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Characterizing phosphorus forms in cropland soils with solution ^{31}P-NMR: past studies and future research needs

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Abstract

Understanding the forms and dynamics of soil phosphorus (P) is essential to maintain agricultural productivity while minimizing environmental risks. Since it was first used on soil extracts in 1980, ^{31}P-nuclear magnetic resonance spectroscopy (P-NMR) has emerged as the leading technique to characterize extractable soil organic P forms. However, it is still underutilized in agriculture; of the more than 200 soil P-NMR papers published to date, only 44 have been conducted in non-pasture soils used for the production of annual or perennial crops, and only nine of those have linked identified P forms to agronomic parameters such as yield. This paper reviews these prior studies, suggesting gaps in research with respect to cropping systems and geographical regions. In particular, there have been few recent P-NMR studies that have fully identified P forms in African soils, and few studies of permanent crops such as orchards and vineyards. There is a need to link future P-NMR studies of cropping systems to agronomic parameters, and combine P-NMR with other techniques to fully capture P dynamics in cropping systems.

Keywords: Phosphorus, NMR, Soil, Cropland

Introduction

Phosphorus (P) is an essential element for all organisms, and is needed for energy transfer through adenosine triphosphate (ATP), cell membrane structure (phospholipids and lipoteichoic acids), cell reproduction [deoxyribonucleic acid (DNA)] and gene expression [ribonucleic acid (RNA)]. Phosphorus is classified as a macronutrient for plant growth, and is a limiting nutrient in many soils [1, 2]. Unlike nitrogen (N), P cannot be fixed from atmospheric sources; it can only be obtained by uptake from soil via plant roots as orthophosphate (HPO_4^{2-} or $H_2PO_4^-$ depending on soil pH) [3].

In soil, P originates from the weathering of apatites, which are primary P minerals containing calcium (Ca). The orthophosphate released from weathering will move to the soil solution, where it can precipitate as secondary minerals that are generally associated with Ca,

magnesium (Mg), iron (Fe) and aluminum (Al); sorb to soil particles; or be taken up by plants and microbes and converted to organic P forms (immobilization) [3]. Organic P forms can be returned to the soil, where they can sorb to soil particles or be mineralized back to orthophosphate for plant uptake from the soil solution.

Organic P can comprise 35–65% of soil P [4]. Soil organic P forms are grouped into broad compound classes, each of which can contain a number of P forms [4]. Orthophosphate esters are subdivided based on the number of ester linkages. Orthophosphate monoesters have one ester linkage per phosphate, and include sugar phosphates (e.g. glucose 6-phosphate), mononucleotides (e.g. adenosine monophosphate) and inositol phosphates. Inositol phosphates identified in soils include the plant P storage compound phytate (*myo*-inositol hexaphosphate, *myo*-IHP) and several of its stereoisomers (*scyllo*-, D-*chiro*- and *neo*-IHP) [5–7]. Orthophosphate diesters have two esters per phosphate, and include phospholipids, lipoteichoic acids, RNA and DNA. Phosphonates have a carbon (C)–P bond, and include natural compounds such as ciliatine (2-aminoethyl phosphonic

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acid) and agricultural chemicals such as glyphosate [*N*-(phosphonomethyl) glycine]. Orthophosphate anhydrides are linear chains of orthophosphate, linked with energy-rich phosphoanhydride bonds. These can include inorganic and organic compounds, all of which are generally included with organic P due to similarities in dynamics and analysis. Organic orthophosphate anhydrides include ATP; inorganic compounds include polyphosphates, which are chains of two (pyrophosphate) or more orthophosphates. More details on all of these compounds can be found in Condron et al. [4].

The P that is available for plant uptake from the soil solution can be deficient in many regions, requiring fertilization for optimal crop growth [2, 8]. Animal manure (AM) and other biochemically stabilized organic wastes have been applied as fertilizers for centuries; chemical inorganic P fertilizers (CF) were introduced in the late 1800s, and their use has significantly expanded globally since the 1950s [8]. The widespread application of P fertilizers, both AM and CF, has led to concerns with respect to water quality and eutrophication due to excess P from soil that is transferred to water through erosion and runoff [8, 9]. Additionally, CF is produced from rock phosphate, and the long-term sustainability of this non-renewable resource is uncertain [1, 2, 8]. In light of these concerns, there is a need to improve P use efficiency in agriculture, to minimize the need for P fertilizer application.

Many methods have been proposed to improve P use efficiency in crop production. These include targeted fertilization [8], plant breeding for traits such as rooting form, and production of phosphatase and organic acids [1, 2], and enhanced microbial P cycling in the rhizosphere [1, 2]. Key to all of these is an understanding of soil P dynamics, especially those of organic P forms [9, 10]. Historically, organic P forms have received less attention than inorganic P forms, in part because they are more difficult to study [4]. As such, a detailed understanding of the organic P forms contributed to soils from plants, microbes and organic amendments, and the pathways by which these are recycled to plant-available P forms, is lacking relative to that for inorganic P. Advanced spectroscopic tools are required to characterize P forms, the most widely used of which is solution ^{31}P nuclear magnetic resonance spectroscopy (P-NMR). Since its first use in soil science in 1980 by Newman and Tate [11], more than 200 papers have been published that have used P-NMR to characterize soil P forms. However, relatively few of these have studied cropping systems (defined here as non-pasture systems used for annual or perennial crops), and even fewer of these have linked P forms to management practices or to agronomic indicators such as yield or P balance (the difference between fertilizer inputs and crop removal). This has limited the usefulness

of P-NMR studies to guide agricultural practices to improve P use efficiency, including the role of organic P forms in soil fertility. In order to connect P cycling in soil to sustainable management in agriculture, the knowledge gained from past studies needs to be evaluated and used to direct future research. As such, the objectives of this manuscript are (1) to review prior studies using P-NMR to investigate soil P forms and dynamics in field studies of non-pasture cropping systems; and (2) to identify knowledge gaps and suggest directions for future research.

