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Characterizing phosphorus in environmental and agricultural samples by ^{31}P nuclear magnetic resonance spectroscopy

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Abstract

Phosphorus nuclear magnetic resonance (^{31}P-NMR) spectroscopy has advanced our knowledge of organic phosphorus (P) in soils and environmental samples more than any other technique. This paper reviews the use of ^{31}P-NMR spectroscopy for soil, water and other environmental samples. The requirements for a successful solid-state or solution ^{31}P-NMR experiment are described, including experimental set-up, sample preparation, extractants, experimental conditions, and post-experimental processing. Next, the literature on solid-state and solution ^{31}P-NMR spectroscopy in environmental samples is reviewed, including papers on: methods; P transformations; agricultural, forest and natural ecosystem soil studies; humic acid and particle size separations; manure, compost and sludge studies; and water research, including freshwater, estuary and marine studies. Future research needs are also discussed as well as suggestions to improve results, such as increased standardization among research groups.

Keywords: Soil; Water; Sediment; Phosphorus; ^{31}P-NMR spectroscopy; Agriculture; Forestry

1. Introduction

From its first use on soil extracts by Newman and Tate (1980) [1], ^{31}P-NMR spectroscopy has substantially advanced our knowledge of organic phosphorus (P) in environmental samples.

The objective of this paper is to review the use of ^{31}P-NMR spectroscopy for soil, water and other environmental samples. The requirements for successful ^{31}P-NMR experiments with environmental samples will be discussed, followed by a review of the knowledge of P forms and cycling gained by the use of ^{31}P-NMR spectroscopy. It is assumed that the reader understands the basic principles of NMR spectroscopy. More detailed descriptions are available in textbooks (e.g. [2,3]) and review articles [4–10]. For a recent review of P forms and cycling, please see the book by Turner et al. [11] and papers, therein.

The ^{31}P-NMR signals of P compounds (both organic and inorganic) of interest in environmental studies generally fall between 25 and −25 ppm (Fig. 1). These includes: phosphonates, with a C–P bond, at 20 ppm; orthophosphate at 5–7 ppm; orthophosphate monoesters, with one C moiety per P, at 3–6 ppm; orthophosphate diesters (two C moieties per P), including phospholipids and deoxyribonucleic acid (DNA), at 2.5 to −1 ppm; pyrophosphate at −4 to −5 ppm; and polyphosphate at −20 ppm. Although rarely reported for environmental samples, a peak for the terminal P group in the polyphosphate chain should also be present at −4 to −5 ppm (Fig. 1). More information about these P forms can be found elsewhere in this issue, including the paper by Turner et al. [12]. Because ^{31}P is the only naturally occurring P isotope (100% natural abundance), all P species within a sample can potentially be detected by NMR spectroscopy. Thus, if the appropriate acquisition parameters are chosen, the area under each peak is proportional to the number of that particular type of P nucleus, allowing NMR spectroscopy to quantitatively identify different P forms in a sample. Fig. 2

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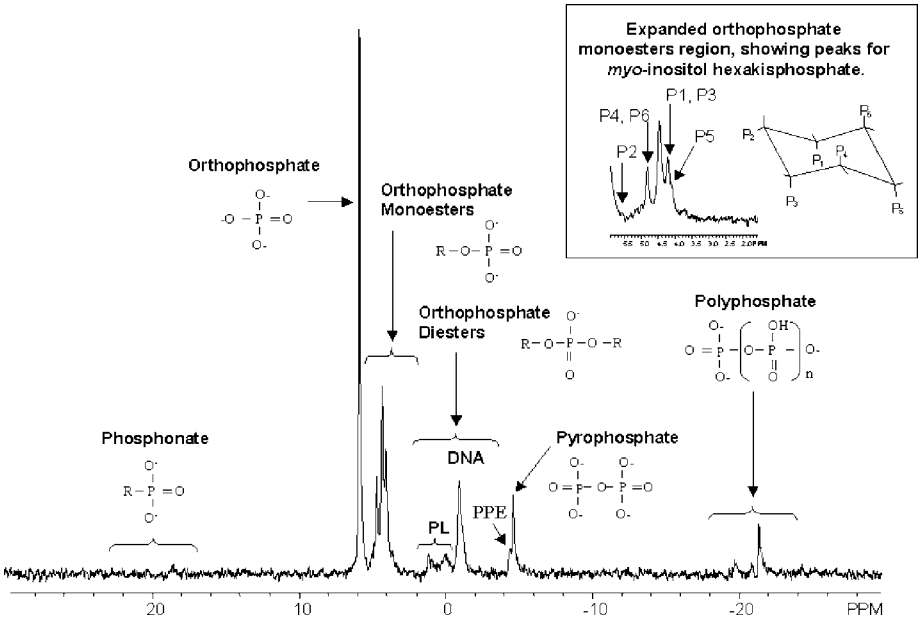

Fig. 1. A solution ^{31}P-nuclear magnetic resonance (NMR) spectrum of a forest floor sample extracted with NaOH–EDTA [24], produced on a Varian Unity ^{INOVA} 500 MHz spectrometer equipped with a 10 mm broadband probe, using a 90° pulse, 0.68 s acquisition, 4.32 s pulse delay, 25 °C temperature, and 7 Hz line-broadening. This spectrum shows the diversity of P species in natural samples, including phosphonates, orthophosphate, orthophosphate monoesters, orthophosphate diesters such as phospholipids (PL) and deoxyribonucleic acids (DNA), pyrophosphate and polyphosphate, with the terminal P in the polyphosphate chain indicated by PPE. The inset shows the expanded orthophosphate monoesters region, indicating the peaks and structure for *myo*-inositol hexakisphosphate (phytic acid).

shows solution ^{31}P-NMR spectra for several extracted environmental samples. These include: forest floor material from grass in an oak-grass savannah in California; an alkaline agricultural soil from Idaho; marine sediment trap material from Monterey Bay, California; a sewage sludge sample from England; and a humic acid extracted from an Australian soil. This gives a good idea of the variation in P forms, and their relative proportions, in environmental samples.

2. Requirements for a successful ^{31}P-nuclear magnetic resonance spectroscopy experiment

To maximize the information obtained from a ^{31}P NMR experiment, it is important to obtain spectra with good resolution and a high signal-to-noise ratio, where spectral resolution describes the sharpness and separation of the peaks and the signal-to-noise ratio (S/N) is the peak height relative to baseline noise. Although these are important concerns for all NMR experiments, they are especially important during ^{31}P-NMR analysis of environmental samples, which are more complicated than studies of purified compounds due to the relatively low concentration of P in natural samples, the range of P species that are usually present, and the natural association of P with paramagnetic ions such as iron (Fe) and manganese (Mn). Analytical conditions must be carefully selected to obtain reliable, quantitative results.

2.1. Experimental set-up

The first choice is whether to use a solid sample (solid-state ^{31}P-NMR) or an extract (solution or liquid-state ^{31}P-NMR). Solid-state ^{31}P-NMR allows samples to be examined directly with minimal preparation or alteration and requires only small sample sizes. However, the technique is limited by the low natural concentrations of P. For example, Kolowith and Berner [13] could only obtain spectra from shale samples with P concentrations of 25 μmol g^{-1} or higher. Spectral resolution is also poor in solid-state ^{31}P-NMR spectra compared with solution ^{31}P-NMR. One reason for this is chemical shift anisotropy, which is the orientation-dependent chemical shift. Nuclei in solutions are in rapid motion, which averages the shielding factor to a single value. In solids, nuclei are fixed in all possible orientations. Shielding is therefore not uniform, and will depend on the positions of the nuclei in the sample and within the magnet. This produces small changes in chemical shifts for nuclei with the same chemical structure, resulting in broad peaks in solid-state spectra relative to solution spectra. This difference in resolution can be seen in Fig. 3, which shows ^{31}P-NMR spectra of a culture-grown algal species, *Thalassiosira weissflogii*. The sample was first analyzed by solid-state ^{31}P-NMR (Fig. 3A, top spectrum), and then was extracted with sodium hydroxide (NaOH) plus ethylenediaminetetraacetic acid (EDTA) and analyzed by solution ^{31}P-NMR (Fig. 3A, lower spectrum). The spectra are plotted on the same scale; the spinning side bands of the solid-state spectrum are outside this spectral

