Identification and quantification of organic phosphorus forms in soils from fertility experiments

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Abstract

The effects of soil type, fertilization type, amount of fertilization, and crop rotation on the composition of organic phosphorus (P) compounds in soils from a number of long-term Swedish experimental plots were investigated with ^{31}P-NMR. Soil types investigated were loamy sand, sandy loam, silty clay loam and clay. P was added to the soils since the 1950:s at 4 different levels, in the form of either manure or mineral fertilizer. Results show that in soils receiving no P addition the majority of the soil P were present in the form of phosphate monoesters (60 – 70 %, depending on soil type), but that a P addition equivalent to the amount of P removed annually by harvest altered this relation so that the soils were dominated by orthophosphate instead. This trend became more obvious with increasing P addition, and at the highest P addition the orthophosphate comprised 70% or more of the total extracted P in all soils. These changes in the soil were entirely due to increase of orthophosphate, since the amounts of monoesters did not change with increasing P additions. This was true both for P additions in the form of manure or mineral fertilizer. Neither did soil type of crop rotation influence the results. Thus, the results indicate that there is no apparent build up of organic P in the soils, but that P addition mainly affects the orthophosphate amounts in the soils, regardless of form or amount of fertilizer.

Introduction

Phosphorus (P) is, as one of the essential macronutrients in the environment, vital in many processes concerning energy and production. However, when nutrient enrichment of a water body exceeds the normal uptake capacity, a state of eutrophication might be induced. Eutrophication means enrichment with nutrients, but is commonly used to describe a state of anthropogenic over-enrichment with unwanted consequences for the ecosystem, ranging from excessive and sometimes toxic algae blooms to anoxia caused by oxygen depletion during decomposition of elevated biomass input to deeper waters (e.g. Kotak et al. 1993, Bennet et al. 2001, Carpenter 2005 and references therein).

The effect of increased P concentrations is especially pronounced in areas where P is the limiting nutrient, which includes most fresh water systems, and likely also some marine and brackish ecosystems (Hecky 1998, Hellström 1998, Boesch et al. 2005). Due to its wide geographical extent and its potential to affect entire ecosystems, eutrophication is regarded as one of the largest threats to the aquatic environment today.

There is thus a need for knowledge regarding the major sources of P input, and calculations indicate that agricultural runoff makes up the major part of the P reaching the surface waters in Scandinavia (Bergström et al. 2007, Brandt et al., 2009). The increased P content of agricultural soils giving rise to this runoff is an important field of research and is affected by various internal and external processes, including the application of fertilizers.

Soils generally contain both inorganic and organic P compounds, both of which are important sources of P to plants (Brady, 1990). Organic P content varies, but comprises a large part of the total P content of the soil (Turner et al 2005), commonly more than 50% (Brady, 1990). Organic P can be transformed through enzymatic reactions (Quiquampoix och Mousain, 2005) or be assimilated directly by microorganisms (Frossard m. fl., 2000), and this pool is thus very active in processes that affects both plant uptake and P losses. Studies on organic P forms in soil has been ongoing for decades, and nuclear magnetic resonance spectroscopy (NMR) is now one of the most used techniques to distinguish between various forms of organic P compound groups. Studies have included a variety of agricultural procedures likely to affect soil P, for example comparisons of manure types (Hansen et al, 2004) and effects of tillage (Cade-Menun et al 2010).

Fertilization is a very important aspect of soil P, and its effects on the organic P components of the soils are not entirely understood. As excessive use of fertilizers may lead to a build up of P in the soil, with increased P losses to the surrounding surface waters as a result, knowledge about the forms in which P is stored in the soil and how these forms are affected by increased fertilization is needed. Previous studies focusing on the effects of P addition on organic P in soils with NMR has mainly looked at the effects of different forms of manure (Hansen et al, 2004; Hill & Cade-Menun, 2009), and rarely on different levels of P additions, whether it is mineral fertilizers or animal manure.

The focus of this study is thus to investigate the influence of different fertilizing regimes and fertilizer types on the soil content of organic phosphorus (P). Four soils of different characteristics (silty clay loam, loamy sand, sandy loam and clay) were investigated with nuclear magnetic resonance spectroscopy (P-NMR). On each of these soil types 4 different levels of fertilizer additions were established since 1950:s, and in addition was each of the additions made in two crop rotations, one with commercially available fertilizer, and one with manure. On the zero P addition soils, were no fertilizer was added, the potential influence of using ley farming on the organic P content of the soil was investigated. Each of the tested set ups were made in replicates. The study was thus aimed both at investigating the impact of various fertilizing regimes on the composition of organic P compounds in the soil, as well as increasing the knowledge on organic P in soils in general.

Methods

Study sites

The sites chosen for the study was a selected number of experiment plots, maintained by the Department of Soil and Environment at the Swedish University of Agricultural Sciences and parts of an ongoing fertilization experiment since the 1950: s. The 4 experiment plots in this study were chosen to give a gradient in soil types and encompass silty clay loam (Vreta Kloster), loamy sand (Högåsa), sandy loam (Fjärdingslöv) and clay (Kungsängen). The characteristics of these soils are well investigated (Börling et al., 2001; Carlgren och Mattsson, 2001; Djodjic et al., 2004; Kirchmann, 1991; Kirchmann et al., 1999). On each of these soils 4 different levels of fertilization was added, corresponding to zero P addition, P addition equivalent to the amount of P removed annually by harvest etc (henceforth called P addition), P addition + 15 kg P/ha, and P addition + 30 kg P/ha. The size of the individual experiment blocks were 125 m^2. All plots received 150 kg N ha^{-1} to ensure N limitation did not occur.

In addition, each of the additions was made in two crop rotations, one with commercially available fertilizer, and one with manure. The field in southern Sweden (Fjärdingslöv sandy loam) was included in 4-yr crop rotations whereas the remaining fields were situated in central Sweden and had 6-yr crop rotation with cereals and oilseeds. Crop residues are removed in the rotation with livestock but incorporated in the rotation without. Cattle manure is applied in the livestock rotation according to rules above whereas triple superphosphate (20 % P) was applied in the rotation without livestock. Fertilization occurs every second year. On the zero P addition soils, were no fertilizer was added, the potential influence of using ley farming on the organic P content of the soil was investigated. Each of the tested set ups were made in replicates. The layout of the study is summarized in table 1.

Sample preparation

The soils collected from each sampling site were air-dried, ground and sieved (<2mm) before analysis. Extraction for ^{31}P-NMR analysis was made following Cade-Menun and Preston (1996). For each extraction, 2.5 g of dried soil was shaken for 16 h at 20°C with a 1:1 mixture of 0.5 M NaOH and 0.1 M Na_2EDTA. After 16 h, the samples were separated from the extraction solution by centrifugation at 8000 rpm for 10 min, and the extract collected. A 5 ml sub sample was collected from the extract for total extractable P analysis, and the remainders of the extracts were frozen and lyophilized. 0.75 g of the lyophilized extracts was redissolved in 0.9 ml 1 M NaOH and 0.1 ml 0.1 M EDTA, which gave a concentration of the original extract of about 30 times, yielding a sufficiently high P concentration in the samples for successful ^{31}P-NMR analysis. The concentrated extracts were frozen until analysis, a procedure proven not to affect the extracted P compounds (Hupfer et al., 2004).

