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Organic phosphorus in Madagascan rice soils

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Abstract

As a first step towards assessing the contribution of organic phosphorus to rice nutrition, the chemical nature of phosphorus in a range of Madagascan rice soils was determined by NaOH–EDTA extraction and solution ^{31}P nuclear magnetic resonance spectroscopy. A considerable proportion of the extractable phosphorus occurred in organic forms (19–44%), mostly as phosphate monoesters, with smaller concentrations of DNA. Inositol phosphates were detected in less than half of the soils, despite their perceived abundance, while phosphate monoesters in the remaining soils consisted of the alkaline hydrolysis products of RNA and phospholipids. Organic phosphorus concentrations were greater in soils rich in organic matter, but there were no apparent differences between soils under conventional flooded rice cultivation and the system of rice intensification. Additional experiments are now required to assess the role of organic phosphorus in the nutrition of rice growing under a range of management and soil conditions.

Keywords: Madagascar; Organic phosphorus; Oxisol; Rice; Soil; Solution ^{31}P nuclear magnetic resonance spectroscopy; System of rice intensification

1. Introduction

Phosphorus deficiency constrains rice production in tropical regions with strongly-weathered soils such as Oxisols and Ultisols (Kirk et al., 1998). Such soils are typically acidic and rich in iron and aluminum sesquioxides, which confers a considerable capacity to retain phosphate (Tiessen and Shang, 1998). This means that phosphate concentrations available to rice plants are naturally very low and are rarely increased by flooding, because anaerobic soils can sorb phosphate more strongly than comparable aerobic soils (Patrick and Khalid, 1974). Improving phosphorus nutrition on infertile soils is therefore an urgent priority if we are to meet the increasing global demand for rice in the twenty-first century (Surridge, 2002).

Almost all studies of the phosphorus nutrition of rice have considered only inorganic phosphate, because this is the form taken up directly by roots. However, organic phosphorus also occurs in soils and offers another potential source of phosphorus for rice plants following enzymatic cleavage. Indeed, there is increasing recognition of the importance of soil organic phosphorus to crops growing in tropical soils (Nziguheba and Bünemann, 2005). Some organic phosphorus compounds are less strongly retained in soil compared to inorganic phosphate, so are potentially more easily accessed by roots (Frossard et al., 1989). Organic phosphorus may therefore be biologically important in infertile soils rich

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in metal oxides that typify those of resource-poor farms in many regions of the tropics (Turner et al., 2006).

There is little information on the contribution of soil organic phosphorus to rice nutrition. Marked depletion of soil organic phosphorus was detected during the growth of flooded rice in soils from India (Basak and Bhattacharya, 1963), Japan (Furukawa and Kawaguchi, 1969), and more recently from various long-term management regimes in South Korea (Lee et al., 2004). In the latter study, organic phosphorus depletion was linked to the enhanced activity of microbes and phosphatases (enzymes involved in the release of phosphate from organic compounds) in cultivated soils. In contrast, a detailed study of phosphate uptake by upland rice grown in a low-fertility Ultisol suggested that organic phosphorus was unlikely to contribute greatly to crop nutrition, despite elevated phosphatase activity in the rhizosphere of several cultivars (Hedley et al., 1994).

Understanding the contribution of soil organic phosphorus to rice nutrition requires detailed information on the chemical nature of the compounds involved. This remains scarce, but can be conveniently obtained using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, which allows multiple compounds to be quantified simultaneously in soil extracts with minimal sample preparation and handling (Condron et al., 1997). The technique was used to determine the phosphorus composition of soils from central Madagascar extracted in 0.5 M NaOH (Zech et al., 1990) and of humic acids extracted from rice soils in the Philippines (Mahieu et al., 2000). In both these studies, phosphate monoesters were the dominant organic phosphorus group, with smaller concentrations of phosphate diesters. However, only relatively small proportions of the soil organic phosphorus were characterized and poor spectral resolution precluded identification of individual compounds in the phosphate monoester region. The latter is of significance due to the marked differences in behavior and potential bioavailability of the various phosphate monoesters (Celi and Barbaris, 2005).

Recent advances in methodology for solution ^{31}P NMR spectroscopy of alkaline soil extracts include improvements in signal identification (Makarov et al., 2002; Turner et al., 2003a; Turner and Richardson, 2004) and the development of a quantitative extraction procedure (Bowman and Moir, 1993; Cade-Menun and Preston, 1996). Soil organic phosphorus can therefore now be quantified and speciated more accurately than was previously possible. Here I report the organic phosphorus composition of a range of rice soils from central Madagascar determined by a standardized NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy procedure. This provides baseline data for future studies on the contribution of organic phosphorus to rice nutrition in low-fertility tropical soils.