Review
Solution ^{31}P-NMR spectroscopy

It is not my intention to review the principles of NMR spectroscopy in this manuscript, because that information can be obtained elsewhere [e.g. 12, 13]. It is also not my intention to give extensive information related to the use of P-NMR in soil and environmental science, as that has been covered in several other review papers [14–17]. However, a brief overview is needed to help understand the strengths and weaknesses of the studies discussed below.

Solid-state P-NMR is rarely used for soil studies; instead, solution (liquid-state) P-NMR is preferred for the improved spectral resolution [14, 15]. Soil total P concentrations are generally in the mg kg^{-1} range; thus, concentrating soil extracts increases the P concentration per NMR sample, significantly improving the NMR response. In the early years of soil P-NMR work, a number of different extractants were used, with little standardization among research groups [14, 15], making it difficult to compare results. In 1996, Cade-Menun and Preston [18] introduced the use of 0.25 M sodium hydroxide (NaOH) + 0.05 M disodium ethylenediaminetetraacetic acid (Na$_2$EDTA) as an extractant, based on a method to measure total soil organic P [19]. Once extracted, solutions are lyophilized to concentrate P. This is now the most widely used extraction procedure for soil and other environmental samples [17]. However, as discussed in detail elsewhere [17], other dissimilarities exist among groups with respect to sample extraction (e.g. soil:extractant ratios), dissolution of lyophilized extracts for NMR analysis, NMR acquisition parameters, and methods to identify and quantify specific P forms. In some early studies, research groups concentrated their extracts with dialysis [e.g. 20, 21]. While this improved the resolution of other peaks by removing most of the orthophosphate, it may have also removed other P forms, and definitely altered the relative proportions of all peaks. Even small changes in preparation methods or parameters can affect the final NMR results, so caution is needed when comparing results from different research groups that use different protocols and parameters.

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The early soil P-NMR work produced spectra that were often very broad, with little resolution of individual peaks. As such, P forms were identified as compound classes only (e.g. orthophosphate, orthophosphate monoesters) [11, 20–28]. There were few attempts to identify specific P forms, and the identification of some compounds such as choline phosphate [23] has since been shown to be incorrect [29]. Advances over time now allow the majority of peaks to be identified with spiking experiments and compound libraries, although it is still difficult to distinguish some specific forms such as mononucleotides [29]. Figure 1 shows spectra from several recent studies of P in cropping systems [30–32]; more information about the samples can be found in the published studies. The P compounds of interest in soil science generally fall between 30 and −30 ppm, and include (from left to right, Fig. 1) phosphonates, from 30 to ~8 ppm; orthophosphate at ~6 ppm; orthophosphate monoesters, at ~7 to 6.1 ppm and ~5.9 to ~2.5 ppm; orthophosphate diesters from 2.5 to −3.9 ppm; pyrophosphate at about −4.2 ppm; and polyphosphates at −4 (end group) and from −5.3 to −30 ppm (mid-chain). More details of the orthophosphate monoester region are shown in Fig. 2, with spectra from published studies [32, 33], and unpublished data (B. Cade-Menun). The spectra are labeled in the manner used by the Cade-Menun research group. More information about the chemical shifts of these and other P compounds can be found in published compound libraries [29, 34].

One of the biggest improvements in recent years involves the identification of peaks for degradation compounds. These are compounds that were orthophosphate diesters in the soil, but which have degraded to orthophosphate monoesters during extraction and analysis. It is generally now agreed that α- and β-glycerophosphates are degradation products from phospholipids, while most mononucleotides (e.g. adenosine monophosphate) originate from RNA [29, 34–37]. There has long been an interest in quantifying total orthophosphate monoesters and total orthophosphate diesters in soil; diesters are thought to be more labile, while many monoesters are more recalcitrant due to strong sorption to mineral surfaces [4]. As such, the ratio of orthophosphate monoesters to orthophosphate diesters (M:D) has been used to predict organic P cycling in soils [4, 23]. In addition, activities of the soil enzymes acid monoesterase, alkaline monoesterase and diesterase are commonly measured [4], and linked back to total orthophosphate monoester and diester concentrations in models [e.g., 38]. However, if corrections are not made for degradation products, the M:D and models are of questionable value for predicting mineralization of organic P forms [37].

Unless compounds labeled with other P isotopes (^{32}P, ^{33}P) have been used in experiments, all P in soils is ^{31}P. As such, P-NMR is a quantitative method, allowing the concentrations of P forms to be determined. This is can be done by measuring the P concentration in the soil extract if the entire extract is used for the NMR experiment, or by measuring the P in the prepared NMR sample after analysis if only a portion of the lyophilized extract is used. In either case, this is done by digestion and colorimetric analysis or by analysis with inductively coupled plasma optical emission spectroscopy (ICP-OES); the percentage of each peak, determined from the spectrum by integration and/or deconvolution, is then multiplied by the P concentration [14, 15, 17]. Concentrations can also be determined by integration against a peak from a standard with a known P concentration spiked into the sample [17].