Analysis

Total P (TP) in the soils was analyzed with inductively coupled plasma – optical emission spectrometry (ICP-OES) on the dried and sieved samples after digestion. Total extractable P was determined using inductively coupled plasma–atomic emission spectrometry (ICP-AES) on the extracts before freezing and lyophilization.

^{31}P-NMR analysis was made on a 400 MHz instrument (Bruker DRX-400), at ambient temperature. An amount of D_2O sufficient to obtain a stable lock signal was added to the extracts before measurement. Spectra were recorded using a 90° observe pulse, acquisition time 1 s, relaxation delay 2.5 s, acquiring around 32000 transients (8 h). Chemical shifts were indirectly referenced to external 85% H_3PO_4 (at $\delta = 0.0$) via the lock signal. Assignment of peaks was done from spectra of sediment extracts spiked with standard solutions ($Na_2HPO_4 \cdot 7H_2O$ for orthophosphate and $Na_2P_2O_7 \cdot 10H_2O$ for pyrophosphate), added to one of the sediment extracts, as well as comparisons with literature (e.g., Cade-Menun and Preston, 1996; Turner et al., 2003; Doolette et al, 2009; Cade-Menun et al, 2010). The resulting spectra were evaluated with Bruker Topspin 2.0, and peaks with a signal to noise ratio exceeding 4 were quantified using the contribution of individual P compound groups relative to total extracted P.

Results

TP in the soils varied between the soils, with the highest amount found in the samples with the highest amount of P addition. For the sandy loam the TP varied from 150 µg P/g DW in the sample without P addition, to about 400 µg P/g DW in the sample with the highest addition, for the loamy sand the TP varied between 300-400 and 600-700 µg P/g DW, for the silty clay loam between 250 and 400-600 µg P/g DW, and for the clay soil between 300 and 500 µg P/g DW (table 2). In addition to this, fertilizing the soils increased the total extractable P by 2-3 times, varying with soil type, with the loamy sand samples yielding the highest results in terms of total extractable P both before and after fertilization. The extraction efficiency was generally high, between 60 and 100 % (table 2).

The ^{31}P-NMR spectra showed that in all cases the P content of the samples was dominated by orthophosphate (Ortho-P) and orthophosphate monoesters (Mono-P). Small amounts of pyrophosphate (Pyro-P) were also found in most samples, while orthophosphate diesters (Di-P) and phosphonates only were found in a few of the samples (figure 1, table 2). Some of the spectra also show enough resolution that a number of individual P compounds may be identified (figure 1). Thus, peaks at 5.34, 4.40, 4.00 and 3.88 may indicate the presence of *myo*-inositol hexaphosphate (*myo*-IP_6), the peak at 3.55 *scyllo*-inositol hexaphosphate (*scyllo*-IP_6), the peak at 4.78 β-glycerophosphate (β-glyc), and the peak at 4.24 may indicate adenosine monophosphate (AMP) (Doolette et al 2009, Cade-Menun et al 2010).

The addition of fertilizer changed the organic P composition of the soils markedly. Mono-P was in general the largest P compound group in the extracts where the soils had not received any P addition. In these samples the Mono-P comprises more than 70% of the total extractable P, with Ortho-P as the second largest P compound group at about 10 - 20%. At the lowest P addition, these two dominating groups are of about equal relative magnitude, but with increased fertilization the relative contribution of Ortho-P increases and is at both P addition + 15 (20) kg P/ha, and P addition + 30 kg P/ha the dominating fraction. At P addition + 30 kg P/ha, Ortho-P comprises more than 60% of the total extractable P in most soils (table 2, figure 2).

The concentrations of the various identified P compound groups follow the relative amounts closely in the case of Ortho-P, which increases from about 80 μg P g^{-1} to 300 μg P g^{-1} in the sandy loam, from about 150 μg P g^{-1} to 400-500 μg P g^{-1} in the loamy sand, from about 50 μg P g^{-1} to about 400 μg P g^{-1} in the silty clay loam and from 100 μg P g^{-1} to about 300 μg P g^{-1} in the clay soil. These increases are all statistically significant ($p<0.01$, two way ANOVA) The amounts of Mono-P, on the other hand, do not vary significantly with increased P addition. In the sandy loam, the amounts of Mono-P are about 100 μg P g^{-1}, in the loamy sand around 170 μg P g^{-1}, in the silty clay loam around 200 μg P g^{-1}, and in the clay soil around 170 μg P g^{-1}. Of the minor constituents, the pyro/poly P varied between 3 and 10 μg P g^{-1}, regardless of soil, while the amounts of Di-P and phosphonates were even lower in the few samples where they were detected (table 2, figure 3).

The ratio between monoesters and ortho-P varied significantly ($p<0.01$, one way ANOVA) depending on the soil type, with the loamy sand having the lowest amount of monoesters, and the silty clay loam the highest. These differences were diminished with increasing fertilizer addition.

No differences due to fertilizer type or use of ley farming on the composition of organic P compounds in the soil could be concluded in the study, except for the loamy sand samples where the Ortho-P amounts were significantly higher ($p = 0,027$) in the samples amended with manure than the samples were mineral fertilizers had been added.

Discussion

General

The most obvious result of the study is the shift in dominance between the major organic P fractions of the soil with added P in the form of fertilization. Fertilization changes the P composition a soil from being dominated primarily by Mono-P to being dominated by Ortho-P, in terms of relative amounts (figure 2). This is important since Mono-P is composed mainly of inositol compounds, and may thus only be available to plants on a longer time frame (Turner et al, 2007), while Ortho-P is readily and immediately available. The third most common P compound group in the extracts, Pyro-P, is although a reactive and plant available compound present in quite small amounts. It should be noted that in this study Pyro-P is discussed together with polyphosphate (Poly-P), as studies have shown that lyophilization might decompose Poly-P into Pyro-P. The lack of Poly-P in the samples of this study might thus not necessarily be due to a lack of these compounds in the soils, but rather an artifact of the sample preparation procedure. The amount of Pyro-P could for the same reason be over-estimated.

In general does Pyro-P not seem to be affected by increased amounts of P added to the soils, as the Pyro-P levels are of the same low magnitude regardless of soil type or amount of fertilizer added (table 2). The type of fertilizer did not either affect the amount of Pyro-P. The low overall levels of Pyro-P in the investigated soils could possibly be explained in same way as the lack of Di-P, by a rapid decomposition due to high microbial activity, as Pyro-P generally is associated with microbes (e.g. Turner et al. 2003).

Thus, the results of the study clearly indicate that addition of P to soils changes the P soil composition towards P forms readily available to plants. The immediate conclusion to be drawn from this is that addition of P, whether as manure or mineral P, is an efficient method for increasing the amount of P readily available to crops.

