

Dissolved phosphorus composition of grassland leachates following application of dairy-slurry size fractions

Bárbara Fuentes¹, Elizabeth Dixon², María de la Luz Mora³, Benjamin L. Turner⁴, and Roland Bol^{2*}

¹ Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile; and Departamento de Ingeniería Química, Universidad Católica del Norte, Antofagasta, Chile

² Sustainable Soils and Grassland Systems Department, Rothamsted Research North Wyke, Okehampton Devon EX20 2SB, United Kingdom

³ Departamento de Ciencias Químicas, Universidad de La Frontera, Casilla 54-D, Temuco, Chile

⁴ Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Panama

Abstract

Appropriate management of P from slurry can increase crop production and decrease nutrient loss to water bodies. The present study examined how the application of different size fractions of dairy slurry influenced the quantity and composition of P leached from grassland in a temperate climate. Soil blocks were amended (day 0 = start of the experiment) with either whole slurry (WS), the > 425 µm fraction (coarse slurry fraction, CSF), the < 45 µm slurry fraction (fine liquid slurry fraction, FLF), or not amended, *i.e.*, the control soil (CON). Deionized water was added to the soil blocks to simulate six sequential rainfall events, equivalent to 250 mm (day 0.2, 1.2, 4.2, 11.2) or 500 mm of rainfall (day 18.2 and 25.2), with leachates collected the following day. The results showed that total dissolved P (TDP), dissolved reactive P (DRP), dissolved unreactive P (DUP), orthophosphate, phosphomonoester, and pyrophosphate concentrations generally decreased with the increasing number of simulated rain events. Total dissolved P was leached in the following order WS > FLF ≈ CSF > CON. Dissolved organic C was correlated with TDP, DRP, and DUP in leachates of all treatments. The highest concentrations of dissolved phosphomonoesters and pyrophosphate (147 µg P L⁻¹ and 57 µg P L⁻¹, respectively) were detected using solution ³¹P-NMR spectroscopy in the WS leachates. Overall, there were significant differences observed between slurry treatments (*e.g.*, relative contributions of inorganic P vs. organic P of dissolved P in leachates). Differences were independent from the rate at which slurry P was applied, because the highest dissolved P losses per unit of slurry P applied were measured in the FLF, *i.e.*, the treatment that received the smallest amount of P. We conclude that the specific particle-size composition of applied slurry influences dissolved P losses from grassland systems. This information should be taken in account in farm-management approaches which aim to minimizing dissolved slurry P losses from grassland systems.



Key words: dairy slurry / leachate / dissolved P / total P / reactive P / unreactive P / dissolved organic carbon / grassland / ³¹P-nuclear magnetic-resonance spectroscopy

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1 Introduction

Dairy slurry is typically surface-applied to UK pastures as an organic fertilizer (Anon, 2000). The phosphorus (P) within it can be taken up by plants and microorganisms, sorbed or otherwise held in the soil, or lost by leaching or runoff. The latter signals some inefficiency in recycling or retention of P in soil–plant systems, which may contribute to the eutrophication of water bodies (Withers and Haygarth, 2007; Blackwell et al., 2010; Granger et al., 2010). Cattle manure contains total P concentrations of between 4 and 18 g P kg⁻¹ (He et al., 2004; Sharpley et al., 2004; Toor et al., 2006; Fuentes, 2009). Of this, inorganic P constitutes 60%–70% (He et al., 2004; Fuentes, 2009), with organic and condensed inorganic phosphates constituting the remainder. Studies have documented the presence of pyrophosphate, phosphomonoesters, phosphodiester, and phosphonates in animal manures (Hansen et al., 2004; Turner, 2004; McDowell and Stewart, 2005). Phosphorus in leachate from grassland soils amended with

dairy slurry or effluent also contains a considerable organic P component. For example, in a freely draining New Zealand silt-loam grassland soil organic P constituted 88% of the total leached P, mainly as phosphomonoesters and phosphodiester (Toor et al., 2003).

