor I. A soil extract more closely approximates the P concentration in the soil solution but such an extract is not easily obtained. Most tests involve extraction with water or dilute salt solution (e.g., 0.01M $CaCl_2$). Under some soil and crop conditions, the method provides a satisfactory prediction of P fertilizer requirement. The approach would appear to be most useful for sandy soils, where the conversion of fertilizer P to insoluble forms is minimal.

P extractable with dilute acid fluoride (HCl-NH_4F)

This widely used extractant is designed to remove easily acid-soluble P forms, largely Ca-phosphates and a portion of the Al and Fe-phosphates. The NH_4F dissolves the latter through formation of fluoride complexes with Al and Fe. In general, the method has been found to be most successful for acids soils (<6.5 pH). Low estimates are obtained with calcareous soils because of neutralization of the acid by $CaCO_3$.

P soluble in $NaHCO_3$

Extraction of soil with 0.5M $NaHCO_3$ (pH near 8.5) (Olsen and Sommers, 1982) has been highly successful for predicting P availability in calcareous, alkaline, or neutral soils. This extractant decreases the concentration of Ca in solution by forming the insoluble $CaCO_3$,

with the result that the concentration of phosphate in the solution is increased. Extraction of soil with 0.5M $NaHCO_3$ also leads to solubilisation of a portion of the soil organic P, and, the amount thus solubilised has been regarded as a quantitative measure of the potential contribution of soil organic P to plant uptake (i.e., labile organic P).

P soluble in NH_4HCO_3-DTPA

This test was developed for simultaneous extraction of P, K, and micronutrient cations. The extractant solution is 1M NH_4HCO_3: 0.005 M diethylenetriaminepentaacetic acid (DTPA) at pH 7.6. The chelating agent (DTPA) is used for chelation and solubilisation of micronutrients (i.e., Cu, Fe, Mn and Zn). The reagent dissolves about half as much as 0.5 $NaHCO_3$.

Anion resin-extractable P

This mild test procedure removes P from soil without chemical alteration or changes in pH. In addition to serving as a test for available P, the approach has been used to asses the availability of residual P and to measure the rate of P released from insoluble forms.

P soluble in dilute HCl-H_2SO_4

Soil test involving mixed acids have been found useful for predicting P availability in soils which fix appreciable amounts of P.

Phosphorus and the agriculture

P management

The response in yield of crop to P application was recognized in the middle of the 19th century. As with other mineral elements, potential crop yield is related to the amount of available P for plant uptake. The first P-fertilization experiments were performed in Europe during the 19th century: England (1843, Broadbalk experiment in Rothamsted) and Germany (1878, Halle), testing the effects of various combinations of inorganic fertilizers (R.R. 2008; Merbach et al., 2000); then continued in N America: Illinois Agricultural Experiment Station (1888), Upper Coastal Plain Research Station Rocky Mountin (1903), and Vineland Campus Ontario (1916).

Inorganic-P reactions in soils and aquatic systems are mainly governed by adsorption on sorbent surfaces (mainly oxides; Sposito, 1989) and precipitation processes (Ca phosphates or Fe and Al phosphates, Lindsay et al., 1989). This implies that only a fraction of P applied to soil remains available for plants uptake. From a practical point of view, it is very difficult to know the proportion of applied fertilizer which remains available for plants and the classical strategy for guaranteeing a sufficient P supply to crops has been to maintain the available-P pool in soil well above critical values for fertilizer response (Sims, 2000). A decision-making process for P input requires methods of P analysis, interpretation schemes of analytical results (evaluation of the P status in relation to plant uptake and production-available P status), and derived P-fertilizer recommendations.

Estimation on plant-available P in soils

Various analytical methodologies have been tested during the last decades in order to study the available-P status of soils, including chemical extraction, anion-exchange resins, cation-anion-exchange resins, resin membranes, ferrihydrite-impregnated paper strip, goethite in dialysis membrane, isotopic exchange, and electro-ultrafiltration. The main limitation in studying the available-P pool in soils has been the absence of a general method adequate for wide application: depending on the dominant P forms in soils, the relationship between P

extracted by plant (available P) and P extracted in the lab is different (Mallarino, 1997). As an example, the relationship between the ratio of plant-available P in soil to P extracted by bicarbonate has been shown to be related to soil pH in a group of soils representative of different agricultural areas of Europe (Delgado and Torrent, 1997). This uncertainty in the relationship between available P and chemically extracted P, the heterogeneity of soil properties and retention capacity, and the absence of a European strategy for managing P input in agriculture, have led to a large variety of analytical methods to study available P in soil: Neyroud and Lischer (2003) reported 16 official methods in different countries of Europe. This clearly contrasts with the situation in N America where the P-input strategies are based mainly on three analytical methods: Olsen, Mehlich-3, and Bray (Kuo, 1996; Sims, 2000).

Nonpoint P losses from agricultural soils

The uncertainties about the efficiency of fertilizers, the low fertilizer prices, and EU subsidies to maximize production after World War II resulted in the use of excessive P rates in crop production. At that time, this was thought necessary to produce enough food in case of another war and was probably only excessive in some areas. In the 1970s, many agricultural soils in Germany and The Netherlands encompassed available P contents that clearly exceeded critical values for P fertilizer response (Behrendt and Boekhold, 1993). In UK, the productive grassland and arable area has accumulated an average P surplus of thousand kilogram per hectare since 1935. In The Netherlands, the relative contribution of agriculture to the total P load has increased in some area up to 90% since 1980s. In the 1990s, P loss from overfertilized soils is described as an extended environmental problem in Europe. In W European countries, although fertilizer-P use has decreased in recent years, the average input is higher than the average off-take and thus the risk of P loss via leaching and erosion is increasing. Switzerland, where concerns related to P loss early appeared (1950s), has developed an effective policy decreasing P load of lake waters: Switzerland fulfilled the international objective of halving P inputs into the Rhine and North Sea, achieving a calculated reduction of 51% between 1985 and 2001. On the other side the contribution of agricultural P to eutrophication in S European countries remains largely unassessed and thus warrants pertinent research at the soil, field, and catchments levels.

To reduce the environmental impact related to agricultural P, an environmentally sound P management must be proposed in Europe, taking into account not only an accurate P supply to crops but also a decrease in P losses from agricultural soils. This revision of the P-input strategy has been proposed previously in N America, where overfertilization with P in many agricultural soils has been ascribed to the classical increase and maintain strategy in P management (Sims et al., 2000). Phosphorus-buffering capacity (PBC) of soils, which can be defined as the soil-solution resistance to a change in P concentration, is considered crucial in determining thresholds levels of available-P content in the soils (Celardin, 2003). The knowledge of the PBC is a prerequisite for the development of more environmentally oriented fertilization-recommendation system in Europe. These recommendations must take into account soil P status, P-retention capacity, and dominant pathways for P loss, which range widely between different areas of Europe.

Phosphorus application as mineral or organic fertilizer

Additions of mineral and organic fertilizers (manure, animal wastes, plant residues) have an influence on STP levels over time, but—even more important for P-loss risk—are immediately available for loss following application (Buczko and Kuchenbuch 2007; Hao et al., 2008). Long-term manure additions reduce the P-sorption capacity, but increase soil organic-matter levels which in turn influence porosity, pore-size distribution, aggregate stability, and infiltration capacity (Allen and Mallarino, 2006). Manure covering the soil surface can reduce erosion rates (McDowell and Sharpley, 2002). Losses of P from source areas through interaction of freshly applied materials with precipitation during runoff events are termed “direct”, “incidental”, or “event-specific” (Hart et al., 2004) losses. The importance and magnitude of such direct losses depend on the application rate, timing of application in relation to runoff events, application method, and availability of the applied materials (Sharpley et al., 2001). The risk of direct P losses can persist for several weeks after application. Since the timing of manure or fertilizer applications is of paramount importance for P loss–risk assessment, it is considered in most P indices. However, because the timing of a specific rainfall-runoff event is usually not predictable, factors for timing of application are in most P indices related in a more general way to the seasons of the year and merged with factors for application (Beegle et al., 2006). Incorporation of fertilizer or

manure into the soil by tillage, injection, or subsurface placement can decrease the potential for P losses by runoff, compared with surface application, by more than 95% (Little et al., 2005). Typically, confined animal producers are concentrated at point-like locations with large numbers of animals. The produced manure is usually applied on a limited area of land in the vicinity. In such cases, the applied amounts of nutrients often exceed the amounts necessary for crop production. The problem is exacerbated by the relatively low N : P ratio of manures (as low as 2.5 for poultry manures, with an average N : P ratio of 4) (Sharpley et al., 1994), which is considerably lower than the N : P ratio of plant uptake (8 for major grain and hay crops). Thus, if manure-application rates are adjusted to meet the N requirements of crops, P will be enriched in the topsoil. Compared to P in manures, overapplication of inorganic-fertilizer P is in most cases not critical, because there is an economic incentive for the farmer to adjust fertilizer applications to the crop demands (Sharpley et al., 1994).

Both added manure or litter and native organic matter (humic materials) have significant effects on subsurface P retention (von Wandruszka, 2006). Manure not only affects sorption and precipitation of P, but often contains significant amounts of the element, which is thereby – deliberately or incidentally – added to the land. Humic materials, the breakdown products of the total biota in the environment, generally are not a major source of P, but they do have a mobilizing effect on it in the subsurface.

It has been shown that P decreases the sorption of organic C to acid mineral soils, suggesting a ligand exchange process at the surface (Kaiser and Zech, 1997). As regards the reverse, i.e. the release of P under the influence of dissolved humic materials, Delgado et al. (2002b) have found that application of humics to the soil increases the recovery of Olsen P in all soils tested, except in those with very high Na content. A recent investigation indicates that strong interactions between Pi and humic materials is predicated on the presence of metal ions that act as cationic "anchors", allowing anionic humates and phosphates to associate (Riggle and von Wandruszka, 2005). Stability constants of humate-metal-P complexes tend to be high, with log K values in the range 4.87–5.92 (Zn- and Mg-anchor, respectively).

Assessing potential environmental impacts of soil phosphorus

Sources of P to fresh water are normally categorized as “point sources” which are generally pipes that drain into rivers and streams from industry or sewage treatment works, and “non-point sources” which are much harder to identify and quantify and these include surface and subsurface losses of P from land uses such as golf courses and urban areas, although agriculture is normally considered the primary nonpoint source (Maguire et al., 2000). However, as P inputs to fresh waters from point sources have been reduced, the relative contribution of nonpoint sources, particularly from agriculture, have become increasingly important (Sharpley et al., 1994). For example, losses of P from agricultural soils in the USA have recently been identified as one of the major causes of decreased surface water quality. Part of the reason for this is that soil test P concentrations are increasing due to more P being added to soils in fertilizers and manure than removed in crops and animal products.

There are several pathways through which P can be lost directly from land to fresh waters, including erosion of soil particles and dissolved P in runoff following rain events. Most research up to the present has concentrated on P losses in erosion and runoff, as these were thought to be the major pathways for P losses from agriculture. However, recently the importance of P leaching has been recognized, especially in sandy soils or where there are preferential pathways such as worm burrows and cracks playing a significant role. Leached P can eventually reach surface waters via subsurface pathways. These losses are influenced by many factors such as manure application, tillage practice, and crop cover, as well as the concentration of P in the soil measured as STP.

Obviously, the more total P that is contained on a soil particle, the more total P will be lost when that soil particle is eroded. However, STP is not the same as total P and various soil testing methods have been developed to measure or estimate the fraction of total P that is important for either plant availability, potential environmental impact or both (Pautler and Sims, 2000).

Nonconventional soil testing techniques

In general, in this group of methods little or no salts are added when shaking soil with a solution, with the aim of dissolving and complexing the compounds (Ca, Al, Fe) to which P is bound. As a consequence, the amount of P brought into solution is much smaller than in conventional soil-P tests, giving an indication of the intensity (or direct plant availability) of P in a soil and not of the capacity of the soil to replenish P taken up by plants on the longer term.

The simplest methods used are based on extracting a soil with either water or with a dilute salt solution (0.01 M $CaCl_2$). In both cases, a relatively low concentration of P is found in the extract. Under the same experimental conditions, concentrations measured in 0.01 M $CaCl_2$ were two to three times lower than in water. A large variation in the soil/solution ratio used can be seen in the literature, especially with water, varying from 1:2.5 to 1:60. In environmental studies much higher soil/solution ratios are sometimes used, for example, a range of between 1:5 and 1:3000 was used in a study in which water-extractable P was correlated with P in runoff. Water-extractable P is used for fertilizer advising in Austria, Belgium, The Netherlands, and Switzerland. The use of $CaCl_2$ for advisory purposes was proposed in the past, but no regular application within agricultural soil testing is known.

For a long time it was thought that there was no limitation in the amount of P a soil could retain before significant leaching occurred. Although this may be the case on agricultural sols when STP is low and P inputs via fertilizers and manures balance output via crops, it is not the case when soil is used for long-term applications of P in excess of crop removal. The capacity of a soil to retain P from waste disposal was evidenced a good correlation in acidic soils between the amount of P adsorbed during 1.2 yr plus oxalate extractable P (*Pox*), and oxalate extractable Fe plus Al [(Fe + Al)-ox]. This type of research was continued on a large number of acidic sandy soils from The Netherlands. The Dutch research on this topic showed that, under the condition that the soil is exposed to a relatively high P concentration (>90 mg P L^{-1}) during a long period (> 1 yr), the P sorption maximum (Qmax) of a soil can be estimated as:

$$Qmax = \alpha(Fe+Al)\text{-}ox$$

Based on various studies, the parameter α equals approximately 0.5 ± 0.1, for regulatory purposes simplified as $\alpha = 0.5$. The actual loading of a soil, or the DPS, can be estimated as

the molar ratio between total amount sorbed and the sorption capacity:

$$DPS\ (\%) = 100 \times [Pox/\alpha\ (Fe+Al)ox]$$

The parameter DPS is correlated with environmental parameters related with mobile P, like water extractable P, P in runoff, and other parameters. Based on the Langmuir equation, describing the relation between the concentration of P in solution (C) and P adsorbed onto the soil (Q):

$$Q = Qmax\ (K\ C/(1+KC))$$

$$C = DPS/(K\text{-}DPS\ K)$$

Thus, a nonlinear relation can be expected between DPS and equilibrium concentration when a sufficient wide range of DPS is sampled. A narrow range of DPS, and/or a large scatter of data, may lead to a *quasi* linear relationship between DPS and equilibrium concentration. At a low value of K, a more gradual slope can be expected than at a high value of K. At lower pH, higher values of K can be expected than at high pH, and at higher ionic strength K increases; also dissolved organic matter can reduce P adsorption and lead to lower values of K. Instead of using oxalate-extractable P, Fe and Al for calculating DPS other parameters have been proposed. Mehlich-1 and 3 have been used to extract P, Al, and Fe for DPS calculations.

Use of soil tests for environmental purposes

Although traditional agronomic soil tests were developed to measure the plant availability of nutrients to crops, there is interest in using agronomic STP as an indicator of the potential for P loss from agricultural land (Styles et al., 2006). The STP concentrations are often readily available, as they estimate crop P requirements, or are relatively cheap to obtain, and it would be convenient if these concentrations were good indicators for environmental P losses.

Several researches have found close correlations between P losses in runoff and agronomic STP, with a greater STP concentration leading to higher P concentrations in runoff. Pote et al. (1999) reported correlation coefficients between STP and dissolved reactive P in runoff. Although many researchers have been successful at identifying links between STP and

dissolved P in runoff, it is more difficult to link STP to total P losses where particulate forms of P dominate.

The environmental threshold

In areas where environmental problems such as eutrophication have been identified and linked to P losses from agricultural lands, regulations are frequently developed to control P applications (Torrent et al., 2007). These regulations can take many forms, but one of the more common approaches is to use a STP threshold, above which applications of P in fertilizers or manures are limited or not permitted. The main advantage of the environmental threshold approach over other more complex P loss assessment tools such as the P site index is that it is quick, cheap, and the STP data may already be available for comparison with the threshold.

The first step in the development of a STP threshold should be to decide what critical concentration of P is acceptable in runoff and leaching. Then experiments can investigate the relationship between STP and the concentration of P in surface and subsurface losses, from which regression analysis can identify the STP concentration at the critical P loss concentration. This concept is based on many studies which show close relationships between STP and P losses in runoff and leaching. As surface waters differ in their sensitivity to P inputs and P losses are influenced by management and site factors as well as STP, these steps are not as straight forward as they may seem at first. Indeed, STP alone will never be able to take into account site and transport factors to provide comprehensive P loss risk assessment in the same way that a more complicated tool such as the P index will.

Sharpley et al. (1993, 1995) stated that sustainable management of P for agricultural production and environmental protection required the identification of soil P concentrations that exceed crop P requirements and have the potential for P enrichment in runoff. Often it is assumed that release of P to leachate or runoff will increase more rapidly, per unit increase in STP, at greater STP concentrations or above the STP threshold. Sims et al. (2000) identified what they termed a change point for 60 mg Olsen-extractable P kg^{-1} above which P losses in drainage increased rapidly per unit change in Olsen P, compared to relatively small changes in P loss in drainage below the change point. Using 0.01 M $CaCl_2$ extraction as surrogate for P in drainage, Hesketh and Brookes (2000) found that the change

point measured using Olsen P varied widely between soils, from 10 to 119 mg Olsen P kg^{-1}. Just as for optimum agronomic STP concentrations, there are slight differences between environmental thresholds among regions. These differences in environmental threshold may result from regional variabilities such as soil and climate, as well as the uncertainties involved in linking STP to P losses. The STP threshold can also act as a screening tool for more complex P assessments, for example, if a filed has a STP concentration above a threshold, then a further evaluation such as a P site index needs to be carried out to decide what P management strategy is permitted.

Keeping agronomic STP below the environmental threshold is an important consideration in developing sustainable agricultural systems, as well as many other areas where human activity impacts the environment.

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Chapter 2 - Phosphorus forms and P-sorption properties in three alkaline soils after long-term mineral and manure applications

Abstract

In area of intensive agriculture continuous applications of mineral and organic fertilizers can lead to an accumulation of phosphorus (P) in the soils and progressive saturation of their sorption capacities. The long-term evolution (44 years) of soil P forms has been studied in a factorial experiment combining three soils (sandy, clay and a peaty) with three types of fertilization (no fertilizer, farmyard manure and mineral fertilizer).

The P distribution across the calcium P (P_{Ca}), iron P (P_{Fe}), iron + aluminium P (P_{Fe+Al}) forms, and the effect of treatments on P availability (oxalate extractable-P, Mehlich-3-P, Olsen-P, water extractable-P) were determined. Two sorption P indices and the degree of soil saturation were calculated as well.

The soil P content showed a marked increase, leading to values exceeding the environmental thresholds. While in absolute terms the more consistent accumulations were observed in clay and peaty soils, the increase of soil P in sandy soils is of particular concern, given their lower P sorption capacity in respect to the other soils.

Soil P increase was affected both by quantity and quality of P inputs. Farmyard manure caused a more consistent increase of soil P, affecting in particular the water soluble and labile P forms. The increase of these readily assimilable and potentially mobile P forms was positively correlated with the amount of high molecular weight humic fractions, thus stressing the importance of the type of incorporated materials and of their evolution in determining soil P behaviour. Among the soil P tests, even if our data did not permit to evaluate the correlation with plant adsorption, Melich-3-P proved to be both a reliable method for agronomic and environmental purposes.

Introduction

In Northern Italy long-term applications of animal manures have resulted in a dramatic accumulation of phosphorus (P) in the soils and progressive saturation of their sorption capacities (Castaldi et al., 2009; Delgado and Scalenghe, 2008), increasing the risk of P losses to aquatic ecosystems. Indeed, most animal manures contain N:P ratios that are much lower than needed for optimal crop growth. Because N is the limiting nutrient in manures, they have been applied at rates designed to meet crop N requirements resulting conversely in a dramatic P over-fertilization (Morari et al., 2010). As a consequence highly manured soils have less capacity to retain phosphate (Sharpley et al., 2001), posing a higher risk for the environment than soils that received manure rarely or not at all (Brock et al., 2007).

For all these reasons considerable emphasis has recently been placed on quantifying aspects of the soil P cycle to establish a threshold in the P content for environmental rather than agronomic purposes (*e.g.*, Torrent et al., 2007; Hao et al., 2008).

Mobility of P is strongly affected by its reactions with soil constituents. Many attempts have been made to describe the sequence of reactions that leads to phosphate retention in acid soils (Börling et al., 2004a,b; Allen and Mallarino, 2006). In acid soils, P is fixed into slightly soluble forms by precipitation and sorption reactions with Fe and Al compounds as well as crystalline and amorphous colloids. Phosphorus sorption is highly correlated with the clay content and with amorphous Fe and Al oxides content (Börling et al., 2001). Nevertheless, there is limited information on the behaviour and availability of P in alkaline soils, especially of the calcareous type (von Wandruszka, 2006; Jalali, 2007; Ige et al., 2008). In calcareous soils, surface adsorption and precipitation are major P retention processes depressing the P availability and mobility. For soils rich in carbonates ($CaCO_3$) the P solubility may be controlled by solid phase dicalcium phosphate or by chemisorption of P on calcite, with the formation of a surface complex of calcium carbonate-P with a well-defined chemical composition (Samadi and Gilkes, 1999). The dynamics of organic P compounds, their relationships with carbonate minerals and their interactions with soil organic matter dynamics have not received much attention in the past (Cross and Schlesinger, 2001). Significant changes in labile inorganic and organic P associated with the long-term use of organic and mineral fertilizers in calcareous soils have been reported

(Romanyà and Rovira, 2007). However, it was not clear whether changes in P availability led to significant variations in the total pool or were associated with the alteration in P sorption mechanisms resulting from changes in soil chemistry and biological processes (Romanyà and Rovira, 2009).

Long-term effects of P application on total P and soil P saturation have not been studied in the Po Valley (Northern Italy). This area is the most extensive plain in Mediterranean Europe covering an area of 46,000 km^2 and is developed on a calcareous matrix (*i.e.*, more than 50% of the soils have a content of total carbonate >10%) which was derived from dolomitic parent material. The area comprises the majority of the Italian livestock activities. Understanding the transformations and distributions of various P forms in calcareous soils receiving continuous manure is essential in developing appropriate P management strategies for sustainable agricultural production and environmental protection.

The main starting hypotheses of this paper are that a) the N-based fertilisation plans adopted so far in Northern Italy have led to P saturation conditions in the soils, b) the nature of the fertilizer plays a pivotal role in influencing the mobility, availability and saturation of P in alkaline soils, and c) the Olsen method (Olsen and Sommers, 1982), which has been extensively used in Italy to test the available soil P content, gives only a partial evaluation of the associated environmental risks.

The objectives of our study were to determine in a long-term experiment (44 years) how P distributes across the different chemical forms in three alkaline soils of the Po Valley and the long-term effects of mineral and manure applications on the P availability and saturation. A secondary objective was to test different soil P test methods for environmental purposes.

Materials and methods

Climate

The long-term experiment is located at the Experimental Farm of the University of Padova (Veneto Region, NE Italy 45°21'N; 11°58'E; 6 m a.s.l.). The local climate is sub-humid, with annual rainfall of about 850 mm. In the median year, rainfall is highest in June (100

mm) and October (90 mm) and lowest in the winter months (50-60 mm). Temperatures increase from January (minimum average: -1.5 °C) to July (maximum average: 27.2 °C). The reference evapotranspiration (ET_o) is 945 mm with a peak in July (5 mm/day). ET_o exceeds rainfall from April to September. The site has a shallow water table ranging from about 0.5-1.5 m in late winter-early spring to 1-2 m in summer.

Long-term trial

This experiment began in 1964 in 4 m^2 open lysimeters, 80 cm deep. The experimental treatments derive from the factorial combination of three types of soil, hereinafter called sandy (SDY), clay (CLY) and peaty (PTY) in relation to their dominant property (Table 1), with six types of mineral, organic or mixed fertilization, organized in two randomized blocks (36 lysimeters) (Giardini, 2004).

The soils were brought from three locations in the Veneto region: SDY from the central coastal area, CLY from the south-western plain and PTY from the southern plain. The original soil profiles were reconstructed in the lysimeters. SDY (Cumulic, Vertic, Endoaquoll fine, mixed, calcareous, mesic – US classification, (SSS, 1998; ARPAV, 2005)) contains predominantly quartz and feldspar and a significant amount of dolomite (16%). CLY (Cumulic, Vertic, Endoaquoll fine, mixed, calcareous, mesic – ARPAV, 2005) has a higher amount of montmorrilonite (16%) than the other soils and a considerable presence of mica (19%) and dolomite (15%). PTY (Typic Sulfisaprists euic, mesic – ARPAV, 2005) has a higher mica content (25%) and 12% of montmorrilonite.

