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Investigations on the Identification of Long Term Input of Phosphorus from Organic Sources by Standard Soil P Analysis

Untersuchungen zur Identifizierung von langfristigen Phosphoreinträgen aus organischen Materialien mittels standardisierter Boden-P-Analytik

Abstract

175 soil samples from pot and field experiments with different types of organic (farmyard manure, meat and bone meal, bone meal) and mineral fertilization were used to evaluate the potential of a method to estimate relative shares of soluble organic P in water, CAL, Olsen and AR extracts/digest, based on the combination of two analytical methods (colorimetry and ICP-OES). The main aim of the study was to investigate if long-term fertilization with organic P sources is reflected by an increased share of extractable (or dissolved) organic P in the soil. The applied method worked well to estimate the relative share of organic P dissolved in water, CAL, Olsen and AR extracts. The estimated share of dissolved organic P decreased in the order P-CAL ≥ P-Olsen > P-water ≥ P-AR. However, long-term organic fertilization was not reflected in a higher share of soluble organic P than mineral fertilization. On the contrary, in some cases, organically fertilized plots displayed even higher shares of inorganic P (soluble in CAL) than minerally fertilized plots. Obviously, the received organic P was not enriched in the soluble organic substance of the soil, but was either transferred quickly into inorganic P forms by microbial soil processes stimulated by the large addition of organic matter from MBM/manure, or it was turned into stable organic P compounds which were not solubilized by the tested extractants. Neither the role of soil pH, nor that of organic carbon with regard to influencing relatives shares or organic P or, rather, the turnover of soluble organic into insoluble organic or into inorganic P in agricultural soils, could be clarified within this study and thus warrants further research.

Key words: Soluble organic phosphorus, soil P extraction methods, soil test P, manure, organic fertilization, mineral fertilization

Zusammenfassung

Anhand von 175 Bodenproben aus Gefäß- und Feldversuchen mit unterschiedlichen Typen organischer (Wirtschaftsdünger, Fleischknochenmehl) und mineralischer Düngung wurde das Potential einer Methode zur Abschätzung relativer Anteile in Wasser, CAL, Olsenextrakt und Königswasser löslichen organischen Phosphors bewertet. Die Methode basiert auf der Kombination zweier analytischer Verfahren, namentlich Kolorimetrie und ICP-OES. Hauptziel der Studie war es zu untersuchen, ob die langfristige Düngung mit organischen P-Quellen sich in einem im Vergleich zu mineralischer Düngung erhöhten Anteil gelösten organischen Phosphors im Boden wider spiegelt. Die eingesetzte Methode eignete sich gut, relative Anteile an organischem P, das sich in Wasser, CAL, Olsenextrakt oder Königswasser löst, abzuschätzen. Die geschätzten Anteile an gelöstem organischen P nahmen in der Reihenfolge P-CAL ≥ P-Olsen > P-Wasser ≥ P-Königswasser ab. Langfristige organische Düngung spiegelte sich allerdings nicht in im Vergleich zu mineralischer Düngung erhöhten löslichen organischen P-Anteilen wider. Vielmehr zeigten in manchen Fällen (insbesondere CAL-Extrakt)

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die organisch gedüngten Flächen sogar höhere Anteile an anorganischem Phosphor als mineralisch gedüngte Vergleichsflächen. Offensichtlich wurde der eingebrachte organische Phosphor nicht in der löslichen organischen Substanz des Bodens akkumuliert, sondern entweder aufgrund der Stimulierung mikrobieller Prozesse durch die hohe Zufuhr organischer Substanz (aus Wirtschaftsdünger oder Fleischknochenmehl) zügig in anorganische P-Formen umgewandelt, oder er wurde in stabile organische P-Verbindungen eingebaut, die sich in den eingesetzten chemischen Extraktionsmitteln nicht lösten. Weder die Rolle des Boden-pH, noch jene von organischem Kohlenstoff im Hinblick auf die Umsetzung löslicher organischer in anorganische oder unlösliche organische P-Verbindungen konnte im Zuge dieser Studie geklärt werden – dies bedarf weiterer Untersuchungen.

Stichwörter: Löslicher organischer Phosphor, Bodenextraktionsmethoden, extrahierbarer P, Wirtschaftsdünger, organische Düngung, mineralische Düngung

1 Introduction

Phosphorus (P) is a major nutrient which is essential for plants and animals and has been intensively investigated. P in soil exists in two basic forms: inorganic and organic (Tisdale and Nelson, 1975). Inorganic P includes apatitic minerals, secondary precipitates formed with Ca, Fe and Al and free phosphate ions. Organic P represents about 50% of total P in soils (Yadav and Verma, 2012; Condron et al., 2005: between 30 and 65% of total P; Richardson et al., 2005: between 30 and 80% of total P). Orthophosphate monoesters are considered the dominant form of organic P in most soils, among which inositol phosphate is the most abundant one, while phosphoproteins, mononucleotides, and sugar phosphates only occur in traces (Condron et al., 2005; Turner et al., 2002; Mahdi et al., 2011). Other organic P compounds in soils include orthophosphate diesters (nucleic acids, phospholipids, and teichoic acid), aromatic compounds, phosphonates and organic phosphate anhydrides, e.g. ATP and ADP (Condron et al., 2005). Geochemical and biological processes are controlling the P cycle and the availability of P in soils. These processes involve solubilization, precipitation, sorption, desorption, leaching, immobilization and mineralization (Soinne, 2009). Plants assimilate P in its inorganic form, i.e. as orthophosphate (HPO_4^{2-}, $H_2PO_4^-$). However, there is wide agreement among researchers that organic P also significantly contributes to the nutrition of plants, once it has been mineralized by biochemical hydrolysis or by plant root exudates such as low molecular organic acids, extracellular as well as cell membrane-bound phosphatase enzymes, or protons to acidify the rhizosphere (Condron et al., 2005, Kovar and Claassen, 2005). While some authors claim that labile organic soil P, comprising compounds such as RNA, lecithin, glucose-6-phosphate (G-6-P), and glycerophosphate, is easily available to plants (Zhang et al., 1994, with further references), no evidence has been provided until now to indicate that dissolved organic P compounds may be directly taken up by plants, although organic P substrates may be hydrolyzed within the root apoplast (Richardson et al., 2005; George et al., 2011).

Farmyard manure is an important source of nutrients including P. P in manure can exist in organic or inorganic form; nevertheless, it is mostly present in an inorganic form (60–90%), with its actual composition depending on livestock type (Barnett, 1994; Leinweber, 1996). Organic P mainly exists in manure in form of inositol P, as well as some more labile compounds (Turner, 2004). Applying manure for a long time can change the extractability and solubility of soil P forms; a continuous addition will lead to an increase in soil pH as well as the input of relatively high amounts of Ca, resulting in a switch from Al- and Fe-P which are predominating in acidic soil to Ca-P reaction products (Sharpley et al., 2004). Continued application of farmyard manure can increase the amounts of organic P in the soil under certain circumstances (Ottabong et al., 1997; Zhang and MacKenzie, 1997, both as cited by Condron et al., 2005; Reddy et al., 2000). Important factors favoring the immobilization of P in the form of stable organic compounds are a high C/P ratio (> 300, e.g. due to large C returns to soils in crop residues, Zhang and MacKenzie, 1997) or a deficiency in available N (Ottabong et al., 1997). On the other hand, the long-term application of manure contributes to a reduction of P adsorption capacity, as organic matter may block active exchange sites and solubilize soil components active in P fixation (Lopez-Hernandez et al., 1979, as cited by McLaughlin, 1984). It may also stimulate the microbes and enzymes which increase the biological transition of organic P; as a consequence, available and soluble P levels may increase in both surface and subsurface soil horizons (Mozaffari and Sims, 1994).

Meat and bone meal (MBM) and bone meal (BM) are produced from animal parts which are not used for human consumption. Both contain a number of valuable plant nutrients including P. P in MBM exists in the form of $Ca_5(PO_4)_3OH$ in the bone fraction, while in the meat fraction it is present in organic form (Jeng et al., 2006). MBM and BM are P- and Ca-rich by-products of the poultry and livestock industries (Baker et al., 1989) and are considered an important source of P for soils that suffer from P-deficiency (Bekele and Höfner, 1993). However, the availability of P to plants from both, MBM and BM, is mainly controlled by soil pH. Investigations indicated that MBM and BM are more effective P fertilizers and lead to higher yields in acid soils than in soils with pH > 6 (Bekele and Höfner, 1993; Surendra et al., 1993; Jeng et al., 2006). Generally, a high share (about 80–90%) of MBM-P and BM-P is soluble in ammonium citrate (Rex, 2009), and only a small percentage is soluble in water (3 and 2%, respectively, see Ylivainio et al. (2008) and Rex (2009)). In other words, the amount of immediately available P is significantly less than the amount of P which will become available later during the vegetation period. While in digested mineral fertilizers, available P that is soluble in water and weak acid varies widely, it is mostly higher than in MBM/BM, e.g. 93% in super and triple superphosphate. In contrast to this, untreated rock phosphate (apatite) displays a lower solubility in weak acid than MBM/BM, ranging around 1–7% in ammonium citrate (Zapata and Roy, 2004).

