Eutrophication from Agricultural Sources- The Impact of the Grazing Animal on Phosphorus, Nitrogen, Potassium and Suspended Solids Loss from Grazed Pastures - Phosphorus Dynamics in Grazed Grassland

Final Report

Environmental Protection Agency

The Environmental Protection Agency (EPA) is a statutory body responsible for protecting the environment in Ireland. We regulate and police activities that might otherwise cause pollution. We ensure there is solid information on environmental trends so that necessary actions are taken. Our priorities are protecting the Irish environment and ensuring that development is sustainable.

The EPA is an independent public body established in July 1993 under the Environmental Protection Agency Act, 1992. Its sponsor in Government is the Department of the Environment, Heritage and Local Government.

OUR RESPONSIBILITIES

LICENSING

We license the following to ensure that their emissions do not endanger human health or harm the environment:

- waste facilities (e.g., landfills, incinerators, waste transfer stations);
- large scale industrial activities (e.g., pharmaceutical manufacturing, cement manufacturing, power plants);
- intensive agriculture;
- the contained use and controlled release of Genetically Modified Organisms (GMOs);
- large petrol storage facilities.

NATIONAL ENVIRONMENTAL ENFORCEMENT

- Conducting over 2,000 audits and inspections of EPA licensed facilities every year.
- Overseeing local authorities' environmental protection responsibilities in the areas of - air, noise, waste, waste-water and water quality.
- Working with local authorities and the Gardaí to stamp out illegal waste activity by co-ordinating a national enforcement network, targeting offenders, conducting investigations and overseeing remediation.
- Prosecuting those who flout environmental law and damage the environment as a result of their actions.

MONITORING, ANALYSING AND REPORTING ON THE ENVIRONMENT

- Monitoring air quality and the quality of rivers, lakes, tidal waters and ground waters; measuring water levels and river flows.
- Independent reporting to inform decision making by national and local government.

REGULATING IRELAND'S GREENHOUSE GAS EMISSIONS

- Quantifying Ireland's emissions of greenhouse gases in the context of our Kyoto commitments.
- Implementing the Emissions Trading Directive, involving over 100 companies who are major generators of carbon dioxide in Ireland.

ENVIRONMENTAL RESEARCH AND DEVELOPMENT

- Co-ordinating research on environmental issues (including air and water quality, climate change, biodiversity, environmental technologies).

STRATEGIC ENVIRONMENTAL ASSESSMENT

- Assessing the impact of plans and programmes on the Irish environment (such as waste management and development plans).

ENVIRONMENTAL PLANNING, EDUCATION AND GUIDANCE

- Providing guidance to the public and to industry on various environmental topics (including licence applications, waste prevention and environmental regulations).
- Generating greater environmental awareness (through environmental television programmes and primary and secondary schools' resource packs).

PROACTIVE WASTE MANAGEMENT

- Promoting waste prevention and minimisation projects through the co-ordination of the National Waste Prevention Programme, including input into the implementation of Producer Responsibility Initiatives.
- Enforcing Regulations such as Waste Electrical and Electronic Equipment (WEEE) and Restriction of Hazardous Substances (RoHS) and substances that deplete the ozone layer.
- Developing a National Hazardous Waste Management Plan to prevent and manage hazardous waste.

MANAGEMENT AND STRUCTURE OF THE EPA

The organisation is managed by a full time Board, consisting of a Director General and four Directors.

The work of the EPA is carried out across four offices:
- Office of Climate, Licensing and Resource Use
- Office of Environmental Enforcement
- Office of Environmental Assessment
- Office of Communications and Corporate Services

The EPA is assisted by an Advisory Committee of twelve members who meet several times a year to discuss issues of concern and offer advice to the Board.

An Ghníomhaireacht um Chaomhnú Comhshaoil

Is í an Gníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaol do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntímid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomh-nithe a bhfuilimid gníomhach leo ná comhshaol na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil agus Rialtais Áitiúil a dhéanann urraíocht uirthi.

ÁR bhFREAGRACHTAÍ

CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaol i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreail.

FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain.
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhuisce agus caighdeán uisce.
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí comhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaol mar thoradh ar a ngníomhaíochtaí.

MONATÓIREACHT, ANAILÍS AGUS TUAIRISCIÚ AR AN GCOMHSHAOL

- Monatóireacht ar chaighdeán aeir agus caighdeáin aibhneacha, locha, uiscí taoide agus uiscí talaimh; leibhéil agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntí a dhéanamh.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Cainníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdéan aeir agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaol na hÉireann (cosúil le pleananna bainistíochta dramhaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaol a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Guaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Ghníomhaireacht i 1993 chun comhshaol na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaimseartha, ar a bhfuil Príomhstiúrthóir agus ceithre Stiúrthóir.

Tá obair na Gníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar imní iad agus le comhairle a thabhairt don Bhord.

Eutrophication from Agricultural Sources - The Impact of the Grazing Animal on Phosphorus, Nitrogen, Potassium and Suspended Solids Loss from Grazed Pastures - Phosphorus Dynamics in Grazed Grassland

(2000-LS-2.1.2c-M2)

Final Report

Authors:

D. Bourke,[a,b] D. Jeffrey,[a] P. Dowding,[a] I. Kurz[b] and H. Tunney[b]

Prepared for the Environmental Protection Agency

by

Department of Botany, Trinity College, Dublin[a] and Teagasc, Johnstown Castle, Wexford[b]

ACKNOWLEDGEMENTS

DISCLAIMER

Details of Project Partners

David Bourke
Teagasc Environmental Research Centre,
Johnstown Castle,
Co. Wexford,
Ireland.
Tel: +353 (0)53 9171200
Email: bourkedo@tcd.ie

Paul Dowding
Department of Botany,
School of Natural Sciences,
Trinity College,
Dublin 2,
Ireland.
Tel: +353 (0)1 8961274
Email: paul.dowding@tcd.ie

David Jeffrey
Department of Botany,
School of Natural Sciences,
Trinity College,
Dublin 2,
Ireland.
Tel: +353 (0)1 8961274
Email: david.jeffrey@tcd.ie

Isabelle Kurz
Environmental Protection Agency,
Johnstown Castle,
Co. Wexford,
Ireland.
Tel: +353 (0)53 9160684
Email: i.kurz@epa.ie

Hubert Tunney
Teagasc Environmental Research Centre,
Johnstown Castle,
Co. Wexford,
Ireland.
Tel: +353 (0)53 9171200
Email: hubert.tunney@teagasc.ie

Table of Contents

Preface: Overview of LS-2 Projects – Eutrophication from Agricultural Sources

The objective of this large-scale integrated research project, commissioned in 2000, was to supply scientific data to underpin appropriate actions or measures that might be used in the implementation of national policy for reducing nutrient losses to waters from agricultural sources. The research, including desk, laboratory, field plot, farm and catchment studies, was conducted by teams in Teagasc, the National Universities of Dublin, Cork and Galway, Trinity College Dublin, University of Limerick and the University of Ulster at Coleraine.

The project was divided into three main sub-projects (Figure 1.1):

- **LS-2.1: Pathways of Nutrient Loss to Water with Emphasis on Phosphorus Losses**, which aimed at quantifying and ranking the magnitude of phosphorus (P) loss from soil, grazed pastures and the application of slurry and fertiliser so as to identify effective mitigation strategies.

- **LS-2.2: Models and Risk Assessment Schemes for Predicting Phosphorus Loss to Water**, which aimed at developing three modelling approaches that explored the sources of P and the hydrology that transports it from land to water.

- **LS-2.3: Effects of Agricultural Practices on Nitrate Leaching**, which aimed at measuring nitrate leaching from an intensively managed dairy farm on a soil type typical of a nitrate vulnerable zone.

The main objectives of the project LS-2.1.2: Eutrophication from Agricultural Sources – Grazed Pastures were to provide an assessment of grazing on P losses under Irish conditions. This report synthesises the results and outputs from the project: LS-2.1.2 – Part B, which aimed at assessing P balances and fluxes. Two separate sections, LS-2.1.2 – Part A and LS-2.1.2 – Part B, dealt respectively with losses of P from grassland under normally good grazing practice and small-plot studies.
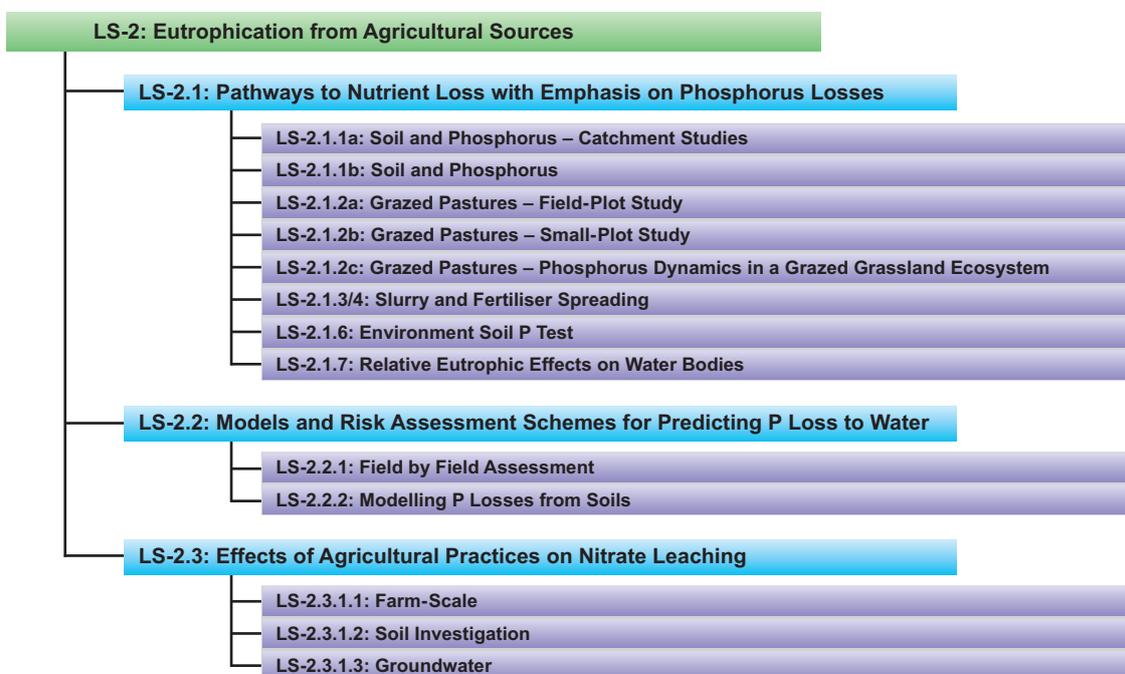

Figure 1.1: Overview of LS-2 projects

Integrated synthesis reports and individual reports from each subproject are available for download on the EPA website: www.epa.ie/downloads/pubs/research/water/.

Executive Summary

The loss of phosphorus (P) from agricultural sources is one of the main causes of eutrophication in P-limited freshwater ecosystems. High losses have been shown from grazed grasslands in particular. The aim of this research was to investigate the pools and fluxes of P in grazed grassland in Ireland. This aim was achieved by laboratory characterisation of P forms and fractions in grassland soils and fresh cattle dung, through field trials set up to examine changes in the P content of dung-pats during decomposition and the effects of decomposing dung-pats on soil P fractions, and by the characterisation of P in overland flow from grazed plots using rainfall simulation.

Phosphorous 31 nuclear magnetic resonance (^{31}P NMR) spectroscopy analysis revealed the presence of inorganic orthophosphate, orthophosphate monoesters (mostly as myo-inositol hexakisphosphate), orthophosphate diesters (as phospholipids and DNA), and pyrophosphates, in a grassland soil. Large variation was found in the P characteristics of dung from beef cattle, in particular, the proportions of the organic and inorganic fractions, explained mostly by the effect of fertiliser P application. 31P NMR spectroscopy analysis revealed the presence of inorganic orthophosphate, orthophosphate monoesters (mostly as myo-inositol hexakisphosphate), orthophosphate diesters and pyrophosphates; again the relative proportion of these forms in the dung was also dependent on fertiliser P application. There was a clear relationship between the P content in the diet of the grazing animals and the P content in the dung. The decomposition of dung-pats at different times of year was explained by varying contributions of rainfall, temperature and biological activity, although all dung-pats had decomposed fully within 90 days of deposition. Significant P was released from the dung-pats (between 19 and 31%) during the first seven days of decomposition because of leaching associated with high water losses from the dung-pats. Physical incorporation of the dung-pat was the principal P release mechanism later in the trial. The decomposing dung-pats had highly significant effects on the concentrations of all soil P fractions (total P, inorganic P, microbial biomass P, and Morgan's P [Pm]) except for the organic P fraction. Pm concentrations increased by between 200 and 450% owing to the presence of dung-pats. The presence of grazing animals increased the concentrations of organic P lost to overland flow.