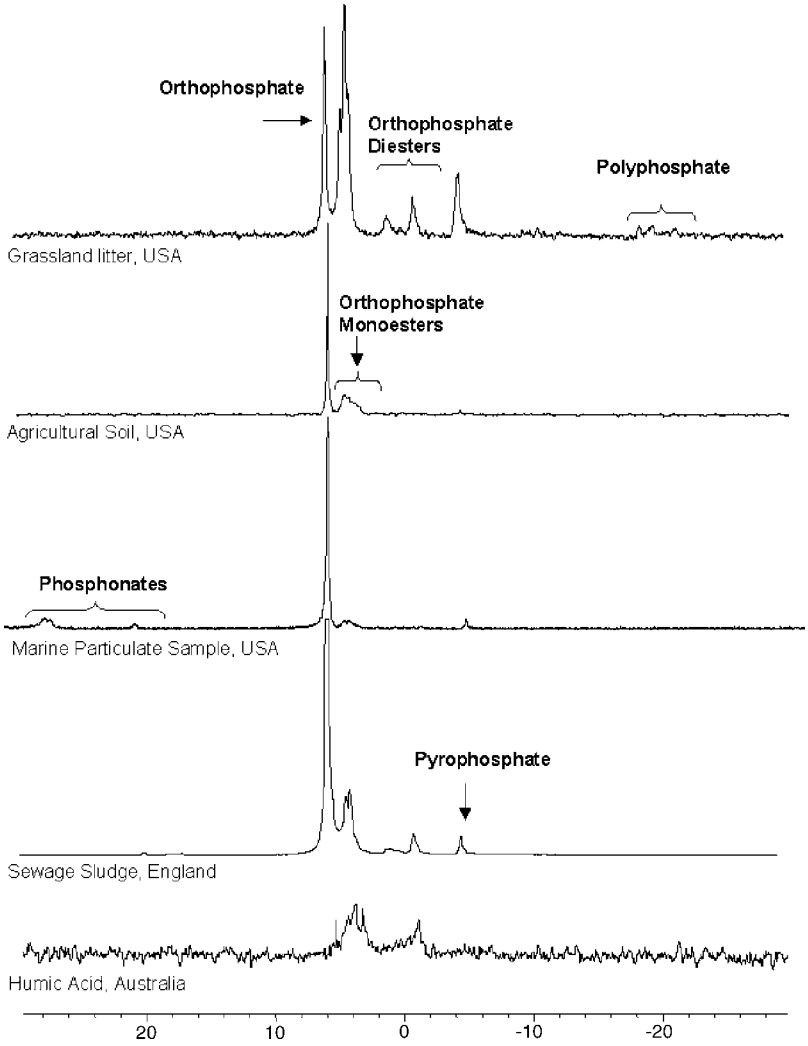

Fig. 2. ^{31}P-NMR spectra of a range of environmental samples. These include: forest floor material from under grass in an oak-grass savannah in California (sample from B. Cade-Menun); an alkaline agricultural soil from Idaho (D. Strawn, University of Idaho); a marine particulate sample from Monterey Bay, California (A. Paytan, Stanford University); a sewage sludge sample from England (M. Smith, Bournemouth University); and a humic acid extracted from Australian soil (N. Mathers, Griffith University). The humic acid was treated with Chelex, while the other samples were extracted with NaOH–EDTA [24]. Spectra were generated by B. Cade-Menun on a Varian Unity ^{INOVA} 500 MHz spectrometer equipped with a 10 mm broadband probe, using a 90° pulse, 0.68 s acquisition, 4.32 s pulse delay and 20–25 °C temperature.

window. The peaks of the solid-state spectrum are broad, each of which overlaps the chemical shifts of several P nuclei. In contrast, peaks in the solution spectra are narrow, allowing P nuclei to be more easily identified. However, peak identification and quantification in solid-state spectra can be improved with processing tools such as spectral deconvolution (Fig. 3B), which is discussed in more detail in Section 3.3.

Sample preparation for solid-state ^{31}P-NMR can be as simple as drying and grinding [13]. Solid-state soil studies have also used dried extracts of soils, in an attempt to remove paramagnetic ions with reagents such as dithionite [14,15], diethylenetriamine pentaacetate (DTPA) [16], or oxalate [17].

For solution ^{31}P-NMR, solid samples must first be extracted. A discussion of the effects of extraction on soil organic P can be found elsewhere in this issue [12]. As with any extraction procedure for soil organic P, there is always the risk of hydrolysis [18]. Thus, extraction times should be minimized where possible. There is little agreement on the most suitable extractant, but those in use include: NaOH [1,19], the cation exchange resin Chelex in water [20,21], NaOH plus Chelex [22], NaOH plus sodium fluoride (NaF) [23], and NaOH plus EDTA [18,24]. These extractants solubilize different concentrations and forms of P from soils [4,25]. Chelex and EDTA are both used to release P from paramagnetic ions, thereby reducing line broadening and improving spectral quality. Chelex is removed after extraction, which removes paramagnetic ions from solution, but may also remove polyphosphate [22–24]. Sodium hydroxide plus EDTA extracts more P than Chelex in water or NaOH, but Fe and Mn remain in solution complexed with EDTA [24,26]. This

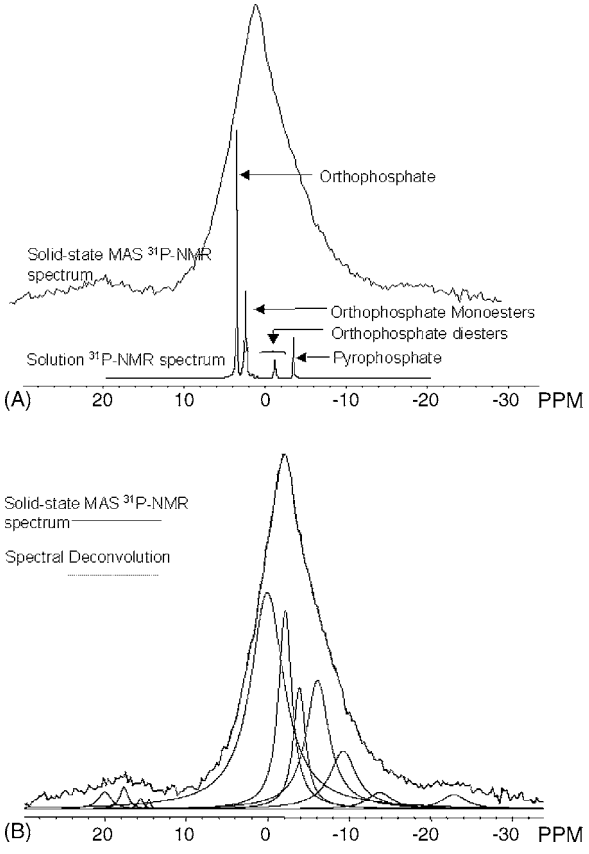

Fig. 3. A comparison of solid-state and solution ^{31}P-NMR spectra for the same samples. (A) Solid-state MAS and solution ^{31}P-NMR spectra (after extraction with NaOH–EDTA [24]) of cultures of the algae *T. weissflogii*. A. Paytan (Stanford University) provided the algal samples. J. Stebbins (Stanford University) produced the solid-state spectra on a 400 MHz Varian VRX/Unity spectrometer with a CPMAS probe and a spinning speed of 6.8 kHz. B. Cade-Menun produced the solution spectra on a Varian Unity INOVA 500 MHz spectrometer equipped with a 10 mm broadband probe, using a 90° pulse, 0.68 s acquisition, 4.32 s pulse delay and 25 °C temperature. (B) The same solid-state spectrum, but with peaks delineated with spectral deconvolution.

hastens relaxation (explained in Section 3.2), but may increase line broadening [25].

Pre-treatments prior to extraction have been used to lower the paramagnetic ion concentration or to increase the P concentration. Such pre-treatments include acid [20,26–28] and dithionite [29,30]. Post-extraction treatments include dialysis [31,32] or exchange resins such as Chelex and Sephadex [33–38].

Solutions are concentrated after extraction by either lyophilization (e.g. [25,39,40]), a stream of nitrogen at 40 °C [41–43], or rotary evaporation [20,37,42,43]. However, given the risk of hydrolysis with higher temperatures [18,25], lyophilization may be the safest method. Water samples have been concentrated by tangential-flow ultrafiltration [46–50].

Samples need to be re-dissolved for solution ^{31}P-NMR, with the final volume determined by the active volume of the NMR probe. For a 10 mm probe (the most common size used for environmental ^{31}P-NMR), the final volume should be 2–3 ml. The amount of dried material that can be redissolved will vary with sample. Sufficient sample should be added to optimize the P concentration in the NMR tube. However, care should be taken to keep the sample from becoming viscous, which will increase line-broadening as molecular tumbling slows. Samples may be redissolved in water, sodium deuteroxide (NaOD) [23], deuterium oxide (D_2O) plus NaOH (e.g. [25]), or deuterated dimethylsulphoxide [30]. Deuterium oxide is used for a signal lock in the spectrometer, and can be added to the sample solution during sample preparation, or in a capillary tube in the NMR tube with the sample [48]. Sodium hydroxide and NaOD are used to adjust the pH to >10 for optimal spectral resolution [30,48,51]. Redissolved samples may be filtered or centrifuged prior to decanting into NMR tube. To prevent hydrolysis, samples should be analyzed as soon as possible after dissolution [12].

2.2. Experimental considerations

2.2.1. Solution ^{31}P nuclear magnetic resonance spectroscopy

High field super-conducting magnets are used for solution P-NMR of environmental samples. The field strength (B_0) of a magnet is usually designated in terms of the frequency of ^{1}H resonance. Thus, a 300 MHz spectrometer has a 7.05 T magnet, a resonance frequency of 300 MHz for ^{1}H, and a resonance frequency of 121.4 MHz for ^{31}P. As B_0 increases, the signal-to-noise (S/N) ratio will increase by $B_0^{3/2}$. Therefore, an NMR experiment using a higher field magnet should produce better S/N than a lower field magnet for the same sample and experimental parameters. However, chemical shift anisotropy will also increase with increasing magnetic field strength. Thus, the increased sensitivity of a spectrometer greater than 600 MHz for ^{31}P-NMR experiments will be offset by a loss of resolution [3].