Espinosa et al. (1999) identified a range of organic P species in leachate from grassland in a temperate climate using ion-exchange preconcentration followed by high-performance liquid chromatography separation, while Turner et al. (2002) used phosphatase enzymes to identify functional groups of organic P compounds in water extracts of a series from pasture soils of Australia. Solution ³¹P-NMR spectroscopy is a convenient method for the characterization of the P forms in grassland soils (*e.g.*, Turner et al., 2003b; Bol et al., 2006; Murphy et al., 2009) and organic wastes (Turner, 2004; McDowell and Stewart, 2005; Fuentes et al., 2009), although

* Correspondence: Dr. R. Bol; e-mail: roland.bol@rothamsted.ac.uk

only a few studies have reported the ^{31}P -NMR composition of soil leachate water or runoff (Toor et al., 2003; Bourke et al., 2009). This is almost certainly due to the disparity between the relatively low P concentrations in runoff water compared to the high P concentrations required for solution ^{31}P -NMR spectroscopy. However, the method was used to detect P compounds in leachate from a soil amended with dairy-shed effluent (Toor et al., 2003). This revealed a remarkable decline in inorganic phosphate in leachate compared to the original effluent, indicating its retention in the soil profile, whereas more mobile organic phosphates passed through the soil and were detected in leachate water. Recently, Cade-Menun et al. (2006) used lyophilization to pre-concentrate river water for detection by solution ^{31}P -NMR spectroscopy.

Møller et al. (2002) showed that the sieving cattle slurry generated a solid fraction with a higher P concentration (1.62–2.04 g L⁻¹) than the liquid fraction (0.63–0.47 g L⁻¹). Analysis of 10 different dairy slurries showed that (80 ± 7)% of total slurry P content was present in the < 45 µm fraction, (5 ± 3)% in the 45–425 µm fraction, and (15 ± 3)% in the > 425 µm fraction (S. Granger, pers. comm.). Variation in physico-chemical and biological interactions of different particle-size slurry fractions in grassland soils influences the loss of organic matter (OM), nutrients, and greenhouse gases (Fangueiro et al., 2007, 2008, 2010). However, no mechanistic information exists on the extent to which individual slurry fractions (with their differing P contents) are transported/retained in grassland soils following surface application. To address this, we compared the quantity and composition of dissolved P leached from a grassland soil amended with whole slurry or two slurry fractions (< 45 and > 425 µm). The finer fraction (< 45 µm) in dairy slurry often contains the majority of P and has a liquid appearance and consistency (i.e., likely to permeate in and through the soil). In contrast, the coarser fraction (> 425 µm) contains more particulate material and has a solid consistency and appearance, so is more likely to remain at the surface when applied. Our aim was to examine how the surface application of different-size dairy cattle-slurry fractions influences the quantity and composition of dissolved P leaching from grassland soil under temperate climate conditions.

2 Materials and methods

2.1 Soil sampling and preparation

Soil samples were collected from a permanent-grassland site at North Wyke Research in Devon, SW England, in November 2007. Soil samples were taken from the Halstow series of noncalcareous Pelosols (corresponding to a Gleyic Cambisol in the FAO classification). Intact soil blocks (25 cm wide, 25 cm long, 6 cm deep) were removed from the top soil, and the vegetation was cut in the laboratory to 1 cm above the soil surface. The soil samples were adjusted initially to 60% field capacity with deionized water. Soil characteristics were: sand 22%, silt 47%, and clay 31%, organic C (OC) 2.9%, pH in water (1:2.5 soil-to-solution ratio) 5.0, cation-exchange capacity (CEC) 20.3 cmol_c kg⁻¹ (Harrod and Hogan, 2008).

2.2 Slurry preparation and fractionation

Dairy slurry was collected from a slurry reception pit on a farm located near North Wyke Research. The slurry was stored at 4°C for 2 weeks prior to fractionation. The particle-fractionation procedure of Fangueiro et al. (2007) was carried out with modifications. Briefly, deionized water (6 L, i.e., 2 × 3 L) was added to 3 L of whole slurry to facilitate the fractionation process, and then the slurry–water mix was successively passed through two sieves (425 µm and 45 µm) to obtain two fractions: fraction 1: > 425 µm (coarse solid fraction; CSF) and < 45 µm (fine liquid fraction; FLF). The 45–425 µm fraction was discarded as the obtained quantity was insufficient to conduct the subsequent leaching study. The fractionation also did not provide enough material of the > 425 µm fraction (CSF) for the full experimental design. Only 125 g of this fraction could be applied, instead of the 313 g by weight as applied in the WS and FLF treatments. We provide values (in italics) for indicative purpose for a CSF* fraction throughout the manuscript: derived by multiplying the actual leachate concentration measurements in the CSF fraction with an application-rate correction factor of 2.51 (equivalent 313 g/125 g). This is for guidance only, as the correction factor of 2.51 assumes that there is a linear relationship between slurry-application rate and concentration rate in the leachate, but this assumption was not tested. Furthermore, soil is not inert medium and many processes in the soil are rate-dependent. However, to enable some comparison of the effects of the three different slurries on the dissolved P losses in the experimental setup a P response factor (P_{rf}) was calculated as follows:

P_{rf} = concentration of P leached on a specific day for one of the three slurry treatments/actual amount of P applied in each of the three slurry treatments.

A higher P-response factor indicates that more P is lost in the dissolved phase per equivalent amount of slurry P applied.

2.3 Leachate experiments

Leachate composition from intact soil blocks (contained in 25 cm × 25 cm × 10 cm deep trays) from three different slurry amendments (i.e., whole slurry, WS; > 425 µm slurry, CSF; and < 45 µm slurry fraction, FSF fraction) were compared to a control treatment (CON) in which no slurry was applied to the soil (see Tab. 1 for their composition). Each treatment was conducted in quadruplicate. The application rate of whole slurry or fractions to each block was equivalent to 50 m³ slurry ha⁻¹. As the amount of dry matter and P varied between slurry treatments, different amounts of inorganic and organic P were added in each treatment (Tab. 1). The amount of water applied to soil–slurry samples and control was adjusted by using the known slurry–dry matter contents.

Six precipitation/wetting events were simulated, by slowly and gently pouring deionized water over the whole of each soil block, with an application rate of 250 mL during the first four events and 500 mL during two final precipitation events. Deionized water was added to soil blocks 0.2, 1.2, 4.2, 11.2, 18.2, and 25.2 d after slurry application. The resulting soil leachates, i.e., the solution which had infiltrated vertically

Table 1: Characterization and application rate of slurry and slurry fractions. Data presented are means and standard deviation of four replicate blocks. Values in parentheses are percentage of inorganic (Pi) and organic P (Po) as a portion of the total P (Pt) for whole slurry and slurry fractions

	Whole slurry	> 425 μm	< 45 μm
Dry matter / %	12.6 \pm 0.13	11.9 \pm 0.06	0.60 \pm 0.03
Concentration			
Pt / $\mu\text{g g}^{-1}$	4624 \pm 62	2506 \pm 24	12158 \pm 212
Pi / $\mu\text{g g}^{-1}$	2760 \pm 51 (60%)	1089 \pm 52 (43%)	7685 \pm 49 (63%)
Po / $\mu\text{g g}^{-1}$	1863 \pm 51 (40%)	1417 \pm 44 (57%)	4500 \pm 226 (37%)
Application rate / g	313	125	313
Amounts of P applied			
Pt / mg	182 \pm 3	37 \pm 1	23 \pm 1
Pi / mg	109 \pm 2 (60%)	16 \pm 1 (43%)	14 \pm 1 (63%)
Po / mg	73 \pm 2 (40%)	21 \pm 2 (57%)	8 \pm 1 (37%)

through the soil, were collected the day following each wetting event (*i.e.*, 1, 2, 5, 12, 19, and 26 d after slurry application). The soil blocks were incubated at room temperature for the whole experimental period. Between each rainfall event, water was added to maintain soil moisture content at 60% of field capacity.

2.4 Slurry and soil characterization

Dry-matter content of the slurry samples (WS, CSF, and FSF) were determined by weighing before and after drying at 85°C overnight. Total P in dried samples was determined by the ignition method at 550°C, and inorganic P was determined in unignited samples, in both cases by extraction in 1 M H₂SO₄. Organic P was subsequently calculated as the difference between P determined in ignited and unignited samples (Saunders and Williams, 1955).