Fertilization treatments considered for this study were as follows: control (UNT); farmyard manure – FYM (40 t $ha^{-1}y^{-1}$); mineral fertilizer – MIN (200 kg $ha^{-1}y^{-1}$ N - 100 P_2O_5 - 240 K_2O). The FYM applied about the same amount of macroelements as MIN and around 3.5-4 t C $ha^{-1}y^{-1}$. Until 1984 there was a two-year maize (*Zea mays* L.) - wheat (*Triticum aestivum* L.) rotation. Thereafter, a variable rotation was adopted between 1985 and 1992, with various horticultural crops. From 1993 to 2002 there was a three-year rotation of tomato (*Lycopersicon esculentum* Mill.) – sugarbeet (*Beta vulgaris* L.) – maize, followed by various horticultural crops, maize and sunflower (*Helianthus annuus* L.) from 2003 to 2007. Apart from fertilization, all plots were treated in the same way in terms of rotation and management (tillage, sowing, harvest, etc.). The top 15-20 cm was dug each autumn

and crop residues were removed from all treatments.

Soil sampling and analysis

In April 2008, samples from the 0 to 20 cm depth were taken from five points and bulked to obtain a sample of about 3 kg per plot. The samples were crushed by rolling pin to break up clods and pass a 2 mm sieve, air dried and stored at low humidity.

Soil pH was measured potentiometrically on 1:2.5 soil/water extracts. Organic C was determined by dry combustion in a CNS Vario Macro elemental analyzer (Elementar, Hanau, Germany) and correcting for the inorganic C. Carbonate content was determined by the calcimeter method and by gravimetric loss of CO_2. Total aluminium (Al_T), calcium (Ca_T), magnesium (Mg_T) and iron (Fe_T) concentrations were determined after digestion with concentrated sulphuric and perchloric acids. Extractable aluminium (Al), calcium (Ca), magnesium (Mg) and iron (Fe) were determined by Mehlich-3 (M3) solutions (Mallarino and Sawyer, 1999; Setter et al., 2009) and limited to Al and Fe plus ammonium oxalate (Ox) (Schwertmann, 1964). The metals in the extracts were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) with a SPECTRO CIROS (Spectro Analytical Instruments, Kleve, Germany). Total P (TP) was determined by two methods: perchloric acid digestion (TPp) (Sparks et al., 1996), and ignition and HCl extraction (TPi) (Chaya, 1996). For perchloric acid digestion, 0.5 g of soil were treated with concentrated H_2SO_4 (6.25 mL) and $HClO_4$ (1.25 mL), and digested at 150 °C. After cooling, the mixture was diluted with distilled water to 50 mL. The P concentration was determined colorimetrically. For the ignition method, comparable samples of soil were extracted with concentrated HCl before (inorganic P, IP) and after ignition (TPi) (Legg and Black, 1955). The organic P (P_{Org}) was calculated as the difference between TPi and IP. For the procedure, 0.5 g of soil were placed in a 50 ml Pyrex beaker, ignited at 550 °C for 1 h, and cooled. After treating with concentrated HCl and heating on a steam plate at 70 °C for 10 min, an additional quantity of concentrated HCl (5 mL) was added. The sample was then left at room temperature for 1 hour, after which the suspension was filtered through Whatman No. 42 filter paper. The supernatant liquid was diluted to 50 mL with distilled water, and the P determined colorimetrically by the malachite green method. The concentration of orthophosphate was revealed by adding 0.4 mL of 17.55 g L^{-1} ammonium

molybdate tetrahydrate in 3.15M H_2SO_4, and 0.4 mL of a dye solution containing 3.5 g L^{-1} of polyvinyl alcohol in 0.3 g L^{-1} of malachite green to 2 mL of the above digested solutions. Absorbency was read after 2 h at 600 nm using a double beam UV-Vis spectrophotometer (Jasco, Tokyo, Japan) (Ohno and Zibilske, 1991; Martin et al., 1999). For the P linked to aluminium (P_{Al}) or to calcium (P_{Ca}), a soil sample (0.5 g) was treated with 25 mL of 1mol L^{-1} ammonium fluoride or 0.25mol L^{-1} sulphuric acid solutions and shaken for 2 h. The solution was then centrifuged, filtered through Whatman No. 42 and the P in solutions determined by the malachite green method. For the P bound to iron and aluminium (P_{Fe+Al}), a soil sample (0.5 g) was shaken for 2 h in 0.1mol L^{-1} NaOH solution (Stevenson, 1986). The extract was then centrifuged and the P content determined by ICP-OES. Extractable-P was determined by ammonium oxalate, Mehlich-3 solutions, Olsen, and water. Acid ammonium oxalate extractable-P (P_{Ox}) was determined by shaking a 1.5 g soil sample with 30 mL of 0.5 mol L^{-1} $(COONH_4)_2$ (pH 3.0) for 2 h in the dark (Schwertmann, 1964). Mehlich-3-P (P_{M3}) was determined by shaking 2.5 g of soil with 25 mL of Mehlich-3 extracting reagent (Mehlich, 1984). Olsen extractable-P (P_{Ols}) was obtained by shaking 1.0 g of soil with 20 mL of 0.5mol L^{-1} sodium bicarbonate solution (pH 8.5) for 30 min (Olsen and Sommers, 1982). Water extractable-P (P_{H2O}) was determined in a soil:water ratio of 1:10 (w/v) at 23 °C for 1 h (Börling et al., 2004a). After filtration through Whatman No. 42 filter paper, P in the extracts was determined colorimetrically by the malachite green method, except for Olsen in which the procedure of Murphy and Riley (1962) was followed.

P Sorption indices and degree of phosphorus saturation

Two single-point P sorption indices (PSI) were determined (Bache and Williams, 1971; Börling et al., 2001). Two grams of air-dried soil (≤ 2mm) were equilibrated with 19.4 and 50mmol P kg^{-1} soil (PSI1 and PSI2, respectively) in 20 ml 0.01mol L^{-1} $CaCl_2$ in plastic centrifuge tubes. Two drops of toluene were added to inhibit microbial activity. The tubes were shaken backwards and forwards at 21 °C for 18 h. Following centrifugation at 3000 rpm for 10 min, the equilibrium concentration in the solution was analyzed colorimetrically by the malachite green method. The amount of P sorbed by the soil was calculated by subtracting the amount of P in the equilibrium solution from the amount added. The PSI

was calculated as the amount of P sorbed by the soil (X) after the single-point addition of P, divided by the logarithm of C (mmol/L) in the equilibrium solution (X/log C).

The degree of phosphorus saturation (DPS), defined as the ratio of extractable or labile P to the adsorption capacity of soil, was obtained for all the samples using the relationship:

DPS = (extractable P/P sorption capacity) * 100 [1]

where the extractable P was either the oxalate-extractable P or Melich-3 P expressed in mmol kg^{-1}, and P sorption capacity of the soil was calculated from oxalate-extractable Fe and Al or from Mehlich-3 extractable Fe and Al also expressed in mmol kg^{-1}.

Statistical analyses

Barlett's test was used on the data to test the homogeneity of variance. Angular transformation was used when required to normalize the data. A two-way completely randomized ANOVA was used to compare treatment effects. The factors considered were soil type and fertilization. The Student-Newman-Keuls test was applied to compare the differences between group means. To identify the structure of the interdependences between the main soil parameters, a joint principal components analysis (PCA) was performed on the following variables: clay, pH, total carbonate, organic carbon, humic carbon with apparent molecular weight above 60 kDa (HF1) and less than 30 kDa (HF3) (Lugato et al., 2010), P_{Ca}, P_{Al}, P_{Fe+Al}, P_{Ox}, P_{M3}, P_{Ols} and P_{H20}. The standardized variables were submitted to PCA; rotated orthogonal components (varimax method of rotation) were extracted and the relative scores were determined. Only PCs with eigenvalue > 1 were considered for the discussion.

Results

General soil properties

The soils were alkaline, with higher pH in SDY (8.0) than CLY (7.8) and PTY (7.5) ($p<0.05$). Soil reaction varied according to carbonate content, which ranged from 8.6 g kg^{-1} in PTY to 49 and 162 g kg^{-1} in CLY and SDY, respectively. OC was influenced by the type of soil ($p<0.05$) and treatments ($p<0.05$), with higher content in PTY (64.6 g kg^{-1}) than CLY

(19.5 g kg^{-1}) and SDY (9.8 g kg^{-1}), and in FYM (35.5 g kg^{-1}) than UNT (28.3 g kg^{-1}) and MIN (30.2 g kg^{-1}).

Total Ca (Ca_T) and Mg (Mg_T) contents were higher in SDY (23110 and 3813 mg kg^{-1}) than CLY (18788 and 3373 mg kg^{-1}) and PTY (11364 and 2328 mg kg^{-1}), with a significant ($p<0.05$) increase of the ratio Mehlich-3 form/total forms in the order PTY>CLY>SDY (Table 2). Mehlich-3 Ca (Ca_{M3}) was affected by the type of soil, with higher values in CLY than SDY and PTY, whereas no significant differences were observed for Mehlich-3 Mg (Mg_{M3}). Total Al (Al_T) and Fe (Fe_T) showed higher contents in CLY and PTY than SDY, while the extractable forms of the two metals followed PTY>CLY>SDY ($p<0.05$) (Table 2). Only the oxalate Al (Al_{Ox}) didn't differ significantly between PTY and CLY.

FYM increased Ca_{M3} and Fe_{M3} and decreased Al_{M3} with respect to MIN and UNT (Table 2). No significant effect was instead induced by treatments on oxalate Fe (Fe_{Ox}) and Al_{Ox}. Nevertheless MIN showed higher values for Fe_{Ox}/Fe_T as well as Al_{Ox}/Al_T ($p<0.05$).

P forms

The different P forms were significantly affected by the type of soil and treatment. In general, the P forms content followed the order CLY>PTY>SDY and FYM>MIN>UNT. Total P (TP) determined by ignition (TP_i) was always higher than that determined by perchloric acid extraction (TP_p). Both TP_p and TP_i in CLY were twofold higher than those in PTY and fourfold those in SDY (Table 3). Organic P (P_{Org}) was from 0.09 to 0.34 times the TP_i, with the lowest value observed in SDY (67 mg kg^{-1}) and the highest in CLY (953 mg kg^{-1}). Calcium P ranged from 855 mg kg^{-1} in SDY to 3239 mg kg^{-1} in CLY, with an intermediate value of 1317 mg kg^{-1} in PTY. Aluminium P (P_{Al}) didn't differ significantly between SDY and PTY. Only iron and aluminium P (P_{Fe+Al}) deviated from the general trend, with a content in PTY (158 mg kg^{-1}) that was almost tenfold higher than those of SDY (22 mg kg^{-1}) and CLY (12 mg kg^{-1}).

Concerning treatments, FYM positively influenced P_{Org}, whereas both FYM and MIN affected inorganic P (IP) (Table 3). In particular, the manured soils showed a P_{Org} value 1.4-fold higher than untreated soils. Nevertheless, the ratio P_{Org}/TP_i was not affected by FYM. The TP_p, TP_i, P_{Ca} and P_{Al} were also affected by treatment with the sequence FYM>MIN>UNT (Table 3). No significant differences between FYM (77 mg kg^{-1}) and

MIN (81 mg kg^{-1}) were instead observed in P_{Fe+Al}.

Extractable P forms

Large variations were observed in extractable P forms. Olsen-P (P_{Ols}), Mehlich-3 P (P_{M3}) and Oxalate-P (P_{Ox}) were in the range 10-108 mg kg^{-1}, 18-216 mg kg^{-1} and 76-1965 mg kg^{-1}, respectively (Table 4). Water extractable-P (P_{H20}) ranged between 1.4 and 22.5 mg kg^{-1}. CLY and PTY always showed higher extractable forms than SDY. Conversely they had lower P_{M3}/TP_i, P_{Ols}/TP_i, as well as P_{H2O}/TP_i, than SDY (Table 4). Treatments also influenced the P extractable forms with a significant interaction with the soils ($p<0.05$). In particular, FYM induced increments over UNT of 7.3-fold for P_{M3}, 6-fold for P_{Ols}, and 1.5-fold for P_{Ox} (Table 4). In the case of P_{H2O}, FMY had a value 6-fold higher than UNT for SDY and PTY, and 16-fold the UNT for CLY (Table 4). Mineral fertilization also significantly increased ($p<0.05$) the P extractable forms but with lower magnitude than FMY. The difference between FYM and MIN progressively reduced from P_{H20} to P_{Ox}, down to the extreme case of CLY where no significant differences were observed in P_{Ox} content (Table 4).

Both P_{M3} and P_{Ols} showed a weak linear correlation with P_{Ox} ($R^2=0.31$), while P_{M3} and P_{Ols} were strongly related ($R^2=0.71$). As concerns the relationships with P_{H20}, solely P_{M3} and P_{Ols} were correlated with P_{H20}; P_{M3} was able to explain more than 90% of the P_{H20} variability, while P_{Ols} only 58%. Three clusters of data were instead recognizable in the P_{Ox}-P_{H20} plot (Fig. 1), corresponding to the three different soils and highlighting the lack of an overall significant relationship between the two variables.

Three factors were extracted by PCA, accounting for 89% of the variance (Table 5). Factor 1 explained 46% of the variance and was correlated (factor loadings > 0.8) with P_{Al}, P_{M3}, P_{Ols} and P_{H2O}. Factor 2 explained 25% and was positively correlated with OC and P_{Fe+Al} and negatively with pH, $CaCO_3$ and HF3. Clay, P_{Ox} and P_{Ca} were possibly correlated with Factor 3 (18% of the variance). Plotting PC1 and PC2 (Fig. 2), the soils resulted as well separated. In particular, PTY was characterized by high values of OC and P_{Fe+Al}, while SDY and CLY by high pH, HF3, $CaCO_3$, and P_{Ca}. Extractable P forms (Mehlich-3, Olsen and water), P_{Al} and HF1 differentiated the soils according to the type of treatments, with high

values in FYM, intermediate in MIN and low in UNT.

P Sorption indices and DPS

Both PSI were affected by the soil type, with higher values in CLY and PTY than SDY (Table 4). In the case of low P addition (PSI1) no significant differences were observed between CLY and PTY, whereas with high P addition (PSI2) the index in PTY was twofold (4.01) that of CLY (1.94). Treatments affected PSI1, but only for FYM in CLY and PTY, while no differences were observed in SDY (interaction soil x treatment significant at 5%).

The DPS1 ((P_{Ox}/(Fe_{Ox}+Al_{Ox})*100) values ranged from 8 to 55, with the highest in sandy soils amended with FMY and the lowest in UNT of peaty soil (interaction significant at 5%) (Table 4). According to the treatments, the index followed the order FMY>MIN>UNT. The same order was also followed by DPS2 ((P_{M3}/(Fe_{M3}+Al_{M3})*100) but with strictly higher values up to 181.

Discussion

General soil properties

CLY and SDY were alkaline, as most Veneto soils have developed from calcareous parent materials (Nardi et al., 2005), whereas in PTY, which was acidic at the beginning of the experiment, the freeing of cations due to the mineralization of soil organic matter have enhanced the soil reaction to alkalinity (Morari et al., 2008). The importance of carbonates in soil composition was also highlighted by M3 extractions that exhibited higher values in both Ca and Mg than Al and Fe. It should be noted that Ca_{M3} to Ca_T ratio was in the progression PTY>CLY>SDY thus suggesting that in SDY and CLY the calcium carbonate minerals, dolomite and calcite, were the mostly present components for specific adsorption (Tunesi et al., 1999). In PTY, the high values of Fe_{Ox} to Fe_T ratios were probably due to the presence of OC that enabled the formation of poorly crystalline Fe oxides (Bertrand et al., 2003; Samadi and Gilkes, 1999). Moreover, PTY and CLY had a high Al_{Ox} to Al_T ratio that

is related to the presence of amorphous forms of aluminium oxides (Ryan et al., 1985; Bertrand et al., 2003). Indeed, both Fe_{Ox} and Al_{Ox} correlated well with OC and clay (data not shown). It is of interest to note that among treatments, FYM enhanced the availability of both Ca and Fe, as evidenced by the higher values of M3 extractions than the other treatments. Conversely, MIN enhanced the amount of amorphous Fe and Al oxides (ratio Ox/T) in respect to both UNT and FYM. These forms have been identified as important P sorption sites (Sparks et al., 1996).

P forms

Both FYM and MIN have increased the TP content in all soils and among the P forms P_{Ca} was the most affected, especially by FYM (Brock et al., 2007; Ige et al., 2008; Hao et al., 2008). The difference obtained by the two methods in determining the TP is probably due to the increased solubility of inorganic P compounds following ignition (Condron et al., 1990) and to an incomplete extraction of organic P compounds by perchloric acid (Condron et al., 2005). Nevertheless, our soils have P_{Org} to TP values similar to those found in other calcareous soils (Bertrand et al., 2003). Of the three soils, CLY and SDY contributed most strongly to the overall positive relationship between TP and $CaCO_3$, and TP with P_{Ca} (data not shown). Calcium carbonate is a very strong complexing agent for P: in calcareous soils P is mainly precipitated as calcium phosphates or co-precipitated with carbonates, thus the calcium P was one of the most important P forms in SDY and CLY (Bertrand et al., 2003; Jalali and Ranjbar, 2009). In spite of this, the strong correlation between OC and P_{Fe+Al} evidenced the contribution of organic matter on the P pool in peaty soil (Stevenson, 1986) (Fig. 2).

Extractable P forms

Both agronomic and environmental aspects should be taken into consideration to evaluate the extractable P results. Indeed, soils considered optimum in soil test P from a crop production point of view may still provide environmentally significant quantities of soluble and sediment P in surface runoff (Sharpley et al., 2003).

Water extracted the least amount of P, while oxalate reagent extracted the highest amount; however, of all the chemical P extractants, Olsen extracted the least amount of P in accordance with many authors (*e.g.*, Ige et al., 2006). Indeed the Olsen extractant has less ability to remove P from soil than acidic extractants (Sims, 2000a). This is due to the fact that as the pH of the extracting solution decreases, more of the phosphate associated with calcium is dissolved. The ability of M3 to extract more P than Olsen could account for the relatively high value of extractable P_{M3} set for optimum crop production. In fact, the value of P_{M3} generally set for optimum crop growth and yield (45-50 mg P kg^{-1}) is higher than the critical values used for P_{Ols} (10 mg P kg^{-1}) (Sims, 2000a,b; Mallarino and Sawyer, 1999). Both the P_{Ols} and P_{M3} indicated that soils' agronomic P levels in our experiment ranged from low to excessively high (Sims, 2000 a,b). The different relationships observed between the extractable P forms indicated that the methods extracted a different proportion of available P, with a higher correlation between M3 and Olsen than oxalate and Olsen or oxalate and M3.

Even if extractable P forms were not tested in terms of plant absorption, these first results seem to indicate that P_{M3} could be an alternative to the traditional P Olsen test for agronomic purposes in Northern Italian soils. It is worth to remember that P_{H2O} represents an imperfect index from crop production perspective since some forms of labile P are not immediately soluble in water but potentially available to the plant during the growing season (Huoba et al., 1997; Ige et al., 2006).

Concerning the environmental aspects, FYM and to a lesser extent MIN, exceed the threshold values of 20 mg kg^{-1} for P_{Ols} and 150 mg kg^{-1} for P_{M3} (McDowell et al., 2001; Sims et al., 2002), thus evidencing the potential risk of P loss with leaching and runoff. Since P_{H2O} has been used by many authors as the P dissolved in water which represents the most labile and mobile form of P in soil, the strict association found between P_{H2O} and P_{M3} (Figs. 1 and 2) strongly suggests that also P_{M3} may be a good indicator of environmental P loss. Our results also showed the increment of labile P forms induced by the treatment and in particular by FYM (Fig. 2). In this context, in CLY and PTY P_{H2O} was mostly affected by P_{Al}. Moreover, in the same soils, FYM also enhanced the HF1 in opposition to the UNT and MIN. These results provide evidence that FYM has a significant effect on P (von Wandruszka, 2006). FYM, that is generally known to affect both sorption/desorption and

precipitation of P, has been found to increase the high molecular size HF (Lugato et al., 2010) which had a strict correlation with the P_{H2O}, thus resulting in a great mobilizing effect. This mobility, due to the strong interactions between P and humic materials, can be related to the presence of metal ions that act as cationic anchors allowing anionic humates and phosphates to associate (Riggle and von Wandruszka, 2005). Our results agree well with those obtained by Delgado et al. (2002) who found that application of humics to soil increases the recovery of labile P such as Olsen P. Moreover the competition of organic molecules for the soil retention sites or the coating of the soil particles by organic molecules could block the P retention sites (Hue, 1991). The FYM effects are confirmed by the higher ratios between labile P forms and total P (Table 4) while P_{Ox}/TP did not vary according to fertilization. This can be explained by the stronger extraction by oxalate that can recover also the more recalcitrant forms (e.g calcium phosphates or P co-precipitated with carbonates), particularly in clay soils.

P sorption properties and DPS

Differences in PSIs were due to variation in the physico-chemical properties of the soils with a P adsorption capacity low in SDY and high in PTY. In particular this seems to be related to the different OC content and the presence of poorly crystalline Fe oxides and amorphous Fe and Al oxides. Organic matter acts directly increasing the adsorptions sites and indirectly inhibiting the crystallization of Al, which in turn can increase P sorption as non-crystalline Al sorbs more P than well-crystallined (Borggaard et al., 1990). It is worth noting, when comparing the two PSI indices, that PSI2 was not significantly affected by treatment. Actually, since P sorption is evaluated by the difference between initial and final concentrations, its accuracy decreases as the amount of P added increases (Börling et al., 2001) and by consequence with fertilization (Ige et al., 2008). The reduction in the PSI1 values with manure application has been hypothesized to be due to the competition of organic molecules for the retention sites (Hue, 1991). Anyway, the low PSI values in SDY and the effect of treatment in reducing PSI1 is well reflected by estimating the degree of P saturation. Indeed, lower DPS values were found in PTY than SDY. In the latter, treatment increased the DPS values with a maximum of 181 induced by FYM. Theoretically, DPS is

not expected to exceed 100%; however, long-term use of FYM caused increases of calcium P phases as mentioned above. This implies that calcium phosphates become stable and that under these conditions large accumulations of P can occur independently of Fe and Al oxides. The high DPS values were in accordance with those of Leinweber et al. (1997) and Koopmans et al. (2003) who found values up to 179 and 208, respectively, in heavily manured fields. Anyway, DPS values of >25 are associated with a great risk of P loss in leaching or runoff.

Conclusions

Our data confirmed the tendency to a higher P saturation after long term application of mineral and organic fertilisers. This can lead to P losses to water bodies, even if the interaction with pedological condition has to be considered. While in absolute terms the more consistent accumulations were observed in clay and peaty soils, the increase of soil P in sandy soils is of particular concern, given their lower P sorption capacity.

The use of organic fertiliser should be carefully evaluated due to the risk of excessive P inputs when N-based fertilization plans are adopted. Moreover, the amount of soluble and labile P forms resulted higher after farmyard manure applications, thus increasing the environmental risk.

From the management stand point it is then necessary to identify soil P tests able to assess at the same time forms available for plant adsorption and those more prone to losses in the environment. In this sense, Melich-3-P appears a good indicator for both agronomic and environmental purposes.

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Table 1 - Main physical and chemical characteristics of the top layer (0-20 cm) at the beginning of the experiment (1964).

Parameters	Clay	Sand	Peaty
Sand (2 mm-50 μm) (g kg^{-1})	250	934	380
Silt (50-2 μm) (g kg^{-1})	230	60	136
Clay (<2 μm) (g kg^{-1})	520	6	484
pH	7.9	8.1	4.9
$CaCO_3$ (g kg^{-1})	26	139	0
OC (%)	1.45	0.17	10.5
N (%)	0.15	0.01	0.67
C/N	10	12	16
Olsen P (mg kg^{-1})	161	26	100
Total P (mg kg^{-1})	2800	500	1100

Table 2 - Calcium, magnesium, iron, aluminium contents and their extractable forms in the 0-20 cm layer: mean values per soil type, treatment, and soil per treatment interaction.