^{31}P NMR spectroscopy analysis revealed the presence of inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters, phosphonates, and pyrophosphates in the overland flow from grazed plots, while only inorganic orthophosphate, orthophosphate monoesters, and pyrophosphates were found in the overland flow from non-grazed plots. These results offer insights into the P dynamics within grazed grassland and the potential P losses from grazed grassland.

1   Introduction

Phosphorus (P) is a major nutrient that has many important roles and influences in production agriculture and all parts of the biosphere (Ritter and Shirmohammadi, 2001). The fate of P and P cycling in the environment are important factors in understanding the potential for, and impacts of, P transport through grassland-based catchments.

A combination of biological, chemical and physical processes, influenced by environmental conditions (e.g. temperature and moisture), together with the history and intensity of land use and management, determines the forms, dynamics and mobility of P in the soil–plant–animal–water system (Condron, 2002). Figure 1 illustrates these processes, including precipitation-dissolution and adsorption-desorption, which control the abiotic transfer of

P between the solid phase and soil solution, and biological immobilisation-mineralisation processes that control transformations of P between inorganic and organic forms (Frossard et al., 2000). The pools and fluxes of P in a grazed grassland system can be conceptualised in a 'black box' input–output, event-driven model. The principal inputs are fertiliser P, P in animal feeds and deposition of atmospheric P. The principal outputs are agricultural product, i.e. meat and milk, and losses to water. Within the 'black box' there are a number of sub-models that relate to the biological, chemical, physical and hydrological drivers that will determine P dynamics within, and losses from, grazed grassland. The major pools and associated transformations (fluxes) of P in the soil–plant–animal system are presented conceptually in Figure 2.
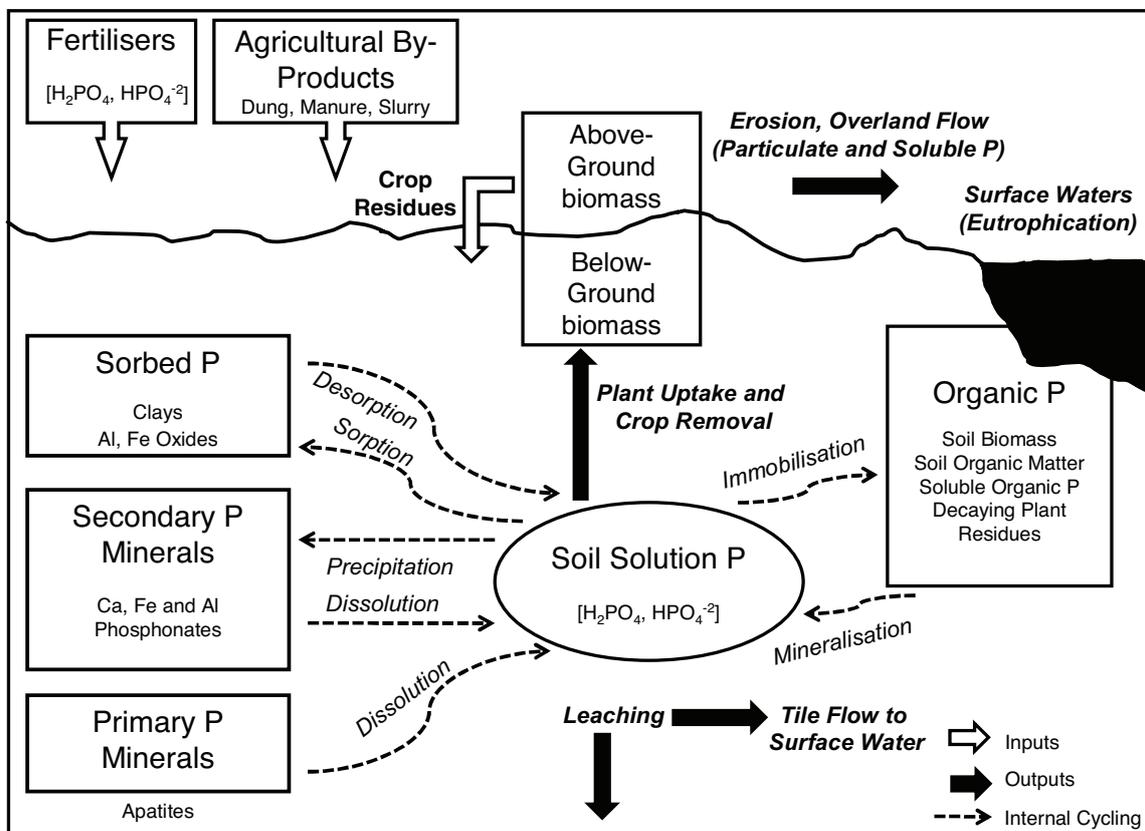
Figure 1: Forms and dynamics of P in the soil-plant system.
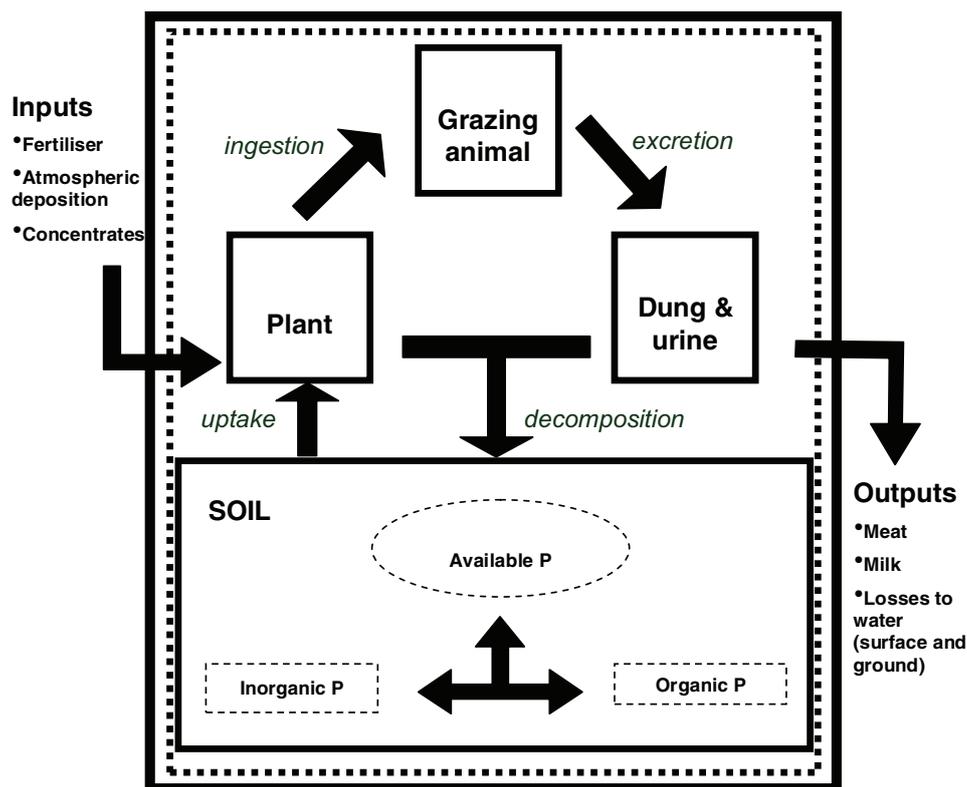
Figure 2: Conceptual illustration of P pools and fluxes in a grazed grassland ecosystem.

From an agronomic viewpoint, rates of diffuse P transfer from grassland soils are traditionally considered generally small – between 0.5 to 6 kg P ha^{-1}yr^{-1} (Ryden et al., 1973; Roberts et al., 1989; Haygarth and Jarvis, 1997; Heathwaite and Dils, 2000; Kurz, 2002; Kurz et al., 2005a; 2005b), but this P loss can have a significant impact on water quality and ecosystem health through accelerated eutrophication.

In Ireland, environmentally significant P losses have been shown to occur from grassland soils. A recent study at the Teagasc Environmental Research Centre, Johnstown Castle, showed that dissolved reactive phosphorous (DRP) loss to water from an intensively grazed grassland field was over 4.77 kg P ha^{-1}yr^{-1} (Kurz, 2002). This field received 30 kg fertiliser P ha^{-1}yr^{-1} and had a soil test P (STP) of 17 mg Pm l^{-1}. Kurz (2002) showed that P losses of this magnitude could be significant in agronomic terms with more than 20% of the P applied in fertiliser being potentially lost to water each year.

Grazing animals in the grassland ecosystem are seen to have three main impacts: (i) defoliation, which affects herbage yield, herbage composition and botanical composition (Korte and Harris, 1987); (ii) treading and subsequent compaction of the soil (Drewry et al., 1999); and (iii) dung and urine deposition, returning to the soil nutrients obtained from the herbage within the grassland and/or from imported feed stuffs (Whitehead, 2000).

The presence of the grazing animal is said to increase the rate at which P and other nutrients are recycled through the grassland ecosystem (Whitehead, 2000). The grazing animal uses only a proportion of the nutrients it ingests and returns between 60 and 99% of these nutrients in the form of dung (Haynes and Williams, 1993). The extent to which the recycling of nutrients in grassland is increased by the presence of grazing animals is influenced by the stocking density, the proportion of time the animals spend in the field and by the concentration of P in the herbage consumed. The

concentration of the total P in the dung has been shown to be dependent on the concentration of P in the diet (Barrow and Lambourne, 1962; Haynes and Williams, 1993). Often, 10–35% of dairy cow excreta is deposited off field, in milking sheds, yards and roadways (Nguyen and Goh, 1994). These off-field losses may be compensated for by supplementary feeds, high in P and other nutrients. The nature of dung and urine deposition creates an uneven distribution of nutrients within the grazed ecosystem. Table 1 presents some of the characteristics of dung and urine patches in terms of their potential impact on the grassland soil environment.

Distribution heterogeneity of excreted nutrients was described by Barrow (1987) as either 'small' or 'large scale', the first resulting from random deposition over a given area, and the second attributed to grazing animals congregating in certain areas of the field, such as water- and feeding-troughs and areas providing shelter. Overall, Whitehead (2000) calculated that approximately 6% of the grazed area will be covered by dung every year, and as much as 30% of the grazed area would be affected by its proximity to the dung-pat.

Generally, there is limited information available on the interaction between soil, plants, animals and environmental conditions in terms of P mobilisation and losses from the grazed grassland ecosystem. Specifically, knowledge on seasonal changes of various P fractions and other soil properties, and on the influence of their controlling factors – biological, physico-chemical and environmental – in Irish grassland soils is inadequate. Much of the research that has been carried out into the decomposition of dung-pats has concentrated on nitrogen cycling, sward composition and the effects of invertebrates on the breakdown of the dung-pat (Castle and MacDaid, 1972; MacDiarmid and Watkin, 1972; Holter, 1979; Dickinson and Craig, 1990; Yokoyama et al., 1991). Less is known about the changes in nutrients, especially P, in dung-pats, and the effect of the dung-pat on soil P fractions and associated processes during the decomposition period. Information on the forms of P contained in manures and more specifically dung is variable at best and often not available.

Table 1: Typical values of the characteristics of dung and urine of grazing animals (cattle and sheep) that may influence nutrient dynamics in the grazed grassland ecosystem (Whitehead, 2000).