Chemical measurements and soil tests have been developed to assess the concentration of plant-available P in soil. Thomas and Peaslee (1973) preferred to use the term "extractable" P instead of available P, because the extracted amount does not measure the available P quantitatively, but gives an indicator of the ability of soil to replenish the soil P required by plants (Black, 1993). As the properties of soils differ widely and due to the variety of P forms in the soil, different soil tests may be best suitable to reflect the amount of available P in a given soil. Extractants found throughout the world comprise a long list, including water, carbon dioxide saturated water, acids, bases, salts, buffered solutions, electro dialysis and ion exchangers (Indiati et al., 1997). However, even with strong extractants it is difficult to extract and quantify different pools of soil organic P, since a large part of it is strongly sorbed to mineral components. Furthermore, organic P compounds are often chemically modified by the extractants (Turner et al., 2005).

Extracted P can, among other methods, either be measured colorimetrically or by using inductively coupled plasma optical emission spectroscopy (ICP-OES). The colorimetric method is based on the reaction of PO_4 and molybdate to form 12-molybdophosphoric acid in an acid solution and sequential reduction to a blue heteropoly compound, where the intensity of the blue corresponds with the concentration of P (Rieman and Beukenkamp, 1961). This method, which was developed by Murphy and Riley (1962), is considered the most widespread analytical method for the detection of inorganic phosphorus, because the coloring agent only reacts with the dissolved reactive phosphorus (the inorganic orthophosphate (PO_4) compound), while the dissolved unreactive phosphorus (often interpreted as organic P) does not react with this agent (Soldat et al., 2009; Regan et al., 2010, as cited by Matula, 2010). On the other hand, inductively coupled plasma optical emission spectroscopy (ICP-OES) is used in most laboratories for soil testing because it is faster, its costs have decreased, and it can measure many chemical elements in the same extractant (e.g., P, K, Ca, Mg, and micronutrients). With this analysis method all P forms, organic and inorganic, can be measured (Mallarino, 2003; Ziadi et al., 2009; Matula, 2010, 2011; Paz-Ferreiro et al., 2012). Many studies demonstrated that ICP measures more P in the same extract than the colorimetric method does. P measured by ICP is sometimes up to 50% higher than P measured colorimetrically (Nathan and Sun, 1998; Elliason et al., 2001; Nathan et al., 2002; Mallarino, 2003; Pittman et al., 2005; Ziadi et al., 2009; Matula, 2010, 2011). Most of these studies agree that this additional amount is related to organic P (Nathan and Sun, 1998; Nathan et al., 2002; Ziadi et al., 2009; Matula, 2010; Paz-Ferreiro et al., 2012).

In a previous study (Shwiekh et al., 2014), we have confirmed that the amount of organic P extractable by soil P tests such as Olsen and water may be estimated by subtracting P measured colorimetrically (equals dissolved reactive or ortho-P) from P measured by ICP-OES (equals total dissolved P) in a lab experiment with pure chemicals. The aim of this study is now to evaluate the potential of this method to estimate organic P dissolved by four standard P extraction methods (CAL, Olsen, water and aqua regia (AR)) in soils fertilized with different organic P sources for a long time, and to investigate if long-term fertilization with organic P sources is reflected by an increased share of extractable (or dissolved) organic P in the soil.

2 Material and methods

2.1 Selection and description of soil samples

A total of 175 surface soil samples with varying chemical and physical characteristics, a soil pH ranging from 4.1 to 7.4, and total C ranging from 0.64 to 3.4% were involved in this study. They came from 3 different origins:

I- 78 Samples from a pot experiment investigating the performance of rendering by-products as P fertilizers, conducted by the Institute of Crop and Soil Science (JKI), Braunschweig, Germany in 2010 and 2011. 39 Pots were filled with 6 kg of soil (classified as Dystic Cambisol/Orthic Luvisol) which was collected in April 2010 and sieved to < 1 cm before filling it into the 10L-Mitscherlich-pots. Four kinds of fertilizers were used: Meat and bone meal (org-MBM), bone meal (BM), rock phosphate (RP) and monocalciumphosphate (MCP). The pots were arranged randomly with three replicates for each treatment. Additionally 3 pots were used as control with no P application (zero P). Two crops were used in this pot experiment: in the first year ryegrass (*Lolium perenne* L) and in the second year maize (*Zea mays* L). The chemical characteristics of the soil are summarized in Tab. 1. The fertilizer application rates are given in Tab. 2.

II- 20 soil samples from a long term field experiment in Braunschweig (Julius Kühn-Institute), Germany (10° 27' E; 52° 18' N), investigating the influence of fertilization with meat and bone meal on various soil microbiological parameters (experimental site "FV 10"). The soil type is a Cambisol with a loamy sand soil texture (< 6.5% clay;

Tab. 1. Chemical parameters of the soil (group I) used for the pot experiment

Soil parameter	Unit	
pH		5.23
N (Total)	%	0.05
P_{CAL}	mg/kg	19.7
K_{CAL}	mg/kg	81.5
Mg (Available)	mg/kg	32.0
C (Total)	%	1.04

Tab. 2. Type, P and N contents of fertilizers applied to the soils of group I

P source	Abbreviation	P content %	N content %
Meat bone meal	Org-MBM	5.4	8.5
Bone meal	BM	10	4.0
Rock phosphate	RP	12	–
Monocalciumphosphate	MCP	23	–

> 47% sand). The samples were collected within 5 years since 2008 (4 samples/year). The field was fertilized either with MBM or with calcium ammonium nitrate and triplesuperphosphate.

III- 77 samples were collected in 2011 from 4 different regions in Germany during a field sampling campaign within the framework of the EU project "Baltic Manure" (14 fields). These regions differ in the dominating soils and dominant parent material. The sampled fields were either fertilized with manure or with soluble mineral fertilizers. Hence, the soils vary significantly in their characteristics such as the total and available content of P, soil texture and pH. The properties of these soil samples are compiled in Tab. 3.

2.2 Methods for P-extraction

The four applied soil P tests are:

I- Calcium acetate lactate method (CAL), according to Schüller (1969), P was extracted with 0.05 M Ca-acetate ($Ca(C_2H_3O_2)_2$) + 0.05 M Ca-lactate ($Ca[CH_3CH(OH)COO]_2$) + 0.03 M acetic acid (CH_3COOH)

II- Sodium bicarbonate extraction method (Olsen) (Olsen et al., 1954), 100 ml of solution (0.5 M $NaHCO_3$) buffered to pH 8.5 with 1 M NaOH/1 L water

III- Water extraction method (van der Paauw et al., 1971)

IV- So-called total P was determined in an aqua regia (AR) digest according to AbfKlärV (1992).: the solution was composed of 3:1 (v/v) HCl: HNO_3. AR is considered as a digestion method, but for the easier way of expression it was included here when the term "extraction methods" is used.

2.3 Methods for P-analysis and estimation of relative share of extractable organic P in soils

Analysis of extracted P was performed colorimetrically by using the ascorbic acid – ammonium molybdate method based on Murphy and Riley (1962). The second analytical method used to measure the extracted P was inductively

Tab. 3. Description of soil samples collected within the EU project "Baltic Manure" (group III)

Field	pH range	Type of fertilizer	Sampling depth (cm)	Soil type	Region
1	6.7–7.0	mineral (DAP)	10	–	Dithmarschen
3	6.8–7.0	cattle slurry	10	–	Dithmarschen
4	5.7–7.0	cattle slurry	10	–	Dithmarschen
7	6.8–7.0	solid cattle manure	10	–	Plön
8	5.7–7.0	solid cattle manure	10	–	Plön
9	5.5–6.4	pig slurry	10	–	Hanover
10	5.9–6.7	mineral (DAP)	10	sandy loam	Hanover
11	5.6–6.1	no fertilization since 1997	10	sandy loam	Hanover
12	5.9–6.5	mineral (PK)	10	loamy sand, sandy loam, clayey loam	Hanover
A	5.4–6.1	mineral	10	loamy sand, sandy loam, clayey loam	Rostock
F	4.9–5.4	solid cattle manure	10	loamy sand, sandy loam, clayey loam	Rostock
H	5.5–5.7	cattle slurry	10	sand, loamy sand, sandy loam	Rostock
I	5.6–7.0	dry chicken manure	30	sand, loamy sand, sandy loam	Rostock

coupled plasma-optical emission spectrometry, using an icap 6000 ICP-OES instrument (Thermo Fisher). As outlined in Shwiekh et al. (2014), estimating extractable organic P in soil samples was done by combining the two forms of P analysis: P measured by ICP-OES (P-ICP) was considered as total dissolved P and P measured by colorimetry (P-Col.) was considered as dissolved inorganic P, hence the organic P was estimated by subtracting P-Col. from P-ICP and expressing estimated extractable organic P in % of total P extractable by the given soil P test.