2. Methods

2.1. Soils and sampling locations

Soil was taken from the plough layer of thirteen rice fields at six locations in the central and eastern region of Madagascar (Fig. 1). Multiple samples were taken from each field and bulked. Soils in the region are mainly Oxisols (Soil Survey Staff, 1999), being acidic with low cation exchange capacities and high soluble aluminum and iron concentrations (Dobermann, 2004). Details of each sample location are reported in Table 1.

Anjomakely and Ambatofotsy are both close to the capital Antananarivo (mean monthly temperature 15–22 °C, mean annual rainfall 1250 mm) (Stoop et al., 2002). Beforona lies near the coast approximately 50 km to the east of Moramanga and is the wettest and warmest of the sites sampled (mean monthly temperature 23–32 °C, mean annual rainfall 2000–3500 mm). The three northerly locations (Imerimandroso, Ambaiboho, and Anosiboribory) are close to Ambatondrazaka in the Lac Aloatra region (mean

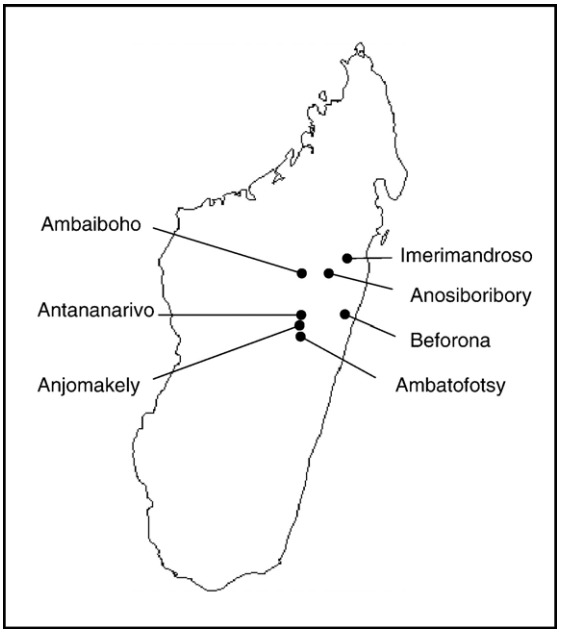

Fig. 1. Map of Madagascar showing the six sampling locations.

Table 1
Descriptions of thirteen Madagascan rice soils

Location	Latitude/longitude	Cultivation	Details
1. Ambaiboho	17°57′ S., 48°28′ E.	Conventional	Received compost, manure, and mineral fertilizer; yield approx. 3.5 t ha^{-1}; sampled dry.
2. Ambaiboho	17°57′ S., 48°28′ E.	Conventional	Received compost, manure, and mineral fertilizer; yield 3.5 t ha^{-1}; sampled wet but not flooded.
3. Ambaiboho	17°57′ S., 48°28′ E	SRI[†]	Yield after 4 y of SRI was 6 t ha^{-1}; received compost, manure, and mineral fertilizer; sampled wet with young rice plants.
4. Ambatofotsy	19°08′ S., 47°57′ E.	Conventional	Long-term yield 2 t ha^{-1} per crop; sampled dry just prior to flooding
5. Ambatofotsy	19°08′ S., 47°57′ E.	SRI	Yield after 13 y of SRI was 6–12 t ha^{-1} per crop; sampled dry just prior to flooding.
6. Anjomakely	19°03′ S., 47°55′ E.	Conventional	Long-term conventional rice production with two crops per year; received compost; sampled dry.
7. Anjomakely	19°03′ S., 47°55′ E.	SRI	Three years of SRI following long term conventional rice production; received compost; sampled dry.
8. Anosiboribory	17°77′ S., 47°78′ E.	Conventional	Located at the Centre Multiplication Semences; soil received organic fertilizer and was sampled wet just prior to planting.
9. Anosiboribory	17°77′ S., 47°78′ E.	Fallow	An organic soil at the Centre Multiplication Semences, previously under grass but recently tilled and limed in preparation for rice production.
10. Beforona	18°97′ S., 48°58′ E.	SRI	Four years of SRI at the Centre de Diffusion pour l'Intensification Agricole (CDIA); daily wet/dry cycles; sampled wet just after planting.
11. Imerimandroso	17°43′ S., 48°58′ E.	Conventional	Received organic fertilizer; yield 3 t ha^{-1}; flooded when sampled with young rice plants.
12. Imerimandroso	17°43′ S., 48°58′ E.	SRA[‡]	Received mineral fertilizer; yield 4–5 t ha^{-1}; sampled wet but not flooded.
13. Imerimandroso	17°43′S., 48°58′ E.	SRI	Yield after 3 y of SRI 6 t ha^{-1} (3.5 t ha^{-1} in first year); received organic fertilizer; flooded when sampled with young rice plants; weekly flooding/drying.