Dung and urine characteristics	Cattle	Sheep
Defecations per day	11–16[a,b,c,d]	7–26[a,b]
Weight of DM per defecation (g)	250–380[a,b,d]	6–34[a,b]
Weight of faecal DM per day (kg)	2.7–5.9	0.2–0.6[b]
Area covered by single defecation (m^2)	0.05–0.09[a,b]	0.007–0.025[a,b]
Area affected by single defecation (m^2)	0.25–0.54[b]	0.015–0.075[a,b]
Urinations per day	8–12[a,b]	15–20[a,b]
Volume per urination (l)	1.6–2.8[a,b]	0.10–0.18[a,b]
Volume of urine per day (l)	12–34	1.7–3.6
Area covered by urine patch (m^2)	0.16–0.50[a,b]	0.03–0.05[a,b]
Area affected by urine patch (m^2)	0.5–2.5[b]	0.06–0.15[a,b]

[a]Haynes and Williams, 1993; [b]Nyguyen and Goh, 1994; [c]MacDiarmid and Watkin, 1972; [d]Marsh and Campling, 1970.

2   Aim and Objectives

Reviewing relevant literature at the beginning of this study helped to identify many fundamental gaps in the knowledge of the P pools (various P forms and fractions in the soil, herbage and dung) and P fluxes (changes in the size of and movement between these P pools) in grazed grasslands. Few studies were found that were concerned specifically with the impact of the grazing animal on P dynamics in Irish grazed grasslands, while the international literature did not always fill in the gaps in the knowledge – particularly in relation to the characteristics of P in freshly deposited cattle dung and its role in the P cycle of grazed grasslands. There was a fundamental need to extend our understanding of the P cycle in grazed grasslands as a source of P that could potentially be lost to water.

Most recent studies of P in Irish grasslands have either focused on (i) the hydrological pathways of P loss (overland and subsurface flow) and the concentrations of P transferred in these pathways (Kurz, 2002; Tunney et al., 2002; Kurz et al., 2005a; 2005b; Doody et al., 2006; O'Reilly, 2006) or (ii) on soil P chemistry and physico-chemical processes such as desorption (Daly et al., 2001; Daly et al., 2002). In general, therefore, the focus to date has been aimed at understanding the variation in P chemistry of the major soil types in Ireland and the relationships between soil test phosphorous (STP) and P concentrations in overland and subsurface flow (Jordan et al., 2005; Styles et al., 2005) and the development of P-risk evaluation schemes for the identification of critical source areas (Hughes et al., 2005). The effects of management (grazing, fertiliser application) and other aspects of the P cycle, most notably organic P (Po), have often been overlooked.

It is important to note that a review of the literature since the experimental work of this study ended has revealed many publications similar to much of the subject matter of the current study – particularly in countries with intensive animal-production agriculture. These publications recognise that

similar gaps in the knowledge were identified, i.e. fundamental data were required concerning grazed grassland as a potential source of P as water quality continues to deteriorate and the need for mitigation measures grows.

The current study was one of three subprojects in LS-2.1.2, which had the remit of investigating grazed grasslands as a source of and pathway for P loss to water. A previous study (Kurz et al., 2005a; 2005b) demonstrated increased concentrations of P in overland flow shortly after the presence of grazing animals, and identified the need for further research to quantify and explain the role of the grazing animal. The other subprojects (LS-2.1.2a and LS-2.1.2b) focused on the losses of P in overland flow from field and small plots as a result of normal grazing activity. The overall findings and recommendations from all three subprojects are discussed in the Phosphorus LS-2.1 Integrated Report.

The current study therefore was required to focus on grazed grasslands as a source of P and was given a general aim of looking at the 'pools and fluxes' of P within grazed grasslands.

Specific objectives were to:

- examine the seasonal dynamics of P fractions and related soil properties in a grassland soil;
- examine dung-pat decomposition and associated P changes;
- examine the impact of decomposing dung-pats on soil available P;
- characterise P forms and fractions in soil samples collected over a three-month period;
- characterise dung-pat P forms and fractions in grazed grassland receiving different P fertiliser inputs;
- characterise P forms and fractions in overland flow samples collected from grazed and non-grazed small plots in a rainfall-simulation experiment.

3   Materials and Methods

3.1   Introduction

The experiments in this report began in August 2002 and ended in July 2004. Table 2 presents details on the experimental number as used in this report, the experimental title, and the name of the experimental field site within the Johnstown Castle Estate (where the experiments took place).

Descriptions are given of (i) the three experimental sites, (ii) the experimental design for the six experiments and (iii) the methods used to analyse soil, dung and water samples.

3.2   Site Descriptions

The three study sites (Cowlands, Red House Field and Dairy Farm) were located at the Teagasc Environmental Research Centre, Johnstown Castle, Wexford, Ireland (302404/116584 UTM) at an altitude of 60–70 m above sea level. The research centre covers an area of about 390 ha with the three sites located less than 1.5 km from a meteorological station. The land use of the three sites was set up as permanent pasture. Mean annual rainfall during the experimental period was 1032 mm with a mean annual temperature of 10.6 $^{\circ}$C as measured at the meteorological station.

3.2.1   Cowlands

The soil has a sandy loam texture and is classified as a gley (Culleton and Diamond, 1972). FAO-UNESCO (1974) classifies the soil as a humic gleysol. Soil test P, measured as Pm concentrations, ranged from 3.2 to 17.6 mg l^{-1} and herbage P concentrations ranged from 0.29 to 0.46%. Soil pH had a mean of 6.2. The grassland consists of a mixture of *Lolium perenne* L., *Agrostis* spp. L. and *Poa trivialis* L. with other species found in lower abundance. The site is described in more detail by Culleton et al. (2000). A long-term grazing experiment (beef cattle) has been in place in the Cowlands since 1968 with its experimental design described in more detail in Section 3.3.5. Details of fertiliser applications for 2004 are provided in Table 3.

Table 2: Details on experimental numbers, titles and field sites.

Experiment no.	Experiment title	Experimental site
1	Seasonal changes in grassland soil P and associated soil properties	Red House Field
2	Dung-pat decomposition and associated changes in P	Red House Field
3	The effect of dung-pats on soil P dynamics	Red House Field
4	Characterisation of P in grassland soils	Red House Field
5	Characterisation of P in fresh dung	Cowlands
6	Characterisation of P in overland flow	Dairy Farm

Table 3: N and K fertiliser applications for Cowlands and P-specific fertiliser applications during grazing season, 2004.

Dates	Fertiliser information	
	Type	Rates (kg element ha^{-1})
16/02/04	Urea	28
1/03/04	K	62
20/02/04	P	According to treatments described in Table 4
2/04/04	Urea	57
6/05/04	CAN	50
28/05/04	CAN	50
22/06/04	CAN	33
16/07/04*	CAN	33
19/08/04	CAN	33

*Dung samples used in Experiment 5 collected during July 2004.

3.2.2   Red House Field

The Red House Field is a 0.5 hectare site located beside the Cowlands site (but not used within the long-term experiment). The mean Pm concentration for this site was 7.1 mg l^{-1} (Teagasc Soil P Index 3), ranging from 4.9 to 10.5 mg l^{-1}. Other site characteristics, including soil and vegetation properties, are as described for the Cowlands site (Section 3.2.1). The Red House Field was subdivided into 302 plots, each 1.5 m^2 in area. Plots were then chosen randomly for use in Experiments 1, 2, 3 and 4 described later (Sections 3.3.1 to 3.3.4). The site was reseeded in 1999, receiving N, P and K at the time. This site received no fertiliser and was not grazed for two years prior to and during the course of the experiments. The site had received light fertiliser (N) dressings in the past with occasional light grazing (used as relief ground for the grazing experiment in the Cowlands). During the course of the experiments the site was managed by regular mowing to simulate grazing, removing the cut grass from the site. No grazing animals were allowed on site during the course of the experiments.

3.2.3   Dairy Farm

The Dairy Farm experimental site and design are described in the Final Report on Section b (LS-2.1.2b) of the Grazed Pastures Project (Kurz and O'Reilly, 2006) with more management details found in Culleton et al. (1999). The soil at the Dairy Farm site has a sandy loam topsoil

and is classified locally as a brown earth (Gardiner, 1962). FAO-UNESCO (1974) classifies the soil as a gleyic cambisol. The Dairy Farm site was grazed rotationally by dairy cows at an estimated stocking rate of 2 LU ha^{-1}. This resulted in grazing at intervals of approximately three to four weeks from April to October each year. The overland flow samples from the small-plot studies (as described in the Final Report of LS-2.1.2b) collected in the dairy farm were used in Experiment 6 (Section 3.3.6).

3.3   Experimental Design

This section describes the approach for each of the six experiments undertaken, examining soils, dung, and water and various interactions of these substrates in the grazed grassland ecosystem (Sections 3.3.1 to 3.3.6). Soil, dung and water sampling, processing and storage and methods of analysis will be described in later sections.

3.3.1   Experiment 1: Seasonal Changes in Grassland Soil P and Associated Soil Properties

Soil nutrient dynamics (total P [Pt], inorganic P [Pi], organic P [Po], [Pm], organic carbon [C], total nitrogen (N), available potassium (K) and available magnesium [Mg]), and associated biological properties (microbial biomass P [MBP]), were examined on 20 plots (1.5 m^2) at the Red House Field site on 19 sampling days in 2003. Soil samples were taken at least once per month, often on a weekly basis as these plots were also used as control plots in the dung-

pat studies outlined in Experiment 3. Soil samples were taken using the Teagasc bucket sampler, taking between 10 and 20 cores (25 mm diameter, 100 mm depth) from each plot on each sampling occasion. The cores were then bulked to form a single sample per plot for analysis. The results from this experiment were also used in Experiments 3 and 4.

3.3.2 Experiment 2: Dung-Pat Decomposition and Associated Changes in P

Fresh dung, collected from a dairy herd in the Dairy Farm, was used to artificially create dung-pats (diameter of 30 cm and 2 kg in wet weight) on 20 plots (1.5 m²) at the Red House Field site. The dung-pats were placed on netlon mesh (7 mm) to allow for easy dung-pat weighing, non-destructive sampling of soil under the dung-pats and free movement of fauna (earthworms and dung-beetles) between the soil and the dung-pats. Four trials (in October, 2002 [OT], and March [MT], August [AT] and September [ST], 2003) were conducted to be representative of dung deposited at different times within the grazing season. Dung-pat decomposition in terms of wet weight (ww), dry weight (dw), percentage organic matter (%OM) and associated nutrient changes (Pt, N, K, Mg, C) in the dung were measured. Dung-pat measurements were made initially on Day 1, and subsequently on Days 7, 14, 28, 60 and 90 after application. Non-destructive (i.e. repeated measures on the same dung-pats used for the entire experiment) composite samples of four dung pats each were used to provide five samples from the 20 plots for analysis on each sampling day. This was done to minimise the impact of sampling on the dung-pats. The results from this experiment were also linked to those in Experiment 3.

3.3.3 Experiment 3: The Effect of Dung-Pats on Soil P Dynamics

Three of the four dung-pat trials (MT, AT and ST) used in Experiment 2 were also used to examine the effect of dung-pats on soil chemical and biological properties during the decomposition period. Measurements of soil Pt, Pi, Po, Pm, C, N, K and Mg and associated biological properties (MBP) were made during the decomposition period as outlined in Sections 3.3.1 and 3.3.2. Soil sampling occurred initially on Days 1, and subsequently on Days 7, 14, 28, 60 and 90 after application. Soil was sampled by lifting the netlon mesh and taking a core (25 mm diameter, 100 mm depth)

from underneath the soil affected by the dung-pat. Soil samples from four plots were composited on each sampling day to minimise the destructive nature of the sampling, forming five samples for analysis. Control samples (Experiment 1) were collected at the time of sampling to allow for non-experimental changes. The samples from this experiment were also used in Experiment 4.

3.3.4 Experiment 4: Characterisation of Po in Grassland Soils

Soil samples collected during Experiment 1, over five sampling days from 23 September to 15 December, were used in this experiment to characterise the Po in an Irish grassland soil. ³¹P NMR spectroscopy and traditional wet chemistry methods were used to examine these samples in greater detail.

3.3.5 Experiment 5: Characterisation of P in Fresh Dung

The nutrients in fresh dung samples were characterised using ³¹P NMR spectroscopy and traditional wet chemistry methods. Fresh dung samples (n = 5) were collected in July 2004 from an individual paddock contained within each of the six P fertiliser treatments of the long-term Cowlands experiment. The Cowlands long-term grazing (beef production) and P cycling experiment was initiated here in 1968. Phosphorus fertiliser was applied annually at three treatment rates: 0, 15 and 30 kg ha^{-1} yr^{-1}. The annual application was made in the spring and was spread on top of the soil. The grazing animals in each treatment were grazed rotationally around six paddocks, with 18–24-day regrowth intervals. Stocking rates were reduced progressively as grass growth rates declined as the year progressed. Over the 35 years, live-weight gain, herbage P, soil P, P loss to water, and botanical composition have been measured as part of this long-term experiment (Culleton et al., 2000).