		Ca			Mg			Fe					Al				
		T	M3	M3/T	T	M3	M3/T	T	Ox	M3	Ox/T	M3/T	T	Ox	M3	Ox/T	M3/T
		mg kg^{-1}			mg kg^{-1}			mg kg^{-1}					mg kg^{-1}				
Soil	Treatment																
Clay	UNT	17986*	8472a	0.47	3561	1183	0.33	28734	6080	150d	0.21c	0.005c	34847	2131a	650.6	0.061	0.0187b
	FYM	19395	8284ab	0.43	3235	1335	0.41	33625	5663	141d	0.17c	0.004c	39262	1818b	535.5	0.046	0.0136b
	MIN	18984	7922abc	0.42	3323	1031	0.31	34586	6707	145d	0.19c	0.004c	38269	2194a	633.2	0.057	0.0165b
Mean		18788*b*	8226*a*	0.44*b*	3373	1183	0.35*b*	32315 *a*	6150*b*	145*b*	0.19*b*	0.005*c*	37459*a*	2048*a*	606.4*b*	0.055*a*	0.0163*a*
Sand	UNT	24552	6115d	0.25	4180	1281	0.31	7087	523	97e	0.07d	0.014b	9656	193c	0.1	0.020	0.00001c
	FYM	23884	8007abc	0.34	3872	1530	0.40	6671	453	112e	0.07d	0.017b	8467	157c	0.6	0.019	0.00007c
	MIN	20893	6229d	0.30	3386	1014	0.30	6304	500	97e	0.08d	0.015b	8075	187c	0.1	0.023	0.00001c
Mean		23110*a*	6784*b*	0.29*c*	3813	1275	0.33*b*	6687*c*	492*c*	102*c*	0.07*c*	0.015*b*	8733*b*	179*b*	0.3*c*	0.021*b*	0.00003*b*
Peat	UNT	11893	6883cd	0.58	1994	846	0.42	23546	10673	347c	0.45b	0.015b	44271	2037a	647.6	0.046	0.0146b
	FYM	12480	7140bcd	0.57	2427	1098	0.45	24309	11371	389a	0.47b	0.016b	45332	2128a	625.8	0.047	0.0138b
	MIN	9719	6726d	0.69	2562	1171	0.46	16608	11099	370b	0.67a	0.022a	28811	2057a	694.5	0.071	0.0241a
Mean		11364*c*	6916*b*	0.61*a*	2328	1038	0.44*a*	21487 *b*	11048*a*	369*a*	0.53*a*	0.018*a*	39471*a*	2074*a*	656.0*a*	0.055*a*	0.0175*a*
Mean	UNT	18144	7157B	0.43	3245	1103	0.35	19789	5759	198B	0.25B	0.011	29591	1454	432.8A	0.042AB	0.0111B
	FYM	18586	7810A	0.44	3178	1321	0.42	21535	5829	214A	0.23B	0.012	31020	1367	387.3B	0.037B	0.0092B
	MIN	16532	6959B	0.47	3090	1072	0.36	19166	6102	204B	0.31A	0.014	25052	1479	442.6A	0.051A	0.0136A

UNT, untreated; FYM, farmyard manure; MIN, mineral; T, total; M3, Mehlich-3; Ox, oxalate. *In the same column differences among soil x treatment, among soil mean (italicized letters) and among treatments (capital letters) were at $P \leq 0.05$.

Table 3 - Forms of P in the 0-20 cm layer: mean values per soil type, treatment, and soil per treatment interaction.

		TPp	TPi	IP	P_{Org}	P_{Org}/TPi	P_{Ca}	P_{Al}	P_{Fe+Al}
		mg kg^{-1}					mg kg^{-1}		
Soil	Treatment								
Clay	UNT	2443b*	3495	2541c	953	0.27abc	2794c	89	6c
	FYM	3162a	4925	4030a	895	0.18bcd	3628a	432	21c
	MIN	2571b	4460	3726b	734	0.16cd	3297b	240	9c
Mean		2725*a*	4293*a*	3433*a*	861*a*	0.20*b*	3239*a*	253*a*	12*b*
Sand	UNT	466f	730	663f	67	0.09d	649g	25	13c
	FYM	674ef	1425	1149e	276	0.19abcd	1093f	123	31c
	MIN	836e	1203	1065e	138	0.12d	824g	115	23c
Mean		659*c*	1120*c*	959*c*	160*c*	0.13*c*	855*c*	87*b*	22*b*
Peat	UNT	917e	1408	955e	453	0.32ab	735g	74	82b
	FYM	1596c	2549	1676d	874	0.34a	1849d	268	178a
	MIN	1162d	2128	1679d	449	0.21abcd	1369e	144	213a
Mean		1225*b*	2029*c*	1437*b*	592*b*	0.29*a*	1317*b*	162*b*	158*a*
Mean	UNT	1276C	1878C	1387B	491B	0.23A	1393C	63C	34B
	FYM	1810A	2967A	2285A	682A	0.24A	2190A	274A	77A
	MIN	1523B	2597B	2157A	440B	0.16B	1830B	166B	81A

UNT, untreated; FYM, farmyard manure; MIN, mineral; TPp, total P by perchlorich acid digestion; TPi, total P by ignition; P_{Org}, organic P; IP, inorganic P; P_{Ca}, calcium linked P; P_{Al}, aluminium P; P_{Fe+Al}, iron and aluminium P. *In the same column differences among soil x treatment, among soil mean (italicized letters) and among treatments (capital letters) were at $P \leq 0.05$.

Table 4 - Extractable P, PSI and DPS values in the 0-20 cm layer: mean per soil type, treatment, and soil per treatment interaction.

		P_{Ox}	P_{M3}	P_{Ols}	P_{H2O}	P_{Ox}/TP_i	P_{M3}/TP_i	P_{Ols}/TP_i	P_{H2O}/TP_i	PSI1	PSI2	DPS1	DPS2
		mg kg^{-1}											
Soil	Treatment												
Clay	UNT	1593b*	28ef	15d	1.4d	0.46bc	0.008f	0.004d	0.0004h	0.91a	2.09	27b	3g
	FYM	1965a	216a	108a	22.5a	0.40c	0.044b	0.022abc	0.0046c	0.85b	1.83	38b	31c
	MIN	1935a	106c	46cd	5.1d	0.44bc	0.024d	0.010cd	0.0012g	0.89a	1.90	31b	13e
Mean		1831a	117a	56a	9.7a	0.43b	0.025b	0.012b	0.0020c	0.88*a*	1.94*b*	32*a*	16*b*
Sand	UNT	76g	18f	12d	1.5d	0.10e	0.025d	0.016bcd	0.0021e	0.74c	1.11	15cd	34c
	FYM	235f	114c	39cd	9.5c	0.16d	0.080a	0.028ab	0.0066b	0.74c	1.07	55a	181a
	MIN	165f	39e	15d	4.7d	0.14d	0.033c	0.012bcd	0.0038d	0.74c	1.61	34b	79b
Mean		159c	57c	22b	5.2b	0.14c	0.046a	0.019a	0.0042a	0.74*b*	1.26*c*	35*a*	98*a*
Peat	UNT	689e	27ef	10d	2.4d	0.49ab	0.019e	0.007d	0.0017f	0.90a	4.21	8d	3g
	FYM	1400c	194b	70bc	18.0b	0.55a	0.076a	0.028ab	0.0071a	0.85b	4.20	16c	21d
	MIN	1011d	87d	83ab	4.5d	0.48abc	0.041b	0.039a	0.0021e	0.88a	3.63	12cd	9f
Mean		1033b	103b	54a	8.3a	0.51a	0.045a	0.025a	0.0036b	0.88*a*	4.01*a*	12*b*	11*c*
Mean	UNT	786C	24C	12C	1.7C	0.35	0.017C	0.009C	0.0014C	0.85A	2.47	17C	13C
	FYM	1200A	175A	72A	16.7A	0.37	0.067A	0.026A	0.0061A	0.81C	2.37	36A	78A
	MIN	1037B	77B	48B	4.7B	0.35	0.033B	0.020B	0.0023B	0.84B	2.38	26B	34B

UNT, untreated; FYM, farmyard manure; MIN, mineral; Ox, oxalate; M3, Mehlich-3; Olsen; TPi, total phosphorus by ignition; PSI, single-point P sorption indices derived from the addition of 19.4 (PSI1) and 50 (PSI2) mmol P kg^{-1} soil; DPS1, $P_{Ox}/(Fe_{Ox}+Al_{Ox})*100$; DPS2, $P_{M3}/(Fe_{M3}+Al_{M3})*100$. *In the same column differences among soil x treatment, among soil mean (italicized letters) and among treatments (capital letters) were at $P \leq 0.05$.

Table 5 - Loadings values of some physical and chemical variables on the axes identified by principal components analysis for the three types of soils. Bold numbers > | 0.8| .

Variable	Factor 1	Factor 2	Factor 3
pH	-0.25	**-0.90**	-0.11
$CaCO_3$	0.03	-0.70	-0.68
Clay	0.06	0.43	**0.88**
OC	0.01	0.97	0.10
P_{Ox}	0.40	0.06	**0.90**
P_{M3}	**0.95**	0.17	0.16
P_{Ols}	**0.82**	0.30	0.23
P_{H2O}	**0.92**	0.11	0.08
P_{Ca}	0.49	-0.27	**0.81**
P_{Al}	**0.85**	-0.01	0.41
P_{Fe+Al}	0.07	**0.89**	-0.09
HF1	0.57	0.13	-0.56
HF3	0.27	**-0.89**	-0.07
Explained Variance (%)	46.45	24.64	17.81

OC, organic C; P_{Org}, organic P; P_{Ox}, oxalate extractable P; P_{M3}, Mehlich-3 extractable P, P_{Ols}, Olsen P, P_{H2O}, water soluble P, P_{Ca}, calcium P; P_{Al}, aluminium P; P_{Fe+Al}, iron and aluminium P; HF1, humic carbon with apparent molecular weight above 60 kDa; HF3 humic carbon with apparent molecular weight less than 30 kDa.

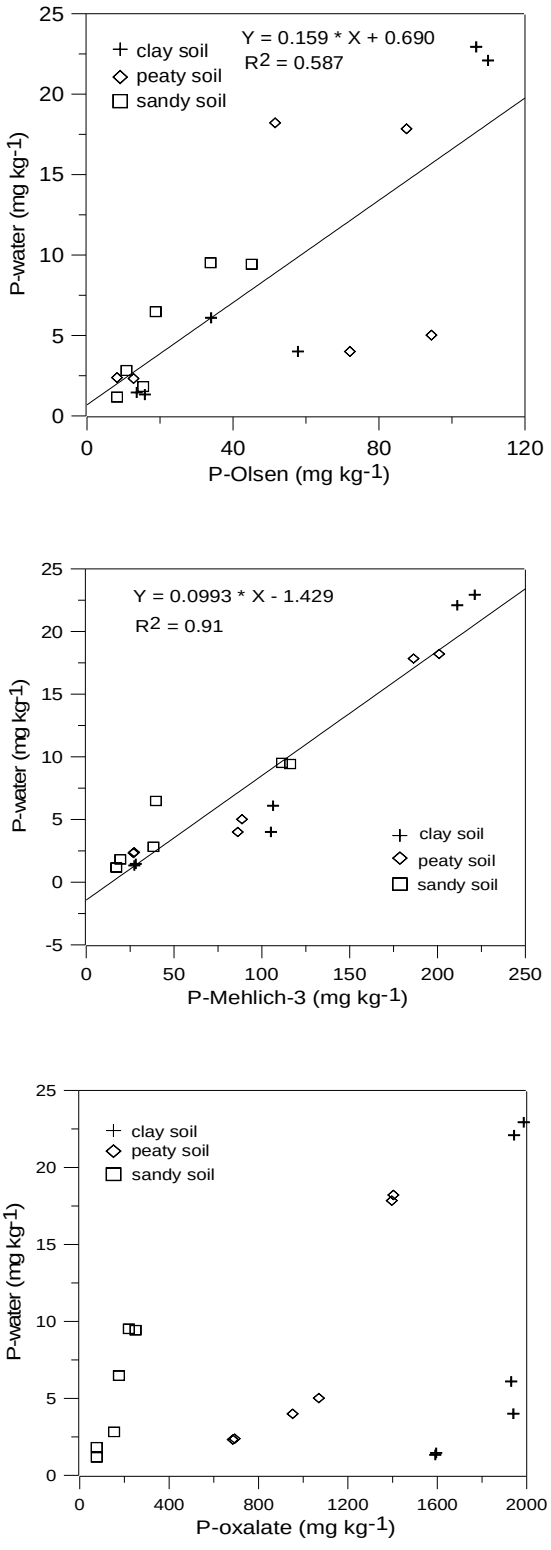

Figure 1 - Relationships between extractable P forms and P_{H2O}.

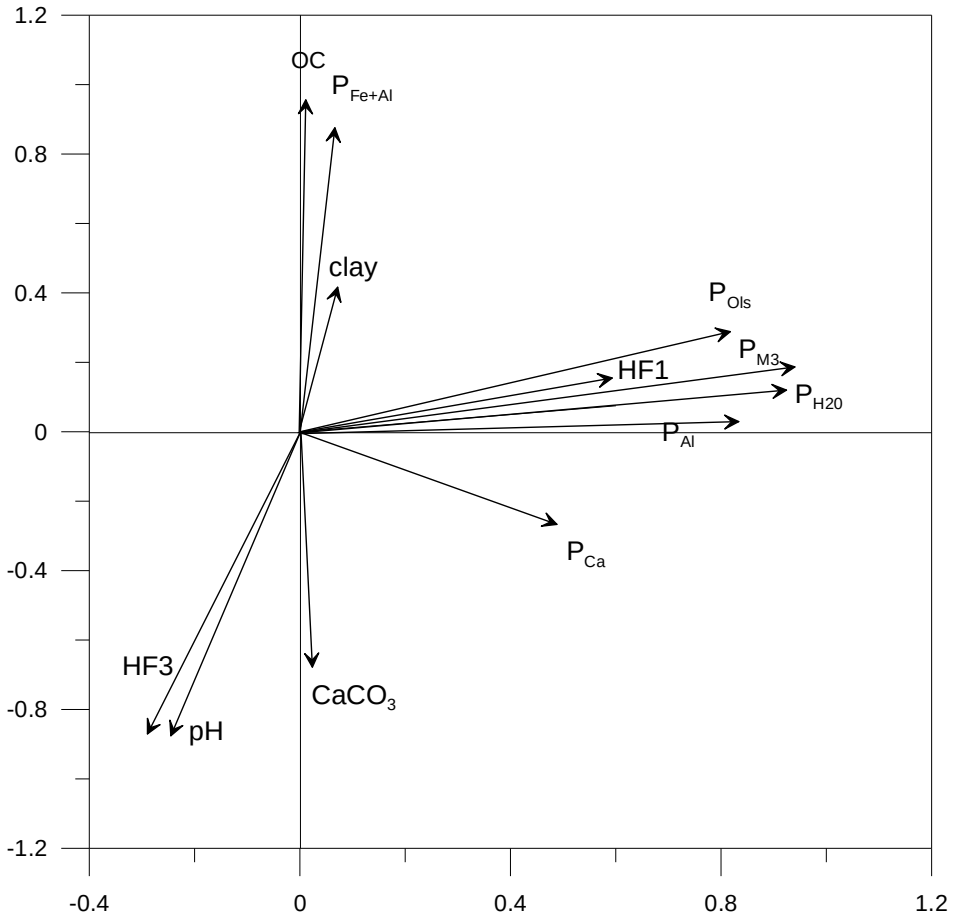

Figure 2a - Variables projected in the plane determined by the first two principal axes.

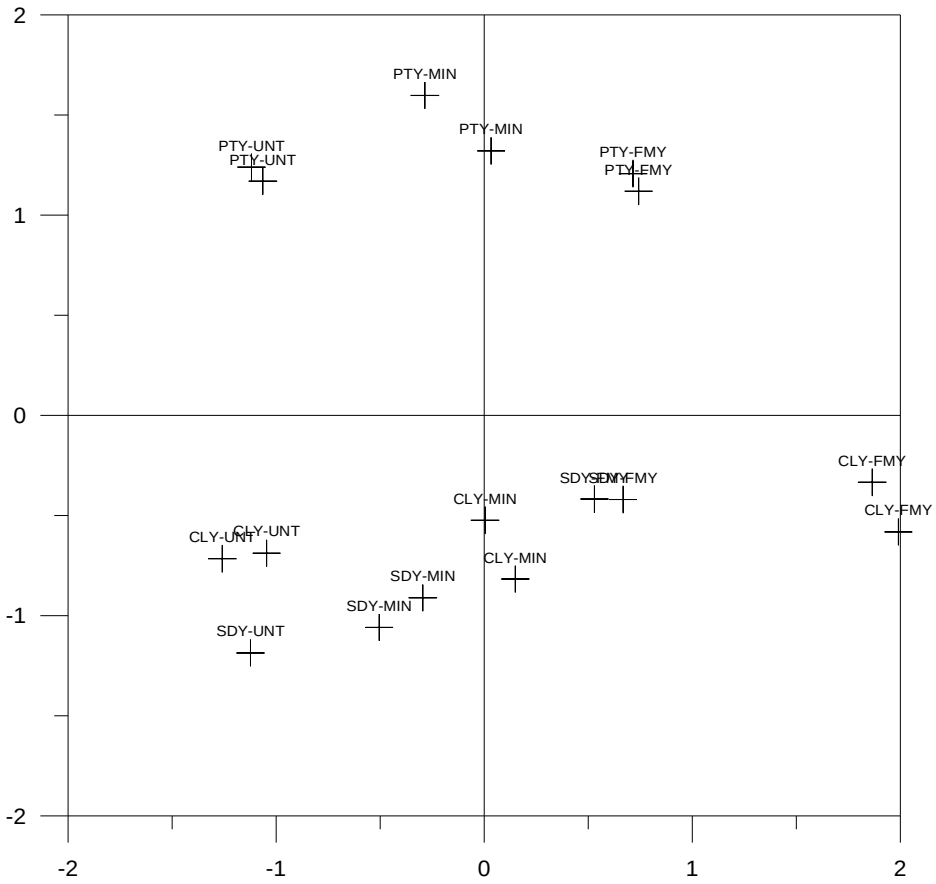

Figure 2b - Positions of the 18 soil samples plotted in the reduced space of the first two principal components after principal components analysis.

Chapter 3 - Phosphorus forms, P-sorption properties and risk in P release in three alkaline soil profiles (0-100 cm) after long-term mineral and manure applications

Abstract

The accumulation of phosphorus (P) in agricultural soils due to fertilization has increased the risk of P losses from agricultural fields to surface and or subsurface waters. In risk assessment systems for P losses, both P release from soil to solution and transport mechanisms need to be considered. In this study, the overall objective was to identify soil variables to predict potential P release from soil to solution. Soils from a long-term experiment began in 1964 were considered. The experimental treatment was derived from a factorial combination of three soils (sandy, clay and peat) with three types of fertilization (no fertilizer, farmyard manure and mineral fertilizer). Soils samples were collected from a depth of 0 to 100 cm, at intervals of 10cm.

The P distribution across the calcium P (P_{Ca}), iron P (P_{Fe}), iron + aluminium P (P_{Fe+Al}) forms, and the effect of treatments on soil test P (STP) (oxalate extractable-P, Mehlich-3-P, Olsen-P, water extractable-P) for P availability were determined. Phosphorus extractable with water was used as an estimate of potential P release from soil to solution. Two sorption P indices were also calculated to estimate the P sorption capacity. In comparison with the control, manure application significantly increased the levels of all P forms with the largest increase occurring in the STP. The potential P release was related with a split line to Olsen-P and Mehlich-3-P for individual soils and a change point at the concentration of 54 mg kg^{-1} Olsen-P both in CLY and PTY, and at a concentration of 10 mg kg^{-1} Mehlich-3-P in CLY, and 170 mg kg^{-1} Mehlich-3-P was determined. Thus, long-term manure application had caused the movement of P throughout the soil profiles and is a potential risk to the environment.

Introduction

In Northern-Italy continued long-term fertilization of the land has lead to available phosphorus (P) concentrations in topsoils that exceed those required for optimal plant growth, thus increasing the potential for P loss to surface waters and leaching and subsequent eutrophication (Pizzeghello et al., 2011). The continued application of manure, in particular, has significant effects on P retention due to competition for P fixation sites among organic acids, and/or the complex of exchangeable Al and Fe by manure components (von Wandruska, 2006). Manure does not itself affect sorption and precipitation of P, but contains significant amounts of the element, which is thereby applied in excess to the land, contributing to the different P pools (Hao et al., 2008). Reactions of P with soil constituents strongly affect the agronomic effectiveness and environmental considerations related to the usage of phosphate fertilizers. Therefore, the knowledge of transformation of P added to the soil and the effects of soil properties on the transformation have become essential for long-term planning of fertilization strategies to sustain crop production and minimize the impact on water quality (Guo et al., 2008). The fate of surplus fertilizer P depends on the transport mechanism, soil type and soil properties such as P sorption capacity and the type of fertilizer added (Eghball et al. 1996). The translocation and distribution of P in the soil profile is important both for plant uptake and for the risk of P losses. If P stays in the root zone, it can be taken up by plants, but if it is transported to deeper layers the risk of P losses occurring increases (Börling et al., 2004). Previous studies have shown that P can accumulate in the subsoil down to a depth of 30-180 cm, following long-term fertilization with organic and inorganic P fertilizers (Sharpley et al., 1993; Kingery et al., 1994). Several authors have studied P sorption capacity in soil profiles. Contradictory results have been obtained, with both higher and lower P sorption capacity in the subsoil compared to the topsoil. Mozaffari and Sims (1994) found that P soil capacity measured either with P sorption isotherms or through a single-point P sorption index, increased with depth. In contrast, Peltovuori et al. (2002) found a decrease in P sorption capacity with depth, estimated by the extraction of Al and Fe with acid ammonium oxalate

solution.

To evaluate the mobility of P in soils with long-term manure application history, a better understanding of the relative contributions of different forms of P is needed. Phosphorus release from soil to water is regulated by the relationship between soil P quantity and solution intensity, namely the partitioning of P bound to soil solid phases and dissolved into solution. This partitioning can be described using sorption isotherms (Torrent and Delgado, 2001). A change point on the sorption curve denotes the conversion of sorptive power. Below the change point, soil P is confined to the high-energy sites, while, above that point, soil P is held in low-energy sites, and hence it is more easily released into water (Syers et al., 1973). The fractions of P extractable by various chemical agents are used as proxies of soil P quantity, and water-extractable P often represents P intensity in solution (McDowell and Sharpley, 2001). Previous studies have found that the concentration of dissolved reactive P in surface runoff or subsurface drainage is closely related to soil test P (STP) concentration in the topsoil (Sims et al., 2000). Furthermore, the relationship between STP and water-extractable P, resembling the relationships between STP and dissolved reactive P concentration has shown some ability to predict soil P loss potential (McDowell and Sharpley, 2001; Maguire and Sims, 2002). Thereby, the conventional soil P test in combination with water soluble P can be used as a rapid and convenient analysis method to evaluate P mobility and therefore serve as an assessment of P nonpoint source in agricultural fields (Li et al., 2010).

The objectives of our study were to determine in a long-term experiment (44 years) how P distributes across the different chemical forms in three alkaline soils of the Po Valley and the long-term effects of mineral and manure applications on P availability and saturation. To assess how and if P moves through the soil profiles, the analyses were carried out at intervals of 10 cm from a depth of 0 to 100 cm. The existence of a change point relationship between P water extractable and STP was also considered to evaluate the practicability of this relationship for environmental risk assessment.

Materials and methods

Climate

The long-term experiment is located at the Experimental Farm of the University of Padova (Veneto Region, NE Italy 45°21'N; 11°58'E; 6 m a.s.l.). The local climate is sub-humid, with annual rainfall of about 850 mm. In the median year, rainfall is highest in June (100 mm) and October (90 mm) and lowest in the winter months (50-60 mm). Temperatures increase from January (minimum average: -1.5 °C) to July (maximum average: 27.2 °C). The reference evapotranspiration (ET_o) is 945 mm with a peak in July (5 mm/day). ET_o exceeds rainfall from April to September. The site has a shallow water table ranging from about 0.5-1.5 m in late winter-early spring to 1-2 m in summer.

Long-term trial

This experiment began in 1964 in 4 m^2 open lysimeters, 80 cm deep. The experimental treatments derive from the factorial combination of three types of soil, hereinafter called sandy (SDY), clay (CLY) and peaty (PTY) in relation to their dominant property, with six types of mineral, organic or mixed fertilization, organized in two randomized blocks (36 lysimeters) (Giardini, 2004).

The soils were brought from three locations in the Veneto region: SDY from the central coastal area, CLY from the south-western plain and PTY from the southern plain. The original soil profiles were reconstructed in the lysimeters. SDY (Cumulic, Vertic, Endoaquoll fine, mixed, calcareous, mesic – US classification, (SSS, 1998; ARPAV, 2005)) contains predominantly quartz and feldspar and a significant amount of dolomite (16%). CLY (Cumulic, Vertic, Endoaquoll fine, mixed, calcareous, mesic – ARPAV, 2005) has a higher amount of montmorrilonite (16%) than the other soils and a considerable presence of mica (19%) and dolomite (15%). PTY (Typic Sulfisaprists euic, mesic – ARPAV, 2005) has a higher mica content (25%) and 12% of montmorrilonite.