[†] The système de riziculture intensive, or system of rice intensification (see Methods for description).
[‡] The système de riziculture ameliorée (SRA), which involves the use of mineral fertilizer and the planting of older seedlings (20 d) compared to the système de riziculture intensive (8 d).

monthly temperature 17–24 °C, mean annual rainfall 1025 mm). At the time of sampling (October 2003), soils from the high plateau near Antananarivo were dry and tilled in preparation for the start of the growing season, whereas most of the remaining soils were wet or flooded and contained young rice plants.

At five locations samples were taken from fields under conventional flooded rice cultivation and the system of rice intensification (SRI). The latter is a novel system developed originally in Madagascar that allows resource-poor farmers to improve yields on infertile soils with reduced irrigation and fertilizer inputs (Stoop et al., 2002; Dobermann, 2004). Key features of the system are the planting of widely-spaced young seedlings, intermittent irrigation rather than flooding during the vegetative phase, and application of compost or other organic fertilizers rather than mineral fertilizer. The system is described in detail elsewhere (Stoop et al., 2002).

2.2. Analytical procedures

Samples were dried for 10 d at approximately 35 °C, gently ground, and sieved (<2 mm). Drying can increase the solubility of soil organic phosphorus (Turner and Haygarth, 2001) and may alter its chemical composition. Changes were considered to be minimal for these soils, although this should be assessed in future studies of flooded soils.

Phosphorus was extracted by shaking 5-g of air-dried soil with 100 mL of a solution containing 0.25 M NaOH and 0.05 M Na_2EDTA (ethylenediaminetetraacetate) for 16 h at 22 °C (Cade-Menun and Preston, 1996). This was an adaptation of the original method (Bowman and Moir, 1993), which involved a 2 h extraction at 85 °C. It is assumed that NaOH–EDTA quantitatively recovers organic phosphorus from soil (Bowman and Moir, 1993; Turner et al., 2005a), although there is no direct method with which to confirm this. The ignition procedure

provides an alternative estimate of soil organic phosphorus, but is inappropriate for well-weathered tropical soils due to marked increases in the solubility of inorganic phosphorus following ignition (Condron et al., 1990).

Extracts were centrifuged at 10,000 ×*g* for 30 min and an aliquot taken for determination of total phosphorus (see below). The remaining solution was frozen at −30 °C, lyophilized, and ground. Each freeze-dried extract (~100 mg) was re-dissolved in 0.1 mL of deuterium oxide and 0.9 mL of a solution containing 1.0 M NaOH and 0.1 M EDTA, and then transferred to a 5 mm NMR tube. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P. Samples were analyzed using a 6 μs pulse (45°), a delay time of 2.0 s, an acquisition time of 0.4 s, and broadband proton decoupling. The delay time used here allows sufficient spin-lattice relaxation between scans for phosphorus compounds in NaOH–EDTA (Cade-Menun et al., 2002), especially given the high iron content of the samples analyzed here. Approximately 30,000 scans were acquired for all samples.

Chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4. The values can vary slightly among spectra due to slight differences in pH, viscosity, and ionic strength, although this is minimized by analyzing samples at a strongly alkaline pH (>13). Signals were assigned to phosphorus compounds or functional groups based on literature reports (Turner et al., 2003a) and signal areas calculated by integration. Spectra were plotted with a line broadening of 1 Hz to preserve resolution in the phosphate monoester region. Concentrations of *myo*-inositol hexakisphosphate were determined by multiplying by six the signal at approximately 5.9 ppm arising from the C-2 phosphate on the inositol ring (Turner et al., 2003b). Quantification of the three other signals from this compound tends to overestimate *myo*-inositol hexakisphosphate due to interference with signals from other compounds. Concentrations of *scyllo*-inositol hexakisphosphate were determined by quantifying the signal at approximately 4.2 ppm (Turner and Richardson, 2004).

Total carbon and nitrogen were determined by combustion and gas-chromatography using a Flash EA1112 CN analyzer (CE Elantech, Lakewood, New Jersey, USA). Soil pH was determined in a 1:2 soil to deionized water ratio. Total soil phosphorus was determined following the digestion of ashed samples (550 °C for 3 h) in 6 M HCl, with phosphate detection by automated molybdate colorimetry (Anderson, 1976). Total phosphorus in NaOH–EDTA extracts was determined by a similar procedure. Plant-available phosphate was estimated by $NaHCO_3$ extraction and molybdate colorimetry (Olsen et al., 1954). Correlation coefficients between soil properties and phosphorus functional groups were calculated in Microsoft Excel 2003. All concentrations are expressed on the basis of oven-dry soil (105 °C for 24 h).