2.4 Statistical analysis

Analyses of variance (ANOVA) were conducted using the Statistical Package for Social Sciences SPSS® version 17.0 and Microsoft Office EXCEL 2007. The risk of rejecting a true hypothesis was set at 5% ($p < 0.05$). Oneway ANOVA and Tukey post-hoc test were used to identify significant differences between results from the various extraction and analytical methods. Relationships between different extraction and analytical methods as well as the impact of soil characteristics on extracted organic and inorganic P were tested by calculating the Pearson correlation coefficient, and quantified by regression analysis (applying SPSS as well).

3 Results and discussion

3.1 Differences in extraction strength between the four investigated methods

As could be expected, the amount of extractable P, measured either by ICP or colormetrically, decreased in the order: AR > CAL ≥ Olsen > water. However, the difference between CAL and Olsen was insignificant (Tab. 4a and 4b). The used extraction solutions are different in their properties and chemical nature; hence, the reaction with soil components and their ability to extract available P from the soil differ, which is the main reason of the variations between the results of these methods (see Neyroud and Lischer, 2003). The strong acidic reagent of AR (3:1, v/v, HCl to HNO_3) is used for estimation of "so-called total" P in soils, since it extracts less than 100% of the true total P concentration (about 80% according to WEPAL, 2000). In contrast to this, the water reagent extracts only small amounts of P (mainly P in dissolved form) which apparently not even reflect all forms of labile P (Wünscher, 2013). In between these two are the Olsen and CAL extracts, which, in addition to water-soluble forms, comprise some forms of labile P that are not immediately soluble in water but are potentially available to the plant later during the growing season (Elrashidi, 2010).

The absolute amounts of extracted inorganic P (P-Col.) tended to be higher in CAL than in Olsen in all four groups (org-M, MBM, BM and min); however, no significant difference between these two methods was found for the minerally fertilized plots. Also, no difference between CAL and Olsen was found for the zero-P plots (Tab. 5).

Some scientists argued about the low ability of Olsen to extract P from organically fertilized soils in comparison to acidic reagents and proved in their studies that the absolute amount of extractable P increased whenever the acidity of the reagent increased (Sims, 2000; Ige et al., 2006). This is explained by the fact that the low pH of the reagent will dissolve more phosphate associated with Ca (Pizzeghello et al., 2011). However, in this study, CAL and Olsen extracted almost the same amounts of P from mineral fertilizers. Some of the minerally fertilized plots had received RP as a fertilizer, which also contains poorly soluble Ca-P, so it was checked if the different chemistry of RP would be reflected in the P extractability of the respective samples, particularly with regard to the Olsen extract. Since for the minerally fertilized samples from groups II and III the exact fertilizer type was not known, only samples from group I (comprising RP and MCP in the category "mineral") were included in this test (Tab. 6). However, there was still no significant difference in extractable P between CAL and Olsen, respectively, neither for the rock phosphates, nor for the highly soluble MCP, so obviously the Olsen extract was fully capable of dissolving the same amount of Ca-phosphate as the CAL extract. Hence, there must be another reason why Olsen performed worse than CAL in extracting available P from the two organic fertilizers and from BM.

Nevertheless, this differentiation brings up some useful information: As could be expected, samples from "RP-plots" displayed the lowest solubilities in CAL, Olsen and water. This observation agrees with Jeng et al. (2006), who also report RP-fertilized plots to display lower P-solubilities than those fertilized with MBM. This can be explained by the fact that the Ca-P in MBM, as well as in BM

Tab. 4a. Descriptive statistics for extractable P measured colorimetrically (n = 175)

	Minimum	Maximum	Mean	Median
	mg/kg			
CAL	11.2	276	73.5	65.6
Olsen	16.0	165	55.7	52.8
Water	2.3	43.1	14.9	13.9
AR	254	1003	535	521

Tab. 4b. Descriptive statistics for extractable P measured by ICP (n = 175)

	Minimum	Maximum	Mean	Median
	mg/kg			
CAL	16.0	285	85.2	72.4
Olsen	22.9	186	65.3	63.7
Water	2.1	46.1	16.3	15.3
AR	279	1030	579	564

Tab. 5. The amount of extractable P (mean) measured colorimetrically differentiated by fertilization type (including the zero P plots)

	CAL	Olsen	Water	AR
	mg/kg			
Org-M	93.6	48.7	16.6	495
min	65.1	59.6	15.2	561
MBM	71.4	59.7	15.4	572
BM	80.4	59.1	10.3	557
zero	41.0	41.5	10.1	382

and manure, is not completely derived from apatite, but also to a considerable amount from different chemical structures, which are obviously easier to break up than the very sparingly soluble apatite in RP (Bationo et al., 1991; Menon and Chien, 1995). MCP-fertilized plots, on the other hand, display the highest solubility in the Olsen-extract. Interestingly, however, their solubilities in water are on the same low level as those for the org-M-, MBM- and BM-plots.

Evaluation of these results must be done with care, however, since the plots are not homogeneous with regard to their soil properties such as soil pH and content of organic matter/organic carbon, which will have a considerable influence on the transformation of the various fertilizers and their different P-forms in the soils (see below). Thus, these observations can only describe visible trends.

3.2 Differences in quantification between the two analytical methods

As expected, the results of P-determinations by ICP exceeded those determined by colorimetry in all four extraction methods (Tab. 7).

Main reason for this difference is the fact that the colorimetric method basically measures inorganic P, i.e., orthophosphate ions, while ICP determines total dissolved P including organic and inorganic P (Matula, 2010). There is much evidence in the literature confirming this finding (Nathan and Sun, 1998; Elliason et al., 2001; Nathan et al., 2002; Mallarino, 2003; Pittman et al., 2005; Jacoby, 2005; Ziadi et al., 2009; Matula, 2010, 2011). A highly significant correlation was noticed between P-ICP and P-Col. for all extraction methods. Fig. 1 presents the regression plots of the two analytical methods showing high R^2 values for all extractants, and the regression slopes display that ICP values were higher than colorimetric ones with about 3, 1, and 2% for CAL, Olsen, and water. In the case of AR, the regression slope is below 1, however, from the large intercept on the y-axis it can be seen clearly that, at least within the range of values occurring here, ICP-derived values exceed those determined by colorimetry in this case as well.

For the two extraction methods Olsen and AR, the ratio of P-ICP/P-Col. was nonlinearly related to P-Col., i.e. the difference between ICP and colorimetry was proportionally larger where soils contained low amounts of extract-

Tab. 6. Absolute and relative amounts of extractable P (mean) measured colorimetrically, differentiated by fertilization type (“min” differentiated further into MCP and RP)

	AR	CAL		Olsen		Water	
	mg/kg	mg/kg	%	mg/kg	%	mg/kg	%
Org-M (n = 45)	495	93.6	18.9	48.7	9.8	16.6	3.4
MBM (n = 27)	572	71.4	12.5	59.7	10.4	15.4	2.7
BM (n = 19)	557	80.4	14.4	59.1	10.6	10.3	1.8
MCP (n = 18)	530	96.5	18.3	96.9	18.3	18.3	3.5
RP (n = 18)	596	27.9	4.7	33.4	5.6	5.3	0.9

Tab. 7. Summary statistics of P_{CAL}, P_{Olsen}, P_{AR} and P_{water} (mg/kg) measured by ICP and colorimetry (n = 175)

	Minimum		Maximum		Mean		Median	
	ICP	Col.	ICP	Col.	ICP	Col.	ICP	Col.
P_{water}	2.1	2.3	46.1	43.1	16.3	14.9	15.3	13.9
P_{CAL}	16.0	11.2	285	276	85.2	73.5	72.4	65.6
P_{Olsen}	22.9	16.0	186	165	65.3	55.7	63.7	52.8
P_{AR}	279	254	1031	1003	579	535	564	521