Fertilization treatments considered for this study were as follows: control (UNT); farmyard manure – FYM (40 t $ha^{-1}y^{-1}$); mineral fertilizer – MIN (200 kg $ha^{-1}y^{-1}$ N - 100 P_2O_5 - 240 K_2O). The FYM applied about the same amount of macroelements as MIN and around 3.5-

4 t C $ha^{-1}y^{-1}$. Until 1984 there was a two-year maize (*Zea mays* L.) - wheat (*Triticum aestivum* L.) rotation. Thereafter, a variable rotation was adopted between 1985 and 1992, with various horticultural crops. From 1993 to 2002 there was a three-year rotation of tomato (*Lycopersicon esculentum* Mill.) – sugarbeet (*Beta vulgaris* L.) – maize, followed by various horticultural crops, maize and sunflower (*Helianthus annuus* L.) from 2003 to 2007. Apart from fertilization, all plots were treated in the same way in terms of rotation and management (tillage, sowing, harvest, etc.). The top 15-20 cm was dug each autumn and crop residues were removed from all treatments.

Soil sampling and analysis

In April 2008, samples from the 0 to 100 cm depth (every 10 cm) were taken from five points and bulked to obtain a sample of about 3 kg per plot. The samples were crushed by rolling pin to break up clods and pass a 2 mm sieve, air dried and stored at low humidity.

Soil pH was measured potentiometrically on 1:2.5 soil/water extracts. Organic C was determined by dry combustion in a CNS Vario Macro elemental analyzer (Elementar, Hanau, Germany) and correcting for the inorganic C. Carbonate content was determined by the calcimeter method and by gravimetric loss of CO_2. Total aluminium (Al_T), calcium (Ca_T), magnesium (Mg_T) and iron (Fe_T) concentrations were determined after digestion with concentrated sulphuric and perchloric acids. Extractable aluminium (Al), calcium (Ca), magnesium (Mg) and iron (Fe) were determined by Mehlich-3 (M3) solutions (Mallarino and Sawyer, 1999; Setter et al., 2009) and limited to Al and Fe plus ammonium oxalate (Ox) (Schwertmann, 1964). The metals in the extracts were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) with a SPECTRO CIROS (Spectro Analytical Instruments, Kleve, Germany). Total P (TP) was determined by two methods: perchloric acid digestion (TPp) (Sparks et al., 1996), and ignition and HCl extraction (TPi) (Chaya, 1996) and the P concentration in the extracts was determined colorimetrically by the malachite green method (Ohno and Zibilske, 1991; Martin et al., 1999). The organic P (P_{Org}) was calculated as the difference between TPi and inorganic P (Legg and Black, 1955). For the P linked to aluminium (P_{Al}) or to calcium (P_{Ca}), a soil sample (0.5 g) was treated with 25 mL of 1mol L^{-1} ammonium fluoride or 0.25mol L^{-1} sulphuric acid solutions and shaken for 2 h. The solution was then centrifuged, filtered

through Whatman No. 42 and the P in solutions determined by the malachite green method. For the P bound to iron and aluminium (P_{Fe+Al}), a soil sample (0.5 g) was shaken for 2 h in 0.1mol L^{-1} NaOH solution (Stevenson, 1986). The extract was then centrifuged and the P content determined by ICP-OES. Soil test P (STP) was determined by ammonium oxalate, Mehlich-3 solutions, Olsen, and water. Acid ammonium oxalate extractable-P (P_{Ox}) was determined by shaking a 1.5 g soil sample with 30 mL of 0.5 mol L^{-1} $(COONH_4)_2$ (pH 3.0) for 2 h in the dark (Schwertmann, 1964). Mehlich-3-P (P_{M3}) was determined by shaking 2.5 g of soil with 25 mL of Mehlich-3 extracting reagent (Mehlich, 1984). Olsen extractable-P (P_{Ols}) was obtained by shaking 1.0 g of soil with 20 mL of 0.5mol L^{-1} sodium bicarbonate solution (pH 8.5) for 30 min (Olsen and Sommers, 1982). Water extractable-P (P_{H2O}) was determined in a soil:water ratio of 1:10 (w/v) at 23 °C for 1 h (Börling et al., 2004a). After filtration through Whatman No. 42 filter paper, P in the extracts was determined colorimetrically by the malachite green method, except for Olsen in which the procedure of Murphy and Riley (1962) was followed.

P Sorption indices

Two single-point P sorption indices (PSI) were determined (Bache and Williams, 1971; Börling et al., 2001). Two grams of air-dried soil (≤ 2mm) were equilibrated with 19.4 and 50mmol P kg^{-1} soil (PSI1 and PSI2, respectively) in 20 ml 0.01mol L^{-1} $CaCl_2$ in plastic centrifuge tubes. Two drops of toluene were added to inhibit microbial activity. The tubes were shaken backwards and forwards at 21 °C for 18 h. Following centrifugation at 3000 rpm for 10 min, the equilibrium concentration in the solution was analyzed colorimetrically by the malachite green method. The amount of P sorbed by the soil was calculated by subtracting the amount of P in the equilibrium solution from the amount added. The PSI was calculated as the amount of P sorbed by the soil (X) after the single-point addition of P, divided by the logarithm of C (mmol/L) in the equilibrium solution (X/log C).

Statistical analyses

Barlett's test was used on the data to test the homogeneity of variance. Angular transformation was used when required to normalize the data. A two-way completely

randomized ANOVA was used to compare treatment effects. The factors considered were soil type and fertilization. The Student-Newman-Keuls test was applied to compare the differences between group means. To identify the structure of the interdependences between the main soil parameters, a joint principal components analysis (PCA) was performed on the following variables: pH, total carbonate, sand, clay, organic carbon, Ca_{M3}, Mg_{M3}, Al_{Ox}, Al_{M3}, Fe_{Ox}, Fe_{M3}, PT_i, P_{Org}, P_{Ca}, P_{Al}, P_{Fe+Al}, P_{Ox}, P_{M3}, P_{Ols} and P_{H20}, PSI1, PSI2. The standardized variables were submitted to PCA; rotated orthogonal components (varimax method of rotation) were extracted and the relative scores were determined. Only PCs with eigenvalue > 1 were considered for the discussion.

Results

General soil properties

The soil type, treatment, and depth significantly affected the soil pH, carbonates, and organic carbon (OC) contents (Tab. 1). The soils have different pH values ranging from 7.2 in PTY to 7.9 in CLY and 8.1 in SDY ($p<0.05$) (Fig. 1). CLY and SDY exhibited a slight increment in pH with an increase of depth, whereas PTY showed two zones of decrement: one at depths of 0-40 cm and the other at 60-80 cm. Accordingly for soil reaction, the carbonates content increased from the upper (0-10 cm) to lower (90-100 cm) layer in the order SDY>CLY>PTY ($p<0.05$) (160-271 g kg^{-1} in SDY, 46-216 g kg^{-1} in CLY, and 11-269 g kg^{-1} in PTY) (Fig. 2). The OC content decreased throughout the profile and was in the order PTY (66.6-15.4 g kg^{-1}) > CLY (19.6-12 g kg^{-1}) > SDY (9.9–5.6 g kg^{-1}) ($p<0.05$) (Fig. 3). In PTY the concentration of OC had a relative minimum value at the layer 40-60 cm (39 g kg^{-1}). FYM had a slight but significantly low pH (7.6) with respect to both UNT (7.9) and MIN (7.8) ($p<0.05$). MIN had less $CaCO_3$ content (108 g kg^{-1}) than UNT (114 g kg^{-1}) and FYM (116 g kg^{-1}) ($p<0.05$), whereas both FYM and MIN increased the OC content with respect to UNT (27.3, 26.4 and 23.8 g kg^{-1}, respectively) ($p<0.05$). In SDY soils, in particular, the amount of OC in the 0-50 cm layer was in the order FYN>MIN>UNT, whereas below the depth of 70 cm no differences were found (soil x treatment x depth interaction, $p<0.000$) (Fig. 3).

Both total Ca (Ca_T) and Mehlich-3 Ca (Ca_{M3}) (Fig. 4 and 5) were high in SDY (21937 and 8676 mg kg^{-1}) and CLY (20539 and 8537 mg kg^{-1}) and low in PTY (13614 and 6485 mg kg^{-1}) ($p<0.05$). Total Mg (Mg_T) (Fig. 6) was high in SDY (5149 mg kg-1) and low both in CLY (3801 mg kg^{-1}) and PTY (3616 mg kg^{-1}) ($p<0.05$), whereas Mehlich-3 Mg (Mg_{M3}) (Fig. 7) decreased from SDY (1229 mg kg^{-1}) to CLY (1082 mg kg^{-1}) to PTY (947 mg kg^{-1}) ($p<0.05$). Nevertheless, the Ca_{M3}/Ca_T was high in PTY (0.57) and low both in SDY (0.47) and CLY (0.44) ($p<0.05$), whereas the Mg_{M3}/Mg_T was high in both PTY (0.32) and CLY (0.30) and low in SDY (0.26) ($p<0.05$). The Ca_{M3} content slightly increased from the upper to the 70-80 cm layer in CLY, whereas in PTY it first exhibited a decrement (0-40 cm) and then an increment (40-60 cm). Nevertheless, the distribution of Ca_{M3} throughout the CLY and PTY profiles was not affected by treatment. In SDY, both MIN and FYM positively affected the Ca_{M3} content in the 0-40 cm layer, and MIN down to the bottom layer (90-100 cm).

Total Al (Al_T) was higher in PTY (40660 mg kg^{-1}) and CLY (38247 mg kg^{-1}) than SDY (10828 mg kg^{-1}) ($p<0.05$) (Fig. 8), whereas total Fe (Fe_T) differed in the three soils (CLY, 26714 mg kg^{-1}, PTY, 21474 mg kg^{-1}, and SDY, 7296 mg kg^{-1}) ($p<0.05$) (Fig. 9). The ratios extractable (oxalate and Mehlich-3)/total forms were in the order PTY>CLY>SDY ($p<0.05$), with values from PTY to SDY having ranges of 2036-180 mg kg^{-1} for oxalate Al (Al_{Ox}) (Fig. 10), 10181-395 mg kg^{-1} for oxalate Fe (Fe_{Ox}) (Fig. 11), 654-5 mg kg^{-1} for Mehlich Al (Al_{M3}) (Fig. 12), and 345-93 mg kg^{-1} for Mehlich Fe (Fe_{M3}) (Fig. 13). Both the Al_{Ox} (Fig. 10) and Fe_{Ox} (Fig. 11) increased in the 0-40 cm layer in CLY and PTY, and then decreased in CLY. Whereas in PTY they exhibited a secondary increment from depths of 40-50 cm to 70-80 cm. The Al_{M3} (Fig. 12) increased in depths of 0-50 cm in CLY, whereas in PTY it increased in the upper 0-40 cm and then again from depths of 80 to 90 cm. In SDY, the Al_{M3} was negligible. The Fe_{M3} content increased from the upper to 60-70 cm layer in CLY, and it decreased in depths of 70-100 cm in PTY. Both oxalate and Mehlich-3 forms well correlated with the clay and OC contents (R^2=, Al_{Ox}-clay=0.93, Fe_{Ox}-clay=0.74, Al_{M3}-clay=0.89, Fe_{M3}-clay=0.60, Al_{Ox}-OC =0.71, Fe_{Ox}-OC=0.91, Al_{M3}-OC=0.73, Fe_{M3}-OC=0.92, n=180 $p<0.000$). Concerning treatment type, FYM decreased the $Al_{M3,}$ and both FYM and MIN increased the Fe_{M3}. Differences were significant in the CLY 0-30 cm layer for Al_{M3}, and in PTY 0-70 cm layer for Fe_{M3}. Nevertheless, for both Al and Fe the ratios oxalate

and/or Mehlich-3 with total forms were affected only by MIN.

P forms

In general, the content of the different forms of P followed the order CLY>PTY>SDY, FYM>MIN>UNT, and decreasing values were found with an increase in depth. Total P (TP) using perchloric acid extraction (Tp_p) was always lower than that by ignition (TP_i), notwithstanding they were both linearly well correlated (R^2=0.88 p≤0.000). TP_i in CLY was 1.7-fold higher than PTY and 2.9-fold higher than SDY ($p<0.05$) (Fig. 14). Calcium (P_{Ca}) was high in CLY (2136 mg kg^{-1}) and low in PTY (908 mg kg^{-1}) and SDY (687 mg kg^{-1}) ($p<0.05$) (Fig. 17), and it was strictly and linearly correlated with TP determined both with perchloric acid extraction (R^2=0.90 p≤0.000) and ignition (R^2=0.87 p≤0.000). Nevertheless, both CLY and SDY had a high P_{Ca}/TP_i value. The aluminium P (P_{Al})/TP_i ratio was high in PTY and low in CLY ($p<0.05$), whereas the iron and aluminium P (P_{Fe+Al})/TP_i ratio was in the order PTY>SDY>CLY ($p<0.05$). Organic P (P_{Org}) was from 0.12 to 0.31 times the TP_i, with the lowest value in SDY and the highest in PTY ($p<0.05$) (Fig. 16). FYM positively influenced all P forms (Tab. 1) giving increments over the UNT of 1.2 for TP_p, 1.3 for TP_i, 1.4 for P_{Ca}, 1.5 for P_{Org}, 1.8 for P_{Fe+Al} and of 2.6 for P_{Al} ($p<0.05$). MIN also affected the various P forms although to a lesser extent with respect to FYM ($p<0.05$), with the exception of P_{Org} in which MIN did not differ from UNT. FYM increased the P_{Org}/TP_i and P_{Al}/TP_i ratios, whereas MIN decreased the P_{Org}/TP_i and P_{Ca}/TP_i ratios ($p<0.05$). The soil x treatment x depth interaction significantly affected the P_{Al}, P_{Fe+Al}, P_{Org} up to TP (Tab. 1). Treatment highly increased the P_{Al} content in the 0-30/40 cm layer with values of 4.4-1.9 (PTY), 4.7-4.4 (SDY) and 4.9-2.6 (CLY) fold higher UNT for FYM and MIN, respectively (Fig. 18). FYM increased the P_{Fe+Al} content in the CLY 0-40 cm with a maximum increment of 3.5 fold higher UNT across the entire profile in SDY with values ranging form 28.6 to 15.1 mg kg^{-1} in the FYM treated respect 11.8-4.8 mg kg^{-1} in the UNT (Fig. 19). P_{Fe+Al} accumulated in PTY at depths 30-40 cm and 60-70 cm and the major values were obtained using FYM (Fig. 19). FYM increased the content of P_{Org} in the 0-40 and 80-100 cm layers of SDY, and from the surface to 60-70 cm layer in PTY (Fig. 16). FYM also affected the total P content in the surface layers (0-40 cm) of CLY and PTY and across the entire profile in SDY (Fig. 14 and 15). MIN also affected the total P in SDY and

PTY (Fig. 14 and 15).

Soil test P

The extractable-P was in the order: oxalate > Mehlich-3 > Olsen. Both oxalate-P (P_{Ox}) and Mehlich-3 P (P_{M3}) were high in CLY (1261 and 49 mg kg^{-1}, respectively), low in SDY (105 and 24 mg kg^{-1}, respectively), and exhibited intermediate values in PTY (771 and 46 mg kg^{-1}, respectively) ($p<0.05$) (Fig. 20 and 21). The content in Olsen-P (P_{Ols}) was high both in CLY and PTY (28 and 27 mg kg^{-1}, respectively) and was low in SDY (15 mg kg^{-1}) ($P<0.05$) (Fig. 22). Nevertheless, when considering the ratio with TP_i, P_{Ox}/TP_i was high both in PTY and CLY, and was low in SDY; P_{M3}/TP_i was in the order PTY>SDY>CLY, whereas the P_{Ols}/TP_i was high both in PTY and SDY and low in CLY. Treatment affected all extractable-P forms (Tab. 1). In general, FYM, compared to UNT, showed increments 4.1 fold higher for the Olsen-P, 5.2 fold higher for Mehlich-3-P, and 1.2 fold higher for oxalate-P. MIN also induced increments although of lower intensity than FYM. On the other hand, considering the P_{Ox}/TP_i ratio, FYM determined a decrement with respect to MIN and UNT. Concerning the interactions among factors, FYM induced significant increments in the distribution of the Olsen-P throughout the CLY profile, with values from 8 to 10 fold higher in the upper (0-30 cm) layers (117 versus 17 mg kg^{-1} Olsen-P for FYM and UNT, respectively) to values 5 fold higher down to the (90-100 cm) lower layer (20 versus 4 mg kg^{-1} Olsen-P for FYM and UNT, respectively) (Fig. 22). The differences in P_{Ols} between FYM and UNT in SDY profiles were statistically significant down to the deeper layers. In PTY, both MIN and FYM showed accumulation in the Olsen-P content down to a depth of 40 cm with an increment as high as 7 fold UNT in the upper (0-20 cm) layer (Fig. 22). Noticeably, in PTY a second marked accumulation was induced by MIN at depths of 60-70 cm. Both Mehlich-3 and oxalate-P distribution across soil profiles had similar patterns to Olsen P (Fig. 21 and 20). Of note however, in SDY, FYM induced very high increments in the Mehlich-3 P down to the 80-100 cm layer (24 fold higher UNT) (Fig. 21). Concerning correlations, Olsen-P was linearly well correlated ($R^2=0.75$, $p\leq0.000$) with P_{M3}, whereas both P_{Ols} and P_{M3} were poorly correlated with the oxalate-P ($R^2=0.56$, $p\leq0.000$). In spite of this, oxalate-P was strictly correlated with TP_i ($R^2=0.88$, $p\leq0.000$).

P Sorption indices

Both PSI were affected by the soil type, treatment and depth (Tab. 1). The low P addition (PSI1) showed very small differences among soils with values ranging 0.74-0.88-0.91 mmol P kg^{-1} in SDY, CLY, and PTY, respectively ($p<0.05$) (Fig. 24). FYM affected the PSI1 inducing a slight decrease of this index from 0.86 (UNT) to 0.83 mmol P kg^{-1} ($p<0.05$). The soil x depth did not reveal differences across the soil profiles in CLY and SDY, whereas two increments, at depths of 30-40 and 60-70 cm, were found in the PTY profile (Fig. 24). Both the soil x treatment and treatment x depth were insignificant for PSI1 (Tab. 1). The high P addition (PSI2) showed larger differences than PSI1, with a low value (1.52) in SDY, a high value (4.72) in PTY and an intermediate value (2) in CLY ($p<0.05$) (Fig. 25). FYM increased the PSI2 from 2.68 mmol P kg^{-1} in the UNT to 2.94 to mmol P kg^{-1}, whereas MIN did not differ from UNT ($p<0.05$). For PSI2, both the second and third grade interactions were significant (Tab. 1). In particular, in CLY, both MIN and FYM positively affected PSI2 down to the 90-100 cm layer reaching a value of 2.69 mmol P kg^{-1} for FYM with respect to 1.29 mmol P kg^{-1} for UNT (Fig. 25). In PTY, PSI2 had the same shape as PSI1 but with higher values (Fig. 25). Moreover, in the layers from 30-40 cm to 60-70 cm, PSI2 was affected in the order FYM>UNT≥MIN. However, in SDY there was a slight increase in the PSI2 throughout the soil profiles but without significant differences among treatments (Fig. 25). With relation to correlations, PSI2 strictly correlated with Fe_{M3} (0.90), Fe_{Ox} (0.84), and OC (0.85) ($n=180$, $p<0.000$). Weak but significant correlations were also found between PSI2 and Al_{M3} (0.69), Al_{Ox} (0.67), and clay content (0.49) ($n=180$, $p<0.000$). Of particular importance was the fact that the correlation with Al_{Ox}, Al_{M3}, and clay increased markedly when PSI1 was considered instead of PSI2 (R^2=0.92, 0.93, 0.85, respectively $n=180$, $p<0.000$).

P release

The P extractable in water (P_{H20}) was used to study the release of P throughout the soil profiles. No differences were found among soils, whereas P_{H20} was affected by both

treatment and depth (Tab. 1). Water extractable-P was significantly higher in the topsoil of the fertilized profiles than in the non-fertilized, and among treatments FYM induced higher values than MIN. Nevertheless, soils differed in their behaviour. In CLY (Fig. 23), FYM induced increments in the P_{H2O} content throughout the profile down to a depth of 60-70 cm with values from 17 to 3.4 fold higher UNT, for the upper and lower layer, respectively. In PTY (Fig. 23), FYM increased P_{H2O} down to depths of 30-40 cm reaching a peak of 7.5 fold higher UNT. In SDY (Fig. 23), the peak induced by FYM in P_{H2O} content was of lesser intensity than CLY and PTY, but had an intermediate magnitude when compared with SDY-UNT (i.e., 12 fold higher). Nevertheless, in SDY, a high and quite constant P_{H2O} content was found throughout the profile down to 90-100 cm. Concerning relationships, P_{H2O} exhibited a weak correlation with Olsen-P (R^2=0.57) and a stronger correlation with Melich-3-P (R^2=0.78). Plotting the P_{H2O} content against Olsen or Mehlich-3-P a quantity-intensity relationship typical of a sorption or desorption isotherm was derived. P_{H20} exhibited a change point at a concentration of 54 mg kg^{-1} Olsen-P both in CLY and PTY (Fig. 26), and at a concentration of 100 mg kg^{-1} Mehlich-3-P in CLY, and 170 mg kg^{-1} Mehlich-3-P in PTY (Fig. 27). Change points were not detected in SDY (Fig. 26 and 27) soils because the relationships between P_{H2O} and Olsen-P as well as P_{H2O} and Mehlich-3-P were linear.

Three factors were extracted by PCA, accounting for 83.86% of the variance (Table 2). Factor 1 explained 91.96% of the variance and was correlated (factor loadings > 0.8) with PSI2, Fe_{M3}, OC, P_{Fe+Al}, pH, and Fe_{Ox}. Factor 2 explained 19.18% and was positively correlated with P_{M3} and P_{H2O}, P_{Ols}, and P_{Al}. P_{Ox}, clay, Al_{Ox}, Al_{M3}, P_{Ca}, PSI1, and sand were possibly correlated with Factor 3 (31.77% of the variance). Plotting PC1 and PC2 (Fig. 28), the soils were shown to be well separated. In particular, PTY was characterized by high values of OC, P_{Fe+Al}, and PSI2, while SDY and CLY were characterized by high pH, $CaCO_3$, and P_{Ca}. Extractable P forms (Mehlich-3, Olsen and water), P_{Al}, and PSI1 differentiated the soils according to the treatment type (data not shown), with high values in FYM, intermediate in MIN and low in UNT.

Discussion

General soil properties

SDY and CLY have a constitutional alkalinity, originating from the presence of (calcium) carbonates. The importance of carbonates was confirmed by the Mehlich-3 extractions that exhibited higher values in Ca and Mg than Al and Fe throughout the profiles. The Ca_{M3}/Ca_T ratio, in fact, was in the progression PTY>CLY>SDY, and thus confirmed the importance in SDY and CLY of calcium carbonates as specific adsorption sites (Pizzeghello et al., 2011). In PTY, the neutral reaction was due to the mineralization of the soil organic matter which over a long period had increased the presence of cations and raised the pH (Morari et al., 2008). This result is emphasized in the middle layers of PTY in which the plant root exudates operate (Marschner, 1995). The high values of Al and Fe Mehlich-3 and oxalate forms in PTY and CLY revealed the presence of amorphous forms of Al and poor crystalline Fe oxides (McKeague et al., 1971; Bertrand et al., 2003). These forms are known to be related to the presence of organic matter and/or clay with which they strictly correlated (Börling et al., 2001). The decrease in the extractable forms with depth was also found by Börling et al., (2001) and Lookman et al., (1995) who assumed that higher rates of weathering and higher content of organic matter could promote the formation and stability of amorphous forms of Al and poor crystalline Fe oxides. Treatment affected both M3 and oxalate forms down to the lower layers of soil profiles. Manured soils generally had higher extractable Ca and Mg than non-manured soils, while non-manured soils had higher extractable Al than manured soils (Ige et al., 2008). MIN increased the amorphous Fe and Al oxides. Thus, all these factors are likely to control the P sorption sites in the 0-20 cm layer (Pizzeghello et al., 2011) and throughout the soil profiles.

P forms

The different forms of P highlighted the diverse nature of the soils. This reflects not only the fertilizer history of the soils, but also the contribution of P from the parent materials and

the influence climate has on P forms. While extractions do not enable precise quantification of well-defined forms of adsorbed or precipitated P, they enable identification of major P pools and allow comparison between soils (Stevenson, 1986). As revealed in the 0-20 cm layers (Pizzeghello et al., 2011), the calcium-P fraction dominates in the calcareous soils (CLY and SDY), indicating the importance of relatively stable forms of sparingly soluble Ca phosphates. In contrast, iron and aluminium-P makes a substantial contribution in the organic soil (PTY), suggesting the importance of adsorbed and precipitated forms of P associated with Fe and A1 hydroxides and the solubilisation of organic forms (Pizzeghello et al., 2011).