A probe that can be tuned to the resonance frequency for ^{31}P is also required. This may be a broadband probe, that can be tuned to a range of frequencies, or a quadruple nucleus probe, where the frequencies of four nuclei (^{1}H, ^{13}C, ^{31}P and ^{19}F) are preset. For the same sample and experimental parameters, there should be no difference in the results from these two probe types. Of greater importance is the diameter of the probe, which will determine sample volume. Most broadband or quadruple nucleus probes are 5 mm or 10 mm in diameter. The increased volume by using a 10 mm probe rather than a 5 mm probe will increase the sample P concentration by a factor of 4, requiring 4 times fewer scans for the same sample in the larger tube, and thus reducing the length of the NMR experiment. Most environmental ^{31}P-NMR experiments have used a 500 MHz magnet with a 10 mm broadband probe, although some studies (e.g. [18]) have used 5 mm probes in 600 MHz magnets. However, the higher number of scans needed with smaller diameter probes increases the length of the experiment and thus the risk of hydrolysis.

The range of peaks seen in the spectrum depends on the spectral window. The peak shifts of P forms of interest

in environmental studies generally fall between 25 and −25 ppm (Fig. 1), so the spectral window is set to 50 ppm, centered at 0 ppm. Acquisition times used for environmental ^{31}P-NMR experiments range from 0.17 s for 8000 data points [52] to 1.31 s and 32,000 data points [53]. Pulse angles between 30 and 90° have been used for environmental ^{31}P-NMR, with 90° the most common.

The delay between pulses must be long enough to allow complete relaxation of P species if quantitative results are desired. Cade-Menun et al. [25] determined T_1 values for P species in several extractants, using soil and forest floor samples that were high in Fe and Mn. When the extractant was NaOH–EDTA, T_1 values ranged from 0.137 to 0.918 s. In contrast, when Chelex plus NaOH was the extractant, removing Fe and Mn from solution, T_1 values increased to 0.311–3.1 s. The general rule is that a delay time of $5 \times T_1$ will allow a return to 99.3% of equilibrium [2]. Thus, Cade-Menun et al. [25] suggest that delay times of 1–2 s are adequate for most samples, with longer delay times required for samples from which all paramagnetic ions have been removed (e.g. Chelex-treated samples), or for samples naturally low in paramagnetics, such as calcareous soils and manures [54]. Delay times used for environmental ^{31}P-NMR have ranged from 20 s [1] to 0.2 s [55]. However, there have been no published papers demonstrating that delay times of less than 1 s are adequate for complete relaxation of all P species. Therefore, the results from studies using short delay times may not be quantitative.

To shorten delay times, lanthanide shift reagents have been added to water samples [46]. There has been no reported use of lanthanide shift reagents for soil ^{31}P-NMR experiments. Delay times may also be shortened by using pulse angles of less than 90°, so that the system remains closer to equilibrium at all times [3,6]. However, this will produce a smaller signal per pulse, requiring more scans to achieve the same S/N that would be obtained with a 90° plus and longer delay [3].

About half of the environmental ^{31}P-NMR experiments reported in the literature use proton decoupling to remove scalar coupling of protons to P. This is done with an additional radio-frequency pulse at the frequency of protons, which is inverse-gated, or switched off, during the delay between pulses in order to suppress the nuclear Overhauser enhancements that can distort relative signal area. A detailed comparison of peak intensities with and without proton decoupling has not been done for environmental ^{31}P-NMR, although Turner et al. [18] showed splitting of a phosphonate peak without decoupling, and a single peak with decoupling. As P is not directly bonded to protons, differences in quantification from the use of proton decoupling are likely to be minor.

The use of spinning during sample acquisition is seldom reported for solution environmental ^{31}P-NMR experiments. However, because spinning helps to optimize the magnetic field by averaging field inhomogeneities along the radial (x and y) axes, it can be a simple way to decrease the line broadening observed in many environmental ^{31}P-NMR spectra. Low spinning speeds (10–15 Hz) should be used to avoid spinning side bands.

The number of scans that have been collected during environmental ^{31}P-NMR experiments range from 500 [37] to 110,000 [22]. The number of scans is determined to some extent by the desired S/N, the time required for each scan, the concentration of P in the probe (and therefore, the diameter of the NMR tube used), and the cost and availability of NMR spectrometer time. Signal-to-noise increases as the square root of the number of acquisitions, so four times the number of acquisitions are needed to double the S/N. However, collecting high numbers of scans is not always practical, and brings an increased risk of hydrolysis; using a higher field spectrometer or a wider diameter probe are better ways to improve S/N.

Due to the risk of hydrolysis of some organic P compounds, temperature should be controlled at 20–25 °C, especially for long ^{31}P-NMR experiments [18,25,51]. Temperature will also change the relaxation of nuclei, affecting chemical shift and relative signal intensity [18,51].

2.2.2. *Solid-state ^{31}P nuclear magnetic resonance spectroscopy*

Most solid-state ^{31}P-NMR studies of environmental samples have used cross polarization magic angle spinning (CPMAS) NMR techniques. Magic angle spinning involves spinning the sample rapidly at the magic angle of 57°7′ to B_0. This is called ‘magic’ because at this angle chemical shift anisotropy and dipole–dipole interactions are averaged to zero [6]. Cross-polarization uses a pulse sequence to transfer magnetization from protons to P nuclei, to improve S/N and shorten T_1 relaxation, which can be long in solid samples. A few solid-state ^{31}P-NMR studies of environmental samples have been conducted using Bloch decay experiments and magic angle spinning (MAS), with and without high power decoupling [16,56,57]. Bloch decay uses a single pulse sequence to excite all nuclei in the sample uniformly. There have been no detailed comparisons of cross polarization and Bloch decay experiments for ^{31}P-NMR studies of environmental samples. However, tests with marine sediment trap samples by Benitez-Nelson et al. [58] suggest that Bloch decay is a better choice due to the loss of CP efficiency at the high spinning speeds necessary to position the spinning sidebands outside the chemical shift window of interest. Due to this loss of efficiency, the CP spectrum required the acquisition of four times the number of scans to achieve the same S/N as the Bloch decay experiment.

In contrast to solution ^{31}P-NMR, high field magnets are not advantageous for solid-state ^{31}P-NMR spectroscopy, because increasing the magnetic field increases the strength of spinning sidebands, possibly obscuring features of the spectrum [10]. Solid-state ^{31}P-NMR studies of environmental samples predominantly use 300 or 400 MHz spectrometers (121 or 162 MHz for ^{31}P), spin rates of 5–10 kHz, and spectral widths as wide as 400 ppm (200 to −200 ppm). Experiments have collected as many as 170,000 scans, and have lasted as

long as 48 h. An external standard of 85% H_3PO_4 is used, and hydrolysis from high temperatures is only a concern if the sample is not thoroughly dried prior to analysis.

2.3. Post-experimental processing

The end result of an NMR experiment is the emission signal, which is detected and recorded as a free-induction decay (FID). A free-induction decay is a time-domain signal, because it is recorded as a function of intensity over time. It is converted to a frequency-domain spectrum, with signal intensity plotted as a function of frequency, by Fourier transformation using processing software. Frequency is expressed as chemical shift, in parts per million (ppm), relative to an external standard, with the external standard set at 0 ppm. For solution ^{31}P-NMR, 85% H_3PO_4 is used as an external standard. For many P compounds, particularly orthophosphate, the degree of protonation will vary with pH. The observed chemical shift will be the average of contributions from differently protonated forms of the same P compound. Thus, the chemical shift of these P compounds will change with pH, as is clearly demonstrated by Crouse et al. [51]. Thus chemical shift of orthophosphate in an alkaline soil extract will be downfield from that of orthophosphate in the reference (6 ppm versus 0 ppm). Because chemical shift is relative to a standard and is pH-dependent, it is important to know the pH and standard used when comparing chemical shifts from different studies. The solvent may also be important, for Carman et al. [30] observed chemical shift differences when using deuterated dimethylsulphoxide instead of D_2O for the signal lock.

Other important processing tools are phasing, baseline correction and line-broadening. Phasing removes artifacts from Fourier transformation, and should produce symmetric peaks flanked by flat baselines. Line-broadening uses an exponential multiplication factor, in hertz, to reduce noise and improve S/N. As demonstrated in [4], if the line-broadening is too low, noise reduction is inefficient and S/N will be low; if too high, useful data may be lost. Line-broadening of 10–20 Hz is common for solution ^{31}P-NMR of environmental samples (e.g. [24]), although very clear spectra may use line-broadening of as little as 0.5 Hz [59]. Solid-state samples may require 50–200 Hz line-broadening.

Both an automatic peak-picking routine in the processing software and visual inspection are used to identify peaks. It can be difficult to separate some smaller peaks from background noise for many spectra. Chemical shifts are compared with literature reports to identify P species and compounds. However, when identifying peaks from literature reports, comparisons should only be made with experiments using the same pH. Table 1 shows some of the P compounds identified in solution ^{31}P-NMR studies of environmental samples, while Table 2 shows peak shifts for P minerals determined by solid-state spectroscopy. The overlap of peak shifts for biological and mineral P compounds must be noted. This may complicate the identification of P species in solid-state spectroscopy, because both biological and mineral P forms may be present. To further confirm peak shifts, standards such as methylene diphosphonic acid may be added directly to the sample or included as capillary tube inserts in solution ^{31}P-NMR (e.g. [66]). Nuclei that are equivalent magnetically, such as the two P nuclei in pyrophosphate (P_2O_7), will show only a single peak.