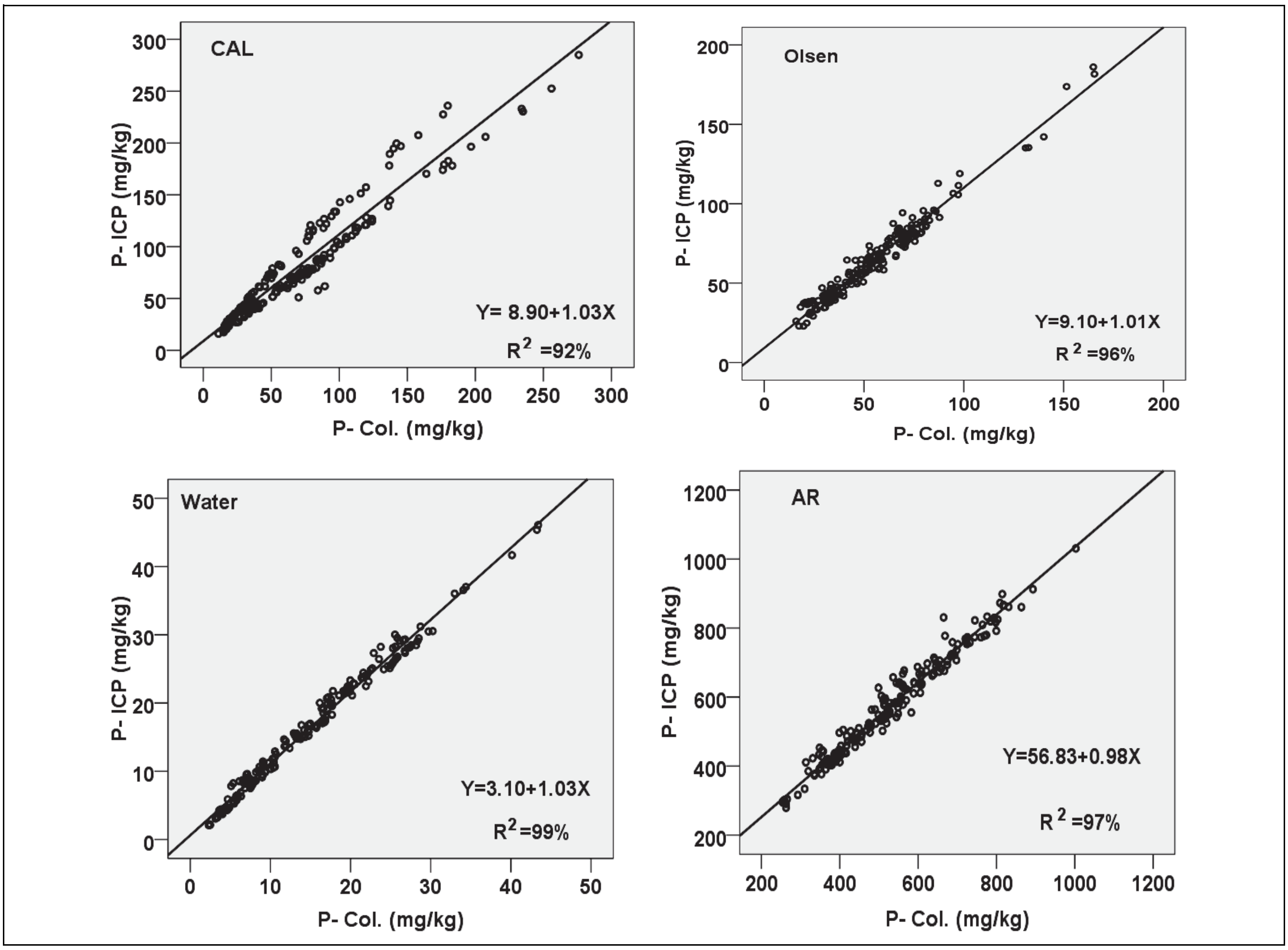

Fig. 1. Relationship between P measured by ICP-OES and P measured colorimetrically in four extraction methods.

able P (Fig. 2). These results matched with the reports by MALLARINO (2003), PITTMAN et al. (2005) and ADESANWO et al. (2013). Both MALLARINO (2003) and PITTMAN et al. (2005) doubted that this relation could be affected by variation in soil pH and organic C. According to ADESANWO et al. (2013), one explanation might be the difference in sensitivity of these two methods, with ICP being the more sensitive one, however, it is surprising then that this phenomenon was not observed in the case of CAL and water extraction.

The plot for CAL (Fig. 2A) shows clearly that there are two subsets of samples, so samples were divided into two groups according to the values of the ratio between P-ICP and P-Col., where the first group ranged from 0.60 to 1.25 and the second one between 1.26–1.70. Yet, as can be seen in Fig. 3, the same results were obtained; i.e. the difference between ICP and colorimetry was not larger where soils contained low amounts of extractable P.

3.3 Comparison of estimated share of dissolved organic P in 4 different soil extracts

Looking at all samples as one big group, the estimated share of dissolved organic P decreased in the order P-CAL ≥ P-Olsen > P-water ≥ P-AR with 18.6, 16.5, 9.8 and 8.4% of the total extractable (= dissolved) P respectively (Fig. 2). The one-way ANOVA with Tukey post-hoc test showed that that the estimates by P-AR and P-water were significantly lower than those by P-CAL and P-Olsen (Tab. 8, Fig. 4).

The low share of organic P estimated for the AR extract may be explained by the fact that the strong acid reagent obviously breaks up most of the organic P compounds into ortho-P, which will then be measured as inorganic P. The relatively low share of estimated organic P in the water extract indicates that water will only extract orthophosphate to a large extent, while it comprises – if at all – only a very small amount of dissolved organic P.

In the next step, the relative share of dissolved organic P in the four soil extractants was compared for different fertilization regimes. Although one may speculate that long-term organic fertilization (org-M, org-MBM) will be displayed by a higher share of organic P in the soil extracts than mineral fertilization, this was clearly not the case for the investigated samples (see Fig. 5). On the contrary, when extracted by CAL, organically fertilized plots displayed even higher shares of inorganic P than minerally fertilized plots (Fig. 5A). This, however, agrees well with the results of various researchers who reported that

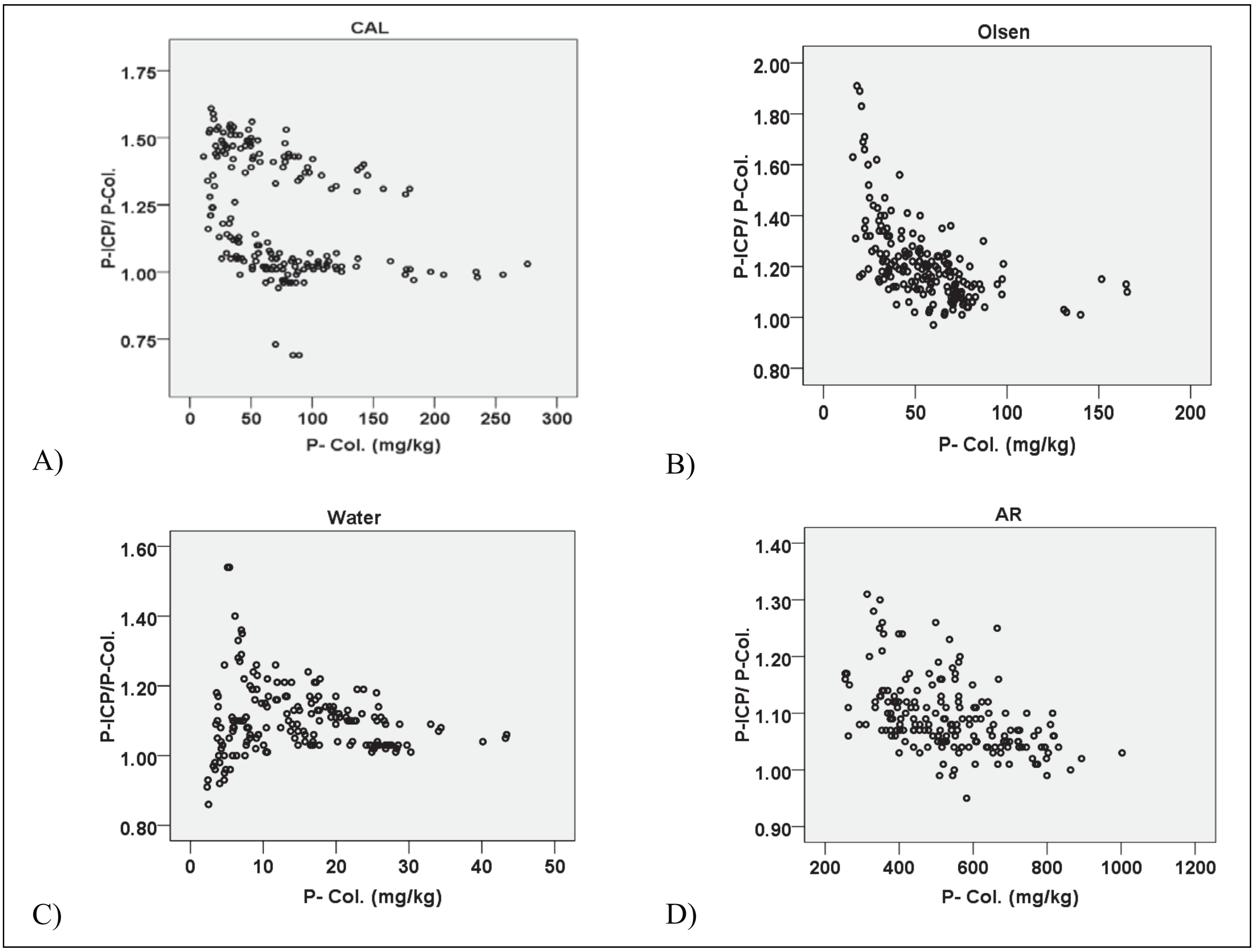

Fig. 2. Relationship between P-Col. and the ratio between P-ICP and P-Col. for (A) CAL, (B) Olsen, (C) water, and (D) AR methods.