2.5 Leachate analysis

Leachate was passed through a 0.45 μm membrane filter (Whatman). Dissolved total C (DTC) and dissolved organic C (DOC) were determined using the Skalar Formacs analyzer. Total dissolved P (TDP) and dissolved reactive P (DRP) were determined by molybdate colorimetry according to Rowland and Haygarth (1997). Dissolved unreactive P (DUP) was determined by difference between TDP and DRP.

2.6 Solution ³¹P-NMR spectroscopic analysis of leachate

Leachate samples from different treatments and rainfall events were frozen at -80°C, lyophilized, and ground prior to ³¹P-NMR analysis. Each freeze-dried extract (\approx 100 mg) was redissolved in 0.1 mL of deuterium oxide and 0.9 mL of a solution containing 1.0 M NaOH and 0.1 M Na₂EDTA, and then transferred to a 5 mm NMR tube. Solution ³¹P-NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ³¹P. 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 for sufficient spin-lattice relaxation between scans for P compounds in NaOH-EDTA (Cade-Menun *et al.*, 2002). Approximately 30,000 scans were acquired for each sample. Chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H₃PO₄. Spectra were plotted with a line broadening of 5 Hz, and signals were assigned to P compounds or functional groups based on literature reports (Turner *et al.*, 2003a). Signal areas were calculated by integration and P concentrations calculated by multiplying the proportion of the spectral area assigned to a specific signal by the total P concentration (mg P L⁻¹) in the original sample. This assumes that all P in the original sample was redissolved in the NaOH-EDTA and measured in the NMR analysis.

2.7 Calculations and statistical analysis

The nutrient data of soil leachates are reported as the means and standard error of the four replicated blocks. The TDP, DRP, DUP, and DOC were evaluated for statistically significant difference between the four treatments and timing of sampling using two-way analysis of variance (ANOVA) with replication. The relationship between DOC and P variables was evaluated by Pearson's correlation coefficient at $p < 0.05$. All statistical procedures (means and standard deviation) were conducted using STATISTICA 6.0 scientific software.

3 Results and discussion

3.1 Slurry and slurry fraction properties

The WS and the CSF fraction had similar dry-matter contents (around 12%), but the FLP was mainly in liquid form (Tab. 1). It has been demonstrated that more than 50% of the particles in slurry are < 45 μm (*i.e.*, the FSF-size fraction) (Fangueiro *et al.*, 2007). Total P in WS was \approx 4.6 mg P g⁻¹ and was predominantly in inorganic form (60%) (Tab. 1). Similar percentages of inorganic P in slurry and manures were reported previously (Haynes and Williams, 1993; Ajiboye *et al.*, 2004;

Hansen et al., 2004; McDowell and Stewart, 2005). Total P in the CSF fraction was lower than in the WS at $\approx 2.5 \text{ mg P g}^{-1}$, but more than half was organic P (57%). This was in contrast with the FSF where most of the P was inorganic P (63%), similar to the WS (Tab. 1). However, total P ($\approx 12.2 \text{ mg P g}^{-1}$) was nearly three times higher in the FSF when compared to the WS (Tab. 1).

3.2 Phosphorus concentrations and P-response factor in the soil leachates

This study showed that P in leachate is modified greatly by the application of slurry particle-size fractions and P load to the soil (Tab. 2). Measured differences in TDP, DRP, and DUP were always highly significant ($p < 0.001$) for the four treatments (WS, CSF, FSF, and CON), the six sampling dates (1, 2, 5, 12, 19, and 26 d) as well as the treatment \times day interaction. TDP measured in the leachates from the WS treatment was always greater than in any other leachates in the experiment (Tab. 2). Indeed, TDP concentrations in leachates in CON were similar for all six rain events and did not exceed $187 \mu\text{g P L}^{-1}$ (Tab. 2). For comparison, Withers and Lord (2002) noted that in the first three winter storms (totaling 67 mm of precipitation) following a single application of 2.6 t ha^{-1} of cattle slurry to a silty-clay-loam grassland soil, TDP concentrations of 25 mg P L^{-1} were recorded in surface runoff and 1.8 mg P L^{-1} in drain flow. The latter value was close to the concentration observed in the first leachate (2.2 mg P L^{-1}) from WS. However, we simulated extreme rainfall events equivalent to 250 or 500 mm of total rainfall. The concentrations of DRP and DUP detected in leachates were not significantly different ($p > 0.05$), as noted previously for this grassland (Turner and Haygarth, 1999) and other soils (Toor et al., 2003; Murphy, 2007). The surface-applied WS application consistently produced elevated concentrations of TDP in leachate, and this effect was evident until the sixth rain event. Immediately following surface application, the DRP concentrations were 1212 , 300 , and $143 \mu\text{g P L}^{-1}$ in soil leachates of WS, FSF, and CSF, respectively (Tab. 2). The DUP concentrations were 961 , 358 , and $242 \mu\text{g P L}^{-1}$ in soil leachates of WS, FSF, and CSF, respectively (Tab. 2).