In 1998, changes were made to half of the plots in each of the original three treatments. Six P fertiliser treatments (described in Table 4) were now in place, 30-30, 30-0, 15-15, 15-5, 0-0, 0-30, kg P ha^{-1} yr^{-1} applied, with the first value of each pair representing the P fertiliser treatment applied between 1968 and 1998, and the second value representing the P fertiliser treatment applied since 1998.

Table 4: Description of P fertiliser treatments in Cowlands experiment.

Treatment No.	Treatment (abbreviation in parenthesis)*
1	Zero P since 1968 **(0-0)**
2	15 kg P ha⁻¹ yr⁻¹ since 1968 **(15-15)**
3	30 kg P ha⁻¹ yr⁻¹ since 1968 **(30-30)**
4	Zero P between 1968-1998; 30 kg P ha⁻¹ yr⁻¹ since 1998 **(0–30)**
5	15 kg P ha⁻¹ yr⁻¹ between 1968-1998; 5 kg P ha⁻¹ yr⁻¹ since 1998 **(15–5)**
6	30 kg P ha⁻¹ yr⁻¹ between 1968-1998; Zero P since 1998 **(30–0)**

*The first value of each pair represents the P fertiliser treatment applied between 1968 and 1998,
and the second value represents the P fertiliser treatment applied since 1998.

The Pt, Pi, Po and Pw fractions of the dung were measured. The water-extractable fractions included total dissolved P (TDP) and dissolved reactive P (DRP), with dissolved unreactive P (DUP) calculated as the difference between the first two fractions (TDP – DRP = DUP). NaOH-EDTA extractable P was also measured in fractions similar to those measured in Pw, TDP, DRP and DUP. The NaOH-EDTA extracts were used to characterise the Po fractions using solution state ^{31}P NMR spectroscopy. Other dung properties and nutrients measured included organic matter (OM), moisture content, dry matter (DM) content, C, N, Mg, and K.

3.3.6 *Experiment 6: Characterisation of Po in Overland Flow*

The P content of overland flow samples collected from grazed and non-grazed small plots was examined using both ^{31}P NMR spectroscopy and traditional wet chemistry methods. This formed an extension of a rainfall simulation experiment designed to examine the impact of the grazing animal on nutrient losses from pasture in overland flow described in the Final Report on Section b (LS-2.1.2b) of the Grazed Pastures Project (Kurz and O'Reilly, 2006). Detailed analysis of the P in overland flow samples from grazed and non-grazed small plots using ^{31}P NMR spectroscopy was required. Further details on the design of this study and on the rainfall simulation can be found in Kurz and O'Reilly (2006).

3.4 Methods of Analyses

3.4.1 *Soil Analyses*

All soil samples were collected using the standard Teagasc bucket sampler to a depth of 100 mm. All soils were sieved to < 2 mm before analysis. Dried soils (oven dried at 40 °C or air dried in a laboratory) were stored in cardboard boxes at room temperature prior to chemical analysis. Field moist soils were stored at 4 °C in a cold room until analysis.

Soil Pt, Pi and Po were measured on oven-dried soils (by extraction of ignited at 550 °C [Pt] and unignited [Pi] samples with 0.5 M H_2SO_4, Walker and Adams, 1958) and were read colorimetrically using an auto-analyser Burkard Series 2000 Colorimeter CF2-X. Po was calculated as the difference between Pt and Pi. Percentage Po was calculated using the Pt and Po values. Pm was extracted from oven-dried soil with Morgan's extracting solution (NaOH and glacial acetic acid, adjusted to pH 4.8) and measured colorimetrically according to the method of Peech and English (1944). Soil K and Mg extracted in Morgan's solution were measured using atomic absorption spectroscopy (Peech and English, 1944). MBP was estimated using chloroform fumigation-extraction (0.5 M K_2SO_4) of soil samples and read using UV absorbance at 280 nm (Turner et al., 2001). The procedure is based on the fact that compounds released after chloroform fumigation from lysed cells absorb in the near UV region. A regression model (Turner et al., 2001) was used to make

the final MBP calculation. OM was measured by loss on ignition of an oven-dried sample. Percentage C (organic) and percentage N (total) were measured using an LECO CN-2000 Dry Combustion Analyzer according to the method of Wright and Bailey (2001). Soil moisture content was measured gravimetrically. Cation-exchange capacity (CEC) was measured by ammonium saturation as described by Chapman (1965). Soil bulk density was estimated according to Jeffrey (1970).

Wet chemistry analyses on NaOH-EDTA and ^{31}P NMR on NaOH-EDTA extracts were carried out according to the method described by Turner et al. (2003a and 2003b). P was extracted from soil in 0.25 M NaOH and 0.05 M EDTA solution for 16 hours at 20 °C. The extracts were then filtered and freeze-dried over an eight-day period. Inorganic orthophosphate was determined in diluted extracts (1:100) by molybdate colorimetry and flow injection analysis. Extracts were diluted to prevent interference from EDTA during colorimetric analysis. Total P was measured by digesting and analysing according to an autoclave method described by Ebina et al. (1983). An automated version of the ascorbic method described by Murphy and Riley (1962) was used to measure reactive P levels. Unreactive (organic) P was calculated as the difference between total and reactive P. Freeze-dried NaOH-EDTA extracts were re-dissolved in 1 ml of 1 M NaOH and 0.1 ml D_2O (for signal lock) and transferred to 7-mm NMR tubes. Solution ^{31}P NMR spectra were obtained using Bruker Avance DPX 400 NMR and analysed using WIN NMR 5.0.

3.4.2   Dung Analyses

Total P, Mg and K were measured on dung samples after digestion in a mixture of concentrated H_2SO_4, H_2O_2 and selenium (catalyst) at 370 °C for 90 minutes. Dung Pt was read on a Vista-MPX ICP-OES. Dung Mg and K were read in an atomic absorption spectrometer, SpectrAA-400. Dung Pi was measured using a modified total inorganic P method for soils, using 0.5 M H_2SO_4 as described by Walker and Adams (1958). Po was calculated as the difference between Pt and Pi. Pw fractions (TDP, DRP, DUP) were made according to the method described by

Pierzynski (2000). OM was measured by loss on ignition of an oven-dried sample. Dung C and N were measured using a LECO CN-2000 Dry Combustion Analyzer according to the method of Wright and Bailey (2001). Dung moisture content was measured gravimetrically. Wet chemistry analyses on NaOH-EDTA and ^{31}P NMR on NaOH-EDTA extracts were carried out according to the methods described by Turner et al. (2003a and 2003b). The P was extracted from dung in 0.25 M NaOH and 0.05 M EDTA solution for 16 hours at 20 °C. The extracts were then filtered and freeze-dried over an eight-day period. Inorganic orthophosphate was determined in diluted extracts (1:100) by molybdate colorimetry and flow injection analysis. Extracts were diluted to prevent interference from EDTA during colorimetric analysis. Total P was measured by digesting and analysing according to an autoclave method described by Ebina et al. (1983). An automated version of the ascorbic method described by Murphy and Riley (1962) was used to measure reactive P levels. Unreactive (organic) P was calculated as the difference between total and reactive P. Freeze-dried NaOH-EDTA extracts were re-dissolved in 1 ml of 1 M NaOH and 0.1 ml D_2O (for signal lock) and transferred to 7-mm NMR tubes. Solution ^{31}P NMR spectra were obtained using Bruker Avance DPX 400 NMR and analysed using WIN NMR 5.0.

3.4.3   Water Analyses

Details on standard water analyses are available in the Final Report on Section b (LS-2.1.2b) of the Grazed Pastures Project (Kurz and O'Reilly, 2006). Briefly, reactive and total P were measured on filtered (0.45μm) and unfiltered samples. Filtering the samples resulted in dissolved and particulate P fractions. Total P was measured by digesting and analysing according to an autoclave method described by Ebina et al. (1983). An automated version of the ascorbic method described by Murphy and Riley (1962) was used to measure reactive P levels. ^{31}P NMR spectroscopy and wet chemistry analyses on NaOH-EDTA extracts of overland flow samples were as described for soil and dung in Sections 3.4.1 and 3.4.2.

3.5   Environmental Conditions

Rainfall, air temperature and soil temperatures (100 mm) were measured at the Johnstown Castle weather station, which, as noted above, is approximately 1 km from the experimental field plots. Soil moisture was measured on site on each sampling day. Soil moisture deficit was calculated from the cumulative balance of precipitation, evapotranspiration and drainage (Schulte et al., 2005).

3.6   Statistical Analysis

Statistical analysis was carried out using MS Excel and SPSS (v12). Summary statistics for all data were calculated. One-way ANOVA was calculated to test the effect of seasonality on soil properties in Experiments 1, and also used in Experiment 5 to examine the treatment effect. The least square difference (LSD) test was then used as a post-hoc test to further investigate significantly different groups within the data from these experiments. Paired and two sample (assuming unequal variances) t-tests were used to examine changes in nutrients under dung-pats after 90 days of decomposition using the control plots to take non dung-pat related changes into account. Simple correlation coefficients (Pearson product-moment) between soil, dung and environmental data were calculated for the baseline study, dung-pat studies and the dung P characterisation experiment. Regression analyses were used to examine rates of decomposition, the associated changes in nutrient, and the relationships between the decomposition variables and the environmental data in Experiments 2 and 3. Trial differences between treatments were examined using the slope coefficients and the 95% confidence intervals.

4 Results and Discussion

4.1 Introduction

Data are presented under the headings used to describe the six experiments outlined in Sections 3.3.1 to 3.3.6. The environmental data concerning the overall project are presented in conjunction with the experimental results in Section 4.2 (Experiment 1). The results and a discussion are presented for each individual experiment. An overall discussion of the experiments follows, together with final conclusions and recommendations for action and research.

4.2 Experiment 1: Seasonal Changes in Grassland Soil P, Associated Soil Properties and Environmental Conditions

4.2.1 Introduction

Knowledge of seasonal variation in the sizes of P pools and fractions, and other soil properties, is important for our understanding of P dynamics in the soil, our evaluation of soil tests for plant-available P and for risk assessment of

P loss from soil to water. Soil P cycling and availability are controlled by a combination of biological processes (mineralisation and immobilisation) and physico-chemical processes (adsorption-desorption and dissolution-precipitation) (Frossard *et al.*, 2000), as well as by changes in environmental conditions such as soil temperature and soil moisture (Chen et al., 2003) and seasonal variations in P demand by plants and competing microbial populations (Tate et al., 1991).