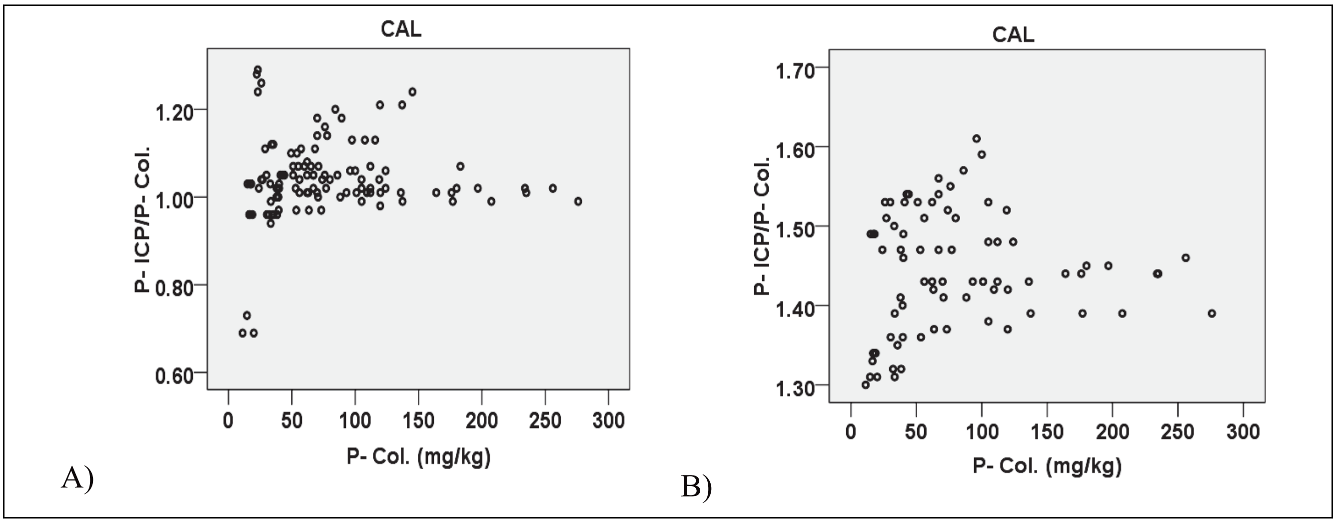

Fig. 3. Relationship between P-Col. and the ratio of P-ICP and P-Col. in the CAL extract for two subsets of samples: A) ratio between 0.60–1.25 and B) ratio between 1.26–1.70.

more inorganic than organic P was found in soil fertilized with manure for a long time in comparison to soils which had received mineral fertilizer: For example, Sharpley et al. (1984) found that there was an increase in the proportion of total P as inorganic P from 34 to 71% with larger manure applications. One reason for this can be seen in the fact

Tab. 8. Descriptive statistics for relative share of organic P (%) estimated by four extraction methods (n = 175). Different letters indicate significant differences at the $p < 0.05$ level

	Minimum	Maximum	Mean	Median
	mg/kg			
CAL	0.5	37.8	18.5a	23.3
Olsen	1.1	47.8	16.5a	15.1
Water	0.1	35.0	9.8b	9.0
AR	0.5	23.7	8.4b	7.2

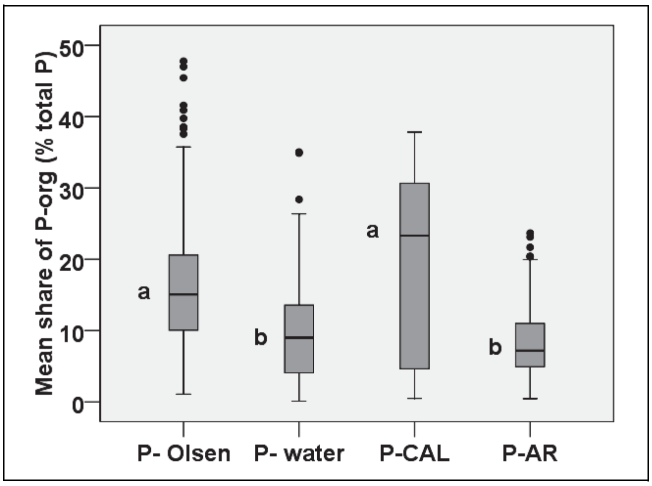

Fig. 4. Comparisons between the relative shares of organic P estimated by four different extraction methods (n = 175). Different letters indicate significant differences between cases at the level $p < 0.05$.

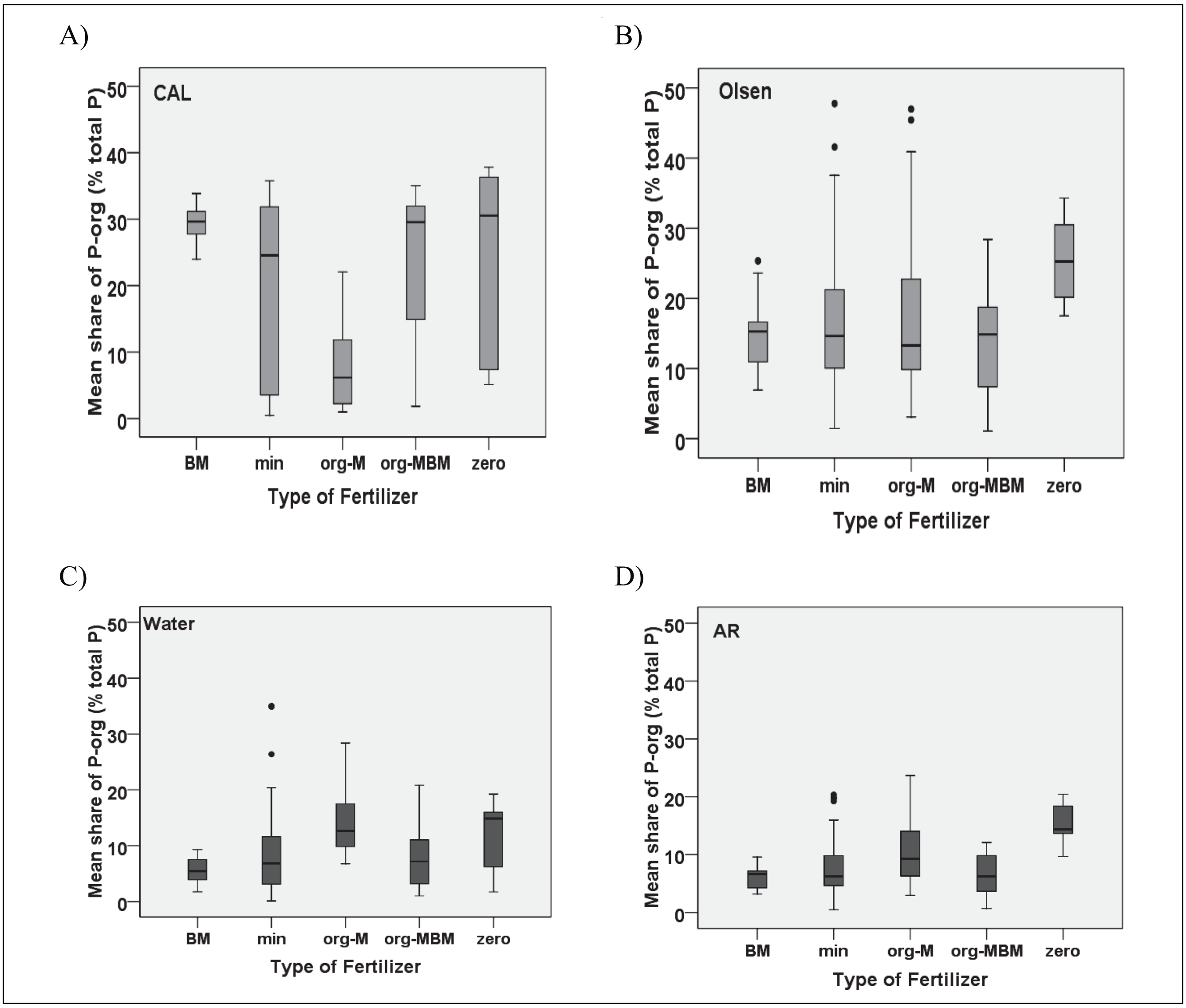

Fig. 5. Comparison between the estimated relative share of organic P, differentiated by fertilization regime, in A) CAL, B) Olsen, C) water and D) AR extract.

that in the first place, between 60–90% of P in manure is inorganic (Barnett, 1994; Leinweber, 1996; Sharpley and Moyer, 2000). In addition to this, the long-term application of manure promotes the activity of microbes and enzymes, thus inducing mineralization of organic P, which further increases the share of inorganic (and soluble) P (Mozaffari and Sims, 1994; Motavalli and Miles, 2002). Depending on the soil pH, chemical hydrolysis of organic P may also play a role in further reducing the organic traces of fertilization (see for example Baldwin, 1998 and Matula, 2010). Similar findings were reported by Adesanwo et al. (2013), who compared STP-analysis by colorimetry and ICP-OES in 30 manured and 30 non-manured soil samples, applying water, $CaCl_2$- and Mehlich 3 extraction methods. They also speculated that chemical transformations of organic P in manured soils might have leveled out differences in organic P contents between manured and non-manured soils, thus accounting for the lack of any effect of manure history on differences between ICP-P and colorimetric P.