If the ^{31}P-NMR experiment was run carefully, with suitable acquisition parameters, then the area under each peak is proportional to the amount of the total sample P found in each P species. Using an integration routine in the processing software, the entire spectrum is integrated, and the integral is then divided into regions representing each peak. After printing the spectrum and integral, the height of the integral for each peak is measured manually with a ruler, and the proportion as a percent of the total height of the integral is determined, which is the percent of total sample P in each P species [4]. Processing software may also be used to determine the value of the integral for each peak, but this only works well for spectra with high S/N and high spectral resolution. For most environmental ^{31}P-NMR spectra, manual determination of areas is necessary.

It can be difficult to determine peak intensities if peaks overlap, but spectral deconvolution, also known as line-fitting, (Fig. 3B) can be used to separate broad peaks into their components, such as in the orthophosphate monoester region of solution [67], or solid-state ^{31}P-NMR spectra [14,57,62]. The deconvolution procedure is part of the processing software, and requires inputs from the operator with respect to the number, shape and chemical shift of deconvoluted peaks. After deconvolution, the calculated spectrum should be carefully compared with the original spectrum. For solution ^{31}P-NMR spectrum, spectral deconvolution works best for very small regions of the spectrum, such as separating orthophosphate monoester peaks to identify *myo*-inositol hexakisphosphate [67]. For solid-state ^{31}P-NMR spectra, as indicated by Fig. 3B, deconvolution may indicate the presence of a number of peaks under one very broad peak. Care should be taken when identifying and quantifying these peaks, and in the subsequent interpretation of results. Peak identification may be more reliable when solid-state ^{31}P-NMR and deconvolution is paired with another technique such as sequential extraction (e.g. [68]).

3. ^{31}P-NMR studies of environmental samples

This section summarizes the literature for ^{31}P-NMR spectroscopy in environmental samples. Due to space constraints, studies of compounds with chemical shifts outside the spectral width of biological P compounds, such as pesticides [64] or biological ^{31}P-NMR studies such as in vivo NMR (e.g. [69]) or mycorrhizal studies (e.g. [70]) are not included. Papers are grouped based on the nature of the research; some papers will fall into more than one group. Care should be taken when comparing the results among studies using different

Table 1
Chemical shift references for alkaline extracts (pH 12) of biological P compounds. Bold type indicates general peak shift ranges; regular type indicates specific chemical shift assignments

Chemical shift (ppm)	Compound	Reference
20	Phosphonates	e.g. [1,19,24]
20	Aminoethyl phosphonates	[18]
18	Phosphonolipids	[25]
12–14	Aromatic phosphonic acid esters	[18]
7.4	Aromatic diesters	[18]
5.7–6.1	Orthophosphate	e.g. [1,19,24]
6–3	Orthophosphate monoesters	e.g. [1,19,24]
5.85, 4.92, 4.55, 4.43	Myo-inositol hexa*kis*phosphate (phytic acid)	[18]
5.4	Glucose-6-phosphate	[18,37]
4.78–4.32	Mononucleotides	[18]
4.85	ß-glycerophosphate	[18]
4.71	Ethanolamine phosphate	[18]
4.14	*Scyllo*-inositol hexa*kis*phosphate	[60]
4.05	Choline phosphate	[17]
3.2	Glucose-1-phosphate	[17]
2.5 to −1.0	Orthophosphate diesters	e.g. [1,19,24]
2.5 to 1.2	Teichoic acids	[26,61]
1.75	Phosphatidyl ethanolamine	[18]
1.57	Phosphatidyl serine	[18]
0.78	Phosphatidyl choline	[18]
0.54	RNA	[18]
0	DNA	[26,61]
−0.37	DNA	[18]
−4	Polyphosphate terminal P group	[18]
−4 to −5	Pyrophosphate	e.g. [1,19,24]
−10	ATP or ADP α-phosphate	[18]
−19 to −21	Polyphosphates	e.g. [1,19,24]
−19.68	ATP β-phosphate	[18]

analytical procedures, including pre-treatments, extractants, or delay times, as these can all affect spectra [24,25].

3.1. Methodological papers

Methodological studies have investigated a number of aspects of environmental ^{31}P-NMR As noted above, a number of extractant have been tested, including: NaOH [1], Bu_4NOH [71], Chelex in water [20,72], NaOH plus Chelex [22,24,25], KOH plus Chelex [33] and NaOH plus EDTA [24,25,59]. Pre-concentration [73], sequential extractants [53,74] and fractionation by gel filtration [37] have also been tested. Peak assignments have been tested by analyzing known compounds [1,18,20,24,27,37,56,57,6–65,75]. Other studies have examined relaxation times [1,25] and the effects of pH [51], temperature [18,25,51], stor-

Table 2
Chemical shift references for P compounds from solid-state ^{31}P-NMR spectroscopic studies

Chemical shift (ppm)	Compound	Reference
9	Dicalcium phosphate dihydrate ($Ca_2HPO_4{\cdot}2H_2O$)	[57,62]
3	Hydroxyapatite [$Ca_5(PO_4)_3OH$]	[63]
3	Octacalcium phosphate [$Ca_8H_2(PO_4)_6{\cdot}5H_2O$]	[63]
−0.4	$Na_4P_2O_7$	[64]
−2	Monetite ($CaHPO_4$)	[63]
−5	Crandallite [$(CaAl_3(OH)_5(PO_4)_2$]	[56]
−8	$Na_3HP_2O_7$	[64]
−9.9	$Na_2H_2P_2O_7$	[64]
−10.2	Brazilianite [$NaAl_3(OH)_4(PO_4)_2$]	[56]
−11	Wavellite [$Al_3(OH)_3(PO_4)_2{\cdot}5H_2O$]	[56]
−13.2	Metavariscite ($AlPO_4{\cdot}2H_2O$)	[65]
−16	Senegalite [$Al_2(OH)_3(PO_4){\cdot}H_2O$]	[56]
−19	Variscite ($AlPO_4{\cdot}2H_2O$)	[56]
−20	Lazulite [$MgAl_2(OH)_2(PO_4)_2$]	[56]
−21.7	Metaphosphate ($Na_6P_6O_{18}$)	[64]
−25	Berlinite ($AlPO_4$)	[56]
−30	Augelite [$Al_2(OH)_3PO_4$]	[56]

age [1,18] and spectral deconvolution during processing [67].

3.2. Phosphorus transformation

Laboratory incubations have been used to examine P transformations. When sewage sludge was incubated with acid and alkaline soils [27], orthophosphate diesters decreased and orthophosphate monoesters increased. However, the opposite was observed when beech leaf litter was incubated with minerals, including Fe-oxide, Al-hydroxide, birnessite and quartz sand [76]. In this case, orthophosphate monoesters decreased and orthophosphate diesters increased, suggesting microbial immobilization of P, which was also observed by Makarov et al. [28] during soil incubations. The organic P composition of soil extracts was more similar to that of microorganisms than plants [77]. However, the ratio of orthophosphate monoesters to orthophosphate diesters is not a reliable indicator of the origins of soil organic P [77]. ^{31}P-NMR spectroscopy has also been used to monitor P transformations after soils were incubated with alkaline phosphatase, acid phosphatase, phosphodiesterase and phytase [33], and to monitor P mineralization in soils amended with poultry litter [78] and sewage sludge [27].

3.3. Studies of agricultural, forest and natural ecosystems

Next grouped are the studies using whole-soil extractions or solid-state ^{31}P-NMR spectroscopy to survey P forms of agricultural, forest and natural ecosystems. Solution ^{31}P-NMR is generally used to look at the relative proportions of P forms, especially organic P, with respect to soil properties, climate, or disturbance. Soil depth has a strong influence on soil P, due to changes in physical and chemical properties. Concentrations of organic P forms are generally higher at the surface of soils in forests and undisturbed ecosystems, and decrease with depth [79,80]. In forests, the greatest diversity of P forms is in the forest floor, while orthophosphate predominates in mineral horizons [22,35,75,81]. In agricultural soils, a decrease in orthophosphate diesters with depth was observed under coffee plantations in Brazil [82], and no change with depth was seen for Brazilian [36] and Chilean soils [83].

Soil pH can affect soil P speciation by its influence on soil chemistry, the soil microbial population, organic matter decomposition, and the extractability of P compounds. Lower pH favors a wider variety of P forms [19], especially phosphonates [41]. Orthophosphate monoesters were negatively correlated to pH and free Fe in acidic to neutral forest soils in Spain [42,84] and in acidic soils after sewage sludge application [27], suggesting stabilization of these P forms by sesquioxides. In high-pH soils, P is very tightly bound, and extraction recovery rates are often lower than in acid soils [54,85].

Moisture and precipitation will also increase soil organic P. Organic P forms are predominant in wetland soils [34,37,86]. Concentrations of orthophosphate diesters and phosphonates increased along moisture gradients in New Zealand [26], Spain [4] and Canada [35,87], and organic P forms were correlated with precipitation in soils from semi-arid regions in the U.S.A. [54]. However, no differences with moisture were observed in a tropical rainforest catena [88].

Increases in organic P forms such as orthophosphate diesters with acidic pH or increased moisture are thought to be due to lower decomposition rates or a reduced microbial population. However, orthophosphate diesters have also been positively correlated with microbial biomass, such as in pasture soils in the UK [89]. This apparent paradox is due to the heterogeneity of orthophosphate diesters as a group: Makarov et al. [28] demonstrated that phospholipids and teichoic acid accumulate in microbially active soil, while DNA accumulates in soils with low microbial activity. This emphasizes the importance of optimal parameters for ^{31}P-NMR of soils, so that all P species can be clearly identified and quantified.