4.2.2 Environmental Conditions

Total rainfall in 2003 was 1020 mm with a maximum daily value of 38.3 mm recorded on 30 October (Figure 3). The year 2003 had an average air temperature of 10.8 °C, typical of Ireland's cool temperate climate (Keane and Collins, 2004) showing distinct summer maxima (20.5 °C) and winter minima (2.1 °C). Figure 4 shows temporal variation in air temperature for 2003. The mean soil temperature to 100 mm in 2003 was 10.3 °C, with a maximum and minimum recorded temperature of 20.1 and 0.8 °C, respectively.
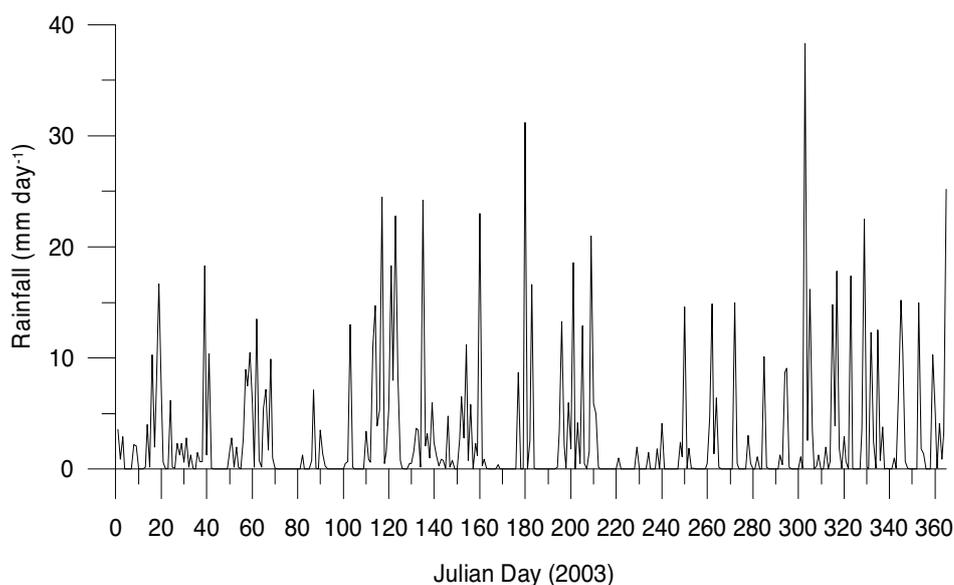
Figure 3: Distribution of average daily rainfall over the period 1 January–31 December 2003 at Johnstown Castle weather station.
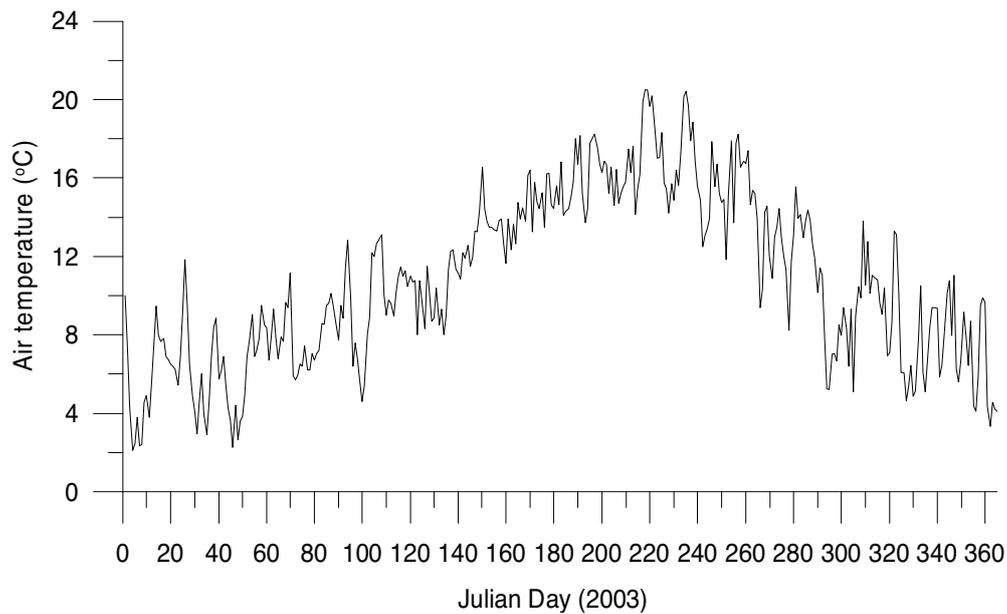
Figure 4: Changes in air temperature over the period 1 January–31 December 2003 at Johnstown Castle weather station.

Soil moisture deficit data (Figure 5) revealed three periods when not enough water was available to maintain the soil at field capacity. Most soil moisture deficits occur in the warmer months and principally from July to early October. Distinct seasonal soil moisture patterns were shown in 2003 (Figure 6). During January and February soil moisture remained at approximately 30%. A dry spell coupled with rising temperatures from early March to early April resulted in decreasing soil moisture content. As expected, there was an overall decrease in soil moisture during the summer months, especially during a dry and warm July and August. Heavy rainfall and decreasing temperatures from the middle of September brought the soil moisture content close to or above 30%, as was seen earlier in the year.
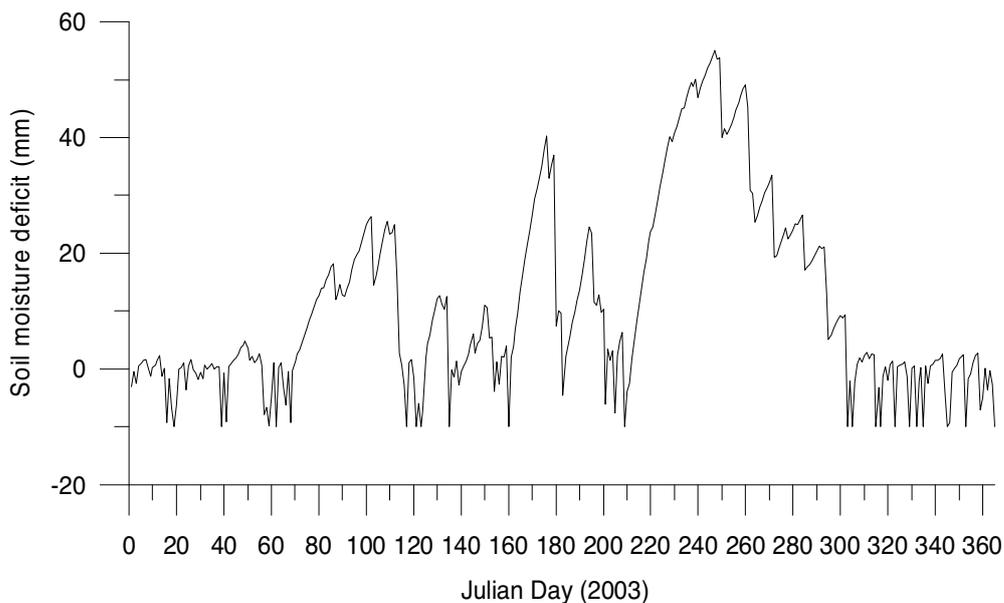
Figure 5: Changes in soil moisture deficit over the period 1 January–31 December 2003 at Red House Field site.

4.2.3 Soil P and Associated Soil Properties

Table 5 shows the summary statistics for the soil phosphorus fractions and associated soil properties measured in the Red House Field site in 2003. The effect of season was found to be significant ($P < 0.001$) for all soil chemical properties (Pt, Pi, Po, Pm, MBP, OM, C, N, K, and Mg) measured in 2003. Figure 7 shows the seasonal fluctuations in the five soil phosphorus fractions measured in the baseline soil plots in 2003. Figure 7a shows the changes in larger Pt, Pi and Po fractions. Soil Pt changed little over the year except for a significant decrease between sampling days 59 and 90, which had fully recovered to typical concentrations by Day 104. The Pi and Po followed a similar trend to Pt in the early part of year, but went on to show distinct seasonal trends. Soil Pi demonstrated strong summer maximum and winter minimum concentrations, while Po showed the converse. Figure 7b shows the changes in Pm and MBP fractions,

again showing distinct seasonal patterns. Both of these labile fractions show very strong summer maximum and winter minimum concentrations.

Table 6 shows the calculated annual turnover of P through the microbial biomass in the baseline soil in 2003. The turnover rate was calculated using the sum of P loss from microbial biomass over the twelve months in 2003. It was estimated that the rate of P loss from the microbial biomass phosphorous (MBP) fraction in the baseline soil over this period was approximately 58 mg kg^{-1} or 50 kg ha^{-1}. The annual turnover rate for this soil was 0.99. This result shows clearly the important role played by the microbial biomass in P dynamics at this grassland site. The microbial biomass is the most active fraction of soil organic matter and represents a dynamic nutrient pool, which responds rapidly to changes in soil environmental conditions.
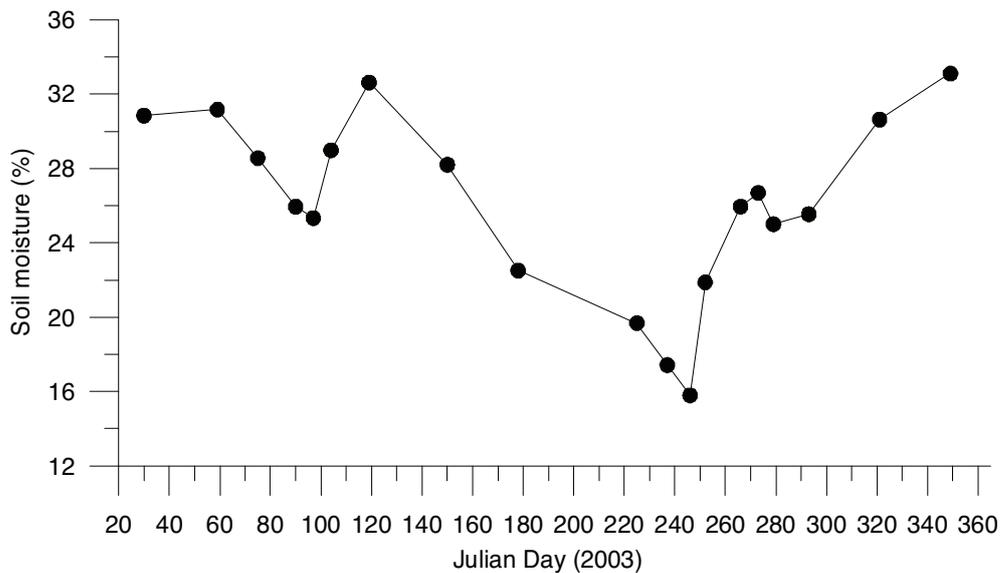
Figure 6: Changes in percentage soil moisture content over the period 1 January–31 December 2003 at Red House Field site.
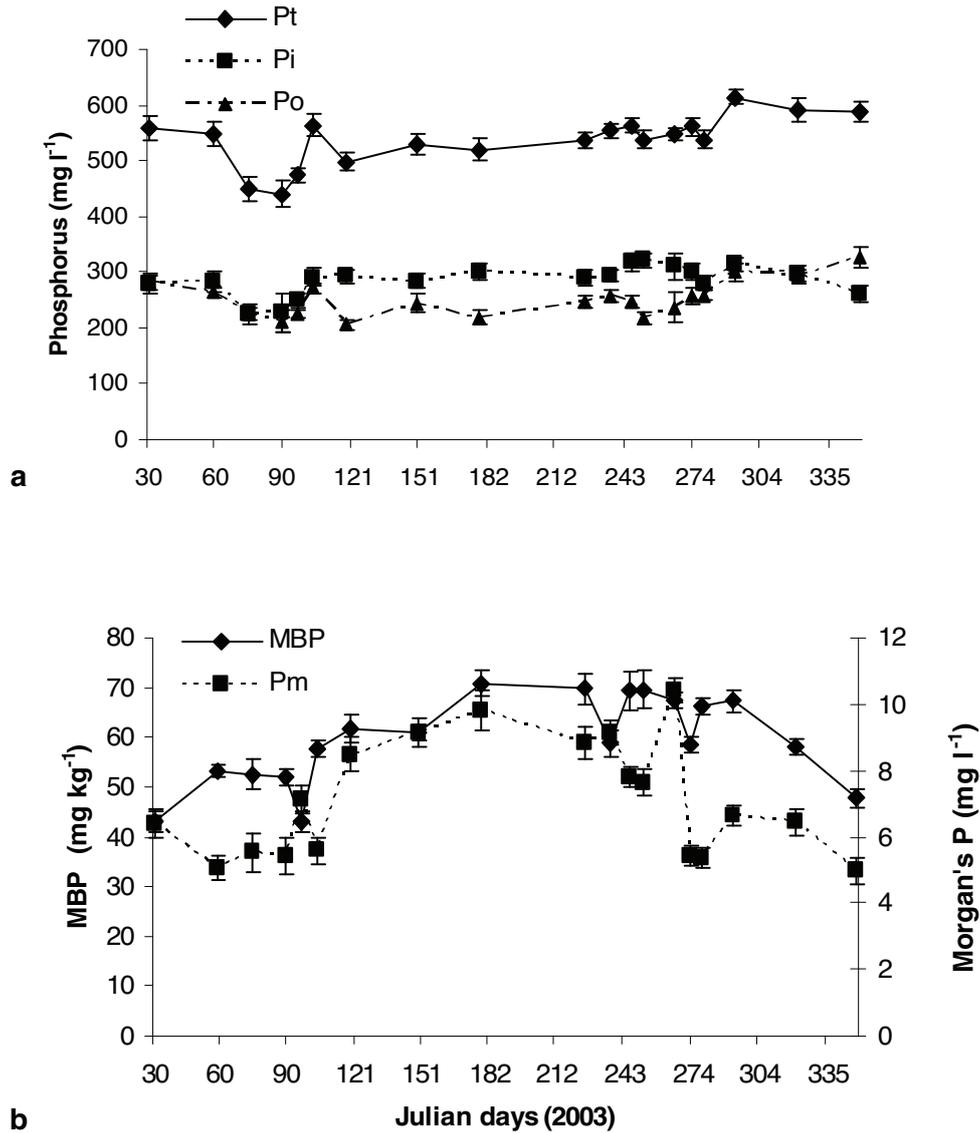
Figure 7: Changes in total, inorganic and organic P (a), and MBP and Pm (b), in the soil over the period 1 January 2003–31 December 2003 (standard errors shown by vertical bars).