The total quantity of P lost from the soil after six rain events from WS reached (597 ± 38) $\mu\text{g P}$ (equivalent to 9.6 mg P m^{-2} or 0.16 g P m^{-3}), while in the FSF, CSF, and CON it reached (180 ± 15) μg , (143 ± 20) μg , and (107 ± 22) μg , respectively. In FSF and CSF the total amount of DRP lost ranged between (88 ± 12) μg and (88 ± 20) μg , while the sum of the soil DRP losses in CON reached (68 ± 15) μg . However, most DRP was lost predominantly from the WS ($[339 \pm 16]$ μg). The DUP collected in WS, FSF, and CSF leachates reached (258 ± 24) μg , (92 ± 12) μg , and (62 ± 8) μg , respectively, whereas the DUP loss in CON was only (39 ± 8) μg . Overall, the percentage of the applied P that was lost in the leachates after the six rainfall events was similar at $\approx 0.3\%$ in all four treatments (WS, CSF, FSF, and CON).

The P concentrations leached from slurry and slurry fractions initially decreased until the third (DRP and DUP; Tab. 2) or the fourth (TDP; Tab. 2) simulated rainfall event. The potential

for TDP, DRP, and DUP loss is therefore greatest for initial events, but declines with time, the latter probably due to the stabilization of slurry-derived P through interactions with soil constituents. Application of the WS and FSF would promote rapid infiltration of the smaller slurry-derived particles into soil, increasing the transfer of the dissolved P to greater soil depth and enhancing the possibility of P leaching. However, the more rapid microbial degradation of these smaller organic particles may also contribute to the overall release of P, causing a further localized enrichment of P concentrations in the deeper soil and ultimately in the leachates. Studies carried out by Fanguero et al. (2007, 2010), using the greatest CO_2 production as an indicator, also showed a rapid biodegradation of the smaller particles ($< 45 \mu\text{m}$) of slurry applied to the soil surface. The authors suggested that the rapid biodegradation is probably a direct result of the higher amount of N contained in this slurry fine fraction.

The data in Fig. 1 shows that the P-response factor for TDP, DRP, and DUP in the first 5 d after application is higher for the FLF application than WS and CSF, suggesting that per mg of P applied by means of this slurry treatment FLF more dissolved P is leached from the grassland in our experimental setup. Beyond day 5, no significant differences were observed in the P-response factor between the three slurry treatments. The initial differential difference in P response between the three slurry treatments is probably related to the fact that the FLF is a more liquid amendment than the other