3. Results

3.1. Soil properties

Carbon concentrations ranged between 11.1 and 153.1 g C kg^{-1} soil, while nitrogen concentrations ranged between 1.14 and 10.04 g N kg^{-1} soil (Table 2). Total carbon and nitrogen were correlated positively ($r=0.98$; $p<0.001$). The largest concentrations occurred in soils from Anosiboribory, although most soils contained <25 g C kg^{-1} soil and <2.6 g N kg^{-1} soil. Phosphorus concentrations ranged between 0.13 and 1.38 g P kg^{-1} soil (Table 2), indicating that at least some soils in the region contained considerable amounts of phosphorus. Total phosphorus was correlated positively with carbon and nitrogen (e.g., for carbon: $r=0.70$; $p<0.01$).

Carbon to nitrogen ratios ranged from 7.3 in a soil under SRI at Imerimandroso to 15.2 in a soil with a large carbon concentration from Anosiboribory, although most values were between 9 and 12 (Table 2). The ratios of carbon to phosphorus and nitrogen to phosphorus were smallest for a soil under SRI at Ambatofotsy (C/P 26, N/P 2.2), while the largest values were for a conventionally cultivated field at Imerimandroso (C/P 111, N/P 13.7).

Bicarbonate-extractable phosphate was <10 mg P kg^{-1} dry soil at all sites, with none detected in soils from Anjomakely and Ambatofotsy. All soils were therefore considered to be phosphorus-deficient in an agronomic context. Concentrations were highest in soils from Imerimandroso, despite these soils containing the least total phosphorus and the largest nitrogen to phosphorus ratios. Soil pH ranged between 4.62 and 5.82 (Table 2) and was not correlated significantly with soil carbon, nitrogen, phosphorus, or their ratios. Textural analysis was not performed, although three Oxisols from the region south of Antananarivo were reported to contain between 19% and 34% clay (Zech et al., 1990).

3.2. Soil phosphorus composition

Extraction with NaOH–EDTA recovered between 34% and 80% of the total soil phosphorus, with the greatest recovery from the organic-rich soils at Anosiboribory (Table 3). Representative solution ^{31}P NMR spectra are shown in Fig. 2. Most of the extracted phosphorus was inorganic phosphate, indicated by the large signal at approximately 6.2 ppm (Fig. 2). Phosphate concentrations

Table 2
Properties of thirteen Madagascan rice soils

Location	Total elements			pH	Elemental ratios			$NaHCO_3$-phosphate
	C	N	P		C/N	C/P	N/P	
	g kg^{-1} soil							mg P kg^{-1} soil
1. Ambaiboho	11.1	1.14	0.337	4.64	9.7	33	3.4	3.3
2. Ambaiboho	16.7	1.58	0.516	5.02	10.5	32	3.1	<1
3. Ambaiboho	20.5	1.80	0.417	4.79	11.4	49	4.3	5.1
4. Ambatofotsy	14.8	1.38	0.531	5.44	10.7	28	2.6	<1
5. Ambatofotsy	18.2	1.56	0.697	5.82	11.7	26	2.2	<1
6. Anjomakely	23.3	2.03	0.305	5.01	11.5	77	6.7	<1
7. Anjomakely	22.6	1.83	0.316	4.62	12.4	72	5.8	<1
8. Anosiboribory	64.3	5.29	0.828	4.98	12.2	78	6.4	<1
9. Anosiboribory	153.1	10.04	1.378	4.74	15.2	111	7.3	3.2
10. Beforona	33.4	3.89	1.128	5.03	8.6	30	3.4	7.1
11. Imerimandroso	20.8	2.59	0.189	4.84	8.0	110	13.7	3.3
12. Imerimandroso	11.1	1.19	0.133	5.17	9.4	84	8.9	4.8
13. Imerimandroso	16.9	2.31	0.188	5.29	7.3	90	12.3	6.9

ranged between 60 and 709 mg P kg^{-1} soil, equivalent to between 54% and 80% of the extracted phosphorus (Table 3). Pyrophosphate, an inorganic polyphosphate with a chain length of two, was detected as the signal at approximately −4.4 ppm in all but the soil with the least carbon. Concentrations ranged between 2 and 11 mg P kg^{-1} soil, which represented up to 4% of the extracted phosphorus (Table 3).

Most organic phosphorus was phosphate monoesters, which occurred as the complex group of signals between 3 and 6 ppm (Fig. 2). Concentrations ranged between 22 and 315 mg P kg^{-1} soil, equivalent to between 16% and 38% of the extracted phosphorus (Table 3). The remaining organic phosphorus was DNA, indicated by the signal at approximately 0 ppm (Fig. 2). Concentrations of DNA constituted up to 77.7 mg P kg^{-1} soil and 9% of the extracted phosphorus, although it was not detected in four soils with relatively low carbon concentrations (Table 3). Where detected, DNA represented between 11% and 23% of the organic phosphorus. Phosphonates, which contain a direct carbon–phosphorus bond, were not detected in any sample.

