Can solid-state phosphorus-31 nuclear magnetic resonance spectra be improved by wet chemical extraction of paramagnetics?

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Abstract

The use of solid-state nuclear magnetic resonance (NMR) spectroscopy to characterise phosphorus (P) in soils has increased recently. However, seldom are solid-state spectra quantitative due to limitations such as interference by paramagnetics. Soils were treated (Ca-EDTA, NaOCl, NaOCl + Ca-EDTA or HF) to remove paramagnetics and the portion of observable P in spectra determined. The fraction of P observable ranged from 18-66% in untreated soils, but HF (45-92%) and Ca-EDTA (21-78%) improved P observability, while treatment with NaOCl did not. Treatment with HF confirmed the detection of organic P as a central resonance around -0.5 ppm with broad SSBs, but removed most of the inorganic P. Although treatment with HF or Ca-EDTA could improve the utility of ^{31}P DP NMR spectra, this needs to be accompanied by %P observable so that conclusions are not based on non-quantitative spectra. Caution should be used when interpreting the output of any ^{31}P DP NMR data, if the % P observable is not reported.

Key Words

Observability, spinning side bands, organic P.

Introduction

Phosphorus is an essential nutrient for plant growth, but the bioavailability of P to plants is determined by the relative quantities of different P species. Traditionally, P speciation has been investigated with a number of wet chemical techniques such as sequential fractionation, but more recently sophisticated spectroscopic techniques such as NMR have been employed (He *et al.* 2007). Solution ^{31}P NMR has emerged as a powerful analytical tool for elucidating soil P processes, but has the disadvantage of requiring the use of extractants that can alter P forms due to hydrolysis. Consequently, there is a question mark over how representative the information is of soil P. Solid state NMR enables the analysis of undisturbed soil samples and as such has been used to validate soil P pools (i.e. species) isolated by wet chemical techniques (e.g., Hunger *et al.* 2005). However, the utility of solid state NMR relies on the accurate determination of P species. Dougherty *et al.* (2005) reported that accuracy of solid state NMR spectra are compromised by factors such as paramagnetic ions that result in only a small fraction of total P in the sample showing up in spectra. Techniques to remove paramagnetic ions do exist (e.g., McDowell and Stewart 2005; Skjemstad *et al.* 1994). Our objective was to determine if these techniques would improve the visibility of P and utility of solid state NMR spectra in different soils.

Methods

Soils

Three soils supporting permanent ryegrass-white clover pasture were chosen for study. These were: 1) a Lismore silt loam (USDA Classification: Typic Dystrocrept) from the 0-7.5 cm depth of topsoil that received 30 kg P/ha/yr; a Taupo sandy loam (USDA classification: Udand) that had been sampled to 10 cm depth and received 50 kg P/ha/yr; and a Wharekohe silt loam (USDA Classification: Aquod) from the 0-7.5 cm depth that received 25 kg P/ha/yr. All soils were air-dried, crushed and sieved < 150 µm before analysis of pH (in water with a 1:2.5 soil to water ratio), organic C (by LECO combustion), total P (after Kjeldahl digestion) and Olsen P.

Extractions designed to help either define or improve the identification of different P species were performed on each soil and defined as the following treatments:

1) Control, unextracted soil;
2) Ca-EDTA, extraction with Ca-EDTA as per the method of McDowell and Stewart (2005) and designed to preferentially remove paramagnetic ions, Fe and Mn without extracting organic P species;
3) NaOCl, extraction with NaOCl as per the method of Siregar *et al.* (2005) and designed to remove

organic matter without affecting inorganic constituents;

4) NaOCl + Ca-EDTA, extraction with NaOCl and then Ca-EDTA; and
5) HF, extraction with 2% HF as per Skjemstad *et al.* (1994) and designed to remove paramagnetics, especially Fe and Mn, from the inorganic phase of the soil.

Solutions and extracted soils were digested using a concentrated HCl:HNO_3 mix (Crosland *et al.* 1995) and P, Fe, and Mn determined by ICP-OES. Inorganic and organic P was determined on sub-samples of each treatment using the ignition method (Saunders and Williams 1955).

Solid state ^{31}P-NMR

Spectra were obtained on a high power ^{1}H decoupling Varian Unity INOVA 400 spectrophotometer with a Doty Scientific supersonic MAS probe at a frequency of 161.9Hz. Air-dried soil samples were packed into 7-mm diameter zirconia rotors and spun at 5 kHz at the magic angle. Phosphorus-21 direct polarization NMR spectra were obtained over a 24 hr period in which time up to 4612 scans had been collected with a 20 s recycle delay between pulses. The recycle delay was sufficient to avoid up to 85% of signal loss due to saturation and incomplete relaxation of nuclei (Dougherty *et al.* 2005). Each spectrum was corrected for a background signal. Spin counting experiments were conducted as per Dougherty *et al.* (2005) using $NH_4H_2PO_4$ as an external standard.

Results and discussion

The soils chosen for study exhibited a wide range of soil P concentrations, Fe, Mn and organic C concentrations, but narrow pH (Table 1). This allowed for the comparison of NMR spectra without pH influencing the distribution and chemical shift of P species.