Soil disturbance, whether natural or anthropogenic, has a strong effect on soil P speciation. Anthills in deciduous forest of Denmark preferentially accumulated organic P forms [90], while no significant differences in P forms were observed between conventional and no till systems in subtropical Brazil [36]. In forests, harvesting increased orthophosphate in surface soils, and increased orthophosphate monoesters with depth [22]. Fire, as a strong mineralizing agent, converts the predominantly organic P forms in forest floor material to orthophosphate [21,72,91]. This orthophosphate is converted back to organic P forms with time, by uptake by trees and conversion to organic forms in foliage, which is returned to the forest floor.

Fertilization often increases orthophosphate and decreases the diversity of P forms for both inorganic fertilizers [19,92,93] and animal manure [66,94], but no differences in P forms were observed in fertilization trials of *Pinus radiata* in New Zealand [95]. Fertilization may also increase organic P forms, especially orthophosphate monoesters [74], although this can depend on soil texture [53]. For manures, orthophosphate concentrations were higher in subsurface soils when dairy manures were applied to high-pH Idaho soils in liquid form from lagoons than in solid form [85]. Phosphorus pollution from a factory increased orthophosphate at depth, but increased orthophosphate monoesters in surface soils, due to increased incorporation of P into plant litter [96], while atmospheric N deposition increased organic P forms in soils from northern England [97].

In New Zealand, application of manure increased organic P forms in soil leachate [98]. Although P in the applied manure was predominantly orthophosphate, P in the leachate was almost all in organic forms. This P was reactive, and could be hydrolyzed with enzymes [98]. Phosphorus in leachates under natural forest floor was also predominantly in organic form [99], with some seasonal variation. These

studies clearly demonstrate that organic P forms in soil can be quite mobile.

Land use change usually alters the relative proportions of orthophosphate monoesters, diesters and orthophosphate. When forests and woodlands were converted to croplands, organic P forms were reduced and orthophosphate increased in soils in Denmark [31], Ethiopia [55], Canada [87], Mexico [100] and Tanzania [94], but no changes in P forms were observed in Khyrgyzia [44] or Brazil [101]. When conifers were planted into grasslands in New Zealand, organic P forms decreased and orthophosphate increased [21,102]. However, conversion of cropland in tropical Cameroon soils to perennial stands of Eucalyptus and tea (*Camellia sinensis*) resulted in only minor differences in P forms, which were predominantly orthophosphate monoesters in all soils [103]. Higher mineralization rates in pasture soils relative to forests were reported in oxisols in Brazil [101], but the reverse was observed in Australia, with lower mineralization of added RNA in pasture than forests soils [104]. Brazilian soils planted with legumes had increased ratios of orthophosphate diesters to orthophosphate monoesters relative to grass-covered soils [105].

Solid-state ^{31}P-NMR spectroscopy of excessively fertilized sandy soils in Belgium identified Ca–P and Al–P forms, which were partially removed by extraction with either water or dithionite, and fully removed by oxalate extraction [14,17]. The predominant P form in calcareous agricultural and marsh soils was identified as hydroxyapatite, a Ca–P compound [15]. Hydroxyapatite was also identified in sludge-amended soils in California, as well as pyrophosphate [106]. In agricultural soils from England, acid soils contained more Al-P forms, while higher pH soils (>5.8) contained more Ca–P forms [67]. Water was shown to preferentially extract mobile Ca–P forms, while 0.01 M $CaCl_2$ extracted mobile Al–P forms [57]. No attempt was made to characterize organic P forms in any of these studies. In contrast, Kolowith and Berner [13] characterized only organic P forms in black shales using ^{31}P-NMR spectroscopy. They demonstrated that orthophosphate esters were the dominant P forms in all weathering stages, which suggests that organic P forms any be resistant to weathering.

3.4. Particle size separations and humic substances

Soil samples can be separated into different fractions based on particle size: sands (0.05–2 mm), silt (0.002–0.05 mm) and clay (<0.002 mm). Smaller soil particles are more reactive, due to increased surface area. Wet or dry sieving may be used for particle size separations, or clays can be flocculated with $CaCl_2$ or $MgCl_2$.

The relationship of particle size to P form is varied. Organic P forms in general increased with decreasing particle size in forest and cropland soils in Denmark and Germany [31], Tanzania [94] and Caucasus [107]. More specifically, orthophosphate diesters, including phospholipids and teichoic acid, were enriched relative to orthophosphate monoesters in the clay fractions of agricultural soils from Germany [93] and Denmark [108], grassland soils from North America [23], sub-humid Ethiopian highland soils [55] and volcanic soils from Chile [109]. In steppe soils from Russia, orthophosphate diester enrichment in clays increased as the ratio of mean annual precipitation to potential evaporation decreased [32]. In contrast, Turrión et al. [44] observed lower relative percentages of orthophosphate diesters than monoesters in the clay fraction of forest soils from Khyrgyzia, and no differences in size fractions in pasture soils, with orthophosphate monoesters the major P form in all soils. Caution should be used in the interpretation of any particle size separation results because the protocols used to separate size fractions may alter the subsequent P forms. Wet sieving can remove soluble inorganic and organic P forms [62,98,99], while chemical flocculation of clays may alter the extractability of P compounds. When Makarov et al. [110] pre-treated a flocculated clay fraction with acid to remove Ca, the concentration of both diesters and monoesters increased, but the monoester:diester ratio increased from 1.4 to 2.9.

Humic substances are high molecular weight, brown-to-black substances formed by secondary synthesis reactions in soil, and are distinct from the biopolymers of microbes and higher plants [111]. They are fractionated based on solubility: fulvic acid (FA) is soluble in acid and alkali; humic acid (HA) is insoluble in acid and soluble in alkali; and humin is insoluble in acid and alkali. Most of the extraction protocols for solution ^{31}P-NMR spectroscopy are analogous to humic substance fractionation, and employ the same alkali extractants, such as 0.1–0.5 M NaOH (e.g. [1,19]). However, humic acids are subsequently precipitated with acid to separate HA, which is precipitated, from FA, which remains in solution. Because humic substances differ from whole soils with respect to chemical properties, some studies have examined P forms in humic substances; these are grouped here.

In general, the range of P compounds in HA and FA is similar to that of whole soils (e.g. [112-114]). The relative proportion of orthophosphate is usually low in HA (Fig. 2), and varies with the pH at which HA is precipitated [115]. Inorganic P forms, including orthophosphate and pyrophosphate, are more common in FA [40,113], although polyphosphates were observed in HA of Siberian soils [116]. Orthophosphate monoesters are the dominant organic P form in both HA and FA [40,112], and HA usually contains more phosphonates and orthophosphate diesters [112-114]. The diversity of P forms in HA will vary with raw humus type [117]. There were more organic P forms in HA under intensive cropping of rice relative to aerated croplands [118], and few organic P forms in HA and FA from earthworm casts [40].

3.5. Manure, compost, sewage sludge and fertilizers

Phosphorus forms have been characterized in various types of manure, to improve their use as fertilizers and to

prevent eutrophication from excess P. Many of these studies were designed to optimize analytical parameters, such as characterization of phytic acid in pig feces and dietary samples [119], pH and temperature requirements to optimize spectral resolution in turkey manure extracts [51], and extractants for dairy and swine manure and poultry litter [59]. Solid-state ^{31}P-NMR spectroscopy of poultry litter showed a complex mixture of organic and inorganic P forms, most of which could not be identified, and a shift from Ca–P forms to Al–P forms after alum amendment [120]. When pig slurry from lagoons was separated into hydrophobic and hydrophilic fractions, only orthophosphate was detected by solution ^{31}P-NMR spectroscopy in the hydrophilic fraction, and no P forms were detected in the hydrophobic fraction [121]. Manure storage conditions can also affect P speciation: Hansen et al. [85] observed a greater relative proportion of phytic acid in solid dairy manure, and a more DNA in lagoon-stored dairy manure.

When urban waste compost was separated into hydrophobic and hydrophilic fractions, no P forms were detected with solution ^{31}P-NMR spectroscopy in the hydrophilic fraction, while poorly resolved peaks for organic and inorganic P forms were detected in the hydrophilic fraction, reflecting the low solubility of this material [121]. Solution ^{31}P-NMR spectroscopy showed that composed fish and crab scrap was predominantly orthophosphate, with traces of monoesters, diesters and pyrophosphate [122]. Solid-state spectra of the same material showed only one broad peak, in the orthophosphate region. Orthophosphate was also the major P form in mature composts from across Canada [123] and Switzerland [124]. Regardless of the input material, solution ^{31}P-NMR spectra of the Canadian composts looked remarkably similar to one another [123], as did the solid-state spectra from the Swiss study [124]. This suggests that the P forms in compost reflect the products of decomposition and microbial synthesis more than the P forms of the starting material.

