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Identification of specific phosphorus compound groups by NMR (nuclear magnetic resonance) spectroscopy

by

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1 ABSTRACT

Only a small proportion of soil phosphorus (P) can be utilised readily by plants, and knowledge of the quantity of readily-available P in the soil is necessary for crop management. Current analytical methods for quantifying this P use different chemical reactions to extract P from the soil. The amount of P in the soil, that is of interest, is then defined by the extractant used (e.g. Olsen's P). Whilst good relationships have been established between Olsen P levels and crop yields, the composition of Olsen P is not known. Knowledge of the actual P compounds (e.g. orthophosphate) or compound groups (e.g. monoesters) could enhance our understanding of P dynamics in the soil increasing the possibilities for manipulation to the advantage of crop nutrition.

The aim of this study was to investigate the use of Nuclear Magnetic Resonance (NMR) spectroscopy to identify different P compounds in soil. The objectives were to investigate the number of different P compounds and/or compound groups in three soil types, and their relative proportion as influenced by extraction method (EDTA, CaCl₂, Olsen), soil type, initial Olsen P level and fertiliser addition.

EDTA extracted more P in total than the other extractants and six compounds/groups were extracted. Orthophosphate (immediately-available) and monoesters (labile) accounted for the majority of the P extracted, but in one soil type (Cholsey) the proportion of diesters (labile) was significantly greater; this soil had the lowest Olsen P. After fertilisation there was an increase in orthophosphate and monoesters, but there was some variability and there was an increase in orthophosphate in an unfertilised Cholsey soil sample, possibly due to mineralisation. The extractant (CaCl₂) is normally associated with representing highly available P forms. NMR identified that this included organic P..

Existing literature was also reviewed relating to P compounds in manures. Orthophosphate was the primary P compound in the manures but there was some indication that poultry manure may have less immediately-available P than dairy manure and the results for pig manure were inconclusive.

This study demonstrated that NMR could be a useful tool to identify specific P compounds or compound groups in manure and soil and so enhance our understanding of what happens to P contained in inorganic and organic fertilisers after application. Identification of different P compounds/groups could assist with explaining why different soil/crop interactions occur in relation to P demand. The study can be used to provide focus for more specific research in relation to crop utilisation of different P compounds/groups, especially organic forms, how these are influenced by soil type, and crop response to fertilisers. The study is potentially a precursor to the development of in-field testing kits.

2 SUMMARY

2.1 Introduction

Only a small proportion of soil phosphorus (P) can be utilised readily by plants, and knowledge of the quantity of readily-available P in the soil is necessary for crop management. Current analytical methods for quantifying this P use different chemical reactions to extract P from the soil. The amount of soil-P that is of interest is then defined by the extractant used (e.g. Olsen P). Whilst good relationships have been established between Olsen P levels and crop yields, the composition of the Olsen P is not known. In particular, it is now known whether readily-available P wholly comprises inorganic P (primarily, orthophosphate - the common form in which plants take up P), or whether Olsen P also contains some organic P, i.e. larger compounds existing from the breakdown of organic matter/plant material and soil microbes.

Knowledge of the actual P compounds in different chemical extracts of soil could enhance our understanding of P dynamics in the soil increasing the possibilities for manipulation to the advantage of crop nutrition. If it were possible to identify actual P compounds and/or compound groups in the soil, then it should be possible to assess whether crops access any particular P compound(s) more than another, and whether this differs between crop types and/or soil types. Nuclear magnetic resonance (NMR) spectroscopy allows the identification of specific P compounds and therefore has the potential to identify and measure the P compounds that make up extracted P (e.g. Olsen P) and residual P, i.e. P remaining in the soil after fertilisation that is not utilised by crops in the same growing season as that of application.

The aim of this study was to investigate the use of Nuclear Magnetic Resonance (NMR) spectroscopy to identify different P compounds or compound groups in soil. Technically, for compounds with very similar structures, the NMR signal is a superimposition of signals from nuclei from a range of similar compounds (usually isomers) within a single compound group. In these instances it is not possible to identify the exact chemical structure of the individual compound, but it is possible to identify the compound group; compound groups have similar biochemical actions hence their activities are more commonly considered by their group name and it is this information that is normally of interest, and is of interest here.