Solution ^{31}P-NMR studies of cropland soils

The P-NMR literature for pasture soils was recently reviewed [39]; as such, this section of the manuscript will review studies that have used P-NMR to characterize P in non-pasture soils used to grow annual or perennial crops. Most commercial crop production relies heavily on fertilization with CF or AM to optimize yields, so improvements in P use efficiencies could have the greatest benefits in these systems.

Studies using P-NMR to characterize P in cropping systems are listed in Table 1. These are grouped by country and listed by date of publication. The soil classifications listed are from the original manuscripts; no attempt was made to convert them to a single classification system. Other information given includes the crops, P fertilization history (kg P ha^{-1} year^{-1}), and the treatments the study was designed to investigate. The degree of peak identification is ranked as B, M or D; B is broad compound classes only (e.g. orthophosphate monoesters); M is moderate, with some more specific identifications, usually of *myo*-IHP; and D indicates spectra with detailed peak identifications, generally confirmed with spiking experiments. Studies that have identified degradation peaks and ideally corrected the calculation of total orthophosphate monoesters for diester degradation compounds are indicated with a C. If agronomic data such as soil test P, yield or P balances were included in the study this is indicated in the final column. Blanks in any column for any paper indicate that the information was not included in the original publication. Some studies included other land uses, such as forests, in addition to cropping systems but the information in Table 1 is for soils from cropland only. Please note that I excluded studies of soils that were identified as "cropland" or "arable" if no specific details about crop rotations were given

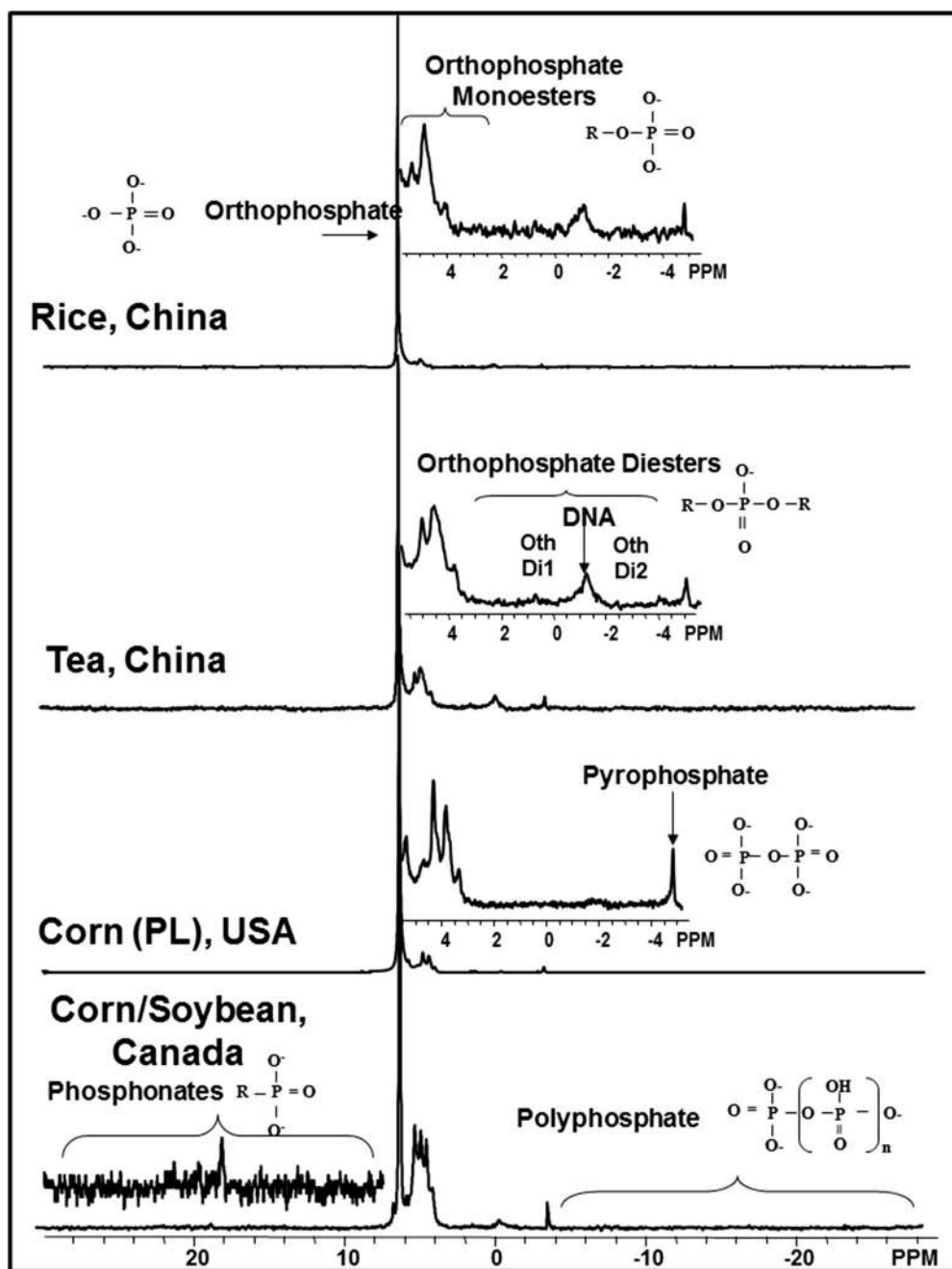Cade-Menun *Chem. Biol. Technol. Agric.* (2017) 4:19