The role played by environmental conditions such as soil moisture (rainfall) and air and soil temperature appears central in the dynamics of all soil P fractions. It is clear that after Day 90, as air and soil temperatures began to rise, the rates of change in the P fractions were at their most dynamic.
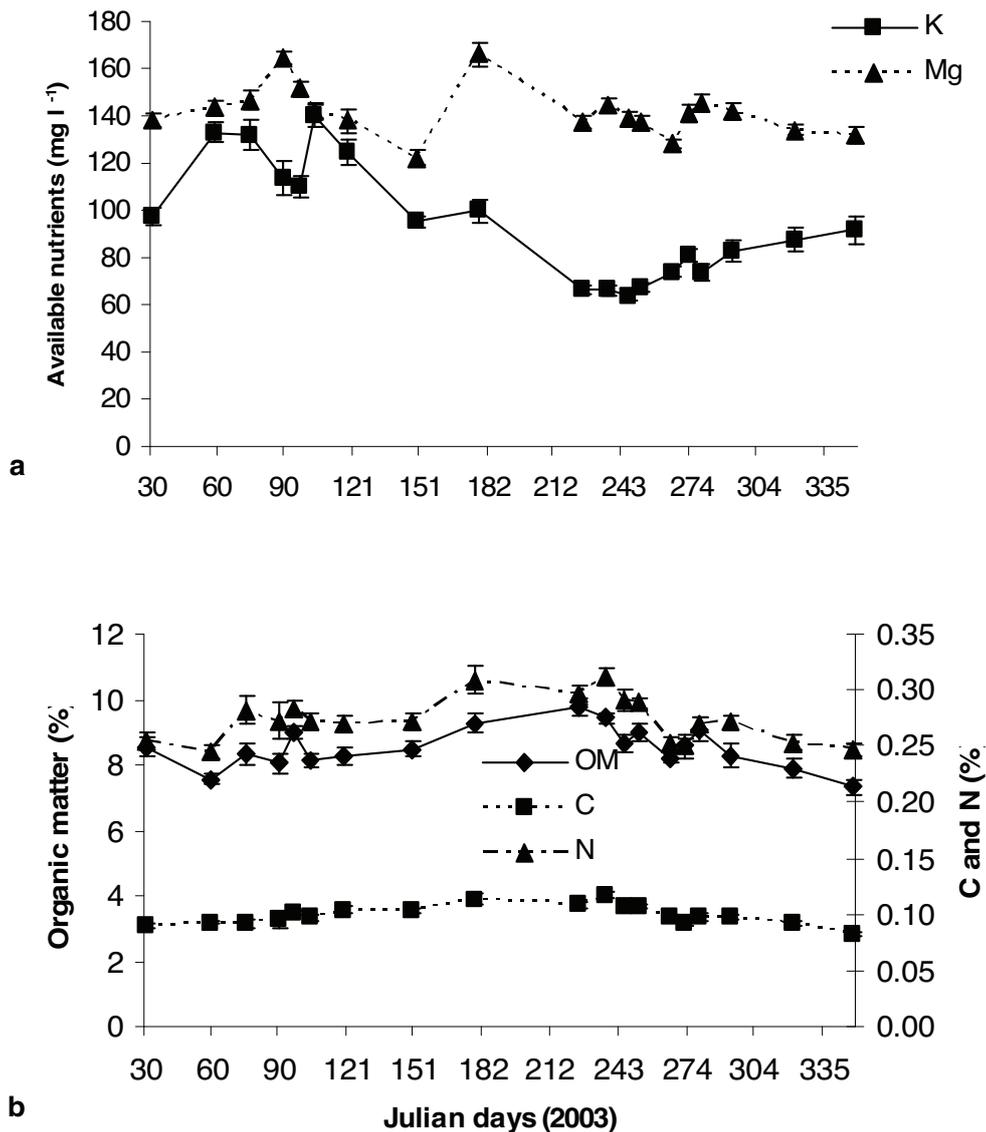
Figure 8: Changes in available K and Mg (a), and OM, organic C and total N (b), in the soil over the period 1 January 2003–31 December 2003 (standard errors shown by vertical bars).

Figure 8 shows the seasonal fluctuations in the other soil properties measured in the baseline soil plots in 2003. Figure 8a shows the changes in the other available soil nutrients measured. The trends displayed are different to the P fractions described. In particular, soil K appears to reach a maximum concentration in early spring, potentially after a winter build-up, which is followed by a constant decrease in K concentrations over the growing season.

The recovery process seems to start again at the onset of decreasing temperatures and winter. Soil Mg does not show distinct seasonal trends like P and K, although there is significant variation between sampling days, but it is not necessarily governed by the same seasonal factors as other nutrients. Figure 8b shows the changes in soil OM, C and N in 2003. All three properties follow very similar trends, distinct summer maxima and winter minima.

Table 5: Mean (standard errors in parenthesis), maximum and minimum values for chemical, biological and physical soil properties determined in a grassland soil over the period 1 January–31 December 2003 at Red House Field site (n = 250). Results of One-way ANOVA included for soil phosphorus fractions and associated soil properties.

Soil Properties	Mean	Minimum	Maximum	One-way ANOVA		
				d.f.	F	Significance[1]
Total phosphorus (mg l^{-1})	536 (5.1)	358	904	18	4.493	***
Inorganic phosphorus (mg l^{-1})	304 (3.9)	131	435	18	2.438	***
Organic phosphorus (mg l^{-1})	252 (3.5)	124	497	18	5.261	***
Percentage organic phosphorus (%)	47.1 (0.5)	23	71	18	3.016	***
Microbial biomass phosphorus (mg kg^{-1})	57.4 (0.9)	2.2	97.3	18	12.370	***
Morgan's phosphorus (mg l^{-1})	7.05 (0.15)	2.8	12.9	18	14.041	***
Organic carbon (%)	3.39 (0.03)	2.45	4.41	18	8.307	***
Organic carbon to organic phosphorus ratio	141 (2)	60	319	18	9.461	***
Organic matter (%)	8.5 (0.1)	6.0	11.8	18	4.885	***
Total nitrogen (%)	0.27 (0.002)	0.17	0.36	18	5.009	***
Organic carbon to total nitrogen ratio	12.54 (0.06)	9.94	16.82	18	5.526	***
Potassium (mg l^{-1})	99.8 (1.8)	54	178	18	31.726	***
Magnesium (mg l^{-1})	141 (1)	93	200	18	8.097	***
pH	6.1					
Soil moisture content (%)	25.8 (1.2)					
Cation exchange capacity (meq ml-1)	15.64					
Clay content (%)	13					
Sand content (%)	59					
Silt content (%)	27					

[1]Significance levels: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 6: Calculated seasonal turnover of soil P through the microbial biomass in top 10 cm of soil in Red House Field site in 2003.

Date	Soil mass (10 cm[1])	Microbial biomass phosphorus			P released (-) from or stored (+) in microbial biomass in 2003	
	kg ha^{-1}	mg kg^{-1}	kg kg^{-1}	kg ha^{-1}	mg kg^{-1}	kg ha^{-1}
30/01/2003	853312	43.14	0.0000431	36.81	10.11	8.62
28/02/2003	853312	53.25	0.0000532	45.44	-0.25	-0.21
16/03/2003	853312	53.00	0.0000530	45.23	-1.12	-0.96
31/03/2003	853312	51.88	0.0000519	44.27	-8.74	-7.45
07/04/2003	853312	43.14	0.0000431	36.81	11.43	9.75
14/04/2003	853312	54.57	0.0000546	46.56	7.01	5.98
29/04/2003	853312	61.58	0.0000616	52.54-0.61	-0.52	
30/05/2003	853312	60.97	0.0000610	52.03	9.84	8.40
27/06/2003	853312	70.81	0.0000708	60.42	-1.06	-0.90
13/08/2003	853312	69.76	0.0000698	59.52	-11.03	-9.41
25/08/2003	853312	58.72	0.0000587	50.11	10.66	9.10
03/09/2003	853312	69.38	0.0000694	59.20	0.13	0.11
09/09/2003	853312	69.51	0.0000695	59.31	-2.03	-1.73
23/09/2003	853312	67.48	0.0000675	57.59	-13.87	-11.84
30/09/2003	853312	53.61	0.0000536	45.75	12.68	10.82
06/10/2003	853312	66.29	0.0000663	56.57	0.94	0.80
20/10/2003	853312	67.23	0.0000672	57.37	-14.80	-12.63
17/11/2003	853312	52.43	0.0000524	44.74	-4.69	-4.00
15/12/2003	853312	47.74	0.0000477	40.74	10.11	8.62
Total annual storage					62.79	53.58
Total annual release					-58.19	-49.66
Turnover rate (annual)[2]					0.99	

[1]The amount of turnover of microbial biomass was calculated based on the soil bulk density in the Red House Field site to soil depth of 10 cm. [2]Turnover rates of microbial biomass phosphorus were calculated as the ratios of total biomass phosphorus loss during the trial to the mean microbial biomass phosphorus concentration during the year (McGill et al., 1986; Chen et al., 2003).

The proportion of soil Pt made up of inorganic, organic and available fractions depends on a combination of factors. These include plant uptake and activity; adsorption-desorption and dissolution-precipitation of Pi; and mineralisation-immobilisation of Po, which is often strongly related to microbial activity and P turnover (Tate et al., 1991; Frossard et al., 2000; Chen et al., 2003). Fertiliser P application also affects these P dynamics but had not been applied to the grassland plots of the current study for a number of years.

The results of the current study showed clearly distinct seasonal changes in the soil properties in 2003 (Figures 7 and 8). The role of the plant (P uptake, seasonal changes in above- and below-ground biomass, competition with microbial populations, senescence, and litter decomposition) was thought to be central in many of the changes in soil properties in the current study, particularly at the beginning of the growing season. The growing season for grassland can be defined by the period during which soil temperature at a depth of 10 cm is consistently above 6 °C, as this is regarded as the critical temperature for grass growth (Keane and Collins, 2004). Based on this assumption, the principal growing season for the Red House Field site was approximately between Days 65 and 305 in 2003.

Saunders and Metson (1971) found considerable P uptake by grasses during the spring flush of growth in New Zealand. In the current study, the 'spring flush period of growth' (between Days 65 and 110) was shown to be one of the most dynamic times of the year in terms of the changes observed in the P fractions (Figure 7). The only significant change in Pt between samples days was recorded during this time, while Pi concentrations decreased significantly in early spring – possibly because of early demand for P by plants. The Pi concentrations of the soil were soon replenished by considerable Po mineralisation, significantly reducing Po concentrations. Overall, the Pi concentration was generally higher in the summer (net mineralisation of Po) than in the winter with an opposite trend seen in the Po fraction (net immobilisation of the Pi). Working with alfalfa plots in Alberta, Dormaar (1972) observed a consistent annual

loss of Po during the period of rapid plant growth in the spring, following a gradual over-winter accumulation of Po similar to the results of the current study. Factors that affect the mineralisation of Po or the immobilisation of Pi include the major influences on microbial activity, such as temperature, moisture, aeration, soil pH, cultivation, presence of growing plants, and fertiliser P additions (Stevenson and Cole, 1999).