Both FYM and MIN affected the P forms in all soils, and in general FYM gave higher increments than MIN. The increments were strongly influenced by soil type and depth. Previous studies have shown that P can accumulate in the subsoil down to depths of 40-180 cm following long-term fertilization with organic or inorganic P fertilization (Sharpley et al., 1993; Eghball et al., 1996; Börling et al., 2004). In our study, the differences in P forms between fertilized and non-fertilized soils were statistically significant down to 40 cm and, in some cases, to even greater depths as for the P_{Org} both in the SDY and PTY soils. The P_{Fe+Al} increment, as well as the TP_i, throughout the profile (0-100 cm) in SDY treated with FYM was consistent with the increase in the OC content. The general increase in calcium P is consistent with manure applications. Manure had a high Ca/P molar ratio (Kalbasi and Karthikeyan, 2004), and continuous addition of manure to soils lead to the accumulation of soil Calcium P (Sharpley et al., 2004; Penn and Bryant, 2008). The long-term fertilisation produced differences between fertilized and non-fertilized soils due to fertilization levels exceeding the crop uptake.

Soil test P

As found in the 0-20 cm layers (Pizzeghello et al., 2011) the different relationships observed between the extractable P forms indicated that different methods extracted a different proportion of available P, with a higher correlation between M3 and Olsen than oxalate and Olsen or oxalate and M3. This confirms that Mehlich-3-P could be an alternative to the traditional Olsen-P test for agronomic purposes in Northern Italy.

The differences in extractable P forms between fertilized and non-fertilized soils were found to be significant in most cases throughout the soil profiles. The differences were probably due to both depletion and fertilization exceeding crop uptake. In fact, both P_{Ols} and P_{M3} indicated that soils' agronomic P levels were higher than those set for optimum crop growth and production (10 mg P kg^{-1} for P_{Ols} and 45-50 mg P kg^{-1} for P_{M3}) (Sims, 2000a,b; Mallarino and Sawyer, 1999) and ranged throughout the profiles from low to excessively high. The excessively high P levels were found down to a depth of 40 cm in all three soils considered. Noticeably, in SDY, both P_{Ols} and P_{M3} decreased with depth, but remained low in the middle layers, and then increased again in the deeper layers consistent with a change in textural properties. In fact, in our experiment, the lysimiters arrived at a depth of 80 cm, so the marked accumulation in Olsen and Mehlich-3 P in the lower layers (80-100 cm) seems to be the result of P translocation throughout the soil profile. These results agree well with other studies in which accumulation has been found in available P and/or some P fractions (i.e., Olsen-P, Alumiun-P, Ca8P) down to a soil depth that varied in accordance with the soil type, and type and time of fertilization (Eghball et al. 1996; Börling et al. 2004; Wang et al., 2007; Guo et al., 2008). In the soils sampled FYM, and to a lesser extent MIN, also exceeded the threshold values of 20 mg kg^{-1} for P_{Ols} and 150 mg kg^{-1} for P_{M3} (McDowell et al., 2001; Sims et al. 2002), thus reconfirming the previous evidence of potential risk of P loss through leaching and runoff (Pizzeghello et al., 2011). It seems that high long term P applications could cause deeper P translocation in soils.

P Sorption

The single-point P addition (PSI) was utilised as an index to estimate the soil P sorption capacity (Börling et al., 2001). Two PSI were considered, one from the addition of 19.4 mmol P kg^{-1} soil (PSI1) and the other from the addition of 50 mmol P kg^{-1} (PSI2). The PSI2 produced higher values and larger differences among soils than PSI1. PSI2 seems more able to estimate the P sorption capacity, as when measuring a PSI the amount of P added should be high enough to exceed the P sorption maximum (Bache and Williams, 1971). Thus, the P sorption capacity was low in SDY and high in PTY. These results are consistent with the

different OC content and the presence of poor crystalline Fe oxides and amorphous Fe and Al oxides. Since fertilization influences the P sorption capacity occupying some sorption sites, PSI2 was expected to be lower in fertilized than non fertilized soils. However, contrary to our expectation and different from the results obtained form the 0-20 cm layer (Pizzeghello et al., 2011), in depths of 0-100 cm FYM increased PSI2. Variations in PSI2 were also found with depth. In particular, PSI2 incremented in the lower layers down to depths of 90-100 cm in CLY-FYM, and in the middle layers in PTY-FYM. In CLY, the increase in PSI2 could be due to precipitation of P with $CaCO_3$ present in the deeper layers, a result which is also corroborated by evidence of high P_{Ca} content. Increments of PSI in deeper layers (below 60 cm), probably due to the precipitation of P with $CaCO_3$, have also been found in fertilized neutral/alkaline soils in other long-term experiments (Börling et al., 2004). Differently, in PTY, the increase in PSI2 appears due to the Al_{Ox} and Fe_{Ox} content. The correlation between PSI2 and Al_{Ox} and Fe_{Ox}, in fact, increased markedly when comparing calcareous (CLY+SDY) (R^2 Al_{Ox}=0.46, Fe_{Ox}=0.42; n=120 $p<0.000$) with organic soils (PTY) (R^2 Al_{Ox}=0.76, Fe_{Ox}=0.74; n=60 $p<0.000$). This suggests the presence in PTY of Fe and Al (hydr)oxides with a lower degree of crystallization and hence a larger surface area which permits more P to be sorbed. PSI and thus P sorption capacity, vary with depth according to soil characteristics and treatment (Sharpley et al., 1984, 1993; Mozaffari and Sims 1994; Börling et al., 2004). From these data, P sorption capacity needs to be estimated in the subsoil as well as in the topsoil to assess the ability of the soil to sorb the P transported in the profile.

Phosphorus release

The P released into water was considered because it represents the P in solution rapidly transported through the soil profile, through preferential flow or following heavy rainfall or snow melt (Yli-Halla et al., 1995). The P_{H2O} content was significantly higher in the topsoil of the fertilized profiles than non-fertilized profiles and it was in a similar range to that reported by Börling et al. (2004). Differing from their study we have found significant differences down to deeper layers, i.e. 60-70 cm in CLY, and throughout the entire profile in

SDY. In FYM treated SDY the high content of P_{H2O} may be due to the increment in OC. In fact, the correlation between OC and P_{H2O} increased when compared to all soils (R^2=0.17, n=180 $p<0.05$) with PTY (R^2=0.41, n=60 $p<0.000$). The high and relatively high P_{H2O} in CLY and SDY (i.e., compared with the respective UNT) well agree with the low P sorption capacity of these soils. On the contrary, the relatively low P_{H2O} in PTY was associated with a higher P sorption capacity.

The quantity of P released into water increased with the increase of Olsen and Mehlich-3 P, and the increases differed markedly among soils. Several studies have demonstrated that the P concentration of leachate and soil test P are closely related once the change point has been reached. This kind of relationship has been used to calculate a change point in STP, above which P release increases more per STP unit than below. This was true for the Olsen-P in CLY and for the Mehlich-3-P in both CLY and PTY. The relationship between P_{H2O} and Olsen-P in PTY instead had a change point in STP above which P release increased less per STP unit than below. These results are difficult to explain. Notwithstanding, soils with low PSC (i.e. SDY) had more water P than soils with high PSC at the same STP level. Our data are in line with the results of several authors that reported change points from 20 to 112 mg kg^{-1} soil for Olsen-P and from 120 to 190 mg kg^{-1} for Mehlich-3-P (Brookes et al., 1997; McDowell et al., 2001; Maguire and Sims, 2002). It is known that the P sorption power of Al and Fe hydrous oxides diminishes with an increasing pH and above pH 5.8 the change point is more greatly influenced by sorbed or precipitated Ca phosphates. Therefore, it is likely that with the range of pH for the soils sampled the change point was more greatly influenced by sorbed or precipitated Ca phosphates. The presence of a change point is in accordance with previous findings with high DPS values (Pizzeghello et al., 2011). Overall, these results provide evidence of a great risk of P loss in leaching or runoff (Leinweber et al., 1997; Koopmans et al., 2003).

Conclusions

The long-term application of mineral or organic fertiliser affects the distribution of P forms in calcareous soils. Compared with non-manured soils, 44 years of applications increased P concentration down to a depth of 40 cm, and in several cases down to a depth of 100 cm, for most inorganic P forms. The results indicate that continuous application of manure P in excess of crop needs will lead to a large accumulation of STP, which poses a potential threat to surface and groundwater quality. Thus, to identify soils vulnerable to P losses, both transport mechanisms and P sources need to be considered. When estimating the P source, the potential P release of the soil is an important variable. In this study, potential P release was related with a split line to Olsen-P and Mehlich-3 P for individual soils. However, soils with high P sorption capacity released less P than soils with low P sorption capacity at the same STP level. STP alone cannot be used to estimate potential P release in risk assessment systems when soils with different PSC are being considered.

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Table 1 - Significance level for the factors and interaction.

Variable	Factor significance (*p*)						
	S	T	D	SxT	SxD	TxD	SxTxD
pH	<0.001	<0.001	<0.001	0.107	<0.001	0.934	0.815
Carbonates	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
OC	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Ca_T	<0.001	0.228	<0.001	0.533	<0.001	0.968	0.960
Ca_{M3}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ca_{M3}/Ca_T	<0.001	0.020	<0.001	0.633	0.001	0.585	0.655
Mg_T	<0.001	0.003	<0.001	<0.001	<0.001	0.802	0.831
Mg_{M3}	<0.001	0.259	<0.001	0.118	0.531	0.224	0.001
Mg_{M3}/Mg_T	<0.001	0.207	<0.001	<0.001	<0.001	<0.001	0.002
Al_T	<0.001	0.031	0.986	0.014	0.021	1.000	0.979
Al_{Ox}	<0.001	0.428	<0.001	0.035	<0.001	0.001	<0.001
Al_{Ox}/Al_T	<0.001	0.001	0.001	0.014	0.040	0.969	0.954
Al_{M3}	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001
Al_{M3}/Al_T	<0.001	0.001	0.173	<0.001	<0.001	0.375	0.003
Fe_T	<0.001	0.049	<0.001	<0.001	<0.001	0.996	0.978
Fe_{Ox}	<0.001	0.329	<0.001	0.102	<0.001	<0.001	<0.001
Fe_{Ox}/Fe_T	<0.001	0.002	<0.001	<0.001	<0.001	0.917	0.417
Fe_{M3}	<0.001	<0.001	<0.001	<0.001	<0.001	0.038	0.001
Fe_{M3}/Fe_T	<0.001	0.014	0.044	<0.001	0.217	0.829	0.982
TP_p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TP_i	<0.001	<0.001	<0.001	0.052	<0.001	<0.001	<0.001
P_{Org}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{Ca}	<0.001	<0.001	<0.001	0.001	<0.001	0.001	0.418
P_{Al}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.050
P_{Fe+Al}	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
P_{Org}/TP_i	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{Ca}/TP_i	<0.001	<0.001	<0.001	<0.001	<0.001	0.028	0.318
P_{Al}/TP_i	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.494
P_{Fe+Al}/TP_i	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
P_{Ox}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{M3}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{Ols}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{H2O}	0.798	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PSI1	<0.001	<0.001	<0.001	0.136	<0.001	0.461	0.095

PSI2	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
P_{Ox}/TP_i	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{M3}/TP_i	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{Ols}/TP_i	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
P_{H2O}/TP_i	0.012	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

S, soil; T, treatment; D, depth.

Table 2 - Loadings values of some physical and chemical variables on the axes identified by principal components analysis for the three types of soils. Bold numbers > $|0.7|$.

Variable	Factor 1	Factor 2	Factor 3
pH	**-0.89**	-0.09	-0.22
Carbonates	-0.53	-0.22	-0.62
Sand	-0.41	-0.04	**-0.86**
Clay	0.36	0.05	**0.89**
OC	**0.91**	0.16	0.22
Ca_{M3}	-0.60	0.01	0.05
Mg_{M3}	-0.38	0.44	-0.22
Al_{Ox}	0.57	0.08	**0.80**
Al_{M3}	0.62	0.04	**0.75**
Fe_{Ox}	**0.82**	0.09	0.50
Fe_{M3}	**0.92**	0.04	0.27
PT_i	-0.09	0.51	**0.81**
P_{Org}	0.26	0.42	0.65
P_{Ox}	0.08	0.41	**0.90**
P_{M3}	0.13	**0.93**	0.17
P_{Ols}	0.14	**0.86**	0.23
P_{H2O}	0.05	**0.92**	-0.03
P_{Ca}	-0.26	0.52	**0.76**
P_{Al}	0.09	**0.86**	0.36
P_{Fe+Al}	**0.90**	0.16	-0.01
PSI1	0.63	-0.13	**0.71**
PSI2	**0.92**	-0.06	0.18
Explained Variance (%)	51.72	21.32	10.82

OC, organic C; M3, Mehlich-3 extractable; Ox, Oxalate extractable; PT_i, Total phosphorus by ignition method; P_{Org}, organic P; P_{Ols}, Olsen P; P_{H2O}, water-extractable P; P_{Ca}, Calcium P; P_{Al}, Aluminium P; P_{Fe+Al}, iron+aluminium P; PSI, single-point P addition of 19.4 mmol P kg^{-1} (PSI1) and 50 mmol P kg^{-1} (PSI2).

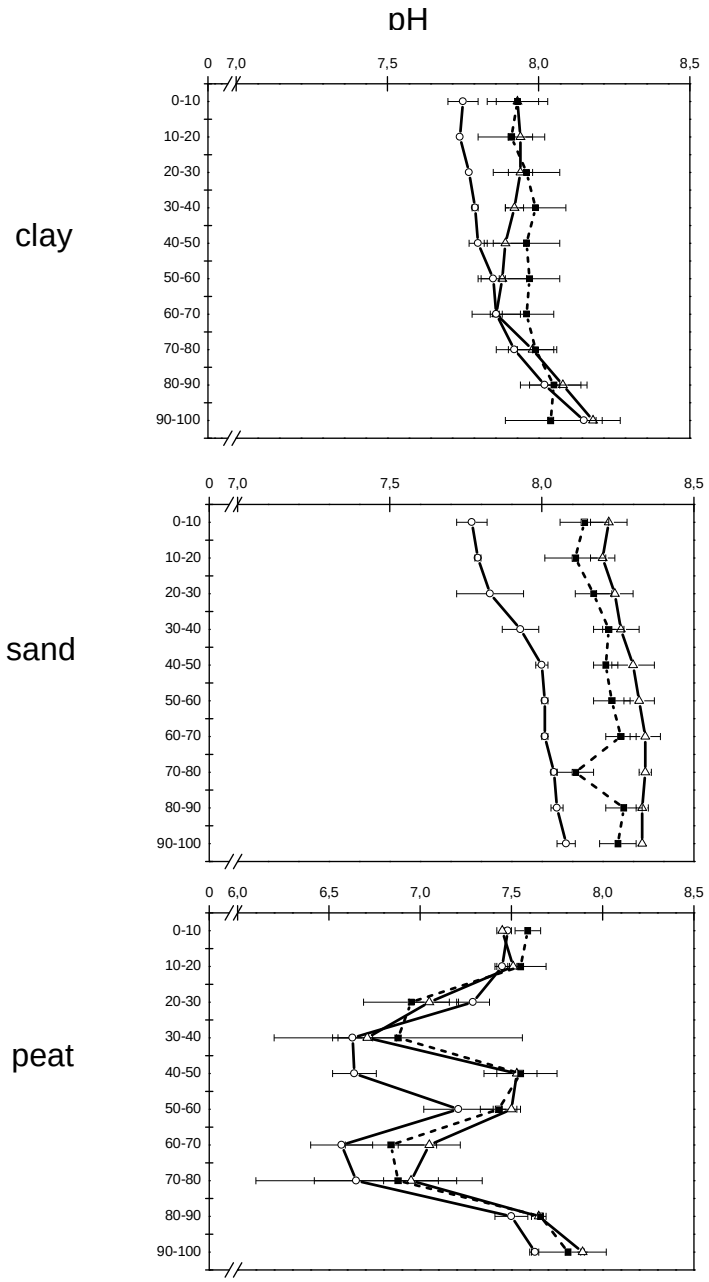

Figure 1 - Soil pH distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

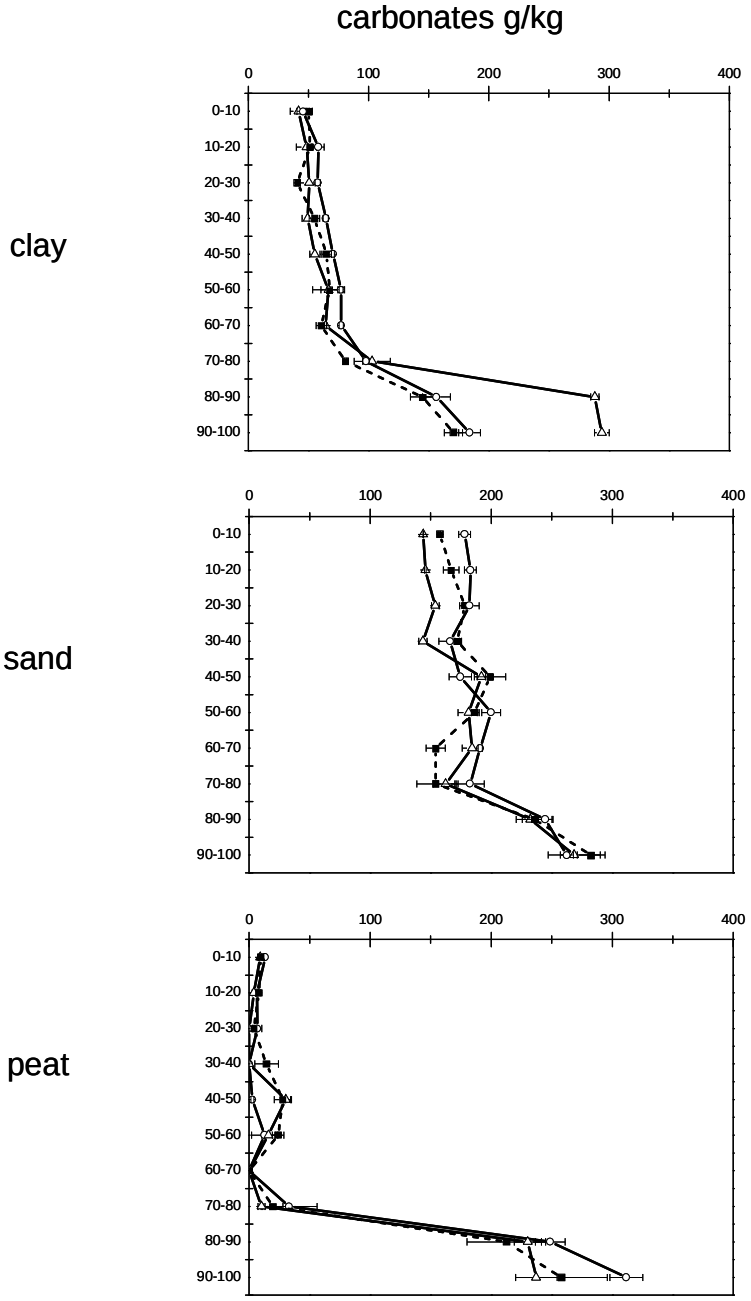

Figure 2 - Carbonates distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

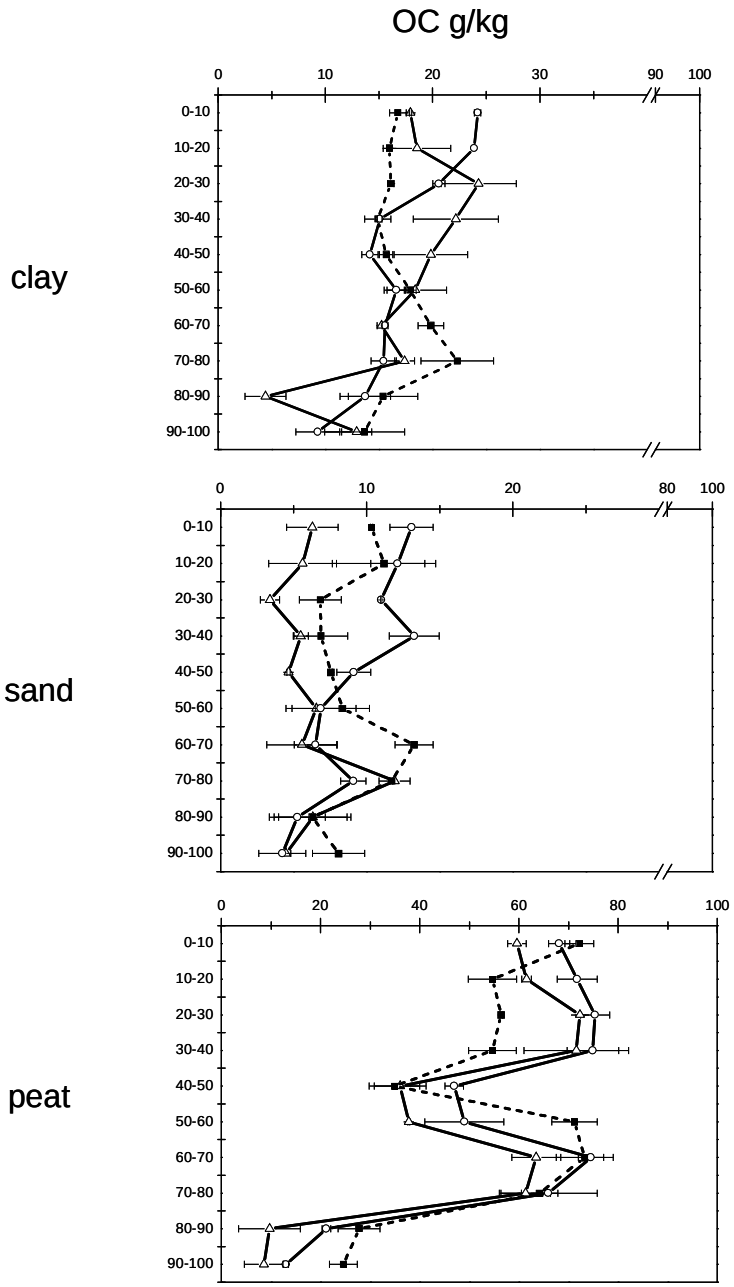

Figure 3 - Organic carbon distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

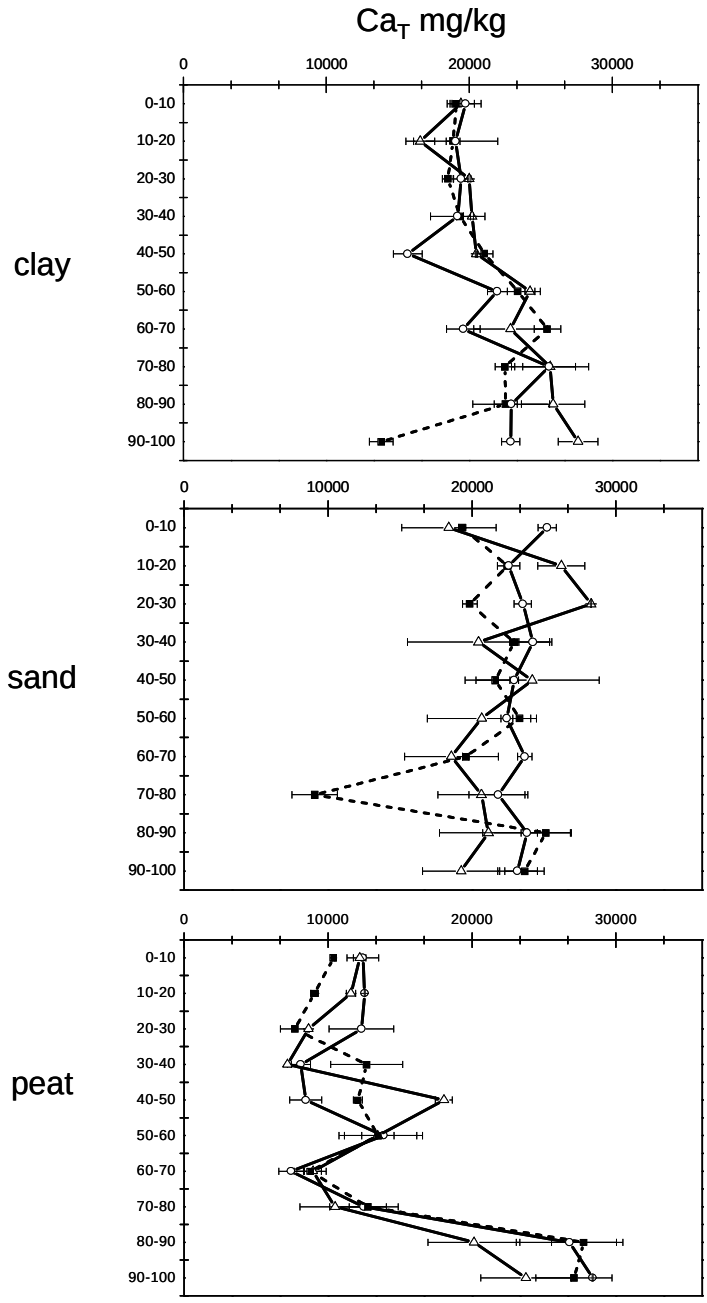

Figure 4 - Total calcium distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