Total organic phosphorus (i.e., the sum of phosphate monoesters and DNA) ranged between 22 and 393 mg P kg^{-1} soil, equivalent to between 19% and 44% of the extracted phosphorus (Table 3) and between 7% and 29% of the total soil phosphorus (Table 4). The ratio of carbon to organic phosphorus ranged between 235 and

Table 3
Phosphorus composition of Madagascan rice soils determined by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy

Location	NaOH–EDTA total P†	Phosphate‡	Phosphate monoesters‡	DNA‡	Pyrophosphate‡	Total organic P‡
	mg P kg^{-1} soil					
1. Ambaiboho	157 (47)	110 (70)	47 (30)	ND	ND	47 (30)
2. Ambaiboho	174 (34)	117 (67)	54 (31)	ND	4 (3)	54 (31)
3. Ambaiboho	192 (46)	125 (65)	57 (30)	7 (4)	3 (1)	64 (34)
4. Ambatofotsy	192 (36)	154 (80)	31 (16)	4 (2)	2 (1)	36 (19)
5. Ambatofotsy	261 (37)	188 (72)	63 (24)	ND	10 (4)	63 (24)
6. Anjomakely	156 (51)	96 (61)	44 (28)	12 (8)	4 (3)	56 (36)
7. Anjomakely	156 (49)	84 (54)	60 (38)	9 (6)	4 (3)	68 (44)
8. Anosiboribory	679 (82)	476 (70)	164 (24)	35 (5)	3 (<1)	199 (29)
9. Anosiboribory	1106 (80)	709 (64)	315 (28)	78 (7)	4 (<1)	393 (36)
10. Beforona	681 (60)	525 (77)	126 (19)	19 (3)	11 (2)	145 (21)
11. Imerimandroso	108 (57)	60 (56)	34 (31)	10 (9)	4 (4)	44 (41)
12. Imerimandroso	92 (69)	68 (74)	22 (24)	ND	2 (2)	22 (24)
13. Imerimandroso	115 (61)	76 (66)	29 (25)	5 (4)	5 (4)	34 (30)

†Values in parentheses are the recovery (%) of the total soil phosphorus.
‡Values in parentheses are the proportion (%) of the NaOH–EDTA total phosphorus.
ND, not detected.

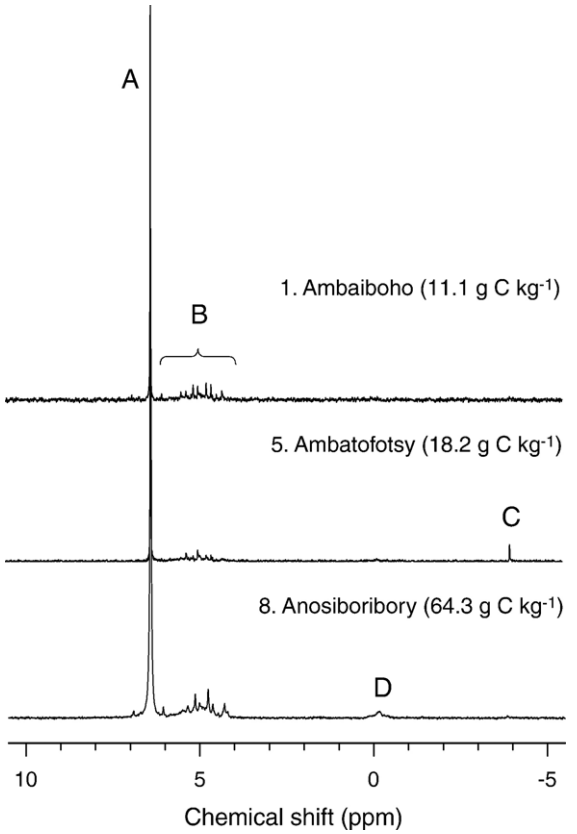

Fig. 2. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of three rice soils from Madagascar. The soils are typical of those containing low, medium, and high carbon concentrations. The labeled peaks are: A, phosphate; B, phosphate monoesters; C, pyrophosphate; D, DNA. Spectra are plotted with 1 Hz line broadening.

505, while the nitrogen to organic phosphorus ratio ranged between 24 and 68 (Table 4). These ratios were much greater than those involving total soil phosphorus (Table 2).