Table 1. Summary of key soil properties and the percentage of P observable by ^{31}P DP NMR in the control (unextracted) soil.

Soil	Olsen P (mg/kg)	Total P (mg/kg)	Total Fe (g/kg)	Total Mn (mg/kg)	pH	Organic C (g/kg)	Observability (%)
Lismore	18	996	26.2	464	5.6	39	18
Taupo	80	1519	16.6	697	5.5	55	64
Wharekohe	18	562	2.8	155	5.6	36	66

The DP spectrum for each soil with or without treatment is shown in Figure 1. The spectra are characterised by one central resonance at about -1 ppm that can be, in some spectrum, differentiated into different peaks each representing a different P species and spinning side bands (SSB) that occur upfield or downfield of the central resonance at distance that corresponds to chemical shift anisotropy. The central resonance has traditionally been deconvoluted via software into peaks that are referenced relative to known compounds. However, the utility of this approach is hampered by whether or not the spectrum is quantitative. If, for example, the majority of P is not visible by solid-state NMR then deconvolution is questionable.

Table 2. The percentage of P (organic P in parentheses), Fe and Mn removed by each treatment and the resulting P observable by ^{31}P DP NMR. Note that although P observable is expressed as a percentage of total P in the sample, inorganic (Pi) and organic (Po) visibility is the product(/100) of % Pi or Po remaining in the sample relative to whole soil and % P observability.

Soil	Treatment	P removed	Fe removed	Mn removed	Observability	Pi visibility	Po visibility
Lismore	Ca-EDTA	6 (12)	10	34	21	20	19
	NaOCl	7 (34)	0	1	20	16	13
	NaOCl + Ca-EDTA	8 (45)	10	27	16	14	8
	HF	42 (10)	23	25	45	37	40
Taupo	Ca-EDTA	4 (8)	14	21	74	67	68
	NaOCl	11 (34)	1	5	60	54	38
	NaOCl + Ca-EDTA	5 (47)	15	16	68	60	38
	HF	51 (32)	23	22	59	17	46
Wharekohe	Ca-EDTA	16 (18)	34	28	78	69	64
	NaOCl	17 (54)	8	11	38	30	17
	NaOCl + Ca-EDTA	14 (56)	19	23	57	38	25
	HF	44 (26)	21	17	92	69	77

The percentage of total P removed by each treatment was greatest in the Wharekohe soil, while HF tended to remove most P (Table 2). Although not wholly specific to either inorganic or organic P, treatments including NaOCl tended to remove more organic P (especially with Ca-EDTA) than either Ca-EDTA or HF, while HF preferentially removed inorganic P. The most Fe and Mn were removed by either Ca-EDTA or HF. For HF and Ca-EDTA, this resulted in an increase in observable P. However, the same was not necessarily true for treatments containing NaOCl, which suggests that paramagnetic compounds, including Fe and Mn, that affect P observability are preferentially associated with the inorganic fraction preferentially removed by HF and Ca-EDTA.

By combining the quantity of Pi or Po remaining in the sample and P observable a dimensionless metric referring to the visibility of either Pi or Po was obtained (Table 2). This assumes that wet chemical techniques are better at defining Po than DP NMR. The resulting values indicated that although HF treatment removed much Pi, the visibility of remaining Pi, and the much enriched Po fraction, was greater in the Lismore and Wharekohe but not the volcanic Taupo soil. In contrast, Ca-EDTA improved observable P compared to the control (and most other treatments), and in turn Pi and Po visibility, but also had the advantage of removing little P compared to untreated soil.