Both solution and solid-state ^{31}P-NMR spectroscopy have been used to speciate P in sewage sludge samples. Both the digestion process and subsequent treatment of sludge will influence P forms. Alternating aerobic and anaerobic conditions in enhanced activated sludge systems affected P storage, with polyphosphates converted to orthophosphate and organic P (including orthophosphate monoesters and diesters) under anaerobic conditions, and back to polyphosphates under aerobic conditions [125–127]. These polyphosphates were located outside the cytoplasmic membrane, and were complexed with metal cations [128,129]. The carbon source added to the reactor is also important: microbes in reactors receiving glucose stored P as polyphosphates and nucleic acids while those in reactors receiving starch stored P as low-molecular weight polyphosphates [129]. The P in anaerobically digested sludge was almost entirely orthophosphate, while half of the P in aerobically digested sludge was organic, including orthophosphate monoesters, DNA and phospholipids [130]. The greatest diversity of P forms, including phosphonates and pyrophosphate, was detected in a commercially available, heat-treated, waste-activated sludge [130]. Hydrophilic fractions of an anaerobically digested sludge from Italy contained orthophosphate, orthophosphate monoesters and pyrophosphate; hydrophobic fractions of the same sludge contained orthophosphate monoesters, phospholipids and DNA [121]. The easy extraction of plant-available P forms such as orthophosphate into water indicates that this sludge will be an effective fertilizer. In contrast, sewage sludge from a Chilean wastewater treatment plant was predominantly orthophosphate [131].

Solid-state ^{31}P-NMR spectroscopy detected Ca-phosphates, Al-phosphates and pyrophosphates in anaerobically digested sludge from California, but only Ca-phosphates in an anaerobically digested and windrow composted sludge [132]. In contrast, only Ca-phosphates could be detected in activated sludge and two anaerobically digested and dewatered sludges from France [63].

Frossard et al. [133] investigated the effectiveness as a fertilizer of chitosan-polyphosphate, which is formed when chitosan is used to remove polyphosphates from wastewater. Solid-state ^{31}P-NMR spectroscopy revealed peaks for pyrophosphate and polyphosphate, which were subsequently biodegraded when incubated in soil. Solid-state ^{31}P-NMR spectroscopy was also used to assess the incorporation of the micronutrients Fe, Mn and Cu into synthetic hydroxyapatite to produce a slow-release fertilizer [16]. The lower signal intensity of the P spectra and reduced relaxation times resulting from the paramagnetic effects of Fe, Mn and Cu show that the micronutrients were incorporated into the hydroxyapatite.

3.6. Freshwater, estuary and marine studies

Relative to terrestrial ecosystems, studies of aquatic P using ^{31}P-NMR spectroscopy are scarce. Solid-state and solution ^{31}P-NMR spectroscopy have been used to characterize P forms in sediments from oceans, estuaries and freshwater systems, and to speciate dissolved and particulate P in the water column. Phosphorus forms have been characterized in lake sediments from Australia [134,135] and Europe [30,136–138]. In general, a range of P compounds was detected, including orthophosphate, pyrophosphate, polyphosphate, and orthophosphate monoesters and diesters [30,137,138]. Phosphonates were not detected in any of these samples. Polyphosphates were detected in most samples, with variations due to seasons [136] and redox conditions [30]. The same range of P compounds, including polyphosphates, were also observed in planktonic seston samples [138].

When river sediments from Canada were extracted with chloroform and methanol [139], a number of phospholipids were identified, including phosphatidyl ethanolamine, phosphatidyl choline and lysophosphatidyl choline. Other, unidentified, phospholipids were also present. Spatial and temporal variability in phospholipid forms and abundance was observed, as well as an inverse relationship between total

dissolved P in the water column and phospholipid concentration in sediments [139].

Dissolved P in lake and river water was characterized after ultrafiltration and reverse osmosis of 250–500 l samples [46–49]. Orthophosphate, phosphonates, orthophosphate monoesters and orthophosphate diesters were detected. Differences in P forms were observed between river and lake samples, as well as seasonal variations.

Very high concentrations of pyrophosphate have been detected in some estuarine sediment [140]. This appears to be related to human impact, because pyrophosphate concentration increased with an increasing degree of urbanization. In another study, no relationship between orthophosphate concentration and the percentage of developed land was detected [141].

Marine sediment samples contain an abundance of P species, including orthophosphate, pyrophosphate, orthophosphate monoesters, teichoic acid, phospholipids and DNA [29,30]. Polyphosphates have not been reported. The presence of phosphonates in some samples but not others may be due to differences in redox conditions [30,58]. The persistence of orthophosphate esters and phosphonates at depth in sediment cores indicates that these P forms are resistant to diagenesis.

Dissolved, high molecular weight P in seawater has been characterized with solid state ^{31}P-NMR spectroscopy after tangential flow ultrafiltration, using 1000–1800 l samples [50,142,143] collected at various depths world-wide. A prominent peak for orthophosphate esters was observed, as well as a smaller peak for phosphonates, representing about 25% of the P. The relative proportion of these two compound classes was invariant with respect to depth and sampling location. To determine a source for the phosphonates, cultures of algae [142] and marine bacteria [50] were examined, as well as commercially available cultures of terrestrial bacteria [143]. Phosphonates were detected only in Gram-positive terrestrial bacteria. Although this may in part be due to the culture conditions of the marine organisms, it suggests that orthophosphate esters in dissolved organic matter from oceans are preferentially mineralized relative to phosphonates. The C–P bond is highly resistant to chemical hydrolysis, thermal decomposition and photolysis [144], allowing phosphonates to persist relative to other P forms. However, it may be mineralized under anoxic conditions [58].

Marine dissolved organic P appears to be chemically distinct from particulate organic P, which also suggests preferential mineralization. In a study of settling particulate matter collected by sediment traps [145], solution ^{31}P-NMR spectroscopy revealed a range of P compounds, including phosphonates, orthophosphate, pyrophosphate, polyphosphate, orthophosphate monoesters and orthophosphate diesters. This research also showed spatial, temporal and depth variations in P forms, and demonstrated that the relative distribution of P forms in particulate organic P differs from that in plankton material.

4. Future research needs

Although the technique of ^{31}P-NMR spectroscopy for environmental samples has been considerably improved and refined after more than 20 years of use, we have only tapped a fraction of the potential [9]. It is an underutilized tool in some fields, especially limnology and oceanography. Although spectroscopy can be used for more detailed studies, such as of P transformations or reaction kinetics, the majority of environmental ^{31}P-NMR papers are surveys of P forms in samples. ^{31}P-NMR can also be linked to NMR studies of other nuclei, such as ^{13}C or ^{1}H (e.g. [22,79,118]), with the potential for two-dimensional NMR spectroscopy (e.g. [146]). Despite improvements in extraction techniques in recent years, we still need to determine if extraction is altering the P forms in samples in any way, such as by hydrolysis [12,18], or if we are preferentially extracting P forms in samples for which recovery rates are low, such as calcareous soils [54]. We should also explore the use of different extractants: those currently in use are designed to extract all of the biological P forms in a sample, but additional information could be gained by also using specific extractants for P forms, such as methanol and chloroform for phospholipids [61,139]. Finally, there is a real need to standardize results among laboratories. The use of different extractants and parameters make it difficult to compare the results of different studies. One option is to select a standard method for extraction, then compare the results from other extractants to this standard with organic P compounds and commercially available reference soils and sediments. Laboratories that regularly conduct ^{31}P-NMR experiments using unusually short parameters such as delay times, pulse angles and pulse widths, should also include experiments with longer parameters, to demonstrate that their results are quantitative. Statements such as ‘no differences were observed’ are insufficient; the results from these trials need to be published. Practices such as resin extraction, dialysis and particle size separations also need to be examined, because they appear to alter or remove P forms, potentially biasing the results [25,90,107].

Although the number of environmental ^{31}P-NMR papers grows each year, we need to increase our access to spectrometers; the majority of experiments are run on a handful of instruments, which contributes to the biases in extractants and analytical parameters. We also must convince our fellow P researchers that ^{31}P-NMR is not an exotic tool for use only under special circumstances, but instead is a powerful tool that can be incorporated into many studies to advance our knowledge of organic P in soils and environmental samples.

References

[1] R.H. Newman, K.R. Tate, Commun. Soil Sci. Plant Anal. 11 (1980) 835.
[2] D. Canet, Nuclear Magnetic Resonance: Concepts and Methods, Wiley, New York, 1996.