These trends, with the dominance of refractory organic P compounds in the soil before fertilization, and dominance of readily available Ortho-P after fertilization, are solely dependent on the increase of Ortho-P content with increased fertilization. The amounts of Mono-P are not affected by fertilization, and remain the same as in the unfertilized soils, regardless of soil type (table 2, figure 3). This is somewhat surprising, as it indicates that no buildup of organic P compounds has occurred in the investigated soils in spite of more than 50 years of continuous fertilization and overfertilization. Even in the cases when the additions of P to the soils were made in the form of mineral fertilizers containing mostly inorganic P, a certain build up organic P compounds due to stimulation of the microbial community could have been expected. The lack of this regardless in which form the P was added could possibly be explained by a fast turnover of the added P; the P is thus taken up by the plants before assimilation into microorganisms have had time to occur.

Di-P only occurred in the loamy sand soil samples, at low amounts and not in all the samples from this site. Since Di-P was found in samples both without any P addition and with the highest P addition, as well as in soils fertilized with manure as well as mineral fertilizers, none of the investigated parameters, except perhaps particle size, seem to affect the amounts of Di-P notably (table 2).

The low amounts of Di-P found in this investigation follow the findings of other studies in the area, and could be the consequence of the soil pH, as it has been shown that adsorption of DNA, which commonly is one of the most abundant forms of Di-P, to soil particles decreases above pH 5 (Khanna et al, 1998). The generally higher pH in the soils of this study (between 6.5 and 7.5) may thus mean that Di-P, being loosely retained in the soils, is being flushed out and depleted relatively quickly. Another potential explanation may be degradation of the Di-P before NMR analysis. The peaks in the NMR spectra corresponding to AMP and β-glyc are assumed to be degradation products of Di-P compounds (Doolette et al., 2009), and the presence of these in the spectra may thus indicate that Di-P does occur in the soils but are decomposed before, or during analysis. Extraction and lyophilization are examples of procedures that may cause degradation, but as there are no direct proofs of these compounds exclusively being decomposition products, the presence of these peaks in the spectra may well mean that these compounds are present in the soils. The lack of Di-P in the soils would in that case most likely be a case of high microbial activity rapidly decomposing the Di-P to other compounds, as Di-P is known to be a rather labile group of P compounds. This is endorsed by the view that both AMP and β-glyc are of microbial origin (Doolette et al., 2009).

Phosphonates occurs in all soils except for Klostergården, but in very low amounts and not in all of the samples from each sample site (table 2). There is no apparent trend concerning the presence of phosphonates in the samples, similarly to the Di-P they seem unaffected by the investigated parameters of fertilizer type and amount. It should be noted that all the identified phosphonates occur at the same shift in the spectra, at about 20.1, which most likely would correspond to aminoethyl phosphonates (Cade-Menun, 2005).

Influence of soil type

The ratio between Mono-P and Ortho-P varied, as mentioned above, significantly depending on the soil type, with the loamy sand having the lowest relative amount of monoesters, and the silty clay loam the highest. These differences were diminished with increasing fertilizer addition. The actual amounts of Mono-P, however, does not change much with soil type, with the exception of the sandy loam, where the Mono-P levels generally are lower than in the other investigated soils.

In addition to this, extractable P varied between the soils and increased by 2-3 times after fertilization. While no obvious correlation between amount extracted P and particle size was found, the coarsest soil, the loamy sand, was the soil showing the highest extractable P both before and after fertilization (table 2).

Influence of manure/mineral/ley

No significant differences ($P > 0.1$, two way ANOVA) due to fertilizer type or use of ley farming on the composition of neither organic nor inorganic P compounds in the soil could be concluded in the study, with the exception of the higher levels of Ortho-P found in the manure amended samples from the loamy sand soil. These trends are the same for all the investigated soil types, and are slightly surprising as it might be expected that an extended period of fertilization with organic material in the form of manure would increase the organic P pool in the soils, as noted above. These observations are following the trends of the total amount of extracted P in the samples, where no indication of a build up of organic P due to increased fertilization is obvious. The reason for this is difficult to ascertain, but the lack of an organic P build up in the heavily fertilized samples may be due to a faster turnover of organic P in these soils due to increased microbial activity induced by the higher total P content.

This lack of apparent influence of the use of manure or mineral fertilizer also means that there are no indications of any possible influences of ley vs. no ley crop rotation. In this study, only the soils where a ley crop rotation was used received manure as P enhancement. This was done to simulate natural conditions where it is expected that mostly farms with cattle would grow ley, and that the presence of cattle would mean that there is a supply of manure to be used on the fields. This obviously means that there is no possibility to distinguish between the potential influences of the use of ley and the use of manure in this study, and since there are no indications of differences between manure and mineral fertilizers, the same must be concluded for ley or no ley crop rotation.

Individual organic P compounds

The most abundant of the identifiable individual organic P compounds were inositol hexaphosphate (IP_6), which constituted a large part of the Mono-P in all samples. IP_6 is a family of compounds characterized by a tendency to be strongly retained in soils, and is thus theoretically not very involved in P cycling. However, recent studies have shown that many microorganisms can utilize IP_6, and that these compounds due to their ubiquity thus very likely have a central role in the environment (Turner et al, 2007).

The highest relative proportions of IP_6 were found in the loamy sand soil samples, where IP_6 constituted 60 to 90% of the Mono-P. In contrast, in the silty clay loam soil the IP_6 only comprised about 20% of the Mono-P. In the clay soil the IP_6 constituted 40% of the total Mono-P, while the spectra from the sandy loam soil in general had to low resolution to make an identification of the peaks in the Mono-P area viable. This is also the soil with the lowest

TP levels, as well as the lowest total extracted amounts of P. In terms of percentage of the total extracted P amount, this means that IP_6 constitutes more than 30 % of all extracted P in the loamy sand soil. As has been noted, the total amount of Mono-P does not seem to be affected by additions of P to the soil, and this is true for IP_6 as well. No indications of changes in the amounts of IP_6 with increasing P additions can be seen for any of the soils, and neither are there any obvious differences due to the type of fertilizer used. This may seem surprising, since various forms of manure have been shown to contain large amounts of IP_6. This related mostly to manure from monogastric animals, however, and is most likely is a consequence of their inability to digest these specific compounds (Turner et al, 2007). In addition, it has been shown that addition of organic P to soils in the form of poultry litter, did not either increase the soil content of IP_6 (Hill & Cade-Menun, 2009). This was hypothesized to be due to microbial decomposition of IP_6 into Ortho-P, which would be consistent with the results of this study. Furthermore could the high levels of IP6 in the soils regardless of the type of fertilizer be attributed to the fact that while addition of organic P in the form of manure would increase the store of IP6, the addition of inorganic P would mean that the store of readily available P in the form of Ortho-P increases, and the utilization of IP6 for nutritional purposes would not be as necessary, thus lessening the decompositional pressure on this group of compounds (Turner et al, 2007).