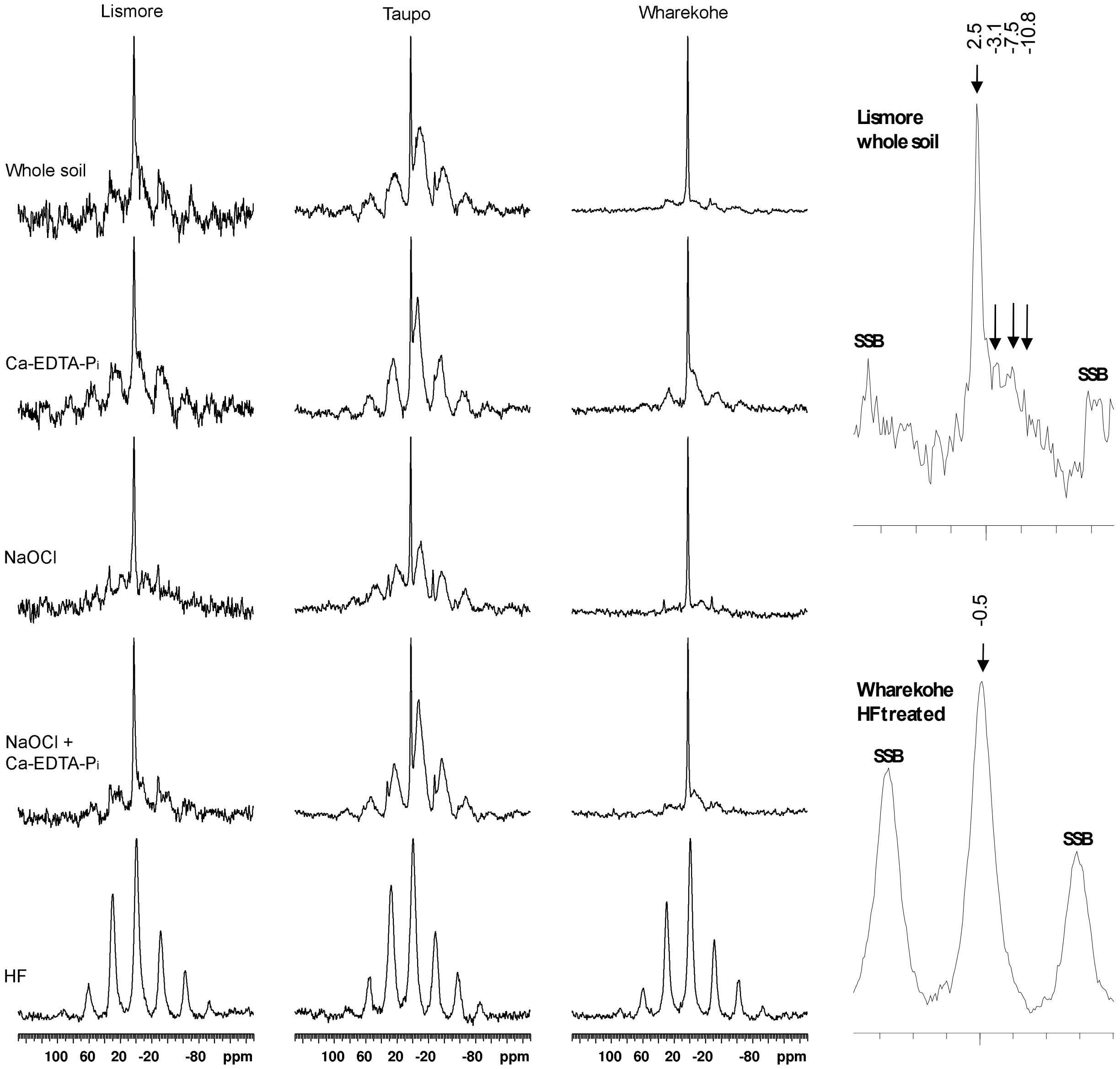

Figure 1. Phosphorus-31 DP NMR spectra of whole soil and soils pre-treated with Ca-EDTA (to remove paramagnetics), NaOCl (to remove organic compounds), NaOCl+Ca-EDTA and HF (to remove paramagnetics from inorganic compounds). Expansions of the Lismore whole soil and Wharekohe HF treated soils are given along with peaks identified at their relative chemical shift (ppm) within the central resonance, provided those peaks exhibited a signal to noise > 5. SSB = spinning side bands.

The utility of these treatments appear to confirm a coarse speciation or confirmation of P into Pi or Po forms. Our data agreed with that of Dougherty *et al.* (2005) by confirming that Po, enriched in the HF treated soil, appeared to have SSB that were much broader compared to spectra dominated by sharp peaks in the central resonance - characteristic of Pi (Figure 1). In a recent spectral analysis of dairy manure, He *et al.* (2009) also showed that SSB were more pronounced in samples after extraction with either water or sodium acetate. However, no consideration was given of the observability or visibility of P within the sample.

Given the use of solid state NMR as a technique to confirm other wet chemical techniques, it is important that peak allocations of P species be correct. Generally, it is good practice to assign a peak to a P species only if the signal to noise ratio is greater than 5. The sample most likely to be quantitative was the Wharekohe HF treated soil (92% observable). However, in this sample only one peak at -0.5 ppm could be assigned (signal to noise ratio of 25). In contrast, several peaks with signal to noise ratios > 5 were able to be separated using deconvolution software on the spectrum of the Lismore untreated soil, but only 18% of P was detectable by the NMR (Figure 1). A similar distribution of peaks was evident for the Taupo and Wahrekohe control soils, but the observability of P, and likelihood for quantitative data, in these samples was greater. This work highlights the potential for inaccurate conclusions if factors such as observable P are not taken into account.

Conclusion

Treatment of soils with Ca-EDTA, NaOCl, NaOCl + Ca-EDTA or HF to remove paramagnetics and improve the quantitation of solid state NMR spectra yielded mixed results. In general, the NaOCl treatments preferentially removed Po, but did not improve the detection (via spin counting) of P by the NMR. Treatment with HF and Ca-EDTA improved P observability compared to the original soil and for HF, confirmed the detection of Po as a central resonance around -0.5 ppm with broad SSBs. The low quantity of P removed and improvement in observability suggests that treatment with Ca-EDTA may be of benefit in generating quantitative data, but requires additional work. We conclude that there is some potential for treatments to improve the quantitation of ^{31}P DP NMR spectra, but caution should be used when interpreting the output of any ^{31}P DP NMR data if the % P observable is not reported.

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