For the other three extraction methods, Olsen, water and AR, the estimated organic P decreased in the following order: org-M > min > org-MBM, however, with no significant differences between org-M and min for the Olsen extractant (Tab. 9). The low amount of estimated organic P in org-MBM plots could be the result of a transformation of MBM-P into a more soluble and thus plant-available form after 2 years of experiment, which is comparable to what Ylivainio et al. (2008) reported about their experiment, which was carried out in non-calcareous soils, i.e. under conditions similar to this study.

Interestingly, a high estimated share of organic P (mean values) was found in the zero P plots, in the case of the Olsen extract these plots even showed the highest share of organic P compared with the other fertilization regimes (25.4% of total extracted P) (Tab. 9). One possible reason could be that mineralization of organic P in P deficient soils may be rather slow since nutrient deficiency will generally lead to a slow growth of roots which in turn decreases the availability of oxygen, one of the important factors that affect the mineralization: according to Harrison (1982), only 1% of organic P is mineralized per year in P deficient soils.

The one-way ANOVA with Tukey post-hoc test used to compare the shares of organic P estimated by the four extraction methods (CAL, Olsen, water and AR) showed no significant differences between water and AR for most fertilizer regimes, except for org-M and zero-P. Between CAL and Olsen, significant differences in the estimated share of organic P were found for minerally fertilized plots and for zero-P (Tab. 9, Fig. 5A–D).

3.4 Effect of soil test P (= STP) class on relative share of dissolved organic P

In the last step it was tested whether the STP category (i.e. the level of P supply) had an influence on the estimated relative share of organic P in the extracts. Soil test P in Germany is classified into 5 categories A-E (Tab. 10).

In Tab. 11 the number of soil samples falling into each of the STP classes, differentiated by fertilization regime is shown.

If the initial speculation about long-term organic fertilization leaving traces in the form of higher shares of extractable organic P had fallen true, this should have shown most clearly in the plots that had received large amounts of organic fertilizers (some of them over a long period of time), i.e. classes D and E of org-MBM or org-M. However, the results displayed the opposite in all four extracts: The average estimated share of organic P in samples fertilized with org-M (for example) in class E was 2.4, 7.9, 9.6 and 8.4% of total extracted P by CAL, Olsen water and AR extracts, respectively. In class A, these averages were 18.1, 39.2, 24.2 and 16.7% (Fig. 6). Comparable relations were found for org-MBM (Fig. 6). Obviously, the received organic P in class E (and D) was not enriched in the soluble organic substance of the soil, but was either transferred quickly into inorganic P forms by microbial soil processes stimulated by the large addition of organic matter from MBM/manure (see Mondini et al., 2008), or it was turned into stable organic P compounds which were not solubilized by the tested extractants. Several researchers recorded a reduction in the amount of organic P in soil even after long application of manure, as mentioned before. Yet, no similar observation has been reported for MBM, since long-term experiments dealing with MBM have been rare, as Bøen and Haraldsen

Tab. 9. Relative share of organic P (%) (mean) estimated by four extractants, differentiated by fertilization types. Different letters indicate significant differences in each fertilizer type at the $p < 0.05$ level

Type of fertilizer	BM	Min	Org-M	Org-MBM	Zero
n	19	74	45	27	10
CAL	29.3a	19.4a	7.6d	23.5a	23.4a
Olsen	14.7b	16.0a	18.0a	13.3b	25.4a
Water	5.7c	8.4b	14.0b	8.2c	11.5c
AR	6.2c	7.4b	10.4c	6.5c	15.4b

Tab. 10. VDLUFA reference values for the soil test P classes A to E, applicable for DL and CAL extracts (Kerschberger et al., 1997)

P-class	mg P/100 g soil	mg P_2O_5/100 g soil
A	≤ 2.0	≤ 5.0
B	2.1–4.4	6–9
C	4.5–9.0	10–20
D	9.1–15.0	21–34
E	≥ 15.1	≥ 35

Tab. 11. Number of soil samples per soil test P class, differentiated by fertilization regime

Fertilization regime	Soil test P class A	B	C	D	E	sum
Zero	6	0	3	1	0	10
Min	3	26	31	10	4	74
BM	0	2	12	5	0	19
Org-MBM	0	6	14	7	0	27
Org-M	6	9	12	8	10	45
Sum	15	43	72	31	14	175

(2013) stated, probably because the restrictions on using MBM as a fertilizer in EU countries were only lifted since 2006.

However, the larger difference between P-ICP and P-Col. in class A may at least in parts be a result of reasons not related to organic P but rather to other factors such as the differences in the sensitivity of these two methods, which are most clearly observed when soil test P is low (see above, chapter 3.2).

In comparison to those plots fertilized with mineral fertilizers (min), the manured plots (org-M) generally did not reveal a higher estimated share of organic P in the four extractants in class A (lowest P supply), confirming that the soluble organic P coming in with the manure transferred quickly into inorganic P by hydrolysis and other processes.

For class E (highest P supply), the estimated share of organic P was significantly higher in the minerally fertilized plots than in the ones fertilized with org-M only in CAL and Olsen, while in water and AR there was a significantly higher share of organic P in the plots fertilized with org-M (Fig. 6). Maybe this could be explained by the