[3] J.K.M. Sanders, B.K. Hunter, Modern NMR Spectroscopy: A Guide for Chemists, 2nd ed., Oxford University Press, New York, 1994.
[4] B.J. Cade-Menun, in: B.L. Turner, E. Frossard, D. Baldwin (Eds.), Organic Phosphorus in the Environment, CABI Publishing, Cambridge, 2004, pp. 21–44.
[5] M.A. Wilson, NMR Techniques and Applications in Geochemistry and Soil Chemistry, Pergamon Press, New York, 1987.
[6] M.A. Wilson, in: K.A. Smith (Ed.), Soil Analysis: Modern Instrumental Techniques, 2nd ed., Marcel Dekker Inc., New York, 1991, pp. 601–645.
[7] H. Knicker, M.A. Nanny, in: M.A. Nanny, R.A. Minear, J.A. Leenheer (Eds.), Nuclear Magnetic Resonance Spectroscopy in Environmental Chemistry, Oxford University Press, Oxford, 1997, pp. 3–15.
[8] C.M. Preston, Can. J. Appl. Spectrosc. 38 (1993) 61.
[9] C.M. Preston, Soil Sci. 161 (1996) 144.
[10] C.M. Preston, Can. J. Soil Sci. 81 (2001) 255.
[11] B.L. Turner, E. Frossard, D. Baldwin (Eds.), Organic Phosphorus in the Environment, CABI Publishing, Cambridge, 2004.
[12] B.L. Turner, B.J. Cade-Menun, L.M. Condron, S. Newman, Talanta 66 (2005) 294.
[13] L.C. Kolowith, R.A. Berner, Global Biogeochem. Cy. 16 (2002) 1140.
[14] R. Lookman, H. Geerts, P. Grobet, R. Merckx, K. Vlassak, K. Eur. J. Soil Sci. 47 (1996) 125.
[15] A. Delgado, J.R. Ruíz, M.D. del Campillo, S. Kassem, L. Andreu, Commun. Soil Sci. Plant Anal. 31 (2000) 2483.
[16] B. Sutter, R.E. Taylor, L.R. Hossner, D.W. Ming, Soil Sci. Soc. Am. J. 66 (2002) 455.
[17] R. Lookman, P. Grobet, R. Merckx, W.H. Van Riemsdijk, Geoderma 80 (1997) 369.
[18] B.L. Turner, N. Mahieu, L.M. Condron, Soil Sci. Soc. Am. J. 67 (2003) 497.
[19] G.E. Hawkes, D.S. Powlson, E.W. Randall, K.R. Tate, J. Soil Sci. 35 (1984) 35.
[20] M.A. Adams, L.T. Byrne, Soil Biol. Biochem. 21 (1989) 523.
[21] L.M. Condron, M.R. Davis, R.H. Newman, I.S. Cornforth, Biol. Fertil. Soils 21 (1996) 37.
[22] N. Gressel, J.G. McColl, C.M. Preston, R.H. Newman, R.F. Powers, Biogeochemistry 33 (1996) 97.
[23] M. Sumann, W. Amelung, L. Haumaier, W. Zech, Soil Sci. Soc. Am. J. 62 (1998) 1580.
[24] B.J. Cade-Menun, C.M. Preston, Soil Sci. 161 (1996) 770.
[25] B.J. Cade-Menun, C.W. Liu, R. Nunlist, J.G. McColl, J. Environ. Qual. 31 (2002) 457.
[26] K.R. Tate, R.H. Newman, Soil Biol. Biochem. 14 (1982) 191.
[27] Z.R. Hinedi, A.C. Chang, R.W.K. Lee, Soil Sci. Soc. Am. J. 52 (1988) 1593.
[28] M.I. Makarov, L. Haumaier, W. Zech, Biol. Fertil. Soils 35 (2002) 136.
[29] E.D. Ingall, P.A. Schroeder, R.A. Berner, Geochim. Cosmochim. Acta 54 (1990) 2617.
[30] R. Carman, G. Edlund, C. Damberg, Chem. Geol. 163 (2000) 101.
[31] G.H. Rubæk, G. Guggenberger, W. Zech, B.T. Christensen, Soil Sci. Soc. Am. J. 63 (1999) 1123.
[32] W. Amelung, A. Rodionov, I.S. Urusevskaja, L. Haumaier, W. Zech, Geoderma 103 (2001) 335.
[33] M.L. Bishop, A.C. Chang, R.W.K. Lee, Soil Sci. 157 (1994) 238.
[34] J.S. Robinson, C.T. Johnston, K.R. Reddy, Soil Sci. 163 (1998) 705.
[35] C.M. Preston, J.A. Trofymow, Can. J. Soil Sci. 80 (2000) 633.
[36] D.S. Rheinheimer, I. Anghinoni, A.F. Flores, Commun. Soil Sci. Plant Anal. 33 (2002) 1853.
[37] H.K. Pant, P.R. Warman, J. Nowak, Commun. Soil Sci. Plant Anal. 30 (1999) 757.
[38] H.K. Pant, K.R. Reddy, F.E. Dierberg, J. Environ. Qual. 31 (2002) 1748.
[39] G. Guggenberger, B.T. Christensen, G. Rubaek, W. Zech, Eur. J. Soil Sci. 47 (1996) 605.
[40] G. Guggenberger, L. Haumaier, R.J. Thomas, W. Zech, Biol. Fertil. Soils 23 (1996) 332.
[41] W. Zech, H.G. Alt, L. Haumaier, R. Blasek, Z. Pflanz. Bodenkunde 150 (1987) 119.
[42] M.C. Trasar-Cepeda, F. Gil-Sotres, W. Zech, H.G. Alt, Sci. Total Environ. 81/82 (1989) 429.
[43] M.I. Makarov, Eurasian Soil Sci. 31 (1998) 778.
[44] M.-B. Turrión, B. Glaser, D. Solomon, A. Ni, W. Zech, Biol. Fertil. Soils 31 (2000) 134.
[45] M.-B. Turrión, J.F. Gallardo, L. Haumaier, M.-I. González, W. Zech, Ann. For. Sci. 58 (2001) 89.
[46] M.A. Nanny, R.A. Minear, Environ. Sci. Technol. 28 (1994) 1521.
[47] M.A. Nanny, R.A. Minear, in: L.A. Baker (Ed.), Environmental Chemistry of Lakes and Reservoirs„ American Chemical Society, Washington, DC, 1994, pp. 161–191, Advances in Chemistry Series 237.
[48] M.A. Nanny, R.A. Minear, Mar. Geol. 139 (1997) 77.
[49] M.A. Nanny, R.A. Minear, in: M.A. Nanny, R.A. Minear, J.A. Leenheer (Eds.), Nuclear Magnetic Resonance Spectroscopy in Environmental Chemistry, Academic Press, New York, 1997, pp. 221–246.
[50] L.C. Kolowith, E.D. Ingall, R. Benner, Limnol. Oceanogr. 46 (2001) 309.
[51] D.A. Crouse, H. Sierzputowska-Gracz, R.L. Mikkelson, Commun. Soil Sci. Plant Anal. 31 (2000) 229.
[52] C.A. Shand, M.V. Cheshire, C.N. Bedrock, P.J. Chapman, D.A. Fraser, J.A. Chudek, Plant Soil 214 (1999) 153.
[53] T.Q. Zhang, A.F. Mackenzie, F. Sauriol, Soil Sci. 164 (1999) 662.
[54] B.L. Turner, B.J. Cade-Menun, D.T. Westermann, Soil Sci. Soc. Am. J. 67 (2003) 1168.
[55] D. Solomon, J. Lehmann, T. Mamo, F. Fritzsche, W. Zech, Geoderma 105 (2002) 21.
[56] W.F. Bleam, P.E. Pfeffer, J.S. Frye, Phys. Chem. Min. 16 (1989) 455.
[57] R.W. McDowell, L.M. Condron, N. Mahieu, P.C. Brookes, S.R. Poulton, A.N. Sharpley, J. Environ. Qual. 31 (2002) 450.
[58] C.R. Benitez-Nelson, L. O'Neill, L.C. Kolowith, P. Pellechia, R. Thunell, Limnol. Oceanogr. 49 (2004) 1593.
[59] B.L. Turner, J. Environ. Qual. 33 (2004) 757.
[60] B.L. Turner, A.E. Richardson, Soil Sci. Soc. Am. J. 68 (2004) 802.
[61] M.I. Makarov, L. Haumaier, W. Zech, Soil Biol. Biochem. 34 (2002) 1467.
[62] R.W. McDowell, P.C. Brookes, N. Mahieu, P.R. Poulton, A.E. Johnston, A.N. Sharpley, J. Agr. Sci. 139 (2002) 27.
[63] E. Frossard, P. Tekely, J.Y. Grimal, Eur. J. Soil Sci. 45 (1994) 403.
[64] L.M. Condron, E. Frossard, R.H. Newman, P. Tekely, J.-L. Morel, in: M.A. Nanny, R.A. Minear, J.A. Leenheer (Eds.), Nuclear Magnetic Resonance Spectroscopy in Environmental Chemistry, Academic Press, New York, 1997, pp. 247–271.
[65] S.J. Duffy, G.W. vanLoon, Can. J. Chem. 73 (1995) 1645.
[66] G.F. Koopmans, W.F. Chardon, J. Dolfing, O. Oenema, P. van der Meer, W.H. van Riemsdijk, J. Environ. Qual. 32 (2003) 287.
[67] B.L. Turner, N. Mahieu, L.M. Condron, Soil Sci. 168 (2003) 469.
[68] R.W. McDowell, L.M. Condron, N. Mahieu, Commun. Soil Sci. Plant Anal. 34 (2003) 1623.
[69] R.B. Lee, R.G. Ratcliffe, Plant Soil 155/156 (1993) 45.
[70] F. Martin, D. Canet, D. Rolin, J.P. Marchal, F. Larher, Plant Soil 71 (1983) 469.
[71] J. Emsley, S. Niazi, Phosphorus Sulfur 16 (1983) 303.
[72] M.A. Adams, Biol. Fertil. Soils 14 (1992) 200.
[73] B.L. Turner, I.D. McKelvie, J. Environ. Qual. 31 (2002) 466.
[74] L.M. Condron, K.M. Goh, R.H. Newman, J. Soil Sci. 36 (1985) 199.
[75] K.H. Dai, M.B. David, G.F. Vance, A.J. Krzyszowska, Soil Sci. Soc. Am. J. 60 (1996) 1943.