This study was undertaken in parallel to the HGCA-funded 'Critical P' project (RD-2008-3554). Soils from the field sites used in the Critical P project and collected by The Arable Group (TAG) were analysed in the project described here. The objectives of the study were to investigate, in soils: a) the number of different P compounds/groups, and b) the relative proportion of the different P compounds/groups, as influenced by extractant, soil type, initial Olsen P level and fertiliser addition.

2.2 Materials and methods

2.2.1 Soil type

All soils were provided by TAG/Rothamsted Research as air-dried samples ground to <2 mm. Three soils with contrasting properties and Olsen P values were selected for analysis by NMR (Summary Table 1).

Summary Table 1 Soil properties & agronomic history

	Great (Gt) Carlton	Caythorpe	Cholsey
Description	Heavy Clay	Sandy Loam	Shallow soil over chalk
Soil texture	Clay Loam	Sandy Loam	Silty clay loam
Sampling depth	22 cm	22 cm	20 cm
Crop 2009	W Barley	Wheat	Wheat
Sampling date	13/05/2009	06/05/2009	10/07/2009
Date fertilised[§]	25/08/09	26/08/09	28/08/09
Date fertilised[§]	02/09/09	07/09/09	07/10/09
Crop 2010	W OSR	Wheat	Wheat
Sampling date	23/03/2010	26/03/2010	21/05/2010
Olsen P (mg/L)*	6 -10	<10	7
Average P	12.3 (10.2 - 13.8)	11.2 (9 - 15.6)	8.7 (8.7 - 8.8)
Average K	95 (87 - 102)	152 (79 - 222)	264 (252 - 281)
(Range) Mg	94 (93 - 95)	110 (95 - 144)	66 (55 - 75)
pH	6.9 (6.6 - 7.1)	6.5 (6.1 - 6.7)	7.6 (7.6 - 7.7)
P added (kg/ha)	261.2	195.4	230.5

[§]Triple superphosphate *As given by farmer

For each soil type, four field replicates were analysed; two did not receive fertiliser, whilst two did receive fertiliser. This means that only six samples in total received fertiliser - two from each of the 3 soil types. In addition, soils were sampled at a time point before any fertiliser addition (T1) and approximately 10 months later when some of the soils had received fertiliser (T2). Replicates used in the current study and their properties are given in Summary Table 2. The same identifiers have been used here as given by the Critical P study (RD-2008-3554) in order to enable cross-reference where applicable.

Summary Table 2 Olsen P content of individual soil replicates & quantity of fertiliser P added

	Identifier	Initial Olsen P (mg/kg)	P added (kg/ha)
Great Carlton	R1:D1	12.4	0
	R1:D4	14.8	261.2
	R2:D2	11.8	0
	R2:D4	14.4	261.2
Caythorpe	R1:D2	11.2	195.4
	R1:D3	7.0	0
	R2:D4	10.2	195.4
	R2:D5	6.4	0
Cholsey	R1:D2	6.0	0
	R1:D9	6.2	230.5
	R2:D3	4.6	0
	R2:D9	6.8	230.5

2.2.2 Extractants

Three extractants were used: 0.01 M CaCl₂ (calcium chloride), Olsen (sodium bicarbonate) and EDTA:NaOH (ethylenediaminetetraacetic acid:sodium hydroxide). The calcium chloride and Olsen extractants are routinely used for quantifying P from soil. EDTA:NaOH (referred to as EDTA) is the standard extractant for NMR; this is a (chemically) strong mixture and it will extract P compounds that are less readily-available, hence it could be representative of residual P. Extracts were performed on sub-samples of the same soil, i.e. the soils were not sequentially extracted. All extracts were freeze-dried prior to NMR analysis and re-constituted in a deuteriated sodium hydroxide buffer.