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Fig. 1 Example spectra from cropping systems from various locations. The rice and tea spectra are from Liu et al. [30], the corn soil, no till, fertilized with poultry litter, is from Georgia, USA [31], and the corn/soybean sample is from Quebec, Canada [32]. *DNA* deoxyribonucleic acid; *Oth Di1, Oth Di 2* orthophosphate diesters other than DNA. Spectra were analyzed without proton decoupling and processed with 7 Hz line-broadening. The full spectra are scaled to the height of the orthophosphate peak; the *insets* were enlarged by different factors, to best show the indicated regions

[e.g. 40–46], as well as incubation studies using soils from crop field [46, 47].

In Africa, there have been two studies from Madagascar and one each from Tanzania, Ethiopia and West Cameroon [24, 26–28, 48]. Four of these studies were published in 2003 or earlier, used NaOH as an extractant [24, 26–28], and identified only broad compound classes. One study used dialysis on sample extracts [26], which likely distorted the relative peak areas. One study from Madagascar [48] used NaOH–EDTA and identified
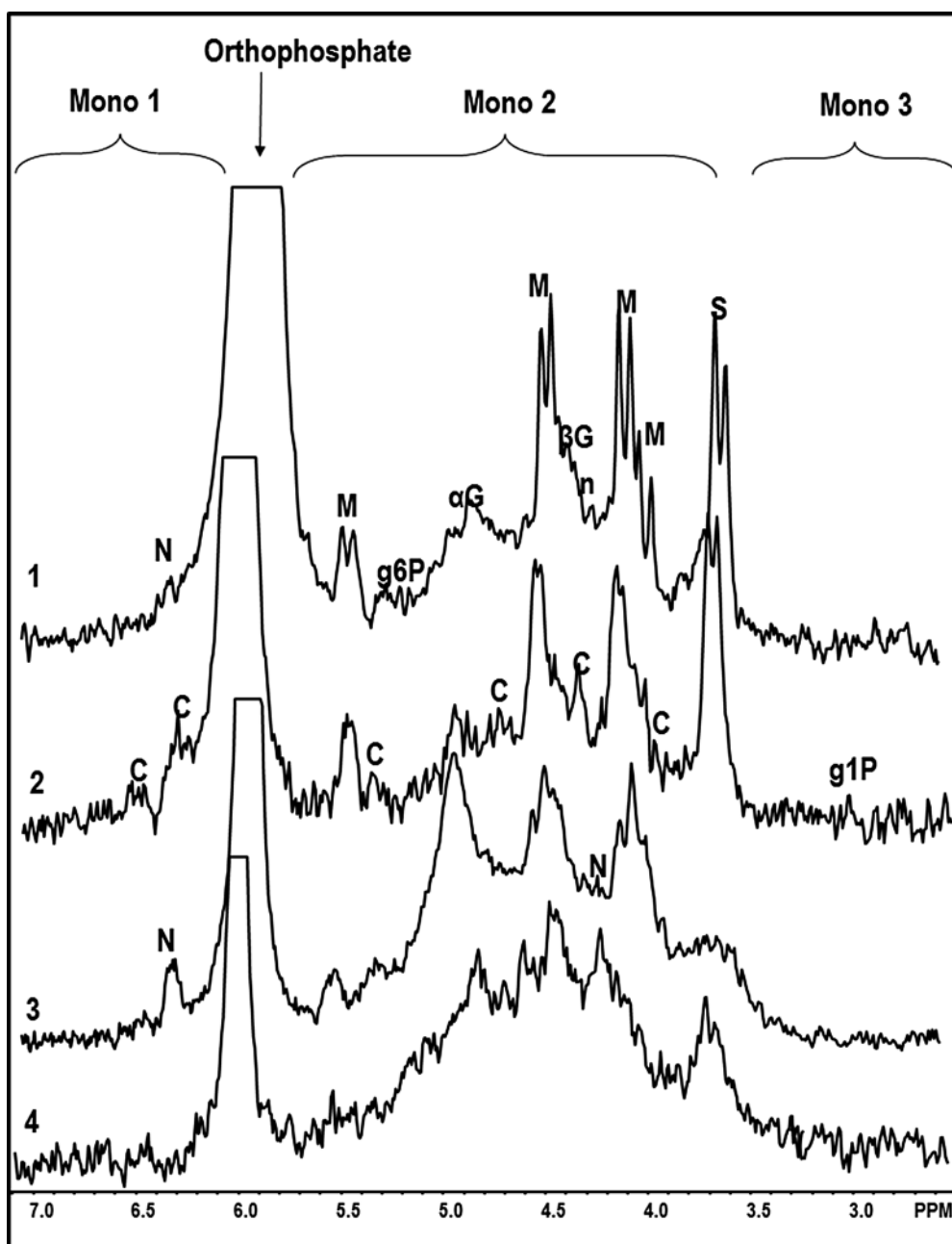
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Fig. 2 Example spectra from cropping systems from various locations, showing details of the orthophosphate monoester region. *1* barley/corn, no till, Prince Edward Island, Canada [33]; *2* corn, France; *3* corn/soybean, no till, Quebec, Canada [32]; *4* Rice, China. Samples *2* and *4* are unpublished spectra. *Mono* general orthophosphate monoester regions; *a-G* α-glycerophosphate; *βG* β-glycerophosphate; *C* D-*chiro* inositol hexaphosphate (IHP); *g1P* glucose 1-phosphate; *g6P* glucose 6-phosphate; *M myo*-IHP; *N neo*-IHP; *n* nucleotides; *S scyllo*-IHP. Spectra were analyzed without proton decoupling, processed with 2 Hz line-broadening, and are scaled to the height of the tallest non-orthophosphate peak shown in all spectra