The most common form of IP_6 identified in most soils, including those of this study, is *myo*-IP_6. Part of this might be due to overestimation when interpreting the NMR spectra (Doolette et al, 2009), but most likely reflects true high amounts of this compound caused by the crops grown (Turner et al, 2002). *myo*-IP_6 is as noted above often considered to be associated with animal manure, and may thus be considered to be an indicator of external input of P to a soil. The other IP_6 compound that could be identified in the soils of this study was *scyllo*-IP_6, a compound which is less known than its stereoisomer *myo*-IP_6, but is thought to be a product of epimerization or microbial processes. It should be noted that while *scyllo*-IP_6 is much less abundant in the soils of this study than *myo*-IP_6, it is easier to identify and quantify due to its position in the NMR spectra.

The two non-IP6 individual compounds identified in the spectra, AMP and β-glyc, are generally believed to be at least partly degradation products of Di-P compounds (Doolette et al, 2009) and likely have a microbial origin (Makarov et al, 2005). In contrast to *myo*-IP_6, the presence of these compounds may thus be an indicator of microbial cycling rather than external input of P. This is tentatively confirmed by the tendency of higher content of AMP and β-glyc in the samples from the soils that received no P addition whatsoever.

Agronomic and environmental relevance

While the most obvious agronomic conclusion of the study is the lack of build up of organic P in the soils in spite of long-term fertilization, other results may be of interest in terms of environmental and agricultural relevance as well. For instance, the increase of Ortho-P generated by fertilization found in this study follows the same trends as the increase in P-AL levels measured in the same soils (Carlgren and Mattsson, 2001; Börling et al., 2004), which might further strengthen the evidence of an increased pool of available P with increased P additions in the form of manure or mineral fertilizers (table 2). The extraction of soil P with the ammonium lactate/acetic acid (P-AL) at pH 3.75 (Egner et al. 1960) is the most common agronomic soil P test in Sweden to determine plant-available P in the topsoil. However, the P-AL does not increase as much as the Ortho-P, which indicates that all of the buildup of Ortho-P due to fertilization might not have the same availability to crops. This discrepancy between Ortho-P and P-AL varies between 1.5 and 11 times, depending on soil type and in which form P was added to the soils. A high discrepancy may indicate that the P is not added in a cost efficient way, as it to a large degree apparently ends up in the unavailable pool of P.

For the sandy loam the discrepancy diminishes with increased P addition to the soil, and the amount of Ortho-P goes from being 4-5 times as much as the P-AL in the unfertilized soils to being about 1.5 of the P-AL at the highest P addition, indicating that in this soil most of the added P ends up in the available pool. The trends in this soil are the same for both manure and mineral fertilizers (table 2). The Fjärdingslöv sandy loam soil has the lowest P sorption capacity of all soils included in this study (Börling et al., 2001), which might explain the above mentioned behavior. For the other investigated soils the trend are quite different, with the Ortho-P to P-AL ratio being around 4 for the clay soil regardless of type and amount of P addition, and a slightly increasing ratio (from 2 to 3) for the silty clay loam with increasing P additions. In fact, it seems that P-AL in some cases rather diminishes with increasing Ortho-P in the over fertilized sample levels, indicating that fertilization at this high initial soil P levels may be an inefficient way of increasing the plant available P pool (figure 4).

The only soil showing different trends for the different types of fertilizers was the loamy sand, in which additions of P in the form of manure did not have any effect on the Ortho-P / P-AL ratio, which stayed at about 6 regardless of P amount added. Addition of P in the form of mineral fertilizer to the loamy sand sites did however make the Ortho-P / P-AL ratio decrease from about 11 in the unfertilized soil to about 4 in the soil with the highest P addition, hence indicating that much of the P added in this form indeed seems available to the crops (table 2). It should be noted, however, that a high Ortho-P / P-AL ratio may not necessarily indicate that all of this difference is unavailable to plants as an underestimation of the plant available P by the P-AL method also is possible.

When discussing environmental relevance of the various P-forms found in this study, consideration should be taken not only to concentrations but also to amounts and availability. Pyro-P levels varies up to 16.2 μg P/g DW (table 2) with an average of 4.7 μg P/g DW. This may not seem consequential but it is equivalent to 57 and 16.5 kg P ha^{-1}, respectively, in the plow layer (0.25 m, assuming a bulk density of 1.4 g cm^{-3}). Considering that the average uptake of P by crops in Sweden is 12–26 kg P ha $year^{-1}$ (Swedish Board of Agriculture, 2009) this P-species could well have an influence on the total P turnover in the soils and P supply to plants in spite of the low concentrations, due to its potential high availability. Consequently, bearing in mind an average P loss in Sweden of about 0.4 kg P ha $year^{-1}$ (Ulén et al., 2007), this same P species may have an influence on the quality of water leaving soil profile in spite their quite low amounts in the soils.

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Table 1. Experimental set up.

Table 2. Results from the analysis of the soil samples (µg g^{-1} DW and %). For total phosphorus (TP), total extracted phosphorus (tot ext P), and the identified P compound groups in the soils both results from the replicate samples are presented, while the presented results for extraction efficiency and relative percentage of the identified P compound groups are based on the average of the replicate analyses.

Figure 1. Examples of typical solution ^{31}P-NMR spectra for some of the soil extracts.

Figure 2. Percentual content of orthophosphate (Ortho-P) and monoester phosphates (Mono-P) in the extracts from the different soils, depending on type of fertilizer (manure/mineral) and amount of fertilizer added (0 = no P addition, A = P addition equivalent to the amount of P removed annually by harvest etc, 15 = A + 15 kg P/ha, 30 = A + 30 kg P/ha).

Figure 3. Amounts of orthophosphate (Ortho-P) and monoester phosphates (Mono-P) in the extracts from the different soils in μg P g^{-1}, depending on type of fertilizer (manure/mineral) and amount of fertilizer added (0 = no P addition, A = P addition equivalent to the amount of P removed annually by harvest etc, 15 = A + 15 kg P/ha, 30 = A + 30 kg P/ha).

Figure 4. Relationship between orthophosphate (Ortho-P) and P-AL for the different soils at the different levels of P addition.

Amount		Description	Variable
4	Sites	Vreta Kloster (silty clay loam), Högåsa (loamy sand), Kungsängen (clay), Fjärdingslöv (sandy loam)	Soil type
2	Crop rotations	With and without ley	Mineral fertilizer/manure Ley/no ley
4	P level	0 P, P addition, P addition + 15 (20) kg P/ha, P addition + 30 kg P/ha	Amount of P added
2	Duplicates	Repetition	Statistics
	In total 4 sites * 2 crop rotations * 4 P levels * 2 replicates = 64 samples		

Table 1.