2.2.3 Phosphorus quantification

Nuclear magnetic resonance works on the principle that different compounds or compound groups have different molecular structures and the nuclei of compounds have a unique resonance when subjected to radio waves in a magnetic field. The output is a spectrum where the position of the peak on the x-axis provides the identity of the compound or group, and the area of the peak provides a measure of the quantity of that compound or group. For EDTA extracts the existing literature was used to assign a P compound or compound group to each peak.

For the Olsen extract, a portion was also analysed by the more standard method of UV spectrophotometry after reaction with molybdate.

2.3 Results

Five P compounds/groups were extracted by calcium chloride in all soil types, although it was not possible to identify the actual groups due to the lack of reference material. The data were quite variable when comparing 'before' and 'after' fertilisation. For example, whilst there was an increase in two P groups in soils that had received fertiliser, there was also an increase in these groups for a replicate of the Cholsey soil that did not receive fertiliser. However, these observations rely on only two replicates, negating statistical analysis so that any extrapolation of the findings must be made with caution. More P was extracted in total by calcium chloride from the Caythorpe soil than for the other soil types. There was a weak effect of soil type on the presence of different P compounds ($p=0.047$) because the Cholsey soil had a higher *proportion* of one of the P groups (although lower absolute quantities) than the other soil types.

EDTA extracted a larger total quantity of P than any other extractant investigated and the largest number of P compounds/groups (six). Approximately three times the total amount of P was extracted from the Caythorpe soil compared to the Cholsey soil using this method. Orthophosphate and monoesters were the dominant P compound and compound group in all soil types. In the Cholsey soil, diesters (P5) contributed to a larger proportion of the total than in the other soil types. There was a significant increase in the proportion of orthophosphate after fertilisation as well as an overall increase in P amounts (with the exception of one Cholsey sample R1:D9).

With the exception of a single sample (Caythorpe R1:D2) Olsen P values measured by Fera using UV were always slightly less than the values provided by Rothamsted with the soil samples supplied. This may partly be due to differences in analytical equipment and operators, which inherently introduces some variability, but it may also be a factor of time as the samples were analysed by Fera several months after they were first analysed by Rothamsted, during which time there may have been transfer of Olsen P to a less available fraction.

Olsen P values were higher in soil samples after fertilisation with increases of at least 2.4, 1.6 and 8.2 times for the Great Carlton, Caythorpe and Cholsey soils respectively. The non-fertilised soils also demonstrated a slight increase in measurable Olsen P which could be a real effect and occur due to mineralisation, or it could reflect the natural variability in P levels in adjacent soil samples and/or variance associated with analytical methods.

NMR analysis of the Olsen P extract produced a single peak for all three soil types which was identified as orthophosphate. The NMR results tally with the UV results in that there is an increase in orthophosphate for the samples that received fertiliser, and, with the exception of a single sample (Cholsey R1:D2) there was also a slight increase in Olsen P in samples at time point two

even when they had not received fertiliser. The Caythorpe soils had the highest levels of Olsen P after fertilisation, despite having the lowest application rate and a lower initial Olsen P (determined by UV) than the Great Carlton soil. The Caythorpe soil had the highest proportion of monoesters (as determined by EDTA) and it is possible that the P in this group may have degraded to orthophosphate which could partly account for this finding.

The two methods of analysis (NMR and UV) of the Olsen P extract were compared by considering the ratio of the P value at time point two compared to the initial value. When Olsen P levels were relatively low, i.e. in replicates that did not receive fertiliser, there was a good correlation between the results of the two different methods. However, after receiving fertiliser, analysis by UV recorded a much greater increase in orthophosphate compared to analysis by NMR.

The orthophosphate from the EDTA extract and that from the Olsen extract (i.e. different extractants, but the same detection method) for the same P compound was also compared. EDTA extracted more orthophosphate from the soils than the Olsen extract, with the exception of a single sample (Cholsey R1:D2) and the effect was far less marked for the Cholsey soil.

The total P extracted by Olsen was similar to, or marginally higher than the CaCl_2 extracts, and there was no influence of soil type indicating the importance of potentially-available organic forms of P.