peaks in detail, including degradation compounds. In Asia, there was one study from Northern Thailand [23] and eight studies from various provinces in China [30, 38, 49–54]. One study used NaOH [25], the remainder used NaOH–EDTA [30, 38, 49–54], and none used dialysis.

Although the majority of studies were published in 2012 or later, only two included detailed peak identifications and corrections for degradation [30, 54]. Four studies from Australia were included [35, 55–57], two of which sampled from the same fields under the same crops [35,

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Table 1 Studies in North America, Europe and South America using ^{31}P-NMR to investigate P in soil under cropping systems, sorted by location

Location	Pub date[a]	Soil[b]	Crop	P fertilization (kg ha^{-1} year^{-1})	Treatment	Peak ID[c]	Agron. data[d]
Africa							
Madagascar [24]	1990	Ferrosol, Andosol	Rice	20	P forms in rice soils	B	
Northern Tanzania [26]	2000	Luvisol	Corn, beans	Various	Forests vs. fertilized (0 CF vs. AM) cropping systems	B	
Ethiopia [27]	2002	Alisol, Nitrisol	Tea, corn, corn/sorghum	Various	Cropping systems; D[e]	B	STP
West Cameroon [28]	2003	Palehumult	Tea plantation, food crop fields	Various	Forest vs. cropping systems (permanent, annual)	B	STP
Madagascar [48]	2006	Oxisols	Rice	Various	Different rice cropping systems; CF, AM, COM	M	STP, Y
Asia							
Northern Thailand [25]	2000	Cambisol	Cabbage	Not given	Cultivated vs. native forests	B	
Shenyan, China [49]	2012	Luvisol	Corn, rice	35 or 47	Land use types, EA	B	STP
Zhejang, China [30]	2013	Ultisol	Rice, tea, vegetables, nursery stock, bamboo	Various	Crops;	D, C	STP
Henan, China [38]	2014	Cambisol	Winter wheat/summer corn	59	Tillage, residue incorporation, aggregates, EA	B	STP
Jilin, China [50]	2014	Phaeozem	Corn–soybean each year	45	Tillage, aggregates	B	STP
Jilin, China [51]	2014	Phaeozem	Continuous corn monoculture	Various	Fertilization, CF and AM, EA	M	STP
Yunnan, China [52]	2015	Ultisols	Vegetables, horticulture	118.5–196.5	Land use, leaching losses; forests vs. open cropland, greenhouses	B	STP
Northeastern China [53]	2015	Gleysol, lithosol	Cropland (winter wheat summer corn), vegetables, orchards		Land use, fertilization, EA, sorption	M	STP
Shandong, China [54]	2016	Gleysol	Greenhouse, double-crop tomatoes	Various	CF, AM; high N fertilizer, leaching	D, C	PB, STP
Australia							
New South Wales [55]	2008	Luvisol	Continuous wheat, wheat–legume rotations	20	Tillage, stubble, rotation, microbial community	B	RP, Y
New South Wales [35, 56]	2009, 2011	Haplorthod, Fragiudult	Vegetables		Pasture vs. conventional and organic vegetables and horticulture	D, C	
New South Wales, Queensland [57]	2014	Vertisol	Grain-fallow-cotton, pulse-fallow-cotton	6 (every 2 years)	Vertisols in Australia	D	STP
Europe, Israel							
Germany, Denmark [20, 21]	1996, 1999	Eutrochept, Hapludalf	Wheat, beet, barley rotation	0–39	Compared fertilized, cultivated fields to native soils; particle size; D	B	STP
Finland [59]	2011	Cambisol	Barley, winter rye, pea, oats rotation	Various	Organic (AM) vs. conventional (CF) rotations	B	STP, PB
Sweden [60]	2013	Cambisol, Phaeozem, Umbrisol	Grain-based rotation	Various	Soil textures, fertilization rates (CF, AM)	M	STP, PB
England [62]	2013	Paleudalf	Winter wheat	0	pH gradient	D	RP
Israel [63]	2014	Chromoxerert	Corn	40 RW, 10 CF	Irrigation, fertilization with CF or RW	D, C	STP