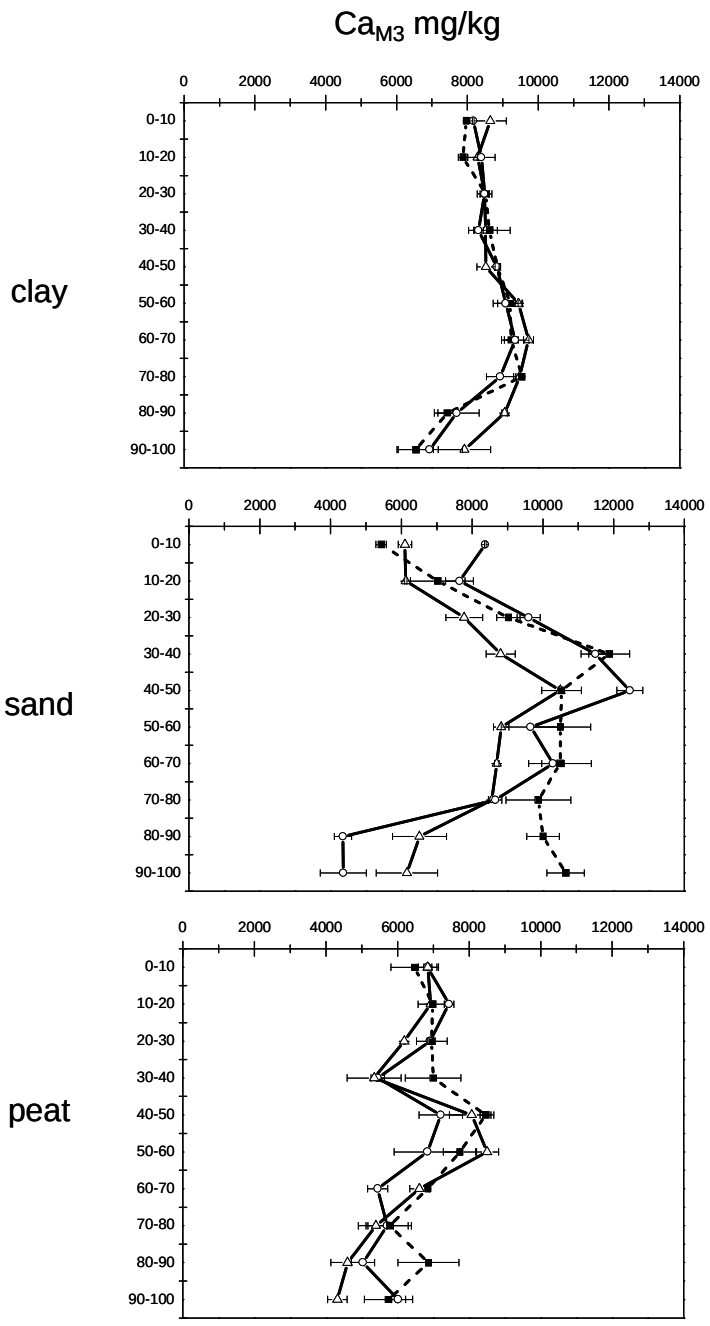

Figure 5 - Mehlich-3-extractable calcium distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

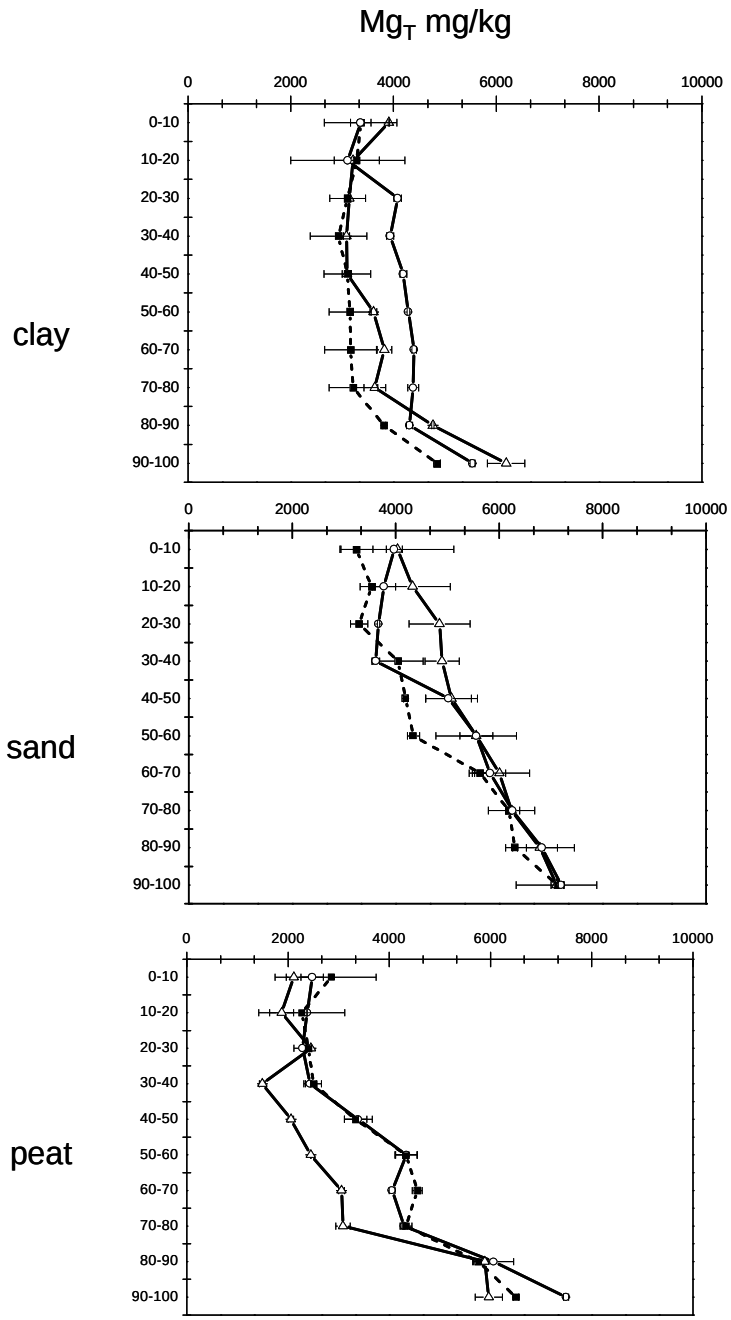

Figure 6 - Total magnesium distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

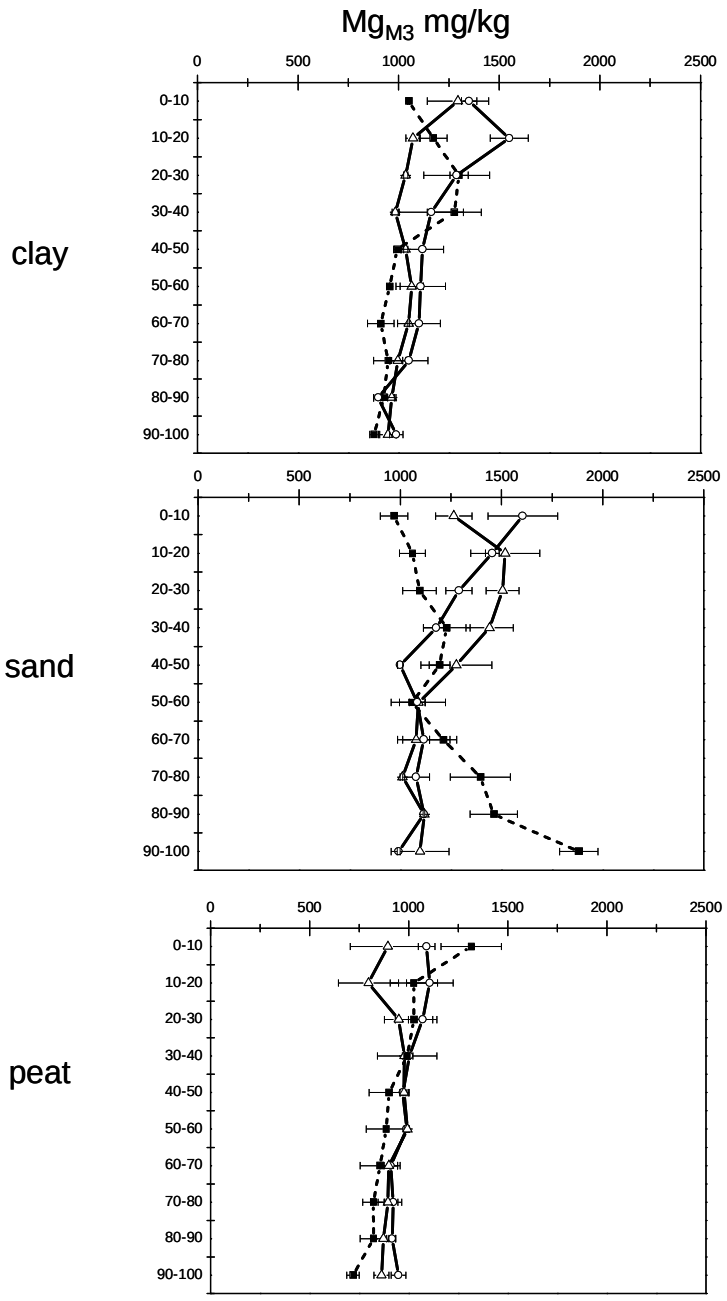

Figure 7 - Mehlich-3-extractable magnesium distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

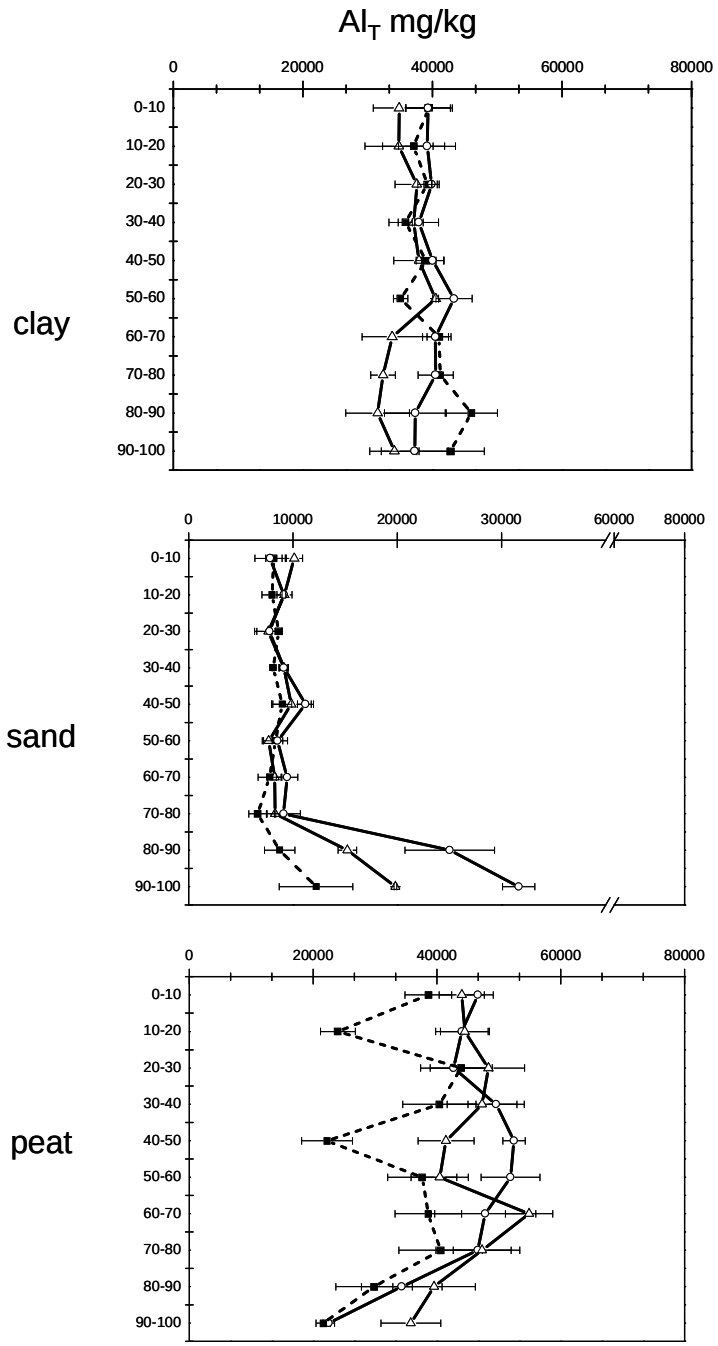

Figure 8 - Total aluminium distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

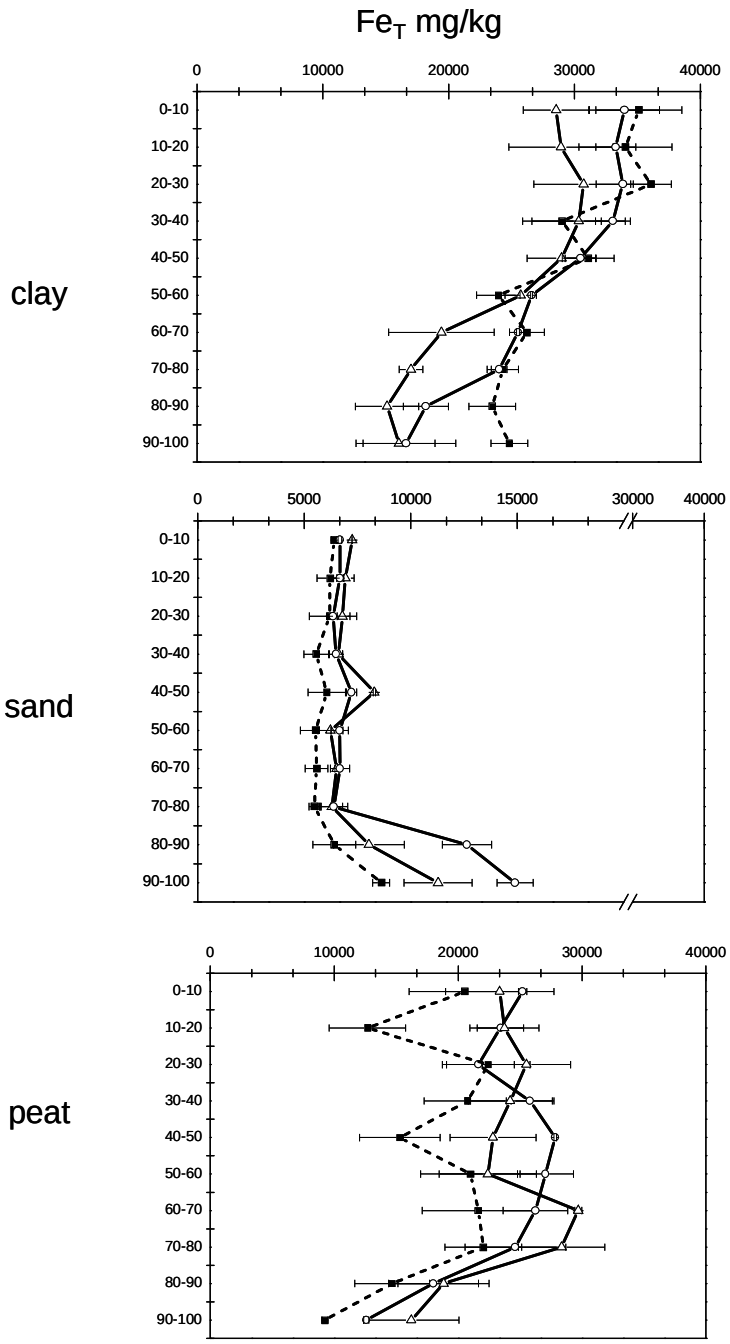

Figure 9 - Total iron distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

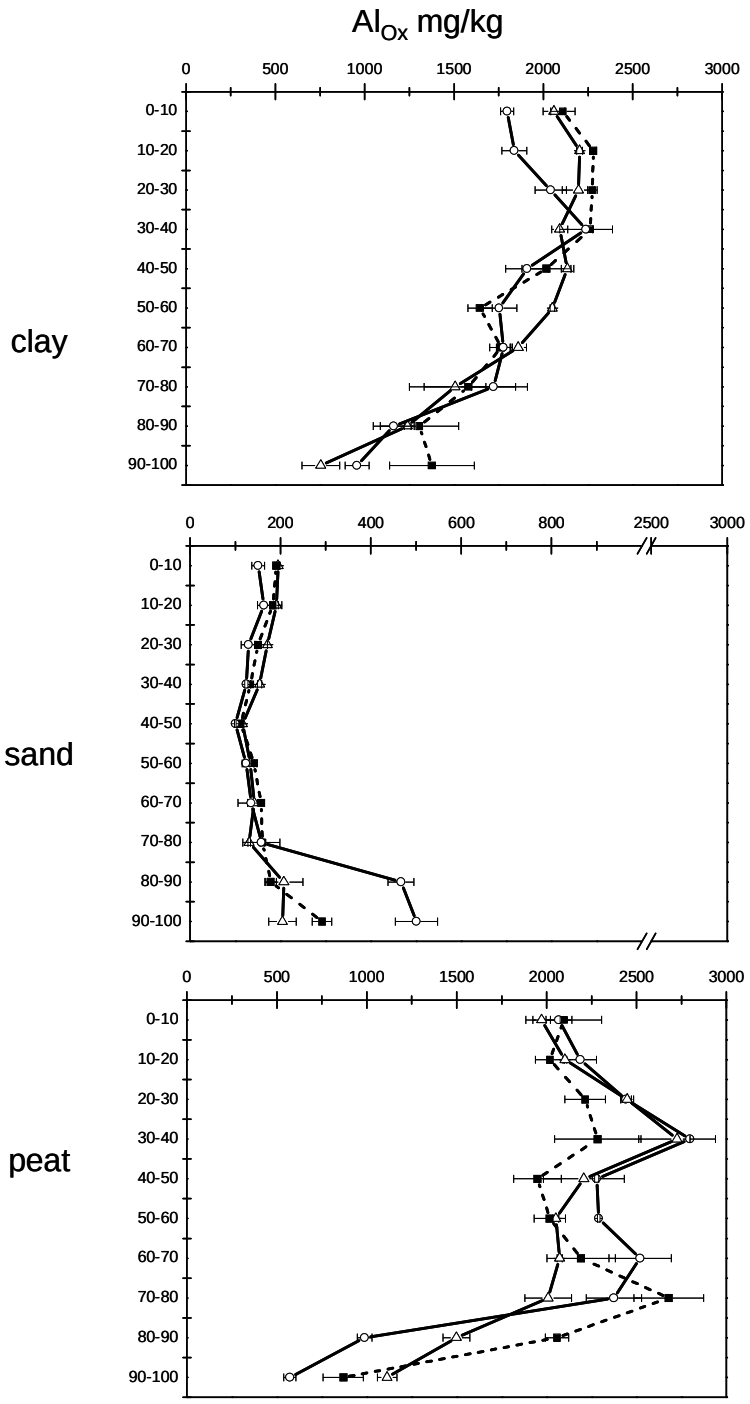

Figure 10 - Oxalate-extractable aluminium distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

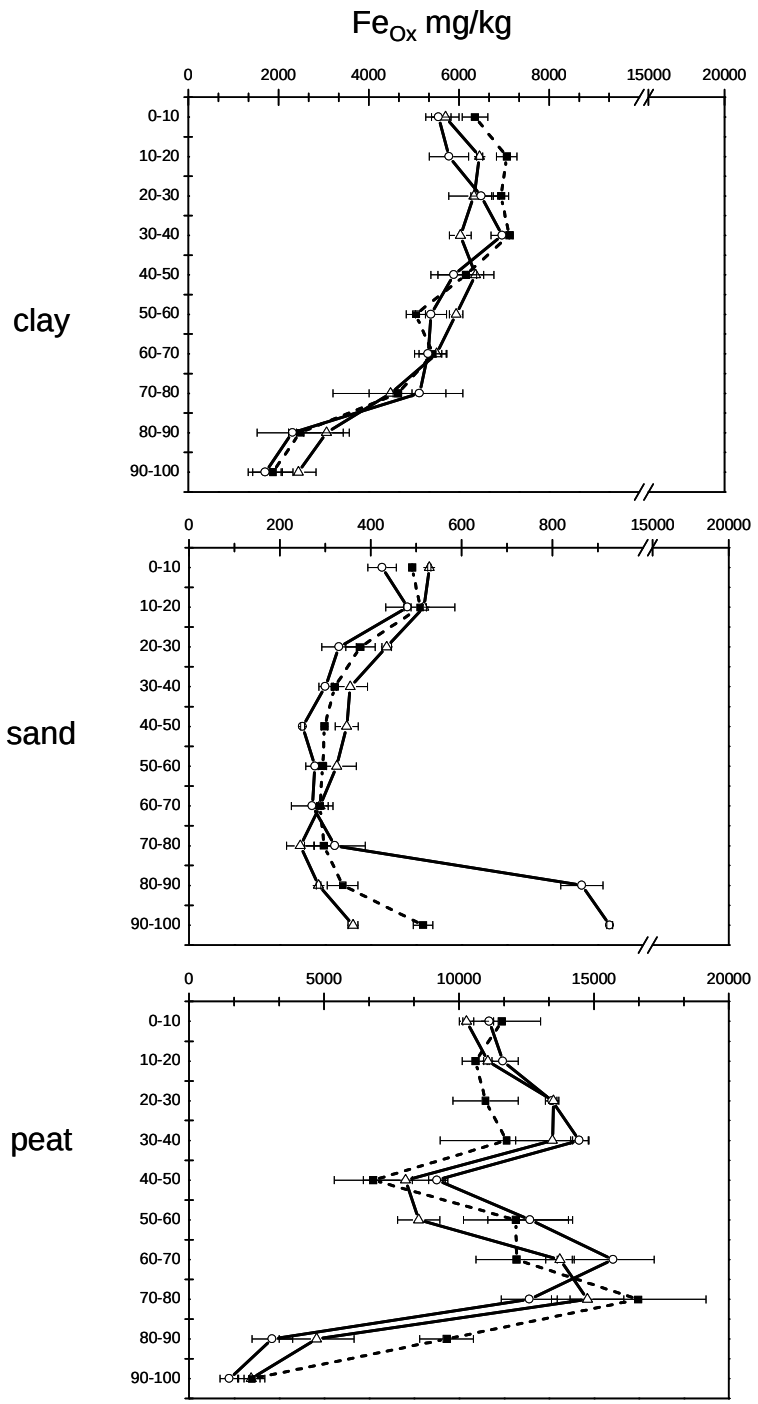

Figure 11 - Oxalate-extractable iron distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