[76] A. Miltner, L. Haumaier, W. Zech, Eur. J. Soil Sci. 49 (1998) 471.
[77] M.I. Makarov, L. Haumaier, W. Zech, O.E. Marfenina, L.V. Lysak, Soil Biol. Biochem. 37 (2005) 15.
[78] D.A. Crouse, H. Sierzputowska-Gracz, R.L. Mikkelson, A.G. Wollum, Commun. Soil Sci. Plant Anal. 33 (2002) 1205.
[79] A. Möller, K. Kaiser, W. Amelung, C. Niamskul, S. Udomsri, M. Puthawong, L. Haumaier, W. Zech, Aust. J. Soil Res. 38 (2000) 1017.
[80] B.L. Turner, R. Baxter, N. Mahieu, S. Sjögersten, B.A. Whitten, Soil Biol. Biochem. 36 (2004) 815.
[81] B.J. Cade-Menun, S.M. Berch, C.M. Preston, L.M. Lavkulich, Can. J. For. Res. 30 (2000) 1714.
[82] I.M. Cardoso, P. Van der Meer, O. Oenema, B.H. Janssen, T.W. Kuyper, Geoderma 112 (2003) 51.
[83] M. Escudey, G. Galindo, J.E. Förster, I. Salazar, A.L. Page, A. Chang, Commun. Soil Sci. Plant Anal. 28 (1997) 727.
[84] F. Gil-Sotres, W. Zech, H.G. Alt, Soil Biol. Biochem. 22 (1990) 75.
[85] J.C. Hansen, B.J. Cade-Menun, D.G. Strawn, J. Environ. Qual. 33 (2004) 1521.
[86] H.K. Pant, K.R. Reddy, J. Environ. Qual. 30 (2001) 668.
[87] L.M. Condron, E. Frossard, H. Tiessen, R.H. Newman, J.W.B. Stewart, J. Soil Sci. 41 (1990) 41.
[88] J.C. Forster, W. Zech, Z. Pflanz. Bodenkunde 156 (1993) 61.
[89] B.L. Turner, N. Mahieu, L.M. Condron, Org. Geochem. 34 (2003) 1199.
[90] S.M. Kristiansen, W. Amelung, W. Zech, Z. Pflanz. Bodenkunde 164 (2001) 49.
[91] B.J. Cade-Menun, S.M. Berch, C.M. Preston, L.M. Lavkulich, Can. J. For. Res. 30 (2000) 1726.
[92] C.N. Bedrock, M.V. Cheshire, J.A. Chudek, B.A. Goodman, C.A. Shand, Sci. Total Environ. 152 (1994) 1.
[93] P. Leinweber, L. Haumaier, W. Zech, Biol. Fertil. Soils 25 (1997) 89.
[94] D. Solomon, J. Lehmann, Eur. J. Soil Sci. 51 (2000) 699.
[95] R.L. Parfitt, K.R. Tate, G.W. Yeates, P.N. Beets, N.Z. J. For. Sci. 24 (1994) 253.
[96] M.I. Makarov, G. Guggenberger, H.G. Alt, W. Zech, Z. Pflanz. Bodenkunde 158 (1995) 293.
[97] B.L. Turner, J.A. Chudek, B.A. Whitton, R. Baxter, Biogeochemistry 65 (2003) 259.
[98] G.S. Toor, L.M. Condron, H.J. Di, K.C. Cameron, B.J. Cade-Menun, Soil Biol. Biochem. 35 (2003) 1319.
[99] K. Kaiser, G. Guggenberger, L. Haumaier, Biogeochemistry 66 (2003) 287.
[100] W. Zech, H.G. Alt, A. Zucker, I. Kögel, Z. Pflanz. Bodenkunde 148 (1985) 626.
[101] L. Chapuis-Lardy, M. Brossard, H. Quiquampoix, Can. J. Soil Sci. 81 (2001) 591.
[102] C.R. Chen, L.M. Condron, B.L. Turner, N. Mahieu, M.R. Davis, Z.H. Xu, R.R. Sherlock, Aust. J. Soil Res. 42 (2004) 198.
[103] Tchienkoua, W. Zech, Agric. Ecosyst. Environ. 100 (2003) 193.
[104] M.T. Taranto, M.A. Adams, P.J. Polglase, Soil Biol. Biochem. 32 (2000) 169.
[105] L.P. Canellas, J.A.A. Espindola, J.G.M. Guerra, M.G. Teixeira, A.C.X. Velloso, V.M. Rumjanek, Pesq. Agropec. Bras, Brasilia 39 (2004) 589.
[106] Z.R. Hinedi, A.C. Chang, Soil Sci. Soc. Am. J. 53 (1989) 1057.
[107] M.I. Makarov, L. Haumaier, W. Zech, T.I. Malysheva, Geoderma 118 (2004) 101.
[108] G. Guggenberger, B.T. Christensen, G.H. Rubæk, Z. Pflanz. Bodenkunde 163 (2000) 151.
[109] M. Escudey, G. Galindo, M. Briceño, A. Chang, J. Chil. Chem. Soc. 49 (2004) 5.
[110] M.I. Makarov, L. Haumaier, W. Zech, T.I. Malysheva, Geoderma 118 (2004) 101.
[111] D.L. Sparks, Environmental Soil Chemistry, Academic Press, San Diego, 1995, pp. 53–80.
[112] M.W. Makarov, Moscow Univ. Soil Sci. Bull. 51 (1996) 15.
[113] M.I. Makarov, G. Guggenberger, W. Zech, H.G. Alt, Z. Pflanz. Bodenkunde 159 (1996) 467.
[114] M.I. Makarov, T.I. Malysheva, L. Haumaier, H.G. Alt, W. Zech, Geoderma 80 (1997) 61.
[115] C.N. Bedrock, M.V. Cheshire, J.A. Chudek, A.R. Fraser, B.A. Goodman, C.A. Shand, Commun. Soil Sci. Plant Anal. 26 (1995) 1411.
[116] V.D. Tikhova, M.M. Shakirov, V.P. Fadeeva, M.I. Dergacheva, Russ. J. Appl. Chem. 73 (2000) 1278.
[117] G. Ogner, Geoderma 29 (1983) 215.
[118] N. Mahieu, D.C. Olk, E.W. Randall, Eur. J. Soil Sci. 51 (2000) 391.
[119] P.A. Kemme, A. Lommen, L.H. De Jonge, J.D. Van der Klis, A.W. Jongbloed, Z. Mroz, A.C. Beynen, J. Agric. Food Chem. 47 (1999) 5116.
[120] S. Hunger, H. Cho, J.T. Sims, D.L. Sparks, Environ. Sci. Technol. 38 (2004) 674.
[121] G. Gigliotti, K. Kaiser, G. Guggenberger, L. Haumaier, Biol. Fertil. Soils 36 (2002) 321.
[122] C.M. Preston, J.A. Ripmeester, S.P. Mathur, M. Lévesque, Can. J. Spectrosc. 31 (1986) 63.
[123] C.M. Preston, B.J. Cade-Menun, B.G. Sayer, Compost Sci. Util. 6 (1998) 53.
[124] E. Frossard, P. Skrabal, S. Sinaj, F. Bangerter, O. Traore, Nutr. Cycl. Agroecosys. 62 (2002) 103.
[125] M. Florentz, P. Granger, Environ. Technol. Lett. 4 (1983) 9.
[126] D. Uhlmann, I. Röske, M. Hupfer, G. Ohms, Water Res. 24 (1990) 1355.
[127] I. Röske, C. Schönborn, Water Sci. Technol. 30 (1994) 323.
[128] W.E. Hill, L.D. Benefield, S.R. Jing, Water Res. 23 (1989) 1177.
[129] S.R. Jing, L.D. Benefield, W.E. Hill, Water Res. 26 (1992) 213.
[130] Z.R. Hinedi, A.C. Chang, R.W.K. Lee, J. Environ. Qual. 18 (1989) 323.
[131] M. Escudey, G. Galindo, K. Avendaño, D. Borchardt, A.C. Chang, M. Briceño, J. Chil. Chem. Soc. 49 (2004) 219.
[132] Z.R. Hinedi, A.C. Chang, J.P. Yesinowski, Soil Sci. Soc. Am. J. 53 (1989) 1053.
[133] E. Frossard, P. Tekely, J.L. Morel, Fert. Res. 37 (1994) 151.
[134] D.S. Baldwin, Hydrobiology 335 (1996) 63.
[135] A. Khoshmanesh, B.T. Hart, A. Duncan, R. Beckett, Water Res. 36 (2002) 774.
[136] U. Selig, T. Hübener, M. Michalik, Aquat. Sci. 64 (2002) 97.
[137] M. Hupfer, R. Gächter, H. Rüegger, Limnol. Oceanogr. 40 (1995) 610.
[138] M. Hupfer, B. Rübe, P. Schnieder, Limnol. Oceanogr. 49 (2004) 1.
[139] E.E. Watts, P.A.W. Dean, R.R. Martin, Can. J. Anal. Sci. Spect. 47 (2002) 127.
[140] P.V. Sundareshwar, J.T. Morris, P.J. Pellechia, H.J. Cohen, D.E. Porter, B.C. Jones, Limnol. Oceanogr. 46 (2001) 1570.
[141] J.N. Halls, J. Coastal Res. SI36 (2002) 340.
[142] L.L. Clark, E.D. Ingall, R. Benner, Nature 393 (1998) 426.
[143] L.L. Clark, E.D. Ingall, R. Benner, Am. J. Sci. 299 (1999) 737.
[144] A.M. Cook, C.G. Daughton, M. Alexander, J. Bacteriol. 133 (1978) 85.
[145] A. Paytan, B.J. Cade-Menun, K. McLaughlin, K.L. Faul, Mar. Chem. 82 (2003) 55.
[146] W.S. Veeman, Geoderma 80 (1997) 225.