Table 1 continued

Location	Pub date[a]	Soil[b]	Crop	P fertilization (kg ha^{-1} year^{-1})	Treatment	Peak ID[c]	Agron. data[d]
Switzerland [61]	2015	Luvisol	8-year, wheat-based rotation	Various	Fertilization; 0, CF, AM, COM, SS; EH	D, C	PB, RP, Y
Germany [58]	2015	Luvisol	Winter wheat, barley	0	Particle size fractions	B	
United Kingdom [10]	2015	Cambisol, Luvisol, Podzol	Winter wheat, oats, barley	Not given	Compared land uses	B	STP
North America							
Mexico [22]	1985	Vertisol	Tree garden (mango, papaya, avocado); Corn	20 (corn)	Unfertilized gardens, fertilized corn (CF)	B	
Saskatchewan, Canada [23]	1990	Chernozem, Gleysol, Luvisol	Cereal rotation	0	Compared to uncultivated native grasslands	B	
Quebec, Canada [64]	1999	Hapludalf, Humaquet	Corn	0, 44, 132	Fertilization rates	B	STP
New York, USA [66]	2005	Fragiudepts, Dystrudepts	Corn or corn/alfalfa	Various	Compared short and long-term AM application; D	B	STP
Pennsylvania, USA [67]	2009	Hapludults, Hapludalf, Fragiudults, Dystrudepts	Various corn-based rotations	29–120	Compared high rates of AM from various sources	M	STP
Delaware, Maryland USA [68]	2009	Hapludult	Corn, soybean	Various	Compared crop soils with AM to poultry feces, composted manure	M	STP
Prince Edward Island, Canada [33]	2010	Podzol	Wheat, barley/soybean, fescue	Various	Tillage	D, C	STP
Quebec, Canada [32]	2014	Humaquept	Corn/soybean rotation	0, 17.5, 35	Tillage, CF fertilization	D, C	STP
Georgia, USA [31]	2015	Kanhapludult	Corn/winter rye, cotton/winter rye	Various	Tillage, CF and AM fertilization	D, C	STP
Saskatchewan, Canada [65]	2015	Chernozem	Continuous monoculture wheat	0, 10	Long-term with and without CF	D, C	STP, Y
Idaho, USA [69]	2016	Haplocalcid	Wheat–potato–barley	Various	Fertilization: CF, high and low AM	D, C	STP
South America							
Rio Grande do Sul, Brazil [71]	2002	Oxisols, Paleudalt	Various, grain-based	Various	Tillage; various crop rotations	B	STP
Minas Gerais, Brazil [72]	2003	Oxisols	Coffee plantations	5–15	Monoculture coffee vs. agroforestry coffee	B	STP
Rio Grande do Sul, Brazil [73]	2005	Oxisols	Various crops	0, 26, 52	Greenhouse cropping experiment, multiple crop rotation cycles	B	RP
South Chile [70]	2011	Palehumult	Lupin–wheat, oat–wheat rotations	80–90	Rotation, tillage, microbial P, enzymes	B	STP, Y
Santa Catarina, Brazil [74]	2015	Cambisol	Apple orchard	11	Land use; CF, between and in apple tree rows	B	
Rio Grande do Sul and Parana, Brazil [75]	2016	Oxisol, Ultisol	Various summer crops, winter cover crop	9, 27, 70	Grazed and ungrazed winter cover crops, CF	D, C	PB, STB

Within each region, publications are arranged chronologically based on date of publication

AM animal manure, *CF* chemical fertilizer, *COM* compost, *EA* measured phosphomonoesterase and phosphodiesterase activities, *EH* enzyme hydrolysis, *RW* recycled wastewater, *SS* sewage sludge; please see the original publications for more details

[a] Year of publication

[b] As reported in the original publication

[c] Peak identification: B, broad (only compound classes); C, monoesters corrected for diester degradation peaks; D, detailed (most peaks identified); M, moderate (some peaks identified)

[d] Agronomic information was included; PB, P balance(input–removal); RP, resin-extractable P; STP, soil test P (Olsen P, Mehlich P); Y, yield

[e] D: extracted sample dialyzed prior to NMR analysis

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56]. Three studies identified peaks in detail, with correction for degradation [35, 56, 57].

In Europe, two studies from Germany and Denmark used the same research plots [20, 21], but both used dialysis and identified only broad peaks. There was a more recent study from Germany [58], one each from Finland, Sweden and Switzerland [59–61]; one specifically from England [62] and one from the United Kingdom in general [10]. The study from Israel is included here too [63]. Three of these studies identified only broad compound classes [10, 58, 59], one identified the majority of peaks [60] and three identified peaks in detail [61–63]. In North America, there was one study from Mexico [22], five from Canada [23, 32, 33, 64, 65] and five from the USA [31, 66–69]. The early studies identified only broad peaks [22, 23, 64, 66]. These used NaOH as the extractant, and one study used dialysis [66]. All remaining studies from North America used NaOH–EDTA as the extractant. The two studies from 2009 identified some of the peaks [67, 68], while the remainder identified peaks in detail. In South America, there was one study from Chile [70] and five from Brazil [71–75]. The majority of studies from South America used NaOH–EDTA as an extractant, although several studies added additional steps such as pre-extraction with resin and a final treatment with Chelex [71–73]. Only one study from South America identified peaks in detail and corrected for degradation [75].

The crops that have been studied include food crops for humans [e.g. wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.)] and livestock [e.g., corn (*Zea mays* L.), forage oats (*Avena strigose* Screb.)], and cash crops [e.g. tea (*Camellia sinensis* L.), coffee (*Coffea arabica* L.)]. In North America, Europe and Australia, P-NMR has been used most frequently to study annual crops, generally rotations based on wheat or corn. In Africa and Asia, more studies have investigated soils in vegetable and rice production. There have been very few studies of permanent plants, with three studies investigating tea [27, 28, 30], one study of coffee plantations [72], and one in an apple orchard [74].