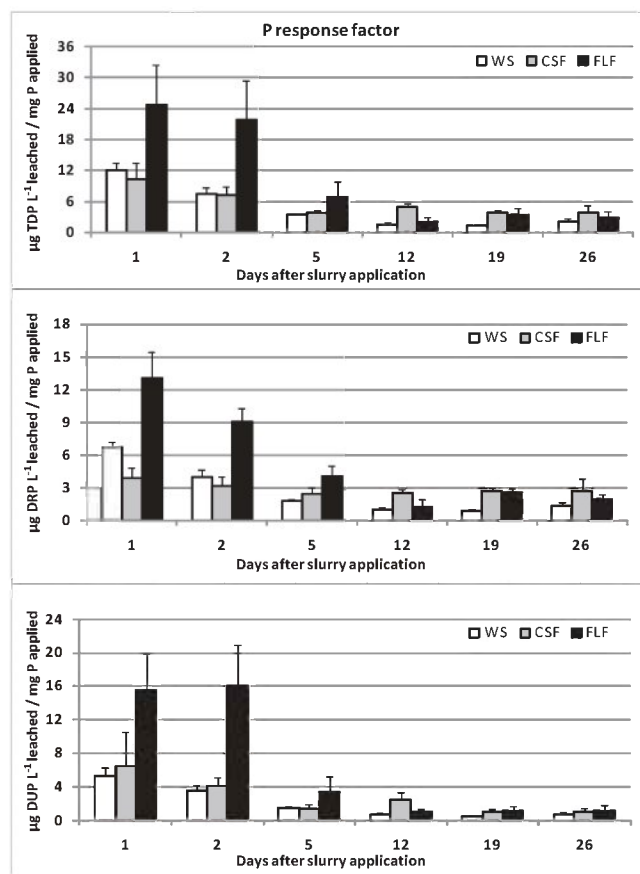


Figure 1: The P-response factor of the different slurries to the soil.

Table 2: Concentrations of dissolved P in leachates from the control-amended soils with slurry and slurry fractions during the experimental period, for total dissolved P (TDP), dissolved reactive P (DRP), and (c) dissolved unreactive P (DUP).

TDP / $\mu\text{g L}^{-1}$					
Treatment day	WS	CSF	CSF*	FSF	CON
1	2173 \pm 223	385 \pm 97	966 \pm 243	657 \pm 108	120 \pm 36
2	1367 \pm 199	271 \pm 53	680 \pm 132	579 \pm 111	187 \pm 40
5	617 \pm 36	143 \pm 17	360 \pm 43	173 \pm 51	170 \pm 75
12	298 \pm 54	184 \pm 22	461 \pm 56	60 \pm 17	128 \pm 18
19	237 \pm 38	140 \pm 18	352 \pm 45	87 \pm 17	104 \pm 18
26	303 \pm 97	142 \pm 46	357 \pm 115	71 \pm 20	179 \pm 70

DRP / $\mu\text{g L}^{-1}$					
Treatment day	WS	CSF	CSF*	FSF	CON
1	1212 \pm 78	143 \pm 32	360 \pm 81	300 \pm 49	77 \pm 27
2	723 \pm 110	118 \pm 29	296 \pm 72	209 \pm 24	103 \pm 26
5	333 \pm 16	90 \pm 20	226 \pm 49	95 \pm 18	87 \pm 29
12	180 \pm 42	88 \pm 8	221 \pm 21	32 \pm 13	45 \pm 10
19	163 \pm 24	100 \pm 11	252 \pm 27	60 \pm 7	84 \pm 18
26	199 \pm 53	102 \pm 35	256 \pm 89	45 \pm 8	116 \pm 40

DUP / $\mu\text{g L}^{-1}$					
Treatment day	WS	CSF	CSF*	FSF	CON
1	961 \pm 147	242 \pm 128	607 \pm 321	358 \pm 86	44 \pm 11
2	645 \pm 94	153 \pm 31	385 \pm 78	370 \pm 96	85 \pm 16
5	284 \pm 30	53 \pm 14	134 \pm 35	79 \pm 36	83 \pm 46
12	118 \pm 31	96 \pm 26	240 \pm 66	28 \pm 7	83 \pm 11
19	74 \pm 16	40 \pm 10	100 \pm 26	27 \pm 11	20 \pm 3
26	104 \pm 43	40 \pm 11	101 \pm 28	26 \pm 13	64 \pm 31

DOC / mg L^{-1}					
Treatment day	WS	CSF	CSF*	FSF	CON
1	38.7 \pm 4.8	19.4 \pm 5.7	48.6 \pm 14.3	16.7 \pm 3.1	4.1 \pm 0.5
2	36.8 \pm 5.2	14.1 \pm 1.7	35.4 \pm 4.2	21.2 \pm 5.1	4.5 \pm 0.2
5	21.4 \pm 2.0	11.1 \pm 1.4	27.9 \pm 3.4	9.3 \pm 2.9	4.4 \pm 0.4
12	10.8 \pm 1.2	6.6 \pm 0.6	16.6 \pm 1.6	5.6 \pm 1.0	18.4 \pm 11.0
19	9.8 \pm 0.7	10.6 \pm 1.4	26.7 \pm 3.4	7.8 \pm 1.2	7.4 \pm 0.5
26	11.2 \pm 2.5	6.9 \pm 0.5	17.3 \pm 1.3	4.6 \pm 0.7	7.4 \pm 0.5

two treatments (WS and CSF), and as such any (dissolved) P contained in it is more likely to move through the soil and be lost through the leaching process.