The concentrations of phosphate monoesters, DNA, and total organic phosphorus were strongly and positively correlated with those of total carbon and nitrogen ($r \geq 0.97$; $p < 0.001$) and total phosphorus ($r \geq 0.77$; $p < 0.01$). Phosphate was most strongly correlated with total soil phosphorus ($r = 0.96$; $p < 0.001$). Pyrophosphate was not correlated significantly with any soil property measured. Soil pH was not correlated with organic phosphorus fractions, but was negatively correlated with the proportion of the total soil phosphorus as organic phosphorus ($r = -0.60$; $p < 0.05$). There were few significant correlations with total element ratios, although organic phosphorus and phosphate monoesters were both significantly positively correlated with the carbon to nitrogen ratio ($p < 0.05$). Bicarbonate-extractable phosphate was not correlated with any soil property or phosphorus fraction determined by NMR spectroscopy.

3.3. Detailed composition of phosphate monoesters

Well-resolved signals in the phosphate monoester region of several spectra allowed detailed information to be obtained on individual compounds. The phosphate monoester regions of two spectra are shown in Fig. 3, one of which contains abundant inositol phosphates, the other of which contains none. In four soils (Beforona, one soil from Anosiboribory, and two from Ambaiboho) concentrations of *myo*-inositol hexakisphosphate ranged between 10.4 and 33.1 mg P kg^{-1} soil, representing between 3.6% and 7.8% of the extracted phosphorus and between 12.2% and 26.0% of the organic phosphorus (Table 5). A trace was detected in the third soil from Ambaiboho. The high organic matter soil from Anosiboribory clearly contained *myo*-inositol hexakisphosphate, but line broadening prevented quantification due to overlap between the C-2 phosphate signal and phosphate. *myo*-inositol hexakisphosphate was not detected in the remaining soils.

Six soils contained quantifiable signals from *scyllo*-inositol hexakisphosphate. Concentrations ranged between 4.0 and 44.3 mg P kg^{-1} soil, which represented between 1.6% and 4.0% of the extracted phosphorus and between 7.1% and 11.3% of the organic phosphorus (Table 5). The ratio of *scyllo*-inositol hexakisphosphate to *myo*-inositol hexakisphosphate ranged between 0.33 and 0.67 for the four soils in which both compounds were detected (Table 5). Concentrations of *scyllo*-inositol hexakisphosphate were strongly and positively correlated with total carbon and nitrogen, organic

Table 4
Organic phosphorus as a proportion of the total phosphorus (%) and the ratios of carbon and nitrogen to organic phosphorus

Location	Organic P/total P	C/organic P	N/organic P
	%		
1. Ambaiboho	14.0	235	24
2. Ambaiboho	10.4	311	30
3. Ambaiboho	15.4	318	28
4. Ambatofotsy	6.7	415	39
5. Ambatofotsy	9.1	288	25
6. Anjomakely	18.4	417	36
7. Anjomakely	21.6	332	27
8. Anosiboribory	24.1	322	27
9. Anosiboribory	28.5	390	26
10 .Beforona	12.9	230	27
11. Imerimandroso	23.1	477	59
12. Imerimandroso	16.6	505	54
13. Imerimandroso	18.0	497	68

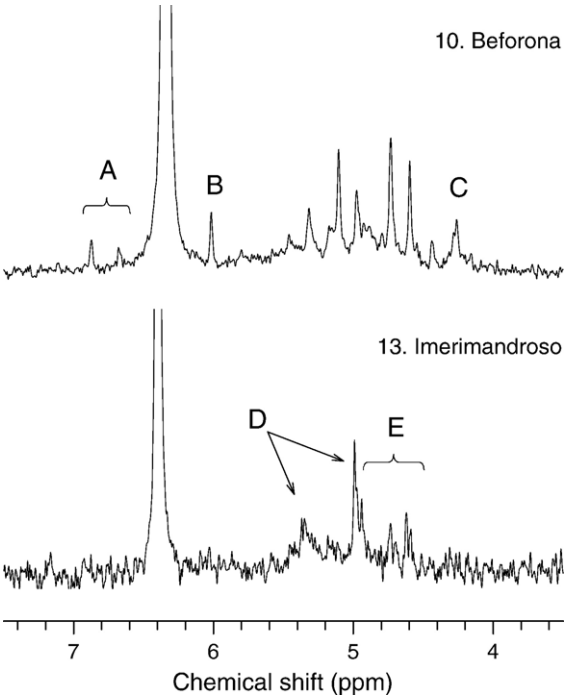

Fig. 3. Solution ^{31}P NMR spectra of NaOH–EDTA extracts of two rice soils from Madagascar, showing detail in the phosphate monoester region. The upper spectrum (Beforona) shows an extract containing inositol phosphates, while the lower spectrum shows an extract containing no detectable inositol phosphates. The labeled peaks are: A, unidentified inositol phosphates; B, the C-2 phosphate of *myo*-inositol hexakisphosphate; C, *scyllo*-inositol hexakisphosphate; D, phosphatidic acid and β-glycerophosphate from the alkaline hydrolysis of phospholipids; E, mononucleotides from the alkaline hydrolysis of RNA. The spectra are plotted with 1 Hz line broadening.

phosphorus, phosphate monoesters, and DNA ($r \geq 0.96$; $p < 0.001$), and less strongly with total soil phosphorus ($r = 0.83$; $p < 0.001$).