Cropping systems have often been included in land use studies, and compared to forests [20, 21, 26, 28, 52, 53] or grasslands [10, 23, 75]. With respect to management practices, fertilization was the most studied practice, and included CF and AM, as well as alternate fertilizers such as compost, sewage sludge and waste water [48, 61, 63]. The next most studied management practice is tillage, with studies in China [38, 50] Australia [55], the USA [31], Canada [32, 33], Chile [70] and Brazil [71].

For improved P use efficiency in agriculture, studies need to not only identify P forms, but also relate both concentrations and changes in P forms to agronomic indicators such as yield or P use efficiency. Of the 44 studies listed in Table 1, many do not even include measures of soil test P (STP), and only nine include information on yield or P balances [48, 54, 55, 59–61, 65, 70, 75]. Of these nine, only six identified peaks in enough detail (M or D) to identify trends in P forms other than orthophosphate [48, 54, 60, 61, 65, 75]. I will focus on these studies in more detail, and they are summarized in Table 2.

The studies that included detailed peak identifications and yield information were studies of rice in Madagascar [48], greenhouse-grown tomatoes in China [54], wheat-based crop rotations in Sweden [60] and Switzerland [61], continuous wheat monoculture in Canada [65], and summer rotations [including corn, soybean (*Glycine max* (L.) Merrill), common bean (*Phaseolus vulgaris* L.)] with a grazed winter wheat cover crop in Brazil [75]. One had only CF [65], one had CF plus grazing of winter cover crops [75], two were fertilized with CF and AM [54, 60], one had fertilization with CF, AM, and compost [48], and one studied fertilization with CF, AM, compost and sewage sludge [61]. Information about soil pH, soil total P and soil test P concentrations is included in Table 2. These ranged considerably for each study depending on the location and treatments. All studies extracted soils for P-NMR with NaOH–EDTA. The recoveries of total P in the NaOH–EDTA extracts ranged from 36 to 96% (Table 2), again varying with location and treatment. The P-NMR results are reported as percentages of extracted P in Table 2 to simplify comparisons among studies. All of the soils in the studies from Madagascar, China and Brazil received some P fertilizers, and all contained high percentages of orthophosphate. The study from Canada included samples from plots that were fertilized from 1967 to 2010, and plots that were fertilized from 1967 to 1995, with no P from 1995 to 2010. The studies from Sweden and Switzerland included plots receiving no P for 30 or 60 years, respectively, plus fertilization at various rates with different fertilizers. The percentages of orthophosphate in the studies from Canada, Sweden and Switzerland varied with P fertilization, and were always lowest in soils receiving no P for the length of the study, as were the soil test P concentrations. The percentages of pyrophosphate and DNA were low across all treatments in all studies. The percentage of *myo*-IHP varied with P treatment, and was highest with the lowest fertilization, while *scyllo*-IHP was present in most samples from all studies, also increasing in plots with lowest fertilizer P inputs. The M:D ratio, after correcting for diester degradation, was over 1 for all but the sites with the lowest fertilization rates in Sweden. The ratio was particularly high in the Switzerland samples because no DNA was detected. The P balance was generally positive for all studies except at sites receiving no P fertilizer. There were generally no

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Table 2 Summary of P-NMR results from selected studies

Location	Madagascar [48]	China [54]	Sweden [60]	Switzerland [61]	Canada [65]	Brazil [75]
Crop	Rice	Greenhouse, double-crop tomatoes	Grain-based rotation	8-year, wheat-based rotation	Continuous monoculture wheat	Various summer crops, winter cover crop
Fertilizers	Different rice cropping systems; CF, AM, COM	CF, AM; high N fertilizer, leaching	Soil textures, fertilization rates (CF, AM)	Fertilization; 0, CF, AM, COM, SS; EH	Long-term with and without CF	Grazed and ungrazed winter cover crops, CF
Soil pH	4.64–5.82[a]	6.91–7.03	5.8–7.5	4.8–5.9	5.4–6.6	5.2–6.4
Total P (mg kg^{-1})	133–1378	685–2446	288–980	442–1150	440–615	841–2531
Soil test P (mg kg^{-1})[b]	<1–6.9 (O)	20–210 (O) 179–1128 (M)	12–223 (A)	114–58 (R)	3–33 (O) 21–103 (M)	22–71 (M)
P recovery (%)[c]	36–82	49–74	48–98	73–96	36–50	60–88
OrthoP (%)[d]	54–80	90.7–93.4	20.4–75.7	53–80	22.6–44.1	71.6–77.6
Pyro P (%)	0–4	0.3–0.8	0–3.2	0.3–0.5	1.0–1.4	2.0–2.3
Myo-IHP (%)	0–5	0.5–1.7	0–24	3.0–6.6	4.6–8.5	6.5–9.7
Scyllo-IHP (%)	0–4	0.1–0.3	1.4–6.6	1.0–2.7	0.8–2.7	2.9–3.9
Other mono (%)	nd[e]	4.2–8.4	nd	7.3–12.2	29.3–34.2	0.7–1.1
DNA (%)	0–9	0.0–2.0	0–3	0	0.3–1.2	0.3–1.6
Other diesters (%)[f]	nd	0.0–0.7	0–17.5	0.7–1.1	2.8–4.5	6.8–9.8
cM:D[g]	nd	1.5–2.0	0.9–5.6	16–25	1.2–1.5	1.3–1.5