The Pm and MBP fractions were affected more by season than any of the other P fractions in the current study (Table 5). Seasonal changes in soil microbial biomass and activity have been explained by a combination of factors, including substrate availability, root growth, microbial competition and community structure, repression of enzyme activity and the accumulation of recalcitrant and toxic compounds (Bardgett et al., 1999). In the current study between Days 65 and 110, a significant increase in Pm coincided with a significant decrease in MBP. Chen et al. (2003) described a similar finding of increased concentrations of labile Pi (bicarbonate and water extractable) in early spring coinciding with a decrease in microbial biomass P and bicarbonate extractable Po.

The relationship between Pm and MBP between Days 65 and 110 suggests increased rates of mineralisation during this key period of the growing season. However, after Day 110, Pm and MBP showed very similar seasonal trends, increasing to maximum concentrations during the warmer summer months, and decreasing again the following winter. It is suggested that the availability of P was strongly linked to microbial activity and turnover of P in this grassland soil. Magid and Nielsen (1992), using arable, grassland and pasture plots, found little or no relationship between labile Pi fractions and soil temperature. They suggested that if biological turnover had been a major cause of the seasonal variation in the Pi fractions then soil temperature would have been significantly related. In their study they did find a significant interaction between soil moisture content and soil temperature in accounting for the variation in total Pi. Perrott et al. (1990) showed, in grazed pastures in New Zealand, a negative correlation (r = -0.60, $P < 0.05$) between MBP and soil temperature. They explained this result by suggesting that bacterial numbers increased during the wetter winter

months, which have much higher nutrient concentrations compared to the drier summer months when fungi may dominate. The relationships between P fractions and other soil properties, and environmental conditions (soil moisture, soil moisture deficit and soil temperature) are crucial to understanding how seasonality affects these properties. Magid and Nielsen (1992) found a strong negative correlation (r = -0.58, P < 0.0001) between various Pi fractions and soil moisture content. Perrott et al. (1990) showed a highly significant (r = 0.82, P < 0.01) positive relationship between soil moisture and MBP in their New Zealand grazed pastures. It is suggested that the interaction between soil moisture (or soil moisture deficit) and soil temperature will be key to understanding many of the fluctuations measured in soil P fractions in the current study and in the wider literature. The interaction of P sources and hydrological pathways leading to P losses requires a fundamental understanding of the processes that govern source P availability.

4.3 Experiment 2: Dung-Pat Decomposition and Associated Changes in P

A large proportion of P in the grazing animal diet is excreted and only relatively small amounts (10–30%) are removed in milk and meat products. The grazing animal contributes significantly to nutrient cycling in grazed grassland by returning large amounts of P, K, Ca, Mg, Na, N and S in dung. Almost all of the P consumed by the grazing animal is deposited on grassland in dung, with as much as 80% of the plant organic P (Po) mineralised to inorganic P (Pi) in dung. Soil P increases under dung-pats is a result of either leaching losses from the dung-pat or the physical incorporation of the decomposing dung-pat. The process of dung deposition leads to the release of these nutrients into soil, with the mechanism of return of each nutrient dependent on its solubility and hence mobility.

Almost 100% of the dung-pats had completely disappeared by Day 90 in all four of the trials, in terms of wet weight, DM and water contents. By Day 90, a heterogeneous mixture of decomposed dung-pat and soil, dung detritus (Aarons et al., 2004a) was all that remained, with analyses in the laboratory therefore limited to samples collected between Days 1 and 60 (Figures 9, 10 and 11). Figure 9 demonstrates the changes in the wet weight (or total mass: water and DM content combined) of the decomposing dung-pats in the four trials. After seven days, 19, 32, 37 and 28% of the dung-pats had disappeared in the OT, MT, AT and ST, respectively. Between Days 8 and 14, the rate of decomposition slowed down in the MT and AT with just a 3% decrease in wet weight in both trials compared to 13 and 15% decreases in the OT and ST, respectively. After 60 days, 9, 12, 18 and 29% of the dung-pats remained on the mesh base in the OT, MT, AT and ST, respectively. Regression models used to describe the
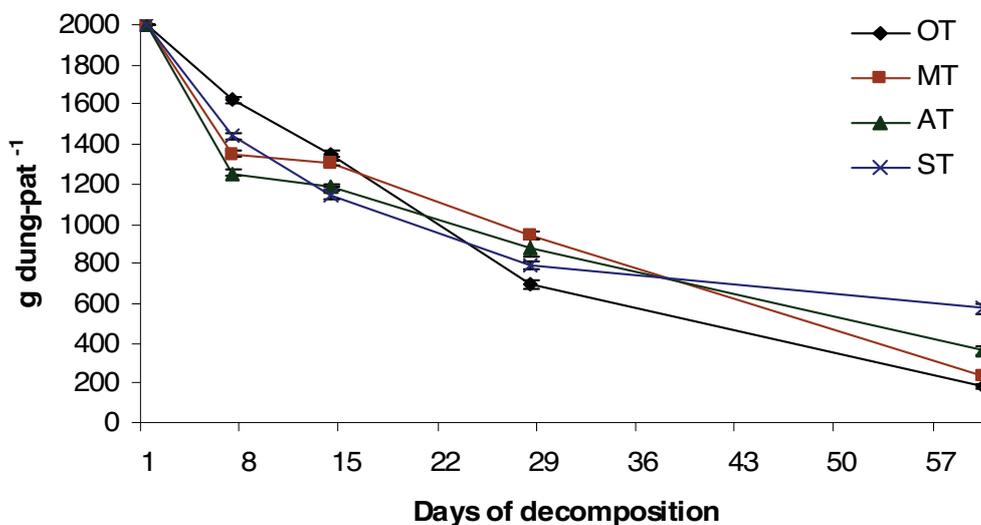

Figure 9: Decomposition of dung-pats in four trials as measured using ww in 2002/2003 in Red House Field site (standard errors shown by vertical bar, n = 20).

rate of decomposition in terms of wet weight for each trial over the first 60 days of decomposition showed that the rates of decomposition were significantly different for all trials ($P < 0.05$), with the OT having the fastest rate and the ST the slowest. All regression models referred to in Section 4.3 for all dung-pat properties were highly significant ($P < 0.001$; $r^2 > 0.88$).

Figure 10 demonstrates the changes in the DM content of the decomposing dung-pats in the four trials. There is a clear DM concentration effect demonstrated, mostly due to the loss of water from the dung-pats at a faster rate than the DM, with the percentage of dung-pat wet weight made up of DM increasing over the 60 days.

DM contents of the dung varied among trials, with Day 1 DM content in the MT being significantly greater (*c.* 30%) than the other three trials. The DM content in the OT followed a very similar decomposition pattern to dung-pat wet weight in the OT. The dung-pats were continually being rewetted by daily rainfall in the early days of the OT, preventing the formation of a hard crust on the surface and maintaining the water content of the dung-pat. The dung-pats in the other trials all formed hard crusts. The DM contents of the other trials were more erratic but all had the common trend of a DM content increase somewhere between Days 1 and 28. The AT and MT began with an initial decrease in DM content, followed by an increase between Days 8 to 14 and Days 15 to 28, respectively. Increases in DM content were measured in the

ST between Days 1 to 7 and Days 8 to 14. Changes in dung-pat DM content as a percentage of the initial DM content remaining showed that, after seven days, 9, 11, and 9% of the dung-pats had disappeared in the OT, MT and AT, respectively, while 15% more DM was measured in the ST. Between Days 8 and 14, further decreases in DM content were measured in the OT and MT while increases of 28 and 4% on the initial DM content were measured in the AT and ST, respectively. A similar increase has been observed in other experiments (Dickinson and Craig, 1990) and is most likely because of the action of earthworms and dung beetles. Their significant role in physical degradation of the dung-pats and incorporation into the soil leads to an initial deposition of soil in the dung-pat. After 60 days, 31, 42, 58 and 59% of the initial dung-pat DM content remained on the mesh base in the OT, MT, AT and ST, respectively.

Much of the DM is composed of lignin, cellulose and hemicellulose, making it difficult to decompose, which is why it remains at a high level until after Day 28 (Haynes and Williams, 1993). By Day 56 it is starting to degrade and the microbes and soil fauna can successfully break down the remaining DM. Regression models that were used to describe the rate of decomposition in terms of DM content for each trial over the first 60 days of decomposition showed that the OT was significantly greater than the other trials ($P < 0.05$), with the AT having the slowest rate, although not significantly different from the MT and ST.
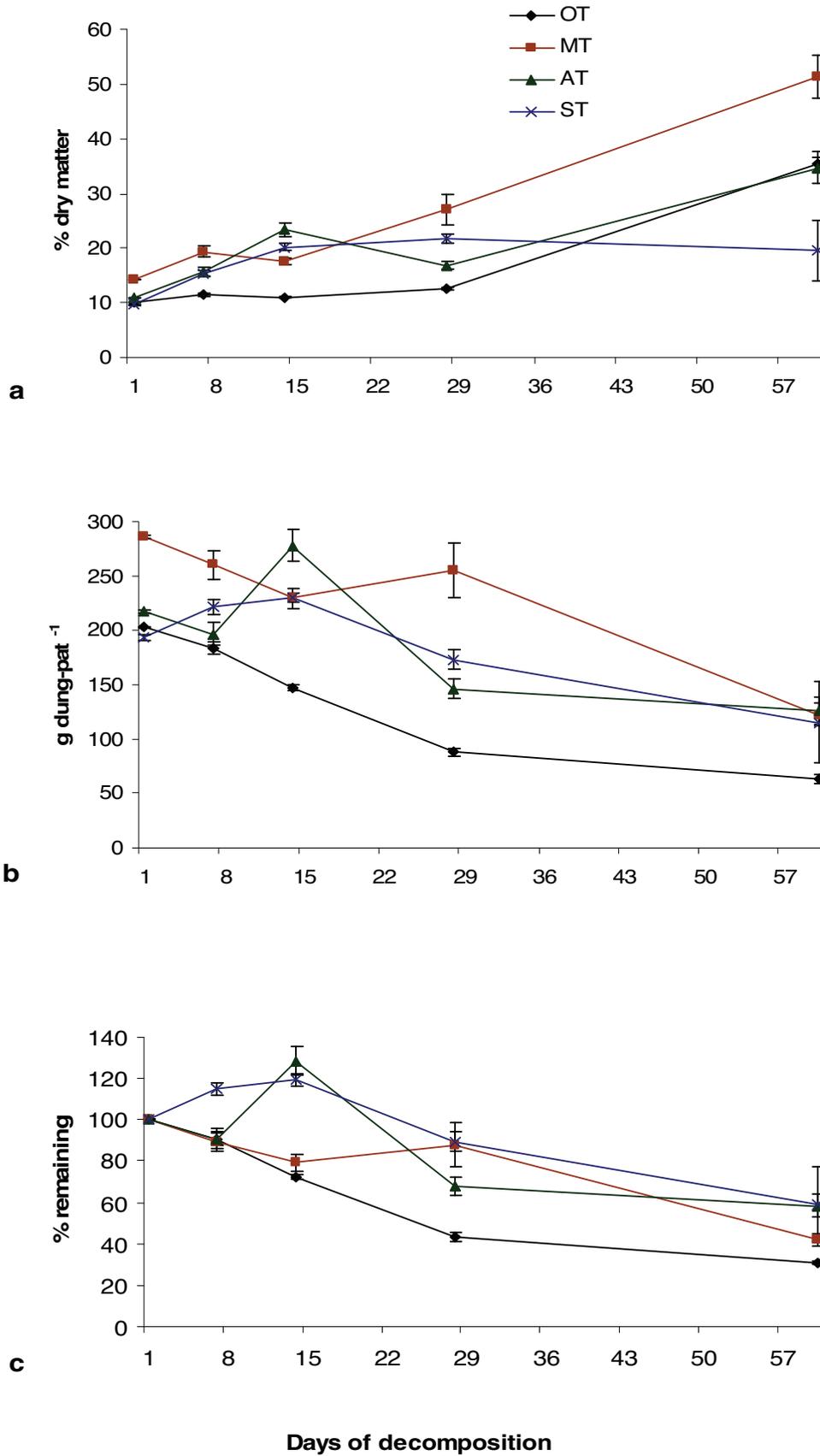
Figure 10: Decomposition of dung-pats in the four trials as measured using DM and displayed as DM concentration (%) (a), DM content (b), and percent remaining (c) in the dung-pats, in Red House Field site (standard errors shown by vertical bars, n = 20).
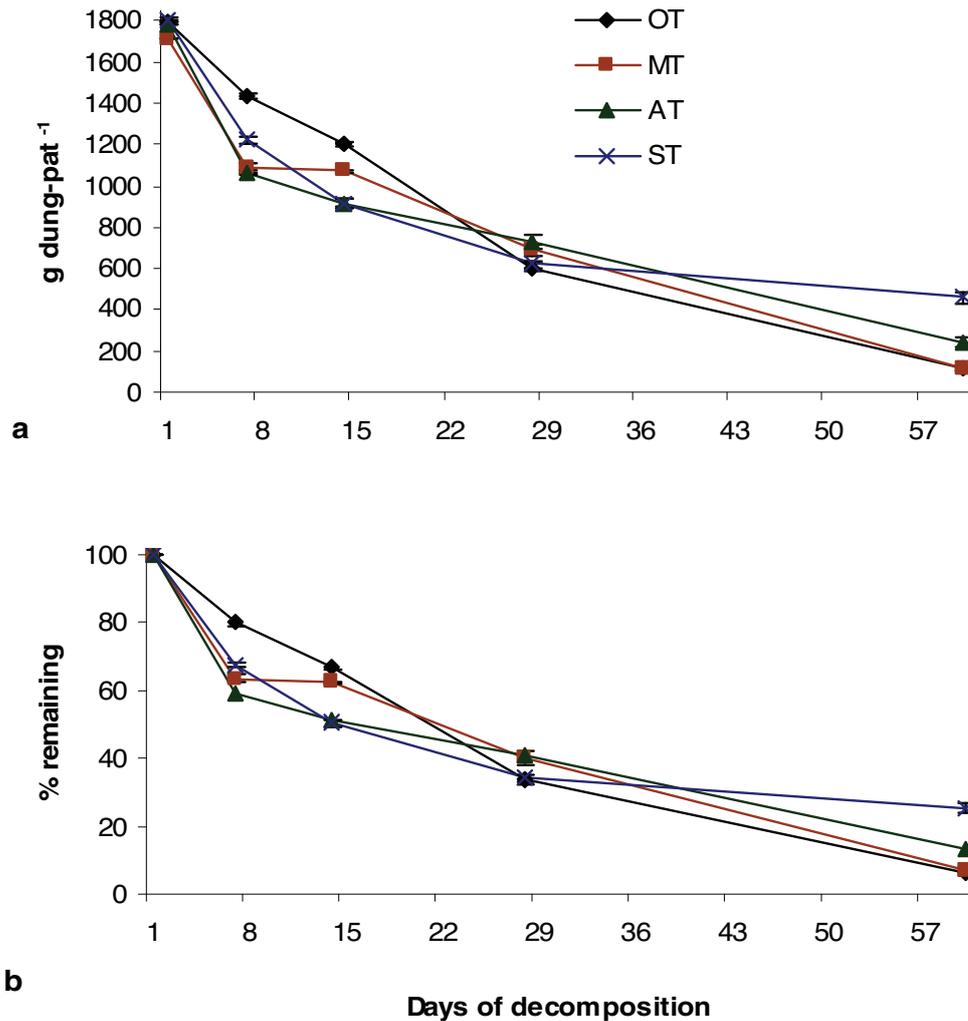
Figure 11: Decomposition of dung-pats in the four trials as measured using water content and displayed as water content (a), and percent remaining (b) in the dung-pats, in Red House Field site (standard errors shown by vertical bars, n = 20).