Signals upfield of phosphate at approximately 6.8 ppm were quantifiable in five soils. These signals probably represent unidentified inositol phosphates because they persist following hypobromite oxidation (Turner and Richardson, 2004). Concentrations ranged between 4.7 and 29.4 mg P kg^{-1} dry soil, equivalent between 3.0% and 7.8% of the extracted phosphorus and between 10.0% and 25.2% of the organic phosphorus (Table 5).

The remaining phosphate monoester signals were characteristic of those originating from the alkaline hydrolysis of phosphate diesters (Turner et al., 2003a). In particular, prominent signals in all samples at 4.9 and 5.2 ppm indicated the alkaline degradation products of phospholipids, while a group of five signals between 4.4 and 4.8 indicated mononucleotide degradation products of RNA (Fig. 3). These signals constituted almost all the phosphate monoesters in soils without detectable inositol phosphates.

4. Discussion

Organic phosphorus was a relatively small proportion of the total phosphorus in a range of rice soils from Madagascar. However, it may have significance for rice nutrition, given that plant-available phosphate concentrations are extremely low and that much of the remaining phosphorus occurs as inorganic phosphate in stable association with metal oxides. There is currently limited information in the literature with which to assess the availability of soil organic phosphorus to rice plants, although it warrants further investigation.

The organic phosphorus was similar in composition to aerobic mineral soils from a range of agricultural environments, including those in the tropics (e.g., Guggenberger et al., 1996; Solomon and Lehmann, 2000). It was also in broad agreement with results from a previous study of three Oxisols from the region south of Antananarivo and three Andisols from a region west of

Table 5
Concentrations of inositol phosphates in Madagascan rice soils determined by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy

Location	*myo*-inositol hexakisphosphate[†]	*scyllo*-inositol hexakisphosphate[‡]	Other inositol phosphates[§]	*scyllo*-to-*myo* ratio
	mg P kg^{-1} dry soil			
1. Ambaiboho	12.3 (26.0)[¶]	4.0 (8.5)	4.7 (10.0)	0.33
2. Ambaiboho	Trace	4.2 (7.8)	13.5 (25.2)	–
3. Ambaiboho	10.4 (16.2)	4.6 (7.1)	11.8 (18.4)	0.44
8. Anosiboribory	24.3 (12.2)	16.2 (8.1)	29.4 (14.8)	0.67
9. Anosiboribory	NQ	44.3 (11.3)	NQ	–
10. Beforona	33.1 (22.8)	10.9 (7.5)	26.5 (18.2)	0.33

NQ, not quantifiable due to line broadening.
[†] Determined by multiplying the C-2 phosphate signal appearing at approximately 5.95 ppm by six (Turner et al., 2003b).
[‡] Determined by integration of the signal at 4.2 ppm (Turner and Richardson, 2004).
[§] Signals upfield of the phosphate signal at approximately 6.8 ppm (Turner and Richardson, 2004).
[¶] Values in parentheses are the proportion (%) of the total organic phosphorus.

Antananarivo (Itasy) (Zech et al., 1990). The latter soils contained relatively high pH (6.0–6.4) and total phosphorus (up to 2910 mg P kg^{-1} soil). However, detailed phosphorus speciation was prevented by poor spectral resolution, to the extent that the separation of signals from phosphate and phosphate monoesters was difficult in some samples.

A similar phosphorus composition was also reported for humic acids in rice soils from a series of long-term field trials in the Philippines (Mahieu et al., 2000). Two fractions were analyzed: a young 'mobile' humic acid fraction and a more recalcitrant 'calcium humate' fraction. Strong correlations were reported subsequently between organic phosphorus fractions observed by solution ^{31}P NMR spectroscopy and various carbon and nitrogen fractions observed by solid-state NMR on the same humic acids (Mahieu et al., 2002). Unfortunately, the humic acids contained only around 20% of the total soil organic phosphorus, so the majority remained unidentified.