The incorporation of OM through slurry and slurry fractions generated high concentrations of DOC in the leachates (20–50 mg C L^{-1}) during the three first rain events (Tab. 2). Measured differences in DOC were always highly significant ($p < 0.001$) for the four treatments (WS, CSF, FSF, and CON), the six sampling dates (1, 2, 5, 12, 19, and 26 d) as well as the treatment \times day interaction. Furthermore, TDP,

DRP, and DUP data all showed significant linear relationships with DOC content in the leachates:

$$TDP = 45.2 \times DOC - 192 \quad (R^2 = 0.82), \quad (1)$$

$$DRP = 24.5 \times DOC - 94 \quad (R^2 = 0.80), \quad (2)$$

$$DUP = 22.0 \times DOC - 102 \quad (R^2 = 0.86). \quad (3)$$

High DOC concentrations were detected in leachates from WS compared with slurry fractions and the control (order was

WS > FSF = CSF > CON). The quantities of DOC in leachates tend to decrease with rain events. The addition of whole slurry resulted in a sevenfold increase in DOC in leachates compared to the control soil. Similar results were reported by *Bol et al.* (1999), who pointed out that $\approx 50\%$ of the C present in leachate was native soil C, suggesting mechanisms such as “priming effects”, “displacement effects”, or “pool substitution”. Dissolved organic C is an important fraction of the soil OM and originates mainly from the degradation of large organic molecules, root exudates, and organic compounds from microbial biomass. The linkages of P to organic C in soil are known to play a key role in the mobilization of P and act directly to increase organic P in leachate. Earlier studies by *Bol et al.* (2006) had already demonstrated that the proportions of dung-derived P and dung-derived C in grassland soils are correlated.

3.3 Solution ^{31}P -NMR spectroscopy

Table 3 shows the results from the solution ^{31}P -NMR analyses of the leachate, and Fig. 2 shows a typical NMR spectrum obtained on the samples. The gross proportion of the

functional P groups was comparable in all treatments, *i.e.*, 73% or more was phosphate, up to 25% being present as phosphate monoesters and up to 13% consisting of pyrophosphate. Clearly, relatively less organic P was present in the leachate samples (0%–25%) as compared the original (source) samples: in WS 40% of the P was organic, in CSF 57% was organic P, and in FSF 37% was organic P. Therefore, our results with respect to slurry applications to grassland soils differ from observations by *Toor et al.* (2003), who found a decline in inorganic phosphate in soil leachates compared to the original dairy-farm effluent. The latter is therefore retained in the soil profile, whereas the relatively more mobile organic phosphates passed readily through the soil and were detected in leachate water. It is not clear if these differences are related to differences in soil type, *i.e.*, freely draining silt loam (*Toor et al.*, 2003) vs. poorly draining clay loam (this study) and thus the type and length of the interactions between various P forms in the amendments and the soil matrix, or other reasons.

The presence and variation in organic P content among treatments and sampling times also seems to exclude the possibi-

Table 3: Distribution of P compounds in leachates from slurry-amended and control soils during the six rain events. Values in italics and subscript in the > 425 μm section are calculated based on 313 g amendment (CSF value $\times 2.51$); di-ester P ($6 \mu\text{g L}^{-1}$) was only detected in the > 425 treatment in rain event 6.