	TP	Tot ext P	P-AL	Extraction efficiency	Phosphonates	Ortho-P	Mono-P	Di-P	Pyro-P
		µg P g^{-1}		%		µg P g^{-1}%			
Sandy loam									
Manure									
0	**316,9**	**176.8**	17	*56.3*	**0.0**	**81,7**	**95,1**	**0.0**	**0,0**
	298,4	**169.7**			**0.0**	**59,8**	**106,3**	**0.0**	**3,6**
					0.0	*40.7*	*58.2*	*0.0*	*1.1*
A	**372,2**	**223,0**	116	*54.5*	**0.0**	**100,0**	**123,0**	**0.0**	**0,0**
	413,9	**268,5**			**0.0**	**151,8**	**112,0**	**0.0**	**4,7**
					0.0	*50.7*	*48.4*	*0.0*	*0.9*
A+15	**459,6**	**287,2**	223	*66.5*	**0**	**170,8**	**108,4**	**0.0**	**8,0**
	485,6	**342,5**			**0,55**	**248**	**89,8**	**0.0**	**4,1**
					0.1	*65.9*	*32.0*	*0.0*	*2.0*
A+30	**642,5**	**411,0**	209	*66.1*	**0.0**	**303,7**	**97,3**	**0.0**	**10,0**
	627,3	**428,7**			**0.0**	**332,4**	**92,6**	**0.0**	**3,7**
					0.0	*75.7*	22.6	*0.0*	*1.6*
Mineral									
0	**312,9**	**148,7**	12	*54.5*	**0**	**53,2**	**93,3**	**0.0**	**2,2**
	288,4	**177,3**			**1,0**	**76,0**	**96,7**	**0.0**	**3,6**
					0.3	*39.3*	*58.6*	*0.0*	*1.8*
A	**331,8**	**196,3**	30	*60.2*	**0.0**	**101,5**	**92,5**	**0.0**	**2,3**
	384,7	**235,4**			**0.0**	**96,8**	**123,1**	**0.0**	**6,4**
					0.0	*46.4*	*51.6*	*0.0*	*2.0*
A+15	**461,7**	**301,7**	103	*62.2*	**0.0**	**182,5**	**114,8**	**0.0**	**4,4**
	552,2	**325,9**			**0.0**	**224,3**	**97,6**	**0.0**	**4,0**
					0.0	*64.7*	*34.0*	0.0	*1.3*
A+30	**533,3**	**397,4**	184	*66.6*	**0.0**	**284,0**	**107,9**	**0.0**	**5,6**
	710,5	**416,8**			**0.0**	**301,5**	**111,3**	**0.0**	**4,0**
					0.0	*71.9*	*26.9*	*0.0*	*1.2*
Loamy sand									
Manure									
0	**527,1**	**444,1**	38	*85.6*	**0.0**	**275,3**	**166,7**	**0,7**	**1,4**
	419,4	**364,6**			**0.0**	**170,1**	**186,9**	**0,0**	**7,6**
					0.0	*54.3*	*44.4*	*0.1*	*1.2*
A	**497,9**	**432,1**	40	*85.7*	**0.0**	**269,7**	**159,3**	**0.0**	**3,1**
	502,9	**425,4**			**0.0**	**243,3**	**182,1**	**0.0**	**0,0**
					0.0	*59.8*	*39.8*	*0.0*	*0.4*
A+15	**734,3**	**733,0**	79	*99.0*	**0.0**	**545,4**	**183,9**	**0.0**	**3,7**
	626,9	**615,7**			**0.0**	**456,9**	**150,5**	**0.0**	**8,3**
					0.0	*74.3*	*24.8*	*0.0*	*0.9*
A+30	**821,7**	**777,4**	92	*96.2*	**0.0**	**590,9**	**177,3**	**3,5**	**5,7**
	756,1	**739,2**			**0.0**	**575,5**	**159,0**	**0,0**	**4,7**
					0.0	*76.9*	*22.2*	*0.2*	*0.7*
Mineral									
0	**483,8**	**410,4**	16	*84.1*	**0,0**	**231,3**	**175,4**	**0,0**	**3,7**
	366,2	**305,6**			**0,9**	**126,7**	**170,3**	**2,3**	**5,3**
					0.1	*48.9*	*49.2*	*0.4*	*1.3*
A	**494,3**	**425,9**	30	*87.8*	**0.0**	**258,5**	**163,9**	**0.0**	**3,5**
	501,7	**448,7**			**0.0**	**271,5**	**172,7**	**0.0**	**4,6**
					0.0	*60.6*	*38.5*	*0.0*	*0.9*
A+15	**633,6**	**622,8**	78	*97.5*	**0.0**	**434,4**	**182,9**	**0.0**	**5,5**
	516,3	**499,6**			**0.0**	**358,6**	**139,6**	**0.0**	**1,5**
					0.0	*70.8*	*28.7*	*0.0*	*0.6*
A+30	**694,5**	**651,6**	114	*90.9*	**1,0**	**476,5**	**168,6**	**0,0**	**5,4**
	689,9	**606,5**			**1,1**	**444,6**	**152,1**	**3,2**	**5,5**
					0.2	*73.2*	*25.5*	*0.3*	*0.9*

Silty clay loam									
Manure									
0	**544,2**	**297,6**	26	*50.9*	**0.0**	**48,6**	**240,9**	**0.0**	**8,1**
	514,6	**242,8**			**0.0**	**59,3**	**174,3**	**0.0**	**9,1**
					0.0	*20.4*	*76.4*	*0.0*	*3.2*
A	**579,7**	**341,7**	29	*59.4*	**0.0**	**108,1**	**223,7**	**0.0**	**9,9**
	590,5	**353,7**			**0.0**	**183,7**	**161,7**	**0.0**	**8,4**
					0.0	*41.8*	*55.6*	*0.0*	*2.6*
A+15	**783,2**	**509,1**	69	*63.9*	**0.0**	**316,9**	**180,9**	**0.0**	**11,4**
	879,6	**551,5**			**0.0**	**410,3**	**135,6**	**0.0**	**5,6**
					0.0	*68.3*	*30.1*	*0.0*	*1.6*
A+30	**751,0**	**374,4**	126	*59.5*	**0.0**	**238,2**	**128,2**	**0.0**	**8,0**
	979,9	**676,9**			**0.0**	**530,8**	**136,6**	**0.0**	**9,4**
					0.0	*71.0*	*27.2*	*0.0*	*1.8*
Mineral									
0	**515,5**	**290,2**	26	*53.3*	**0.0**	**61,5**	**221,4**	**0.0**	**7,3**
	479,5	**241,6**			**0.0**	**50,2**	**185,7**	**0.0**	**5,7**
					0.0	*21.0*	*76.6*	*0.0*	*2.4*
A	**536,1**	**299,7**	29	*53.3*	**0.0**	**83,4**	**208,3**	**0.0**	**8,0**
	577,4	**292,8**			**0.0**	**119,6**	**167,7**	**0.0**	**5,5**
					0.0	*34.3*	*63.4*	*0.0*	*2.3*
A+15	**714,5**	**414,9**	69	*61.4*	**0.0**	**248,4**	**160,5**	**0.0**	**6,0**
	818,7	**530,4**			**0.0**	**377,9**	**147,9**	**0.0**	**4,5**
					0.0	*65.6*	*33.3*	*0.0*	*1.1*
A+30	**794,1**	**545,7**	126	*69.1*	**0.0**	**375,1**	**163,1**	**0.0**	**7,5**
	851,1	**592,1**			**0.0**	**438,1**	**148,4**	**0.0**	**5,6**
					0.0	*71.4*	*27.5*	*0.0*	*1.2*
Clay									
Manure									
0	**688,8**	**312,5**	30	*48.7*	**0,0**	**109,5**	**192,7**	**0.0**	**10,4**
	623,6	**324,5**			**2,7**	**132,9**	**179,5**	**0.0**	**9,4**
					0.4	*38.0*	*58.5*	*0.0*	*3.1*
A	**715,4**	**346,0**	31	*51.3*	**0,0**	**158,0**	**177,8**	**0.0**	**10,2**
	676,9	**367,1**			**2,8**	**191,3**	**162,3**	**0.0**	**10,7**
					0.4	*48.9*	*47.8*	*0.0*	*2.9*
A+15	**855,3**	492,9	89		1,9	309,6	170,6	0,0	10,7
	781,4								
A+30	**883,3**	**527,8**	105	*62.0*	**2,3**	**329,2**	**180,2**	**0.0**	**16,2**
	853,1	**547,7**			**1,9**	**356,6**	**181,2**	**0.0**	**7,9**
					0.4	*63.7*	*33.6*	*0.0*	*2.3*
Mineral									
0	**598,5**	**301,1**	26	*48.1*	**2,0**	**122,3**	**169,6**	**0.0**	**7,2**
	651,2	**298,4**			**0,0**	**117,4**	**172,7**	**0.0**	**8,4**
					0.3	*40.0*	*57.1*	*0.0*	*2.6*
A	**646,7**	**353,8**	38	*53.3*	**2,6**	**188,4**	**156,3**	**0.0**	**6,4**
	685,5	**355,9**			**2,3**	**180,2**	**164,7**	**0.0**	**8,7**
					0.7	*52.0*	*45.2*	*0.0*	*2.1*
A+15	**786,1**	**488,0**	82	*61.5*	**2,8**	**319,8**	**157,4**	**0.0**	**8,0**
	824,0	**502,8**			**2,2**	**344,8**	**147,7**	**0.0**	**8,1**
					0.5	*67.1*	*30.8*	*0.0*	*1.6*
A+30	**866,6**	**542,7**	90	*59.4*	**1,7**	**391,4**	**142,0**	**0.0**	**7,5**
	764,6	**428,8**			**2,7**	**284,9**	**133,6**	**0.0**	**7,5**
					0.5	*69.3*	*28.7*	*0.0*	*1.6*