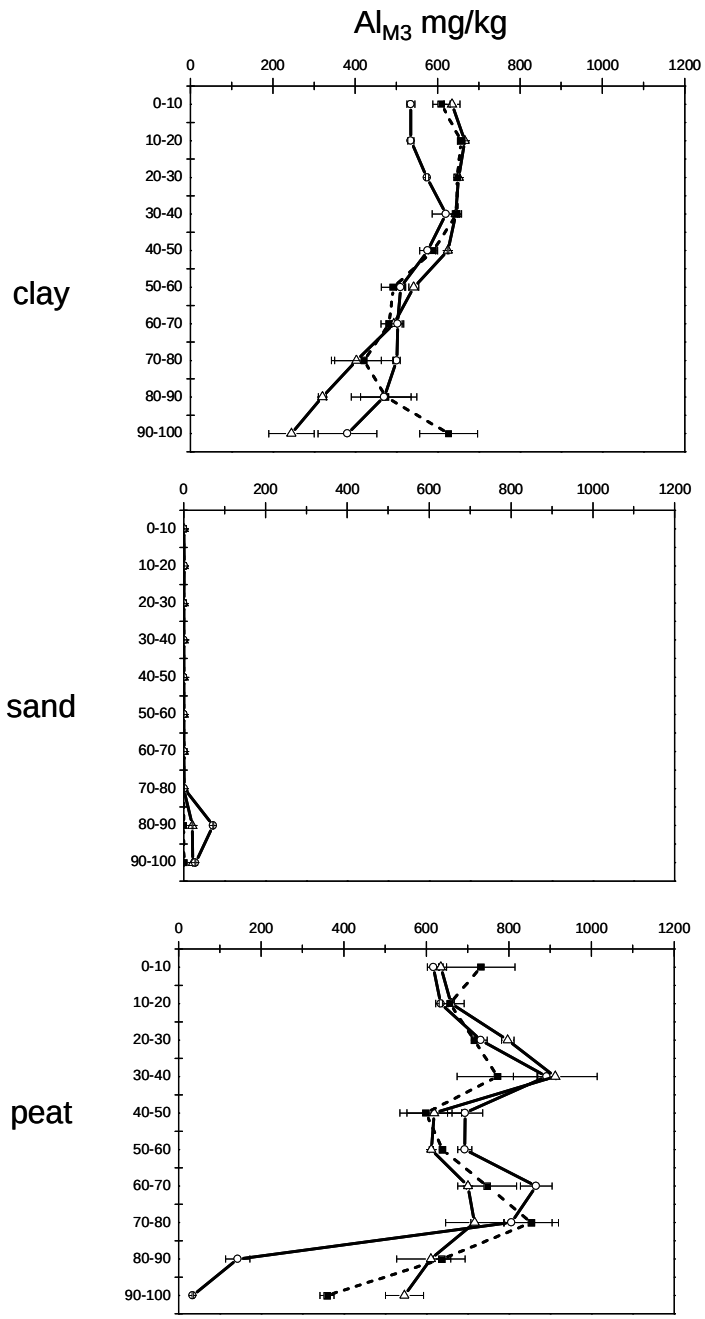

Figure 12 - Mehlich-3-extractable aluminium distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square)**.**

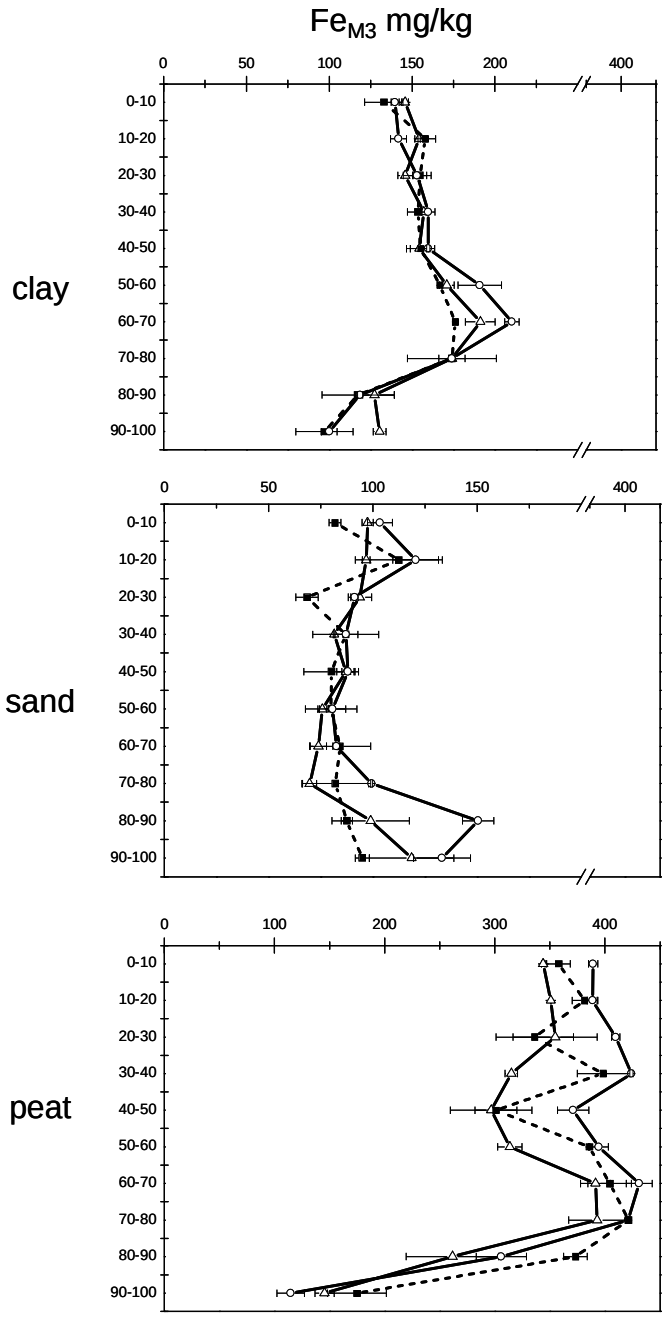

Figure 13 - Mehlich-3-extractable iron distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

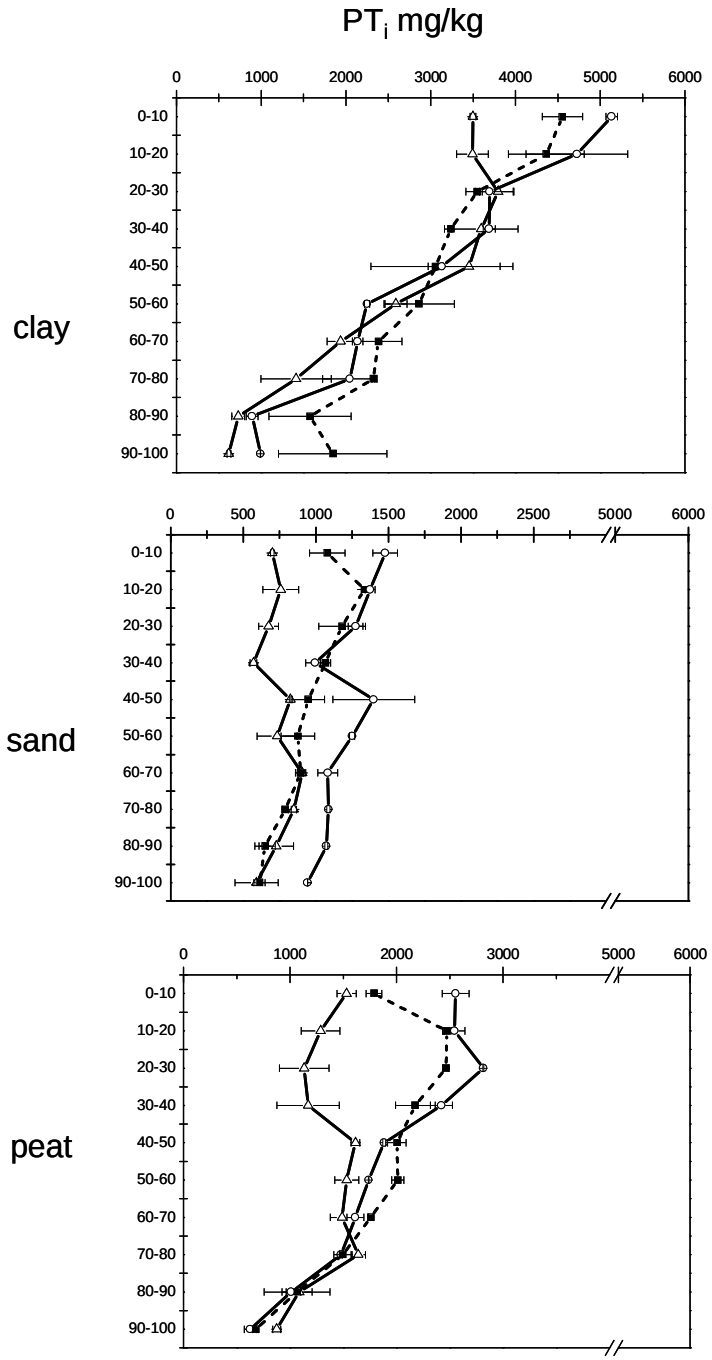

Figure 14 - Total phosphorus (by ignition) distribution in the profiles of the three soils (clay, sandy, and peat) after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

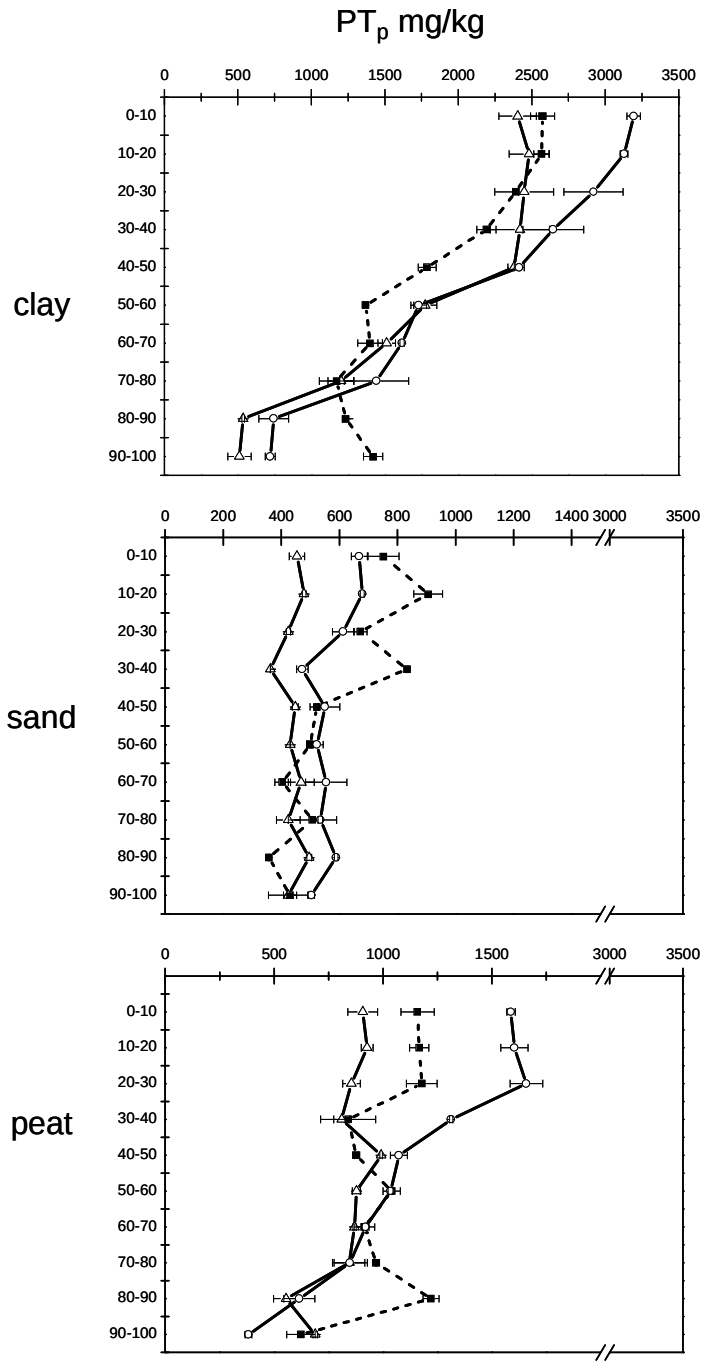

Figure15 - Total phosphorus (perchlorich) distribution in the profiles of the three soils (clay, sandy, and peat) after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

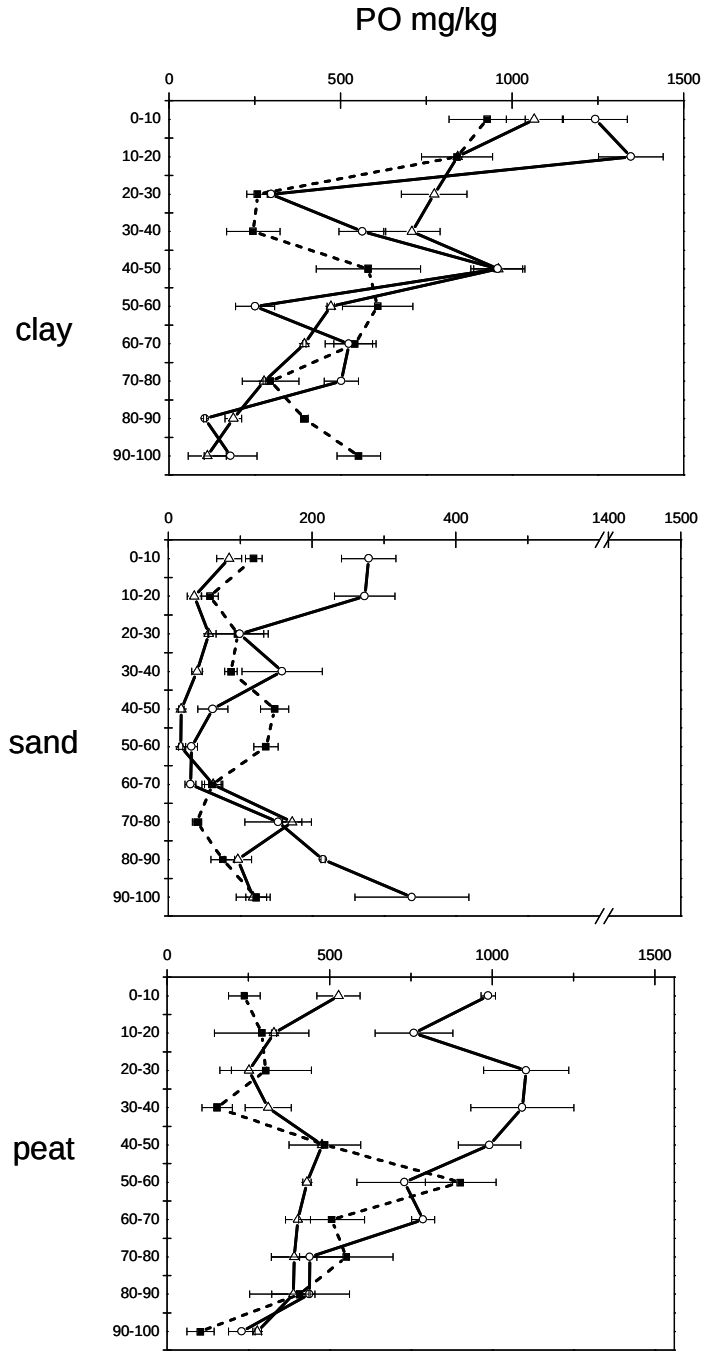

Figure 16 - Organic phosphorus distribution in the profiles of the three soils (clay, sandy, and peat) after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

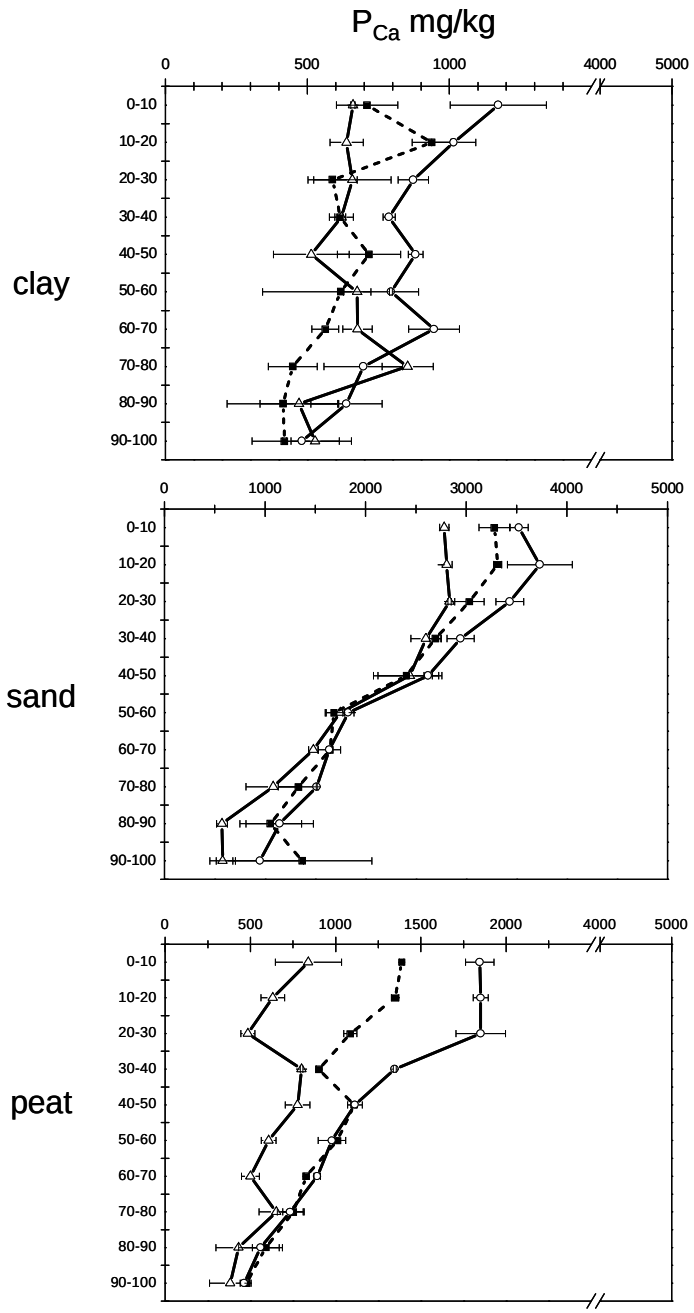

Figure 17 - Calcium phosphorus distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

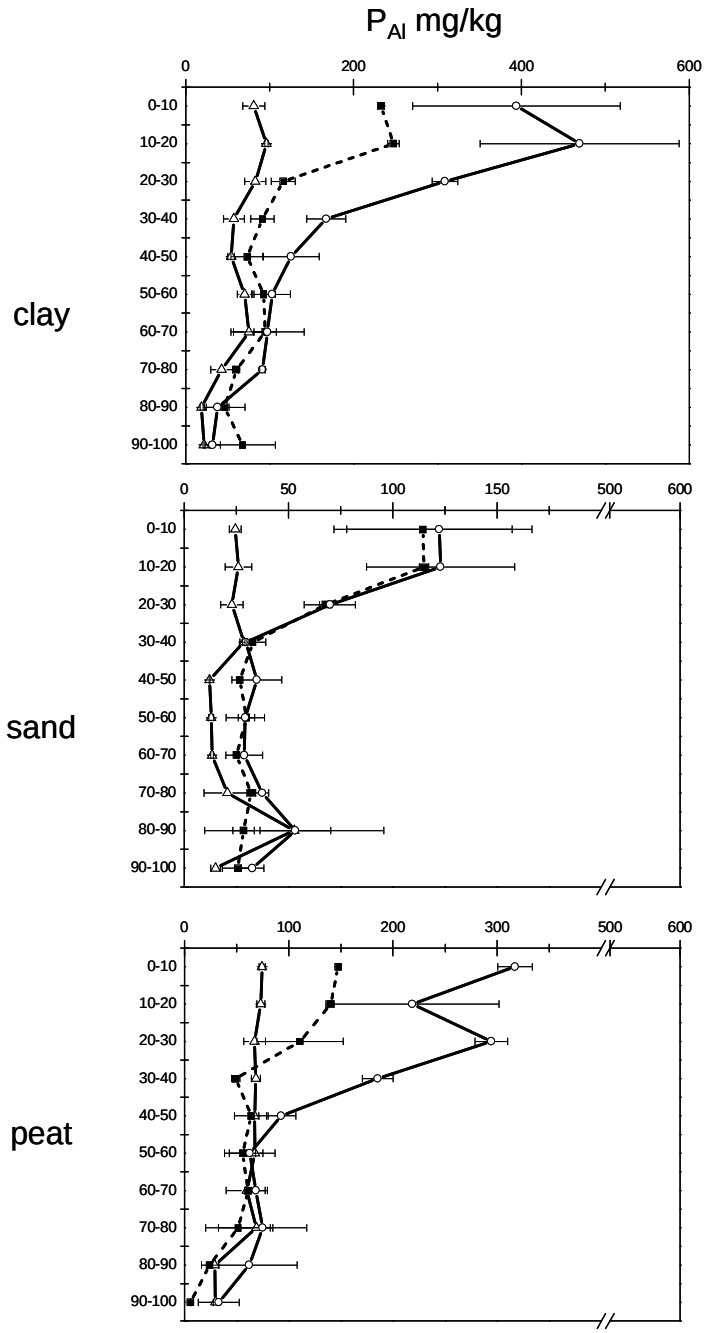

Figure 18 - Aluminium phosphorus distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

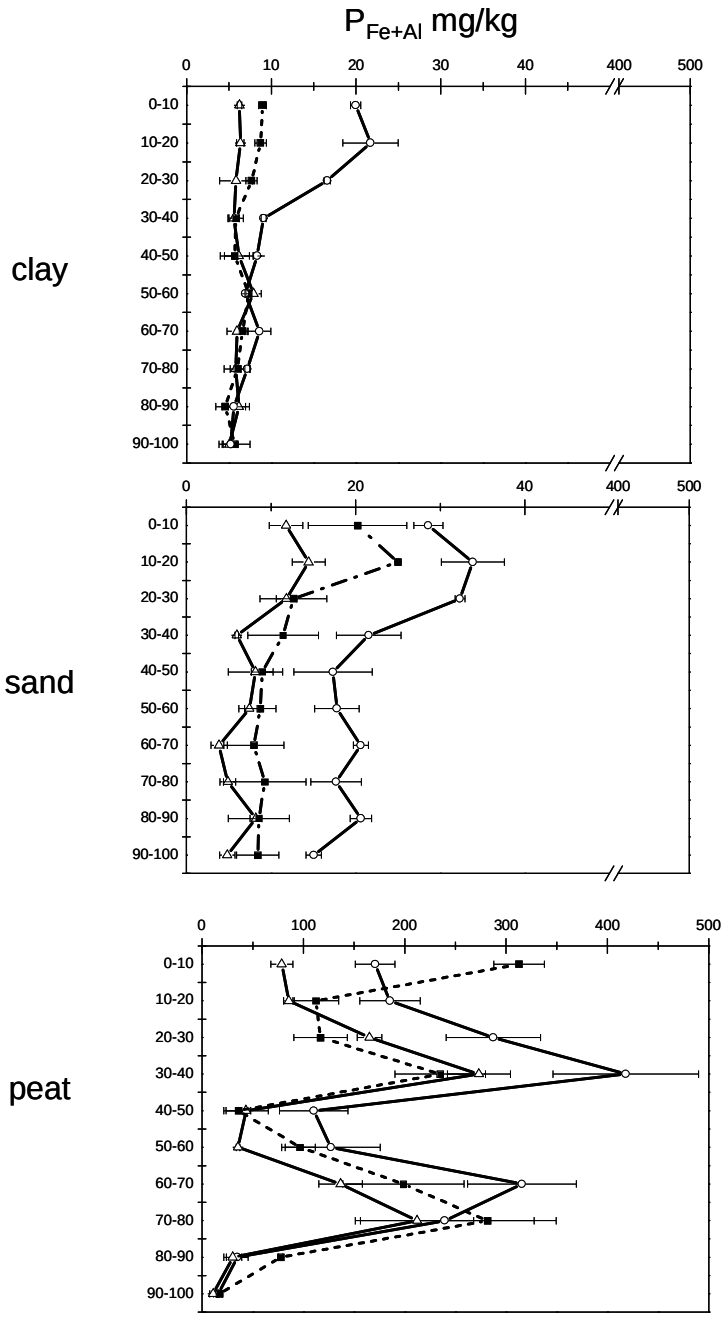

Figure 19 - Iron + aluminium phosphorus distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