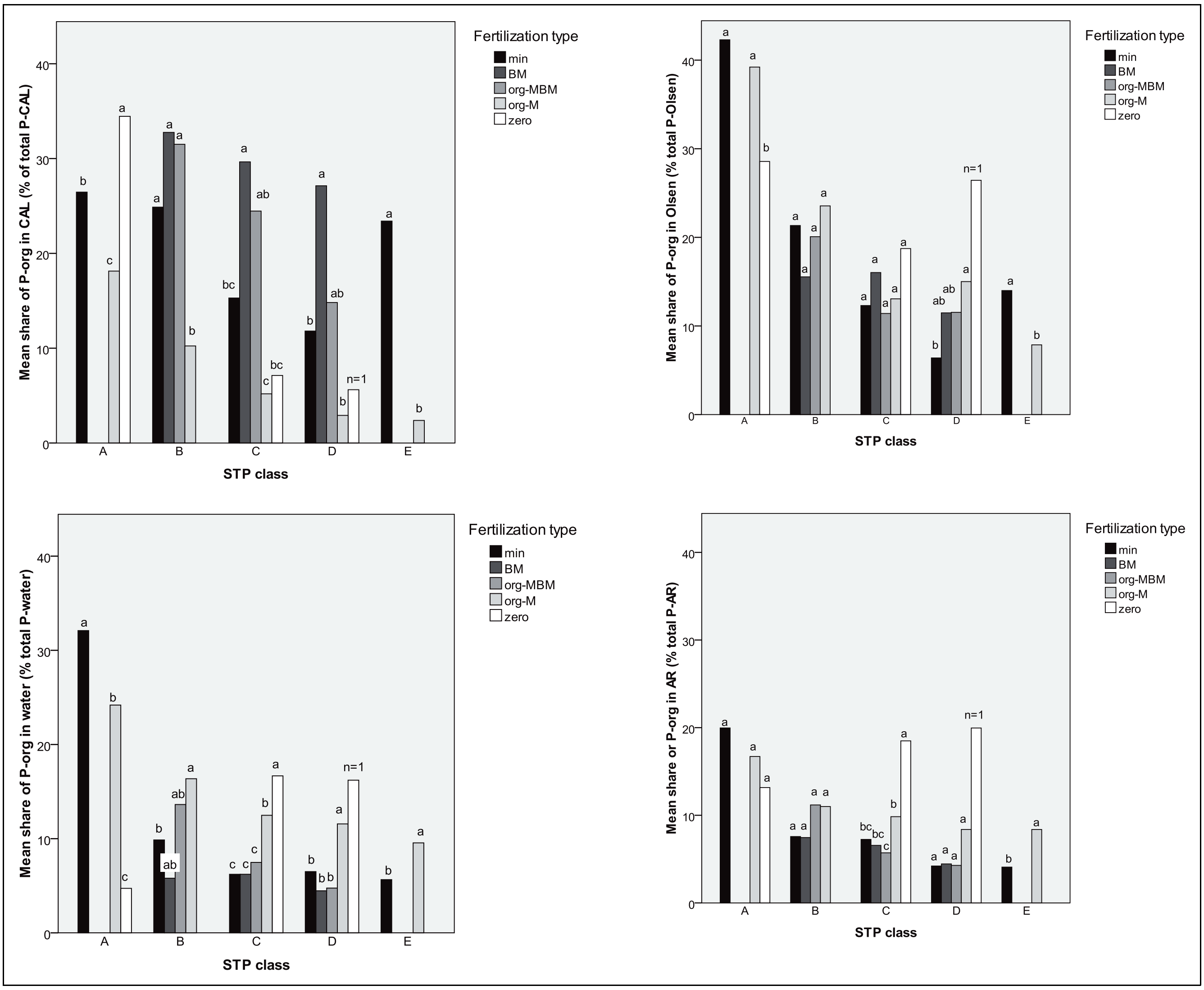

Fig. 6. Comparison between the relative share of organic P estimated for different fertilization types for CAL, Olsen, water and AR extracts, differentiated by STP class. Different letters indicate significant differences between cases (within one STP class) at the level $p < 0.05$. In class D, zero plots were not included into the ANOVA because n = 1; in class E, a t-test between min and org-M was performed.

fact that at high organic P loads, organic P is no longer hydrolyzed completely, but some of it remains as dissolved organic P (water-soluble) or stable organic P (which can only be digested by AR). The reason why not everything is hydrolyzed in this case may be the increase in soil pH coming along with high amounts of organic fertilizer (manure).

3.5 Influence of soil pH on relative share of dissolved organic P

Soil pH is one of the most important factors which may influence the amount of extractable P. As mentioned above, it may have an influence on the strength of hydrolysis, or rather, on the time it takes to hydrolyze organic P into inorganic P. The soil pH values for the 175 samples of this study ranged from 4.1 to 7.4. Looking at all fertilization regimes in total, a strong and significant negative correlation was observed between soil pH and relative share of organic P estimated by the CAL extract (r = −0.83**), and a significant moderate correlation for the water extraction (r = 0.36**). No such correlation was found for the Olsen extract or the AR digest (Fig. 7).

The correlation between soil-pH and estimated share of organic P was also checked for each fertilizer regime separately (Tab. 12).

For the CAL extract, negative and linear correlations existed between soil pH and the relative share of organic P in all five fertilizer regimes, however, the strength of this relationship was different, with the strongest correlation found for zero P fertilization (r = −0.93**) followed by org-MBM, BM and min with moderate correlations, while

Tab. 12. Pearson correlation coefficients between the relative share of organic P, estimated by four extraction methods, and soil pH, differentiated by type of fertilizer (n = 175)

	BM	Min	Org-M	Org-MBM	zero
CAL	−0.823**	−0.793**	−0.566**	−0.881**	−0.933**
Olsen	−0.207	0.050	−0.574**	−0.637**	−0.403
Water	−0.331	0.500**	−0.634**	−0.673**	0.859*
AR	−0.440	0.075	−0.362	−0.756**	0.825**

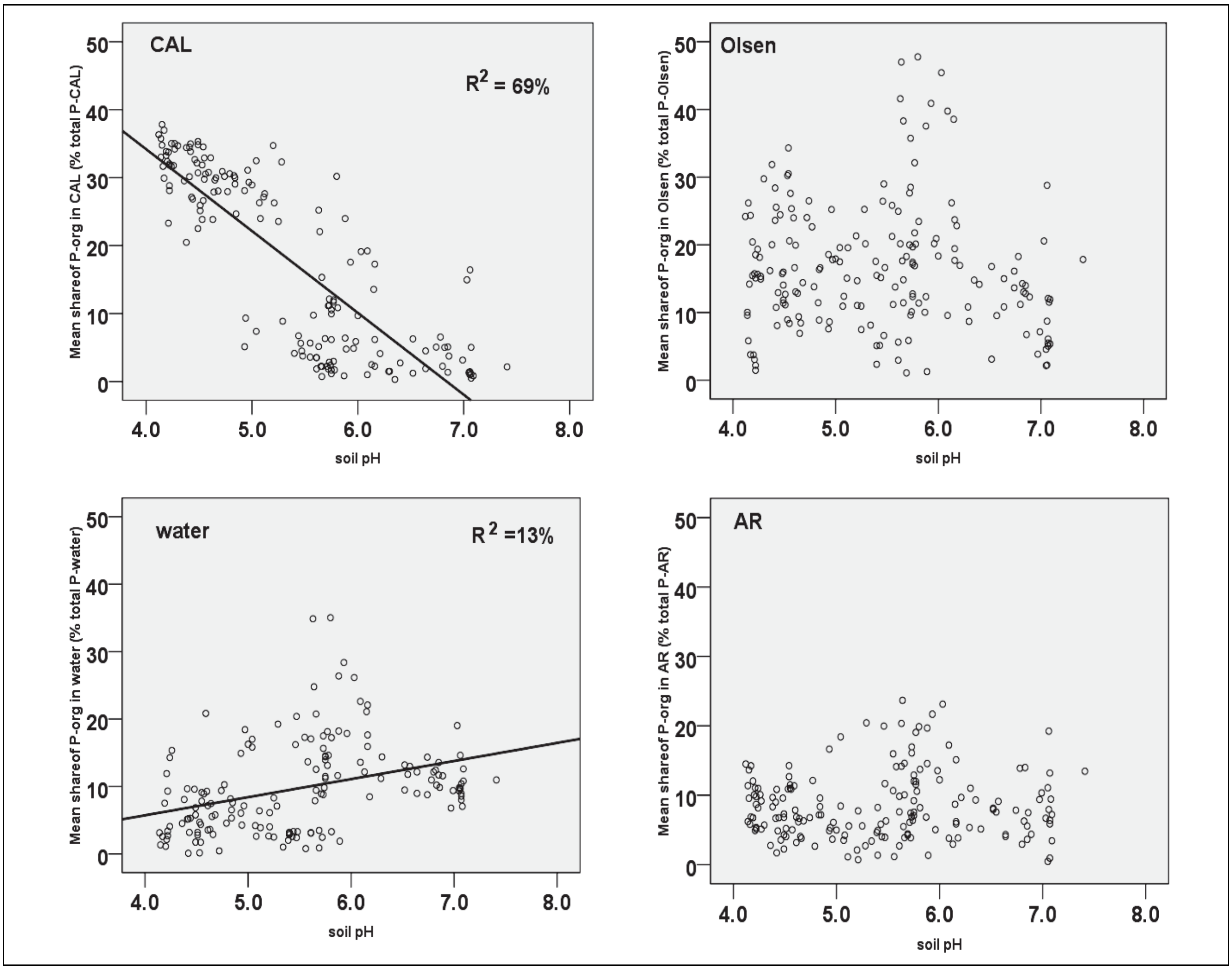

Fig. 7. Scatterplot and coefficient of determination for the relationship between soil pH and relative share of organic P estimated by four extraction methods.

the weakest correlation was found for org-M (r = −0.57). For the Olsen extract there were negative and moderate correlations only for organically fertilized soils, i.e. the variants org-MBM and org-M (r = −0.64**, −0.57** respectively). A similar relationship was found in the investigations by Turner et al. (2003) and Turner and Haygarth (2003): The latter study, which was done for a range of UK pasture soils, confirmed that the concentration of organic P estimated by bicarbonate extractant was controlled by soil pH and this concentration increased when soil pH < 5. Similar correlations were reported by Mallarino (2003), Pittman et al. (2005) and Adesanwo et al. (2013). A negative relationship between estimated share of organic P (which is based on the difference in ICP-P and P-col.) on the one hand and soil pH on the other hand indicates that the estimate increases as soil pH decreases. As pointed out by Adesanwo et al. (2013) (see also: Eckert and Watson (1996), Elliason et al. (2001)), the exact role that pH plays in this respect is so far not clear. The authors speculate that some indirect influence of other parameters (such as the presence of suspended soil particles in the extract) which were not measured here might have an influence.

3.6 Effect of total C % in soil on the relative share of organic P

Usually, the amount of organic P in the soil increases with the C_{org}-concentration, the C/P-ratio can vary between 100 and 1000 (Blume et al., 2010). Reddy et al. (2000) report a significant increase of C_{org} with the organic P content in a soil due to the application of manure or manure and mineral P, which indicates that there might be a relation between the amount of organic P and organic carbon in the soil, thus a relation between the amount of organic P and total carbon in the soil, as the organic carbon is derived from subtracting inorganic carbon from total carbon (Verardo et al., 1990).