Table 2.

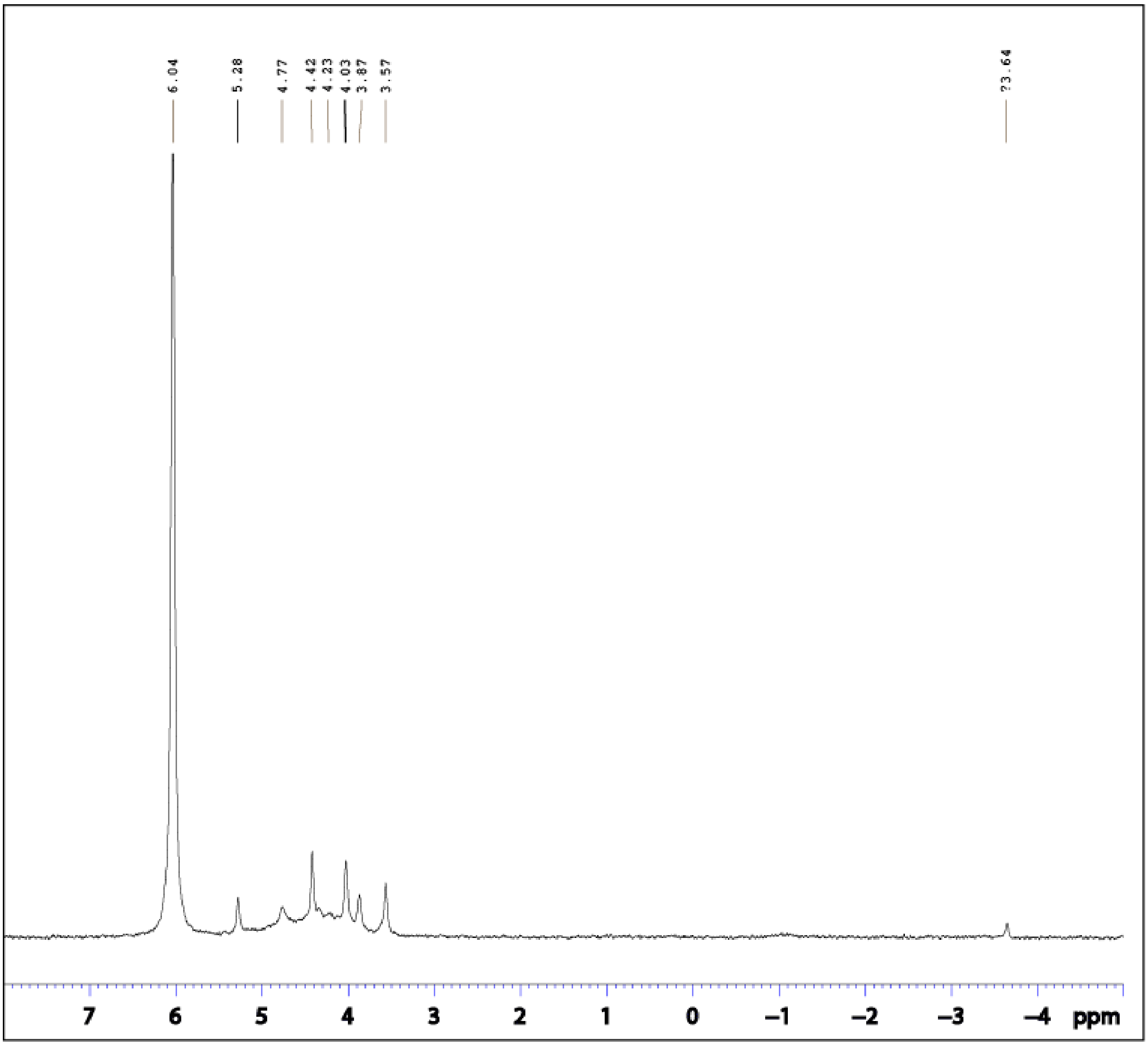
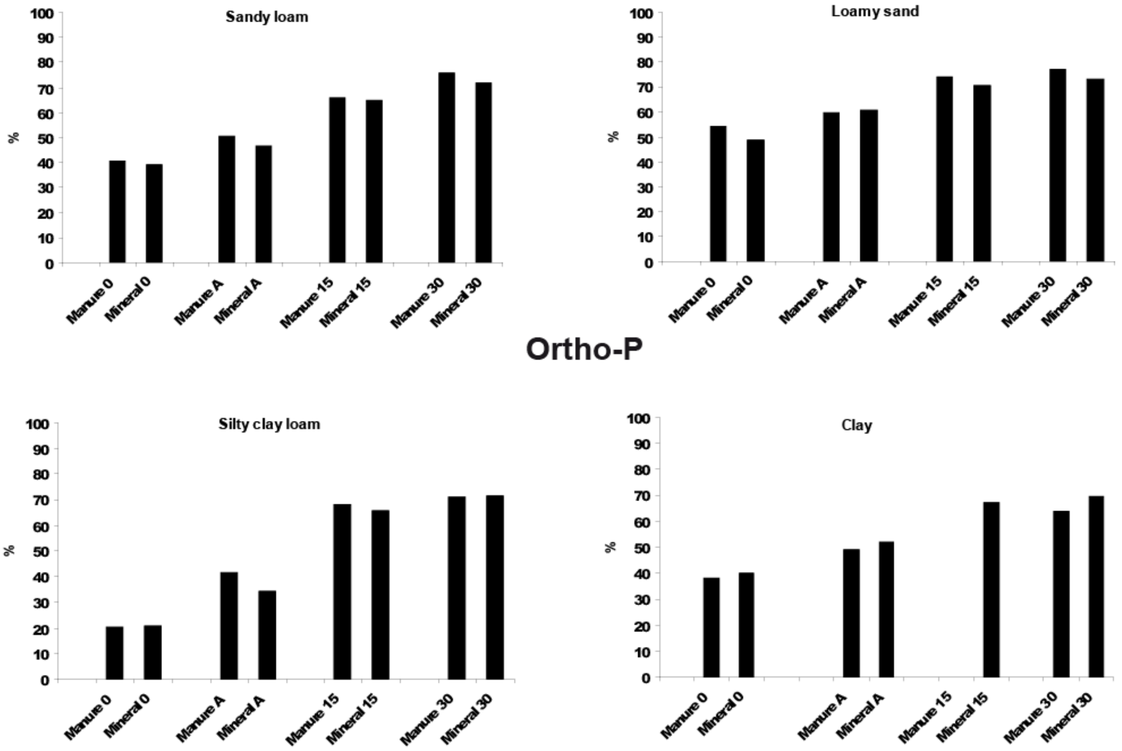