In the current study, inositol phosphates were detected in six soils at similar concentrations to those reported in other environments (Harrison, 1987; Turner et al., 2002). Concentrations vary widely, although much of the early literature must be regarded with caution for analytical reasons (Irving and Cosgrove, 1981; Turner et al., 2002). Data for rice soils is limited, although in a range of soils from Bangladesh, of which some were presumably from rice paddies, inositol hexakisphosphate (stereoisomers were not identified separately) constituted between 18 and 150 mg P kg^{-1} and between 9% and 83% of the soil organic phosphorus (Islam and Ahmed, 1973). There is little quantitative data for *scyllo*-inositol hexakisphosphate in any soils, although concentrations in twenty-nine temperate pasture soils from England and Wales ranged between 11 and 130 mg P kg^{-1} soil and accounted for between 4% and 15% of the soil organic phosphorus (Turner et al., 2005b). In that study, the ratio of *scyllo*-inositol hexakisphosphate to *myo*-inositol hexakisphosphate ranged between 0.29 and 0.79 and was therefore similar to the range reported here for the four soils in which both compounds were detected.

Inositol hexakisphosphates are stable in aerobic soils due to their strong complexation with metals and clay surfaces (Celi and Barbaris, 2005). This means they are relatively unavailable to most crops (Richardson et al., 2005) and there is no evidence to suggest that they are available to rice growing in aerobic soils. It is worth noting, however, that the secretion of organic anions from rice roots under aerobic conditions (Kirk et al., 1999; Huguenin-Elie et al., 2003) could release inositol phosphates from binding sites (Hens et al., 2003).

Inositol phosphates may be more bioavailable in flooded soils. Although information is limited, inositol phosphate concentrations decreased rapidly following submergence of acidic soils from Bangladesh (Islam and Ahmed, 1973) and Japan (Furukawa and Kawaguchi, 1969), with the greatest changes in soils that received lime or were rich in organic matter. Inositol phosphates were also absent in soils from subtropical freshwater wetlands (Turner and Newman, 2005). Anaerobic conditions appear critical, because inositol phosphates in marine sediments from Tokyo Bay were hydrolyzed much more rapidly when incubated under anaerobic conditions compared to parallel samples incubated aerobically (Suzumura and Kamatani, 1995). A caveat is that anaerobic reduction of complexes between iron and inositol hexakisphosphate was reported to form insoluble Fe_4-phytate rather than solubilizing the free inositol hexakisphosphate (De Groot and Golterman, 1993). This could account for the observed decreases in inositol phosphate following submergence of rice soils, but only if, as seems unlikely, the complex remains insoluble in the strong alkali used to extract inositol phosphates.

Alkali-extractable inorganic phosphate is conventionally classified as associated with aluminum and iron (Hedley et al., 1982) and was a considerable proportion of the total phosphorus in the soils studied here. It may therefore be an important source of phosphorus to rice growing on infertile soils. It seems to be a dynamic pool of phosphorus, because marked changes in NaOH-extractable phosphate were detected in response to mineral fertilization of rice soils of Bangladesh (Saleque and Kirk, 1995). Indeed, there is evidence that it contributes to plant nutrition, because it was depleted in the vicinity of rice roots growing on a low-fertility infertile Ultisol (Hedley et al., 1994) and in the rhizosphere of two agroforestry species growing in a Kenyan Oxisol (George et al., 2006). This clearly warrants further investigation.

Despite the numerous management differences between SRI and conventional rice cultivation, there were no clear differences in the phosphorus composition of soils sampled from adjacent fields under the two systems at five locations. This was unexpected, because management can strongly influence the phosphorus composition of rice soils. For example, increasing the submergence time during rice cultivation in the Philippines increased the proportion of phosphate diesters in mobile humic acids from a quarter to almost half of the organic phosphorus, with corresponding decreases in phosphate monoesters (Mahieu et al., 2000).

The similarity in soil organic phosphorus composition under SRI and conventional cultivation does not preclude differences in phosphorus turnover. Accelerated nutrient

turnover is expected under SRI, because compost addition and soil aeration both enhance microbial activity (McLatchey and Reddy, 1998; Stoop et al., 2002). The continual wetting and drying cycles involved in SRI cultivation may also drive organic phosphorus turnover through microbial lysis (Turner and Haygarth, 2001). An accelerated rate of organic phosphorus turnover would explain in part the improved phosphorus uptake by SRI plants compared to those under conventional cultivation in Madagascar (Barison, 2003), although this is difficult to quantify in tropical soils due to their large capacity to sorb phosphate (Bühler et al., 2003).

In summary, organic phosphorus in a range of Madagascan rice soils occurred mostly as phosphate monoesters, with smaller concentrations of DNA. Inositol phosphates were present in detectable concentrations in less than half of the soils, suggesting their relatively rapid turnover in this environment. Soils rich in organic matter contained more organic phosphorus, but there were no apparent differences in the amounts or forms of organic phosphorus between soils under conventional flooded rice cultivation and the system of rice intensification. Additional experiments are now required to assess the role of soil organic phosphorus in the nutrition of rice in a range of soils and cultivation systems.

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