Figure 11 demonstrates the changes in the water content of the decomposing dung-pats in the four trials. After seven days, 20, 36, 41 and 32% of the water content of the dung-pats had disappeared in the OT, MT, AT and ST, respectively. Between Days 8 and 14, a further 13, 2, 8 and 18% of the water in the dung-pats had disappeared in the OT, MT, AT and ST, respectively. After 60 days, 6, 7, 13 and 25% of the initial dung-pat water content remained on the mesh base in the OT, MT, AT and ST, respectively.

The water content in general follows similar trends to the wet weight of the dung-pats in all trials, especially in the early days of decomposition with water leaching out of the dung-pat accounting for much of its reduction in mass. Regression models that were used to describe the rate of decomposition in terms of water content of each trial over the first 60 days of decomposition showed there were no significant differences between the OT and the MT. The AT and ST were significantly different from the OT and MT and also from each other ($P < 0.05$). Again, the overall rate of decomposition was highest in the OT and the MT, followed next by the AT with the slowest rate of decomposition measured in the ST.

Significant changes with time were observed in the concentrations and contents of all nutrients (P, N, Mg and K) measured in this experiment. Data are presented for P only. Figure 12 demonstrates the changes in P in the decomposing dung-pats in the four trials in terms of P concentration and content. The concentrations of P in the
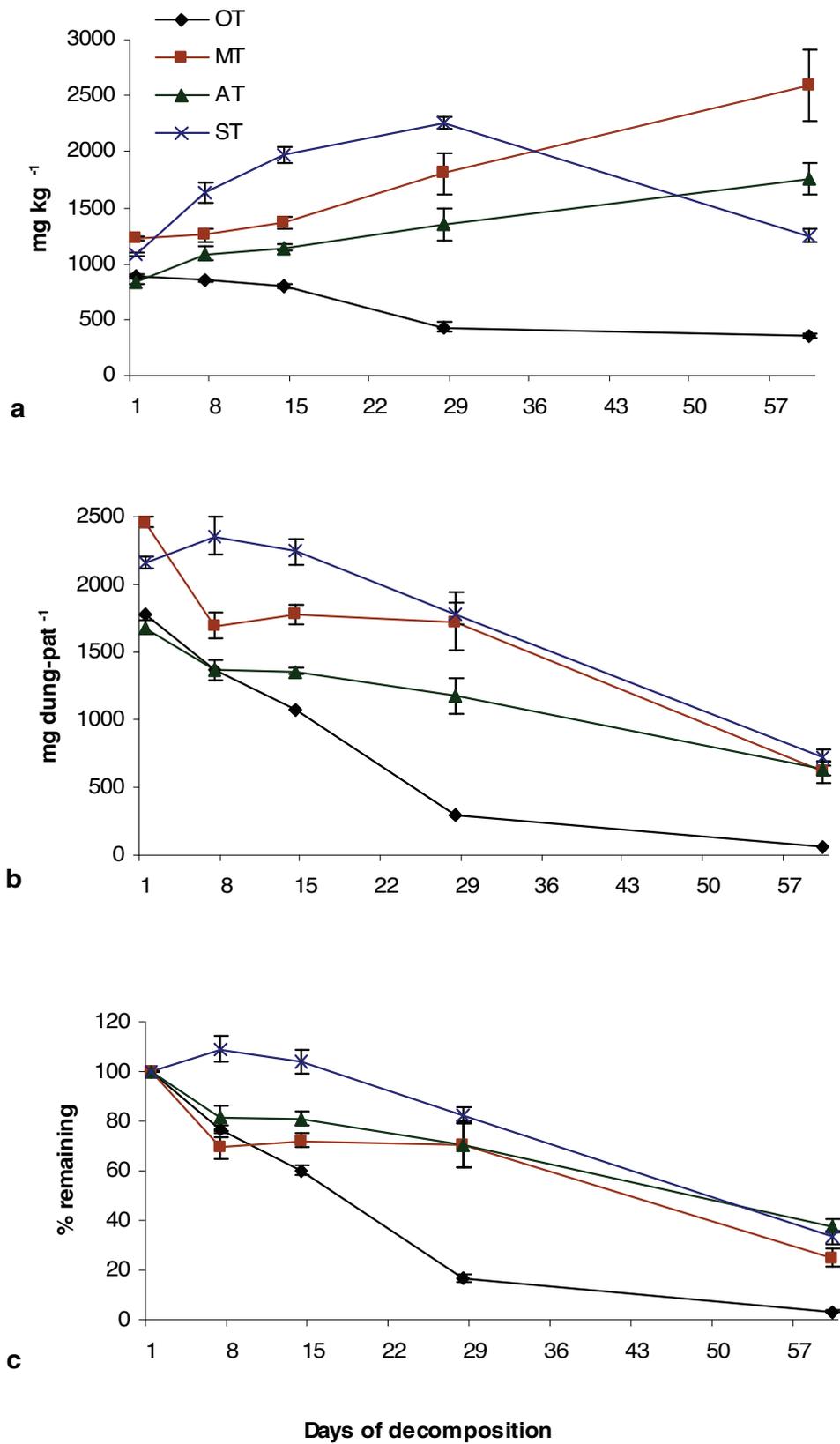
Figure 12: Decomposition of dung-pats in the four trials as measured using P and displayed as P concentration (wet weight) (a), P content (wet weight) (b), and percent remaining (c) in the dung-pats, in Red House Field site (standard errors shown by vertical bars, n = 20).

deposited dung-pats on Day 0 ranged from 0.78% in the August trial to 1.1% in the September trial on a dry weight basis. The P concentration shows similar trends over the 60 days of decomposition as the other nutrients. There is a clear decrease in P concentration in the OT, particularly after Day 14. In the MT and AT there is a continuous increase in P concentration over the 60 days. In the ST there is an increase in P concentration up to Day 28, but then it decreases again between Days 28 and 60; however, the final concentration is still a little higher than the initial. This concentration effect in the ST results in an increase in the P content of this trial over the first seven days (Figure 12b). After seven days, 23, 31 and 19% of the P content of the dung-pats had disappeared in the OT, MT and AT, respectively. There was a 9% increase in the P content of the dung-pats in the ST. Between Days 8 and 14, there was a 17% decrease in P content in the OT, a 3% increase in the MT, no change in the AT and a 5% decrease in the ST, but still remaining 4% more than the initial P content on Day 1. After 60 days 4, 25, 38 and 33% of the initial dung-pat P content remained on the mesh base in the OT, MT, AT and ST, respectively (Figure 12c). Regression models for the rate of decomposition in terms of P content of each trial over the first 60 days of decomposition were very similar to those for N and Mg, with significant differences between the OT and the MT, AT and ST ($P < 0.05$). No significant differences were found between the P decomposition rates of the other trials. The highest rate of decomposition was found in the OT, significantly faster than the other trials.

Figure 13 shows the relationships between the nutrient contents and the DM contents of the dung-pats in the four trials. It shows the variation in the strength of the relationships between the nutrients and the DM, not only between nutrients in the same trial but also for the same nutrient in different trials.

The strongest relationships were all found in the OT, although the relationship between K and DM was much lower than the other three nutrients in the same trial ($r^2 = 0.95$, 0.98, 0.94 and 0.71 for N, P, Mg and K, respectively; $P < 0.0001$ for all). The relationships between P and Mg contents and the DM contents of the dung-pats were not only very similar but had the strongest relationships of the four nutrients in all four trials ($r^2 = 0.98$, 0.75, 0.43 and 0.60, for P in the OT, MT, AT and ST, respectively, and $r^2 = 0.94$, 0.80, 0.50 and 0.53, for Mg in the OT, MT, AT and ST, respectively; $P < 0.0001$ for all). The relationships between N and K contents and the DM contents of the dung-pat were much weaker than those for the other nutrients ($r^2 = 0.95$, 0.54, 0.26 and 0.15, for N in the OT, MT, AT and ST, respectively, and $r^2 = 0.71$, 0.47, 0.37 and 0.25, for K in the OT, MT, AT and ST, respectively; $P < 0.01$ for all, except for both N and K in the ST). The relative strength of these relationships serves as one of the indicators of the mechanisms determining the release of the nutrients from the dung-pats.

Whereas K and N showed almost immediate losses, nutrients such as Mg and P remained unchanged for the most part during the first 30 or so days. Possible mechanisms of entry of nutrients to the underlying soil can be identified by these rates of change. More than 30% of the dung-pats initial wet weight is lost during the first seven days. A significant relationship between K decline and moisture loss indicates that the primary mechanism of entry to the soil for K is through leaching.

P and Mg are more strongly related to DM changes, having stronger bonds with the dung-pat material. P entry into the soil requires physical incorporation and therefore its concentration in the dung pat changes more slowly. The dung-pat needs to be degraded by environmental conditions or biological activity. Earthworms in particular were observed in high biomass under the dung-pats, even in the summer with higher soil moisture contents under dung-pats, and they probably played a large role in incorporating the decomposing material.