Figure 1.


Ortho-P
Sandy loam
Loamy sand
Silty clay loam
Clay
%
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10
20
30
40
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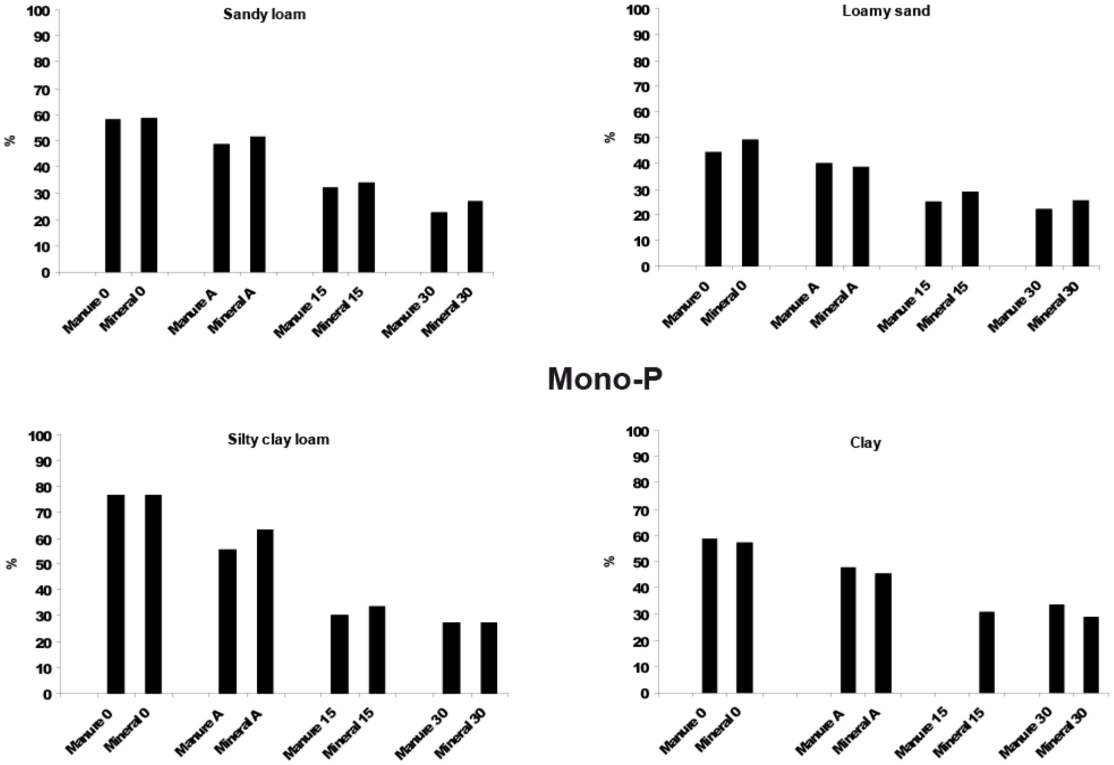
60
70
80
90
100
Manure 0
Mineral 0
Manure A
Mineral A
Manure 15
Mineral 15
Manure 30
Mineral 30


Mono-P
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Loamy sand
Silty clay loam
Clay
%
0
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Mineral 0
Manure A
Mineral A
Manure 15
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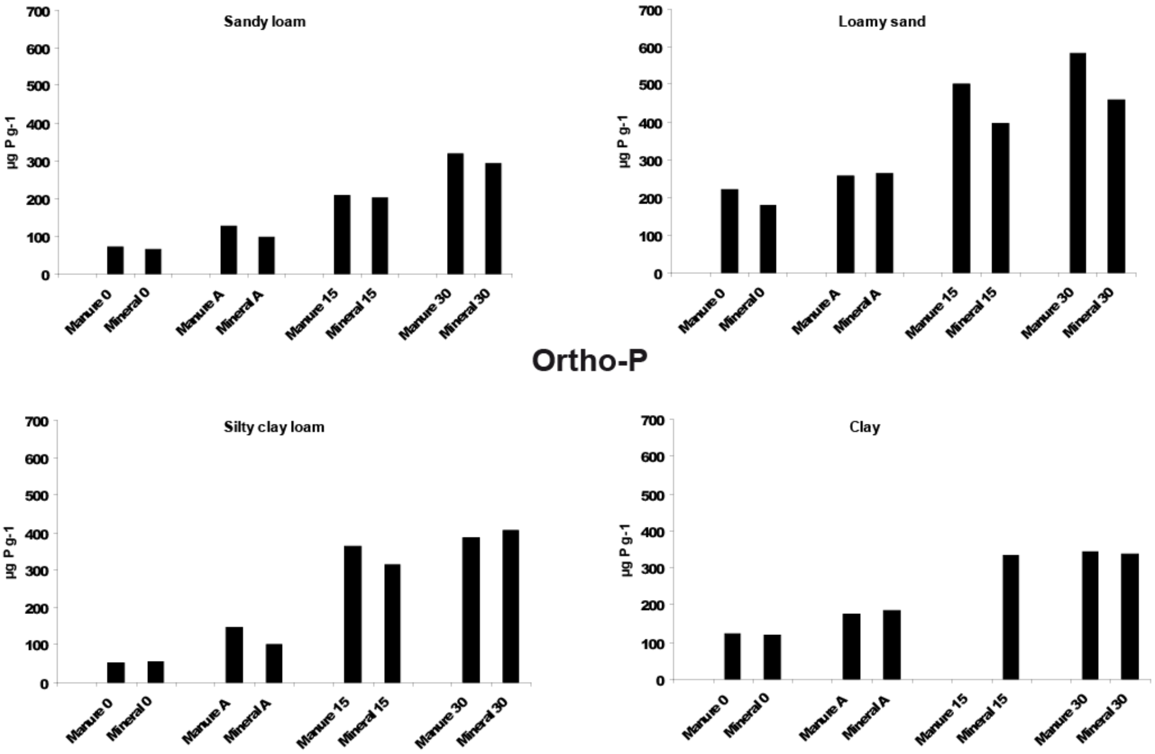
Manure 30
Mineral 30