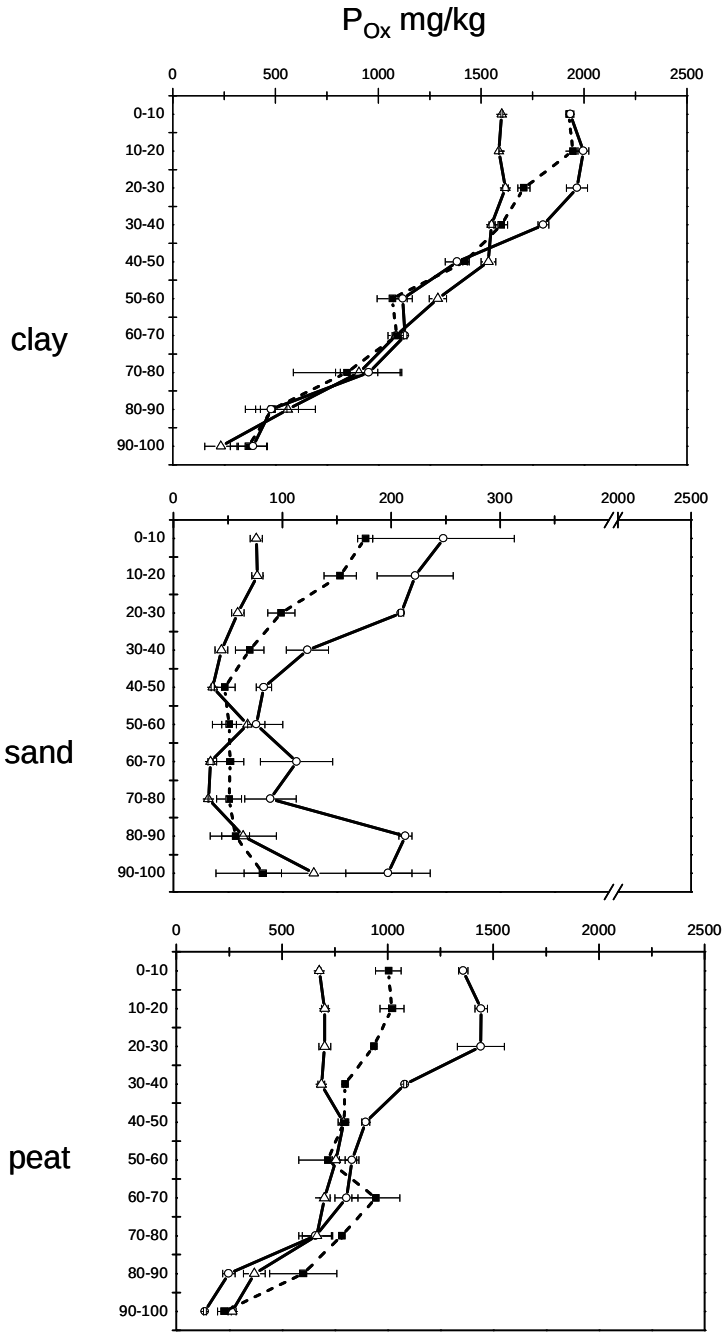

Figure 20 - Oxalate-extractable P distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

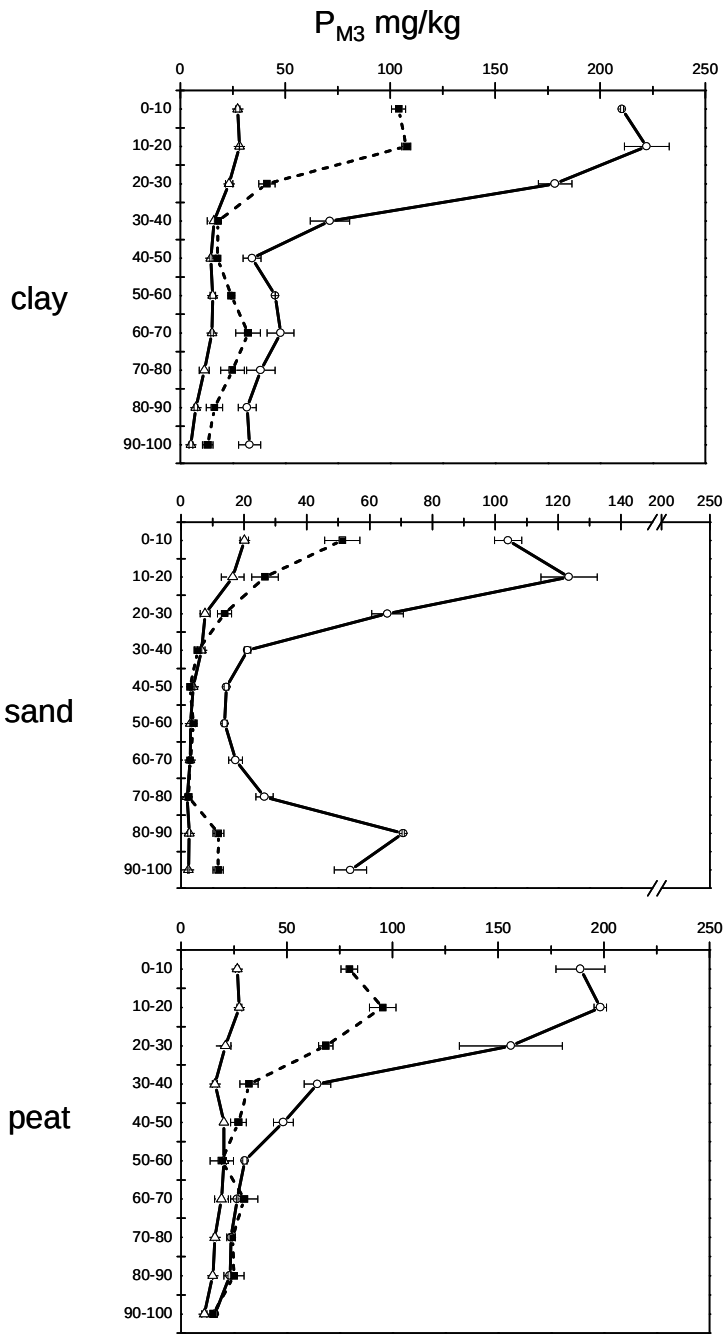

Figure 21 - Mehlich-3-extractable P distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

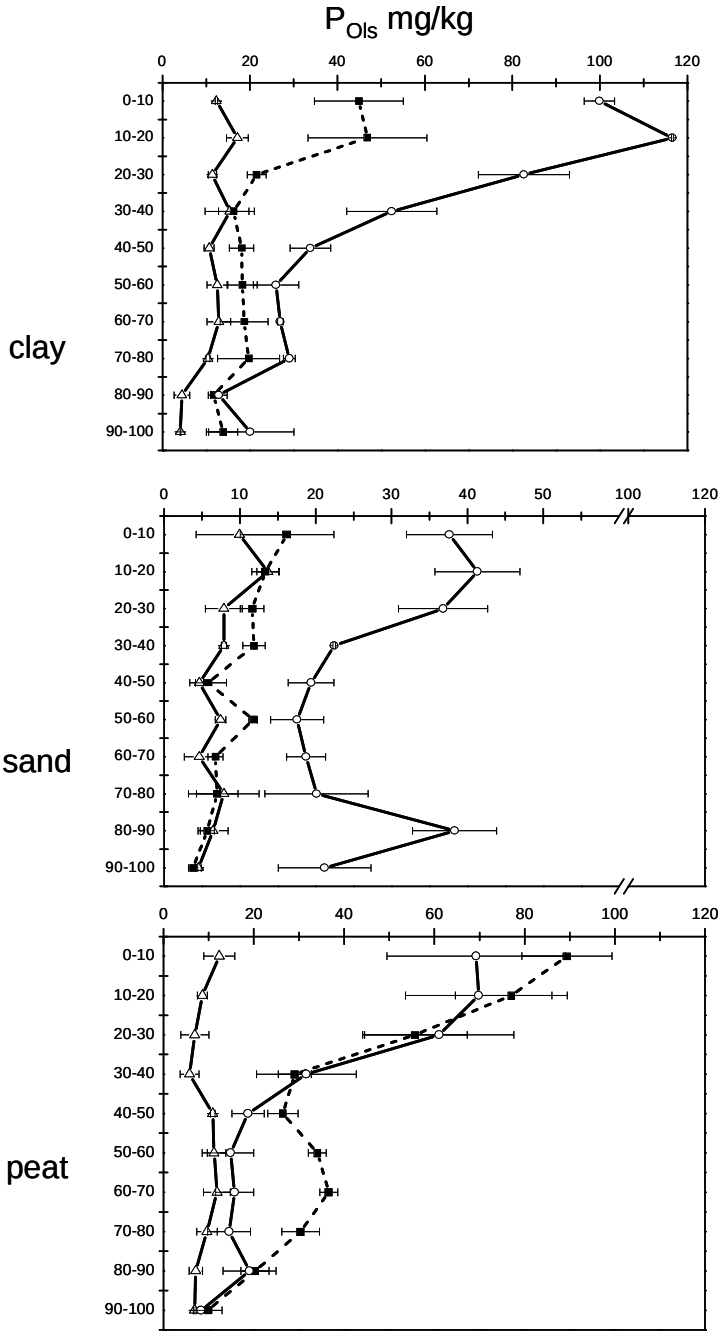

Figure 22 - Olsen-extractable P distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

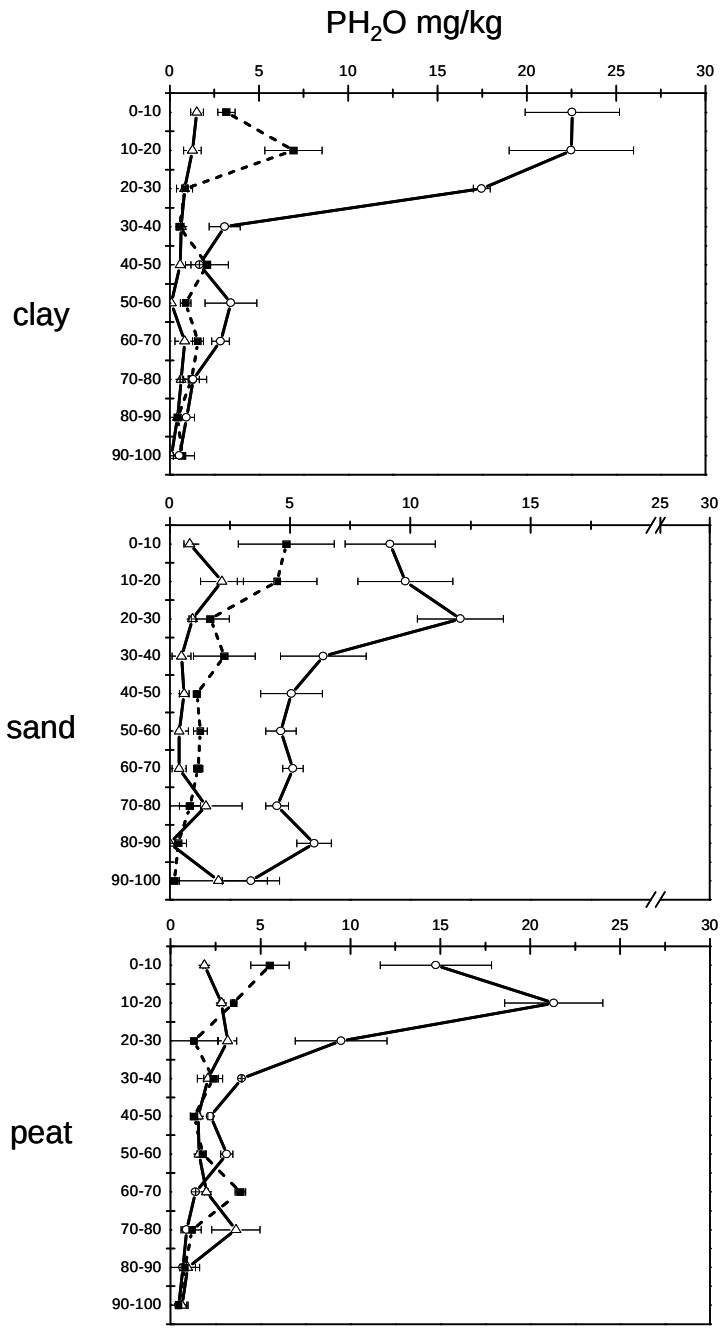

Figure 23 - Water-extractable P distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

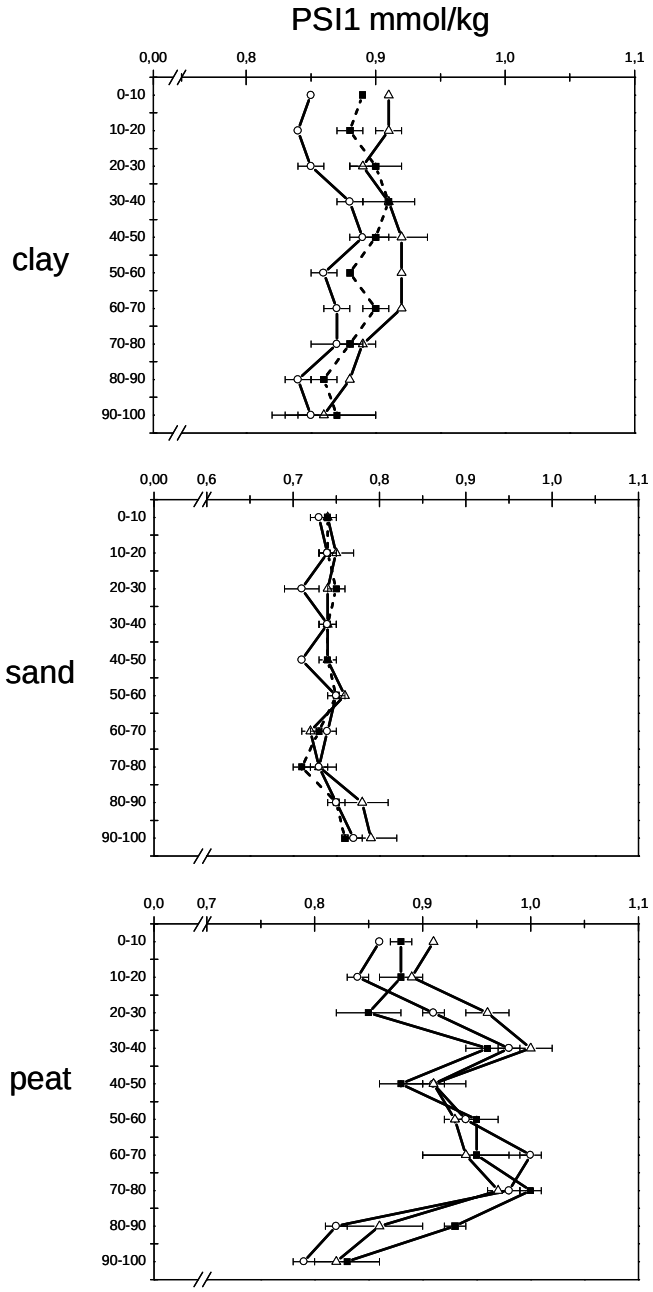

Figure 24 - Single point P addition PSI1 (19 mmol P kg^{-1}) distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

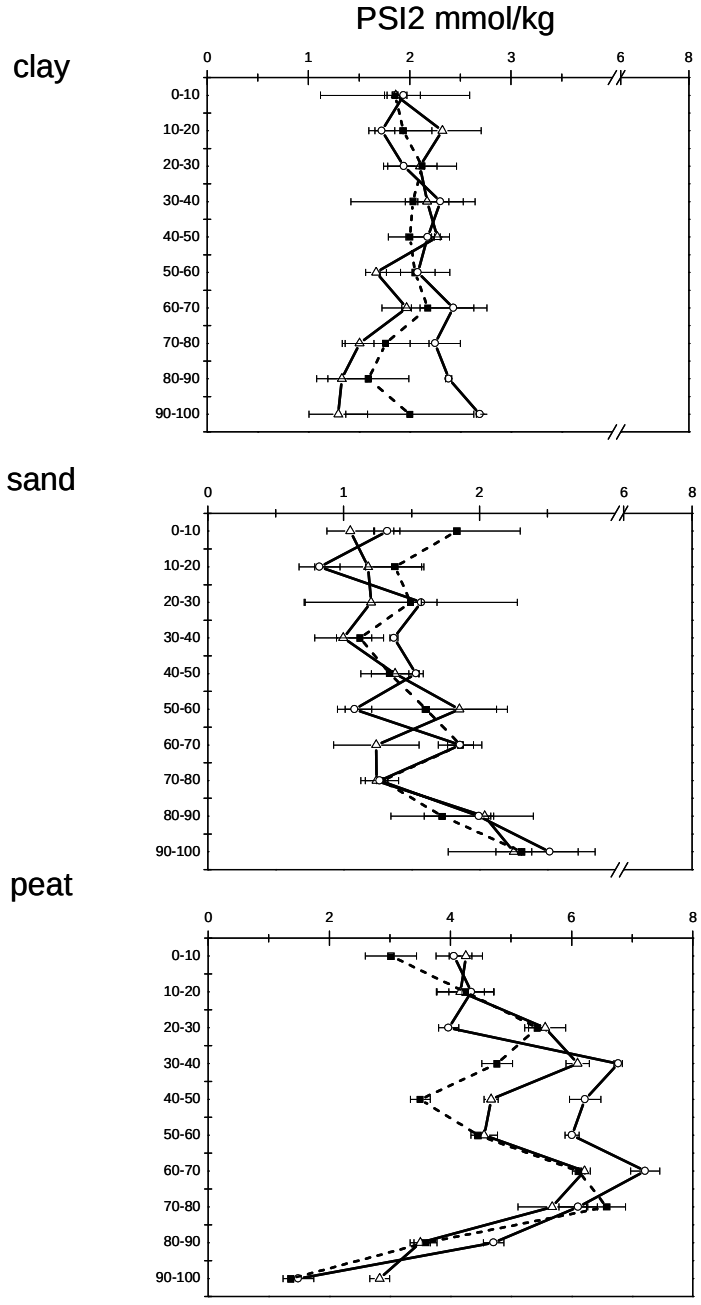

Figure 25 - Single-point P addition PSI2 (50 mmol P kg^{-1}) distribution in the clay, sandy, and peat soil profiles after 44 years of fertilizations. No fertilization (control = triangle); farmyard manure (empty circle); mineral fertilizer (full four-square).

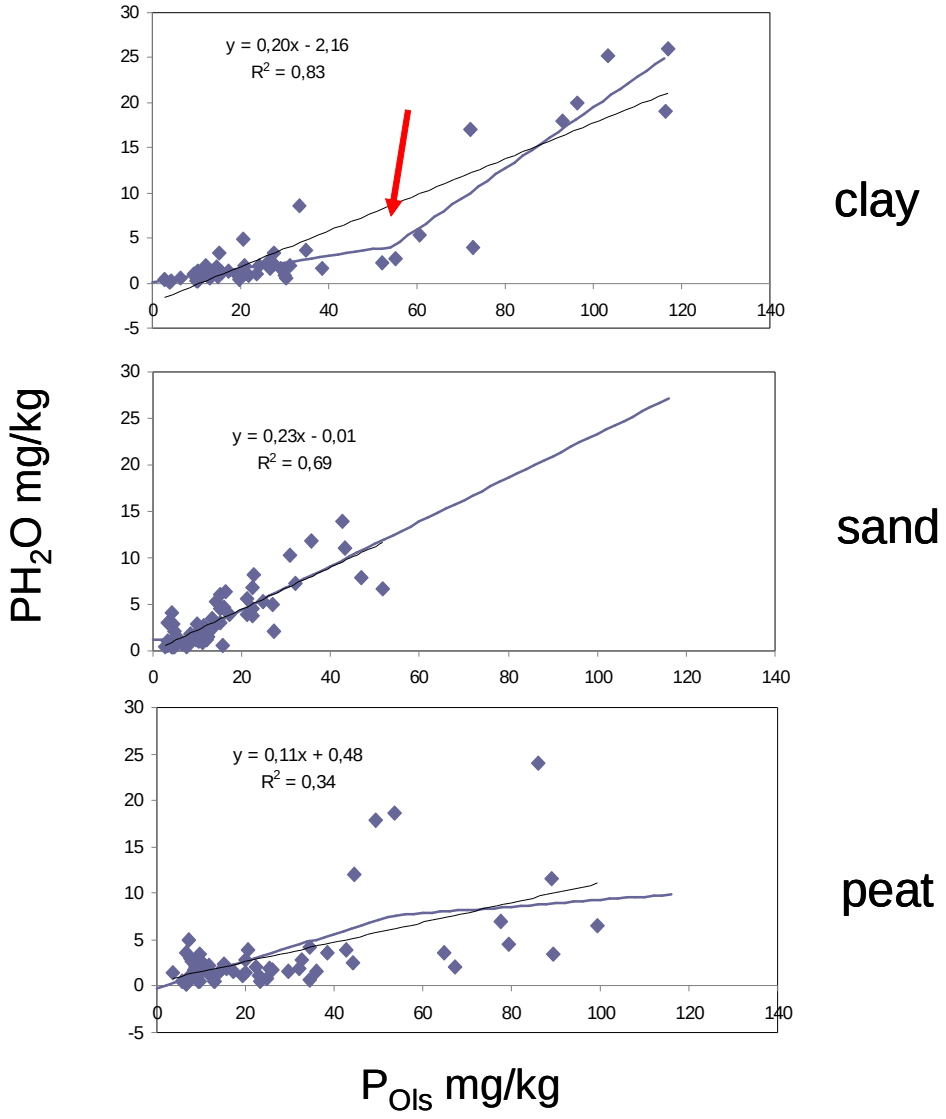

Figure 26 - Olsen-P and water-P relationship in the three soils (clay, sandy, and peat).

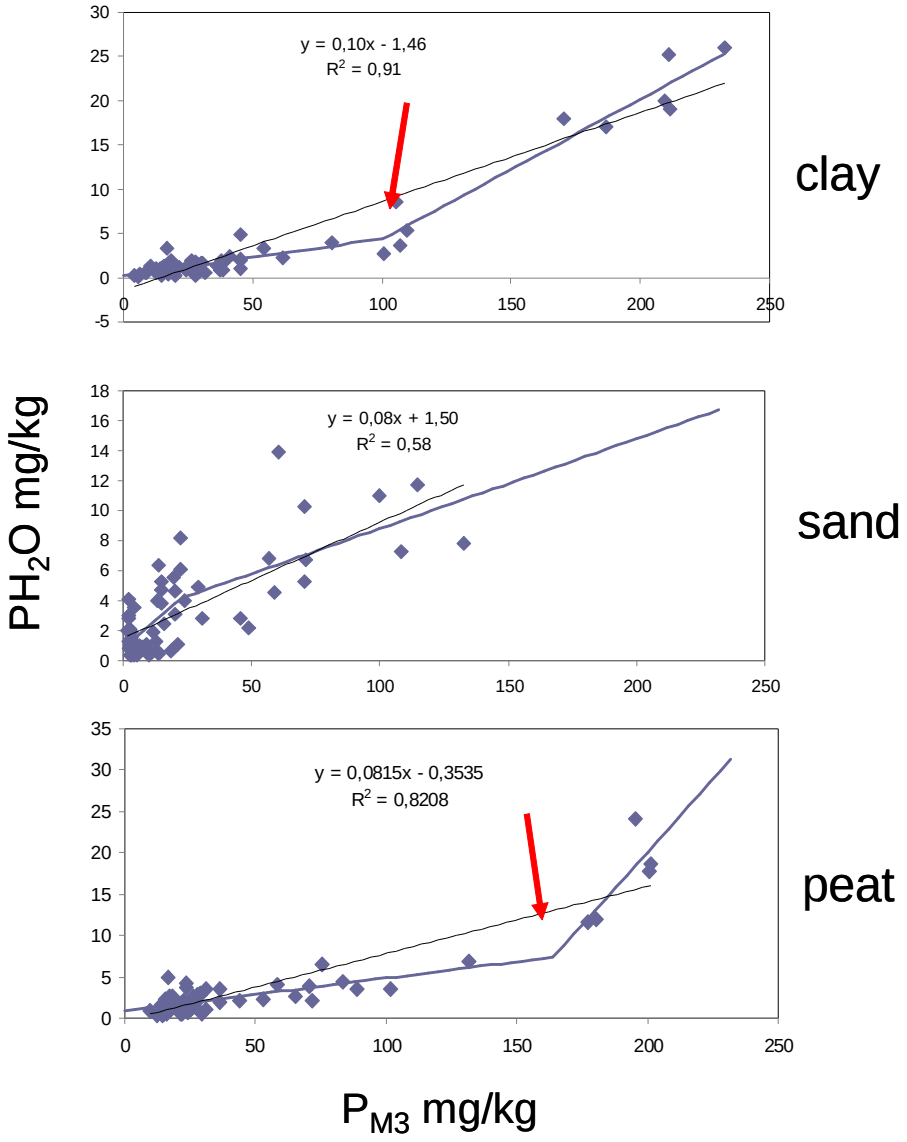

Figure 27 - Mehlich-3-P and water-P relationship in the three soils (clay, sandy, and peat).

Chapter 4 – General conclusion

Several researchers reported that it is not the quantity of phosphorus (P) in a soil that determines how much P can be released, but the degree of soil P saturation. This determines the intensity of the solution P concentration that a soil can maintain and the concentration of P that can be lost in runoff and leaching. Long-term application of mineral or organic fertiliser have affected the soil P status and distribution not only in the upper layers but also down throughout the soil profiles. The high soil P saturation that resulted from the high fertilisation rate was source of available P, which moved into the soil down to the lower layers. This can lead to subsurface P losses to water bodies, particularly in sandy soils.

The use of organic fertiliser should be carefully evaluated due to the risk of excessive P inputs when N-based fertilization plans are adopted. Moreover, farmyard manure strictly incremented the amount of soluble and labile P forms, thus increasing the environmental risk. With respect to the soil test P, the Melich-3-P appears as a good indicator as Olsen P for agronomic purposes and even better in an environmental context. These results evidenced the necessity to assess the risk of P loss from fields into surface and subsurface waters, particularly when the phosphorus addition is high.