AM animal manure, *CF* chemical fertilizer, *COM* compost, *EA* measured phosphomonoesterase and phosphodiesterase activities, *EH* enzyme hydrolysis, *RW* recycled wastewater, *SS* sewage sludge, please see the original publications for more details

[a] Range for all depths and treatments

[b] Soil test P: A, ammonium lactate; M, Mehlich; O, Olsen; R, Resin

[c] Percentage of total P extracted in NaOH–EDTA

[d] Percentage of spectral area

[e] Could not be determined from information in the publication

[f] Calculated from information for α- and β-glycerophosphate and nucleotides as given in paper, if not already calculated in the paper

[g] Corrected for diester degradation compounds

differences among fertilizer types (CF, AM, etc.) within studies; the main differences resulted from differences in P rates applied to soils from each fertilizer. The use of non-CF fertilizers did not build up organic P; organic P was higher in plots not receiving fertilizer, mainly from the drawdown of inorganic P. In Madagascar, there were no differences in yields with treatments. In Canada, fertilizer cessation had also not affected yields, although orthophosphate and STP had decreased. In Switzerland, yields varied among treatments, but P was not the only limited nutrient. For all studies with a 0-P treatment [60, 61, 65], orthophosphate decreased in those plots, but organic P species remained the same or increased. This suggests that crops are drawing orthophosphate down faster than it is being replenished by mineralizing organic P forms. And the relatively low percentages of DNA and P degradation forms from phospholipids and RNA suggest that the P extracted for NMR in these studies is from other sources than microbes [4].

Information such as that which is shown in Table 2 needs to be obtained from a wider range of studies, with respect to soils, crops and fertilizers. This in turn could

be used to enhance P use efficiency, with further testing. For example, does orthophosphate decrease and organic P increase because organic P forms are more tightly sorbed and less available for hydrolysis? If so, then crop breeding or soil microbial inoculation could be used to enhance the production of organic acids or phosphatases to mineralize organic P forms. However, without the detailed information about P forms and their dynamics that can be obtained from the use of advanced studies such as P-NMR, it will be more difficult to develop appropriate strategies to improve P use efficiency.

Future research needs

Solution P-NMR spectroscopy is a powerful technique, producing information not available from other methods. However, the reliability of quantitative data depends on the correct set up of acquisition parameters, e.g. with delay times long enough for full relaxation [17]. This is true for all future P-NMR studies, not just those of cropland soils. Extraction and analytical procedures should also be standardized among research groups, to enhance comparisons of data sets, and all peaks should

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be identified and confirmed with spiking experiments [29]. Calculations of total orthophosphate monoesters and diesters, and the M:D ratio, should be corrected for diester degradation products [37]. And field replicates need to be analyzed by P-NMR as much as possible, rather than compositing samples, to allow statistical analyses of P-NMR results along with other field data.

For studies of agricultural soils, especially those comparing management practices, published papers need to include as much agronomic data as possible. At the very minimum, some measure of STP needs to be included, as well as yield data and P balances. Ideally, P-NMR studies of management practices should also link changes in P forms to agronomic data related to those practices, which has been poorly done in the past. Although tillage has been shown to alter the distribution of soil P forms in several studies from around the world [31–33, 37, 50, 55, 70, 71], these changes in P forms were not linked to yield in any of these studies, making it difficult to determine if changing P forms makes any difference agronomically. Phosphorus NMR studies of agriculture that do not link the identified P forms to agronomic values become esoteric technical studies rather than practical management studies, and are more limited in their wider use.

The majority of studies to date are static studies, capturing the point in time when samples were collected. Sampling needs to be coupled to active changes in crops during the growing seasons such as seed germination (for annual plants) and flowering, and P-NMR measurements should be paired with other techniques such as enzyme assays or microbial community analysis to relate P forms to P dynamics. Long-term studies of cropping systems are available in many countries [e.g., 60–62, 65, 71, 75]; these should be coupled with shorter greenhouse studies to capture P dynamics at different time scales [e.g. 73]. And if orthophosphate is indeed the P form most affected by management, then there is a need to pair P-NMR studies with complementary techniques that better characterize orthophosphate forms, such as sequential fractionations and P-XANES [e.g., 30, 65, 69].

There is a need to understand the inputs into soil from various plant materials (roots, shoots, etc.), and changes in these inputs from changing soil P status [76–78]. And while the P forms in manures have been fairly well-characterized [16] and their dynamics in soil generally well-studied, there is a need for more information on alternate fertilizers, particularly recycled wastes [e.g., 60, 79–81] and their behavior in soil [61, 80]. The range of cropping systems investigated with P-NMR has been limited to date, as have the countries in which it has been used to characterize soil P. There is a particular need to characterize P forms and their dynamics in tropical soils, which sorb P more strongly, and where there are greater concerns with respect to food security [28].

Conclusions

Since its first use in soil science in 1980, P-NMR has been a valuable tool to characterize P forms. In agriculture, there is recognition of the need to improve P use efficiency, arising from concerns about eutrophication from soil P loss and the long-term sustainability of rock phosphate stores from which chemical fertilizers are made. The prior publications reviewed here show that P-NMR can be a valuable tool in studies to improve agricultural P use efficiency, when conducted properly to maximize quantitative information. However, P-NMR studies to date have been limited with respect to cropping systems and geographic locations. Therefore, further research using P-NMR is needed globally on a wider range of crops to fully understand the dynamics in soil of this valuable crop nutrient.

Competing interests
The author declares that she has no competing interests.

Consent for publication
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