Figure 2.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Loamy sand
Ortho-P
Silty clay loam
Clay
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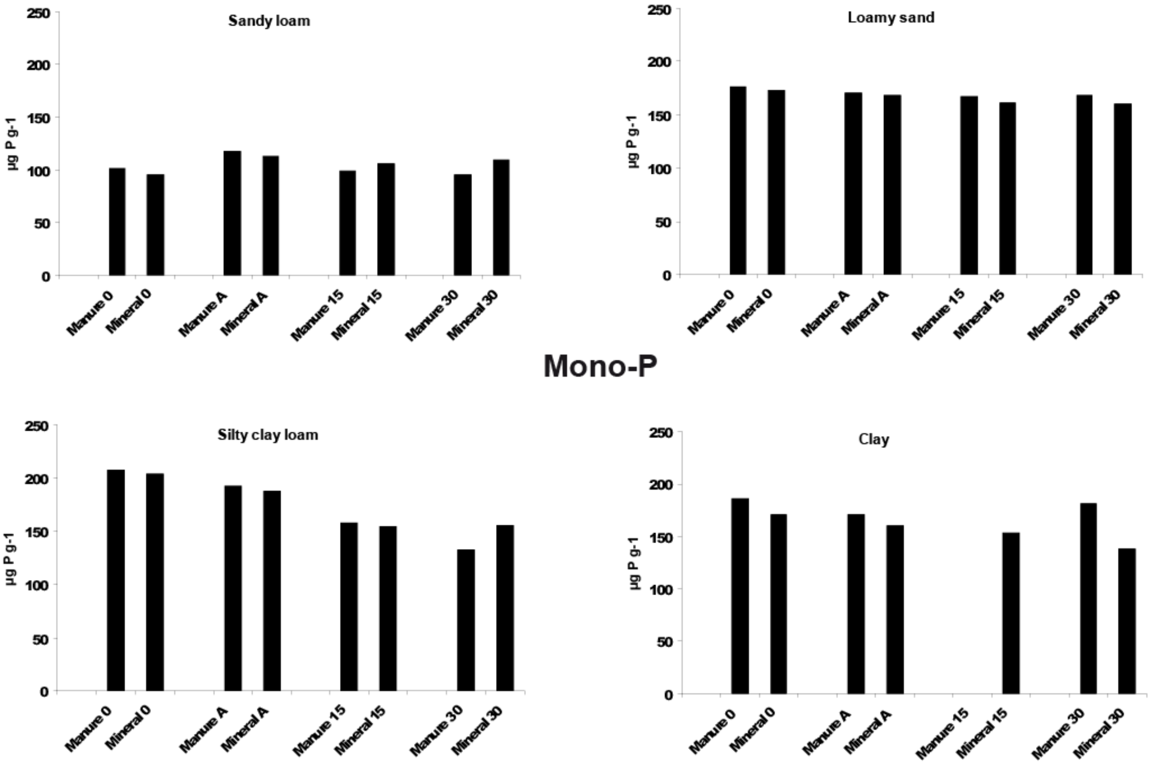
Mineral A
Manure 15
Mineral 15
Manure 30
Mineral 30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Loamy sand
Mono-P
Silty clay loam
Clay
µg P g-1
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50
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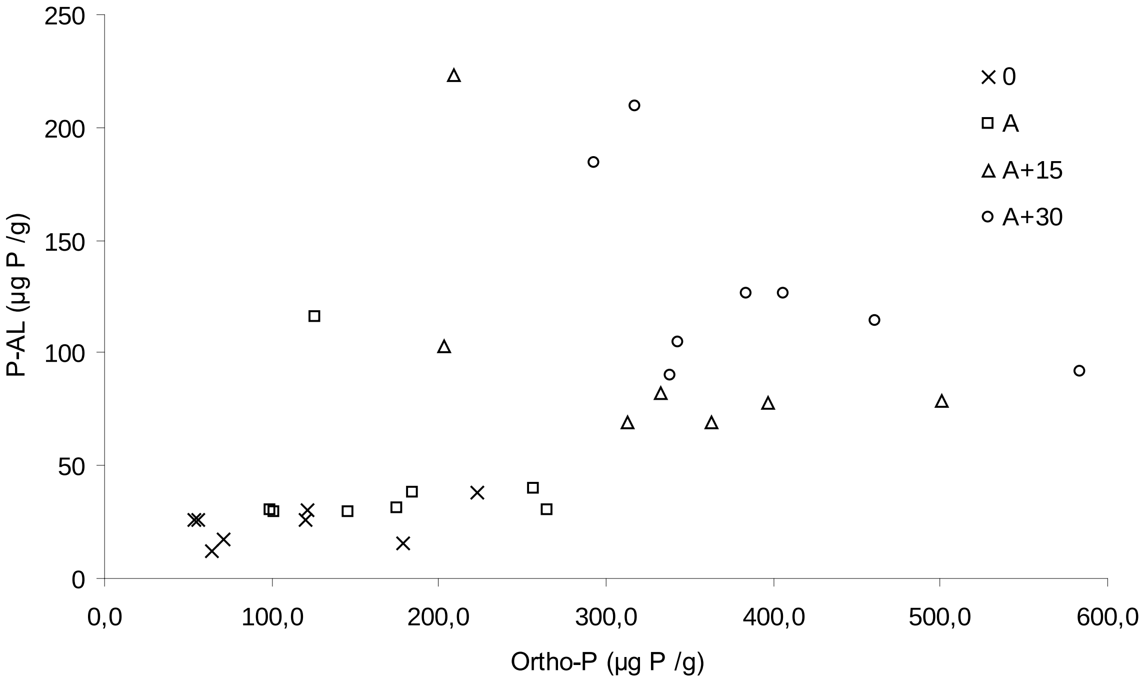
Manure A
Mineral A
Manure 15
Mineral 15
Manure 30
Mineral 30

Figure 3.


250
200
150
100
50
0
P-AL (µg P /g)
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100,0
200,0
300,0
400,0
500,0
600,0
Ortho-P (µg P /g)
× 0
□ A
△ A+15
○ A+30

Figure 4.