2.4 Discussion

The findings of this study have provided insight into the nature of the different phosphorus compounds in soil. A very interesting finding is that the CaCl_2 extract consists wholly of organic P compounds yet this extract is deemed to represent immediately-available P and it is also 'known' that P is 'only' taken up by plants as orthophosphate, i.e. inorganic P. The findings of this study indicate that either, plant-available P contains organic compounds and/or the chemicals used in the more traditional analysis of P (UV spectrophotometry) degrade highly-labile forms of organic P into orthophosphate. The absence of orthophosphate in the CaCl_2 extract may explain why there was no significant effect of time or fertilisation on the quantity of CaCl_2 -extracted P.

The greater increase in orthophosphate in the Olsen extract as determined by UV compared to NMR, particularly for the Cholsey soil, indicated that different entities were being detected. It was postulated that the NMR buffer could have hydrolysed labile forms of organic P extracted by Olsen and/or the acidic chemicals used for UV may have dissolved calcium precipitates.

The high proportion of orthophosphate and monoesters in soils is similar to findings of other workers. However, it should be noted that it is likely that the orthophosphate detected contains, in part, organic P that has been degraded by the extractants and/or buffer. The similarity in

orthophosphate content of the Olsen extract and the EDTA extract in the Cholsey soil indicates that either there are P compounds in the Cholsey soil that are poorly extracted by both Olsen and EDTA, or, in the Cholsey soil, there is a lower content, compared to the other soil types, of the organic P that is contributing to the total orthophosphate extracted by EDTA i.e. orthophosphate + easily-hydrolysable organic P.

A number of studies have been undertaken on P compounds/groups in manures with the majority of studies occurring in the US or New Zealand. The data should be considered in the context that there can be variation in the findings due to different sample collection timings, sampling methods, extraction procedures etc. In cattle manure, orthophosphate was the dominant P compound ranging from 51-77% in fresh excreta and 63–84% in manure/dry excreta. Monoesters ranged from 40–70% in fresh excreta and 12–18% in manure/dry excreta. Diesters were in the range of 2-9% for fresh and dry cattle excreta. This compared to orthophosphate contributions of ~40% in poultry manure or litter with 50-65% being monoesters, particularly phytate as poultry cannot digest phytate present in feed, hence it is excreted. However higher values of orthophosphate in poultry manure have been noted and the content in pig slurry was also variable.

A high quantity of orthophosphate in anaerobic digestate (AD) was reported by others and this could have particular implications in the UK, as AD is being encouraged to reduce the generation of waste. As with manures, the input (feedstock) to the AD will determine the chemical composition of the digestate. In order for animal manures and/or AD to be utilised to their optimum, a better characterisation of the end product is required. In terms of crop nutrition in particular, an understanding is also required of the subsequent interactions with the soil and the influence on other relevant factors such as pH, soil structure, carbon and nitrogen content etc.

Variation in the P groups in manure can be influenced by diet and management practices, and when applied to the soil, further changes will occur. The lack of UK-specific data limits the extrapolation of data to attempt to predict the likely impact on soil P for crop nutrition and/or any potential adverse environmental impact.

2.5 Conclusions

The study has identified some differences in soil types and extractants that can serve as a basis from which further research can be focussed in relation to crop utilisation of different P compounds, especially organic forms, how these are influenced by soil type and response to fertiliser additions in soil. Investigations into the microbial population could assist with understanding the P dynamics. The study is potentially a precursor to the development of in-field testing kits. Key findings are:

- CaCl_2 extracts organic P compounds.

- Olsen P extracts inorganic P. There is a very high probability that Olsen P also extracts highly-labile organic P compounds.
- Measures of orthophosphate by NMR are lower than when analysed by UV spectrophotometry.
- EDTA extracts a higher quantity of P and a greater number of P compounds.
- P is primarily present in soils as inorganic orthophosphate and organic monoesters.
- The alkaline soil had a higher proportion of organic diesters than the other two soil types.
- Orthophosphate comprises a small proportion of P in manures with monoesters and diesters representing a higher proportion.
- Drying/storage affects the P composition of manures/slurry.
- Anaerobic digestate contains a high proportion of orthophosphate.