However, this was not confirmed by the results of this study. While C_{org} was not determined here, correlation tests for C_{total} and estimated share of organic P should give a first indication. When looking at all samples as one group, no visible relationship between the amount of C_{total} and on the amount of estimated relative share of organic P was observed. A very weak but still significant trend towards a positive effect of total C on the estimated relative share of organic P was observed in Olsen, water and AR extracts (r = 0.22***, 0.21** and 0.23** respectively). In contrast, there was a very weak negative effect for CAL, which was, however, not significant (r = −0.08) (Fig. 8).

The correlation between total C and the estimated share of organic P was also examined for each fertilizer regime separately in order to check whether a relationship would be visible for the organically fertilized plots at least (Tab. 13).

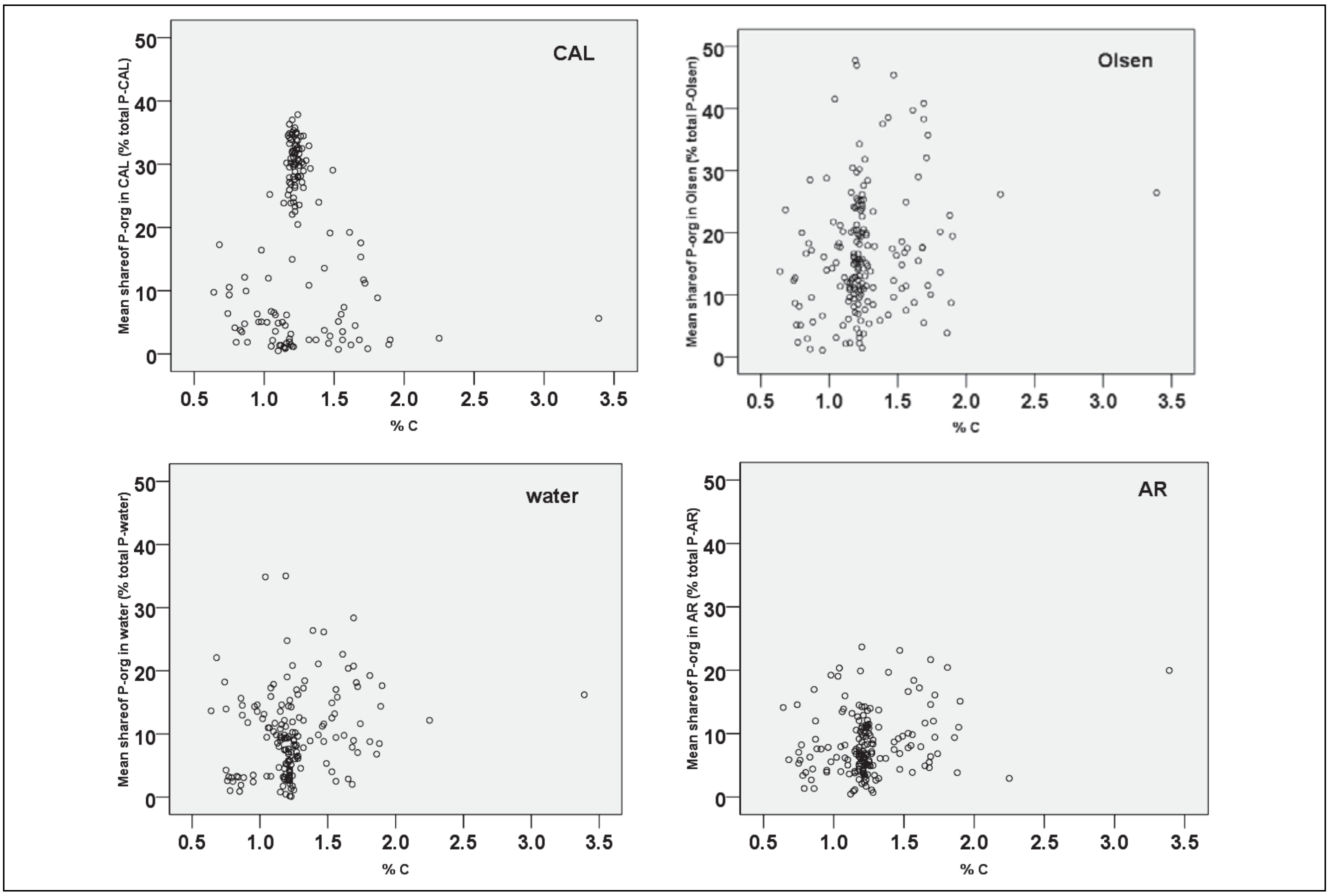

Fig. 8. Scatterplots for the relationship between C% and relative share of organic P estimated by four extraction methods, looking at all samples as one group (n = 175).

Tab. 13. Pearson correlation coefficients between the relative share of organic P estimated by four extraction methods and total C % in the soil, differentiated by type of fertilizer (n = 175)

	BM	Min	Org-M	Org-MBM	zero
CAL	−0.036	−0.133	−0.036	0.958**	−0.658*
Olsen	0.168	0.069	0.193	0.597**	−0.163
Water	−0.108	0.036	0.019	0.708**	0.568
AR	0.369	−0.029	0.030	0.435*	0.680*

Only the org-MBM plots showed significant positive correlations of moderate to high strength between the total C and estimated organic P for all extraction methods, while no significant correlations were found for the manured plots (org-M). Significant and moderately strong correlations were also found for the zero plots, with regard to the estimates from CAL and AR extracts, however, in the case of CAL, the relationship was negative.

Summary and Conclusions

The combination of two analytical methods, colorimetry and ICP-OES, worked well to estimate the relative share of organic P dissolved in water, CAL, Olsen and AR extracts when applied to a set of samples from pot and field experiments comparing various forms of organic and mineral fertilization regimes. Looking at all samples as one big group, the estimated share of dissolved organic P decreased in the order P-CAL ≥ P-Olsen > P-water ≥ P-AR. The low share of organic P estimated for the AR extract may be explained by the fact that the strong acid reagent obviously breaks up most of the organic P compounds into ortho-P, which will then be measured as inorganic P. The relatively low share of estimated organic P in the water extract indicates that water will only extract orthophosphate to a large extent, while it comprises – if at all – only a very small amount of dissolved organic P.

However, as the presented results demonstrated, long-term organic fertilization is not necessarily reflected in a higher share of soluble organic P than mineral fertilization. On the contrary, when extracted by CAL, organically fertilized plots displayed even higher shares of inorganic P than minerally fertilized plots. This agrees well with reports of other researchers in the literature. One reason for this can be seen in the fact that between 60–90% of P in manure is inorganic in the first place. In addition, long-term application of manure promotes the activity of microbes and enzymes, thus inducing mineralization of organic P, which further increases the share of inorganic (and soluble) P. Depending on the soil pH, chemical hydrolysis of organic P may also play a role in further reducing the organic traces of fertilization. Furthermore, chemical transformations of organic P in manured soils might level out differences in organic P between manured and non-manured soils, thus accounting for the lack of any effect of manure history on differences between ICP-P and colorimetric P.

A comparably high estimated share of organic P was found in the zero P plots. This was probably due to a rather slow mineralization of organic P in P deficient soils associated with nutrient deficiency, which generally leads to a slow growth of roots, thus decreasing the availability of oxygen, which is an important factor affecting mineralization rates.

If the initial speculation about long-term organic fertilization leaving traces in the form of higher shares of extractable organic P had fallen true, this should have shown most clearly in the plots that had received large amounts of organic fertilizers, thus ranging in classes D and E. However, the results displayed the opposite in all four extracts. Obviously, the received organic P in class E (and D) was not enriched in the soluble organic substance of the soil, but was either transferred quickly into inorganic P forms by microbial soil processes stimulated by the large addition of organic matter from MBM/manure, or it was turned into stable organic P compounds which were not solubilized by the tested extractants. For class E, the estimated share of organic P was significantly higher in the minerally fertilized plots than in the ones fertilized with org-M only in CAL and Olsen, while in water and AR there was a significantly higher share of organic P in the plots fertilized with org-M. Maybe this could be explained by the fact that at high organic P loads, organic P is no longer hydrolyzed completely, but some of it remains as dissolved organic P (water-soluble) or stable organic P (which can only be digested by AR). The reason why not everything is hydrolyzed in this case may be the increase in soil pH coming along with high amounts of organic fertilizer (manure).

Inconsistent results were recorded when the influence of soil pH on the estimated share of organic P was tested. As already pointed out by other researchers, the exact role that pH plays in this respect is still unclear and needs further investigation. Some authors speculate that some indirect influence of other parameters (such as the presence of suspended soil particles in the extract) which were not measured here might have an influence. Similarly, the role of organic carbon with regard to relative shares of soluble organic P could not be clarified in our study and warrants further research.

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