Treatment	Rain event	Total P	Phosphate	Monoester / $\mu\text{g L}^{-1}$	Pyro-P	Organic P %TP
whole slurry	1	2173	2070	46	57	2
	2	1367	1191	147	30	11
	3	617	508	81	28	13
	4	298	298	0	0	0
	5	237	179	59	0	25
	6	303	272	23	8	8
< 45 μm	1	657	597	36	25	5
	2	579	469	76	34	13
	3	174	143	18	12	10
	4	60	60	0	0	0
	5	87	75	8	5	9
	6	71	61	7	4	10
> 425 μm	1	385 ₉₆₆	346 ₈₆₈	31 ₇₇	10 ₂₄	3 ₈
	2	271 ₆₈₁	197 ₄₉₅	62 ₁₅₅	12 ₃₀	9 ₂₃
	3	143 ₃₆₀	143 ₃₆₀	0 ₀	0 ₀	0 ₀
	4	184 ₄₆₁	184 ₄₆₁	0 ₀	0 ₀	0 ₀
	5	140 ₃₅₂	129 ₃₂₄	10 ₂₅	2 ₅	3 ₇
	6	142 ₃₅₇	125 ₃₁₄	13 ₃₂	2 ₅	4 ₉
Control	1	121	90	14	16	12
	2	187	141	37	10	20
	3	170	170	0	0	0
	4	128	128	0	0	0
	5	104	104	0	0	0
	6	179	179	0	0	0

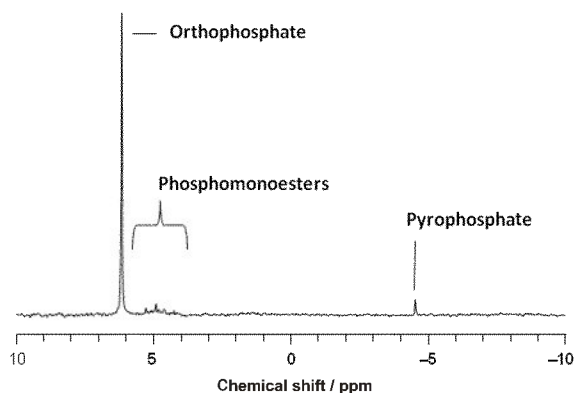


Figure 2: An example solution ^{31}P -nuclear magnetic resonance (NMR) spectrum of a leachate sample (WS; rain event 3), showing the main identified signals. The spectrum was plotted using 5 Hz line broadening.

lity that some organic P forms did not enter solution when preparing the samples for NMR analysis. Clearly the amounts and transfers of slurry- and soil-derived organic P following rainfall are driven by a mix of physico-chemical and biological soil processes that on a daily to weekly temporal time frame show complex behavior of organic-P patterns in leachates.

Phosphomonoesters and pyrophosphate were detected in CON leachates during the first two rain events, the highest concentrations were $37 \mu\text{g P L}^{-1}$ and $16 \mu\text{g P L}^{-1}$, respectively. The former were also detected in leachates from WS (except rain event 4), whereas pyrophosphate was present in the first three leachate samples from WS. Phosphomonoesters and pyrophosphate were generally present in leachates from CSF and FLF. Overall, the highest concentrations of phosphatemonoesters and pyrophosphate were found in WS at $147 \mu\text{g}$ and $57 \mu\text{g P L}^{-1}$, respectively. Phosphodiester were only detected in a single leachate from CSF during rain event 6 at a concentration of $3 \mu\text{g L}^{-1}$. It is likely that the concentrations of phosphomonoesters would be overestimated due to the degradation of some phosphodiester (specifically RNA and phosphatidylcholine) in the alkaline conditions of the NMR analysis (Turner et al., 2003a). No phytate (*i.e.*, myo-inositol hexakisphosphate) was detected in any leachate sample. This was expected, given that (1) dairy manures from pasture-fed cows do not generally contain large concentrations of inositol phosphate (Turner, 2004; McDowell and Stewart, 2005), and (2) phytate is stabilized strongly in soils through interactions with clays and amorphous metal oxides, minimizing its risk of leaching (Espinosa et al., 1999).

Chemical analysis showed that much of the TDP was in the form of DRP. Due to the low concentrations of organic P in leachates, it seems likely that the DUP pool included condensed inorganic P species such as pyrophosphate. Although a low concentration of phosphodiester was detected, it is probable that these compounds (at least in the form of RNA and some phospholipids) were present in leachates and degraded to phosphomonoester during alkaline extraction and ^{31}P -NMR analysis. Similar observations were made by Bol et al. (2006), in that phosphomonoester-to-phos-

phodiester ratios were not fully reflected in the original samples due to hydrolysis of alkali-labile diesters to phosphomonoesters during analysis. The similar predominance of phosphomonoester over diester moieties in leachates from amended soils was reported by Toor et al. (2003) and Bourke et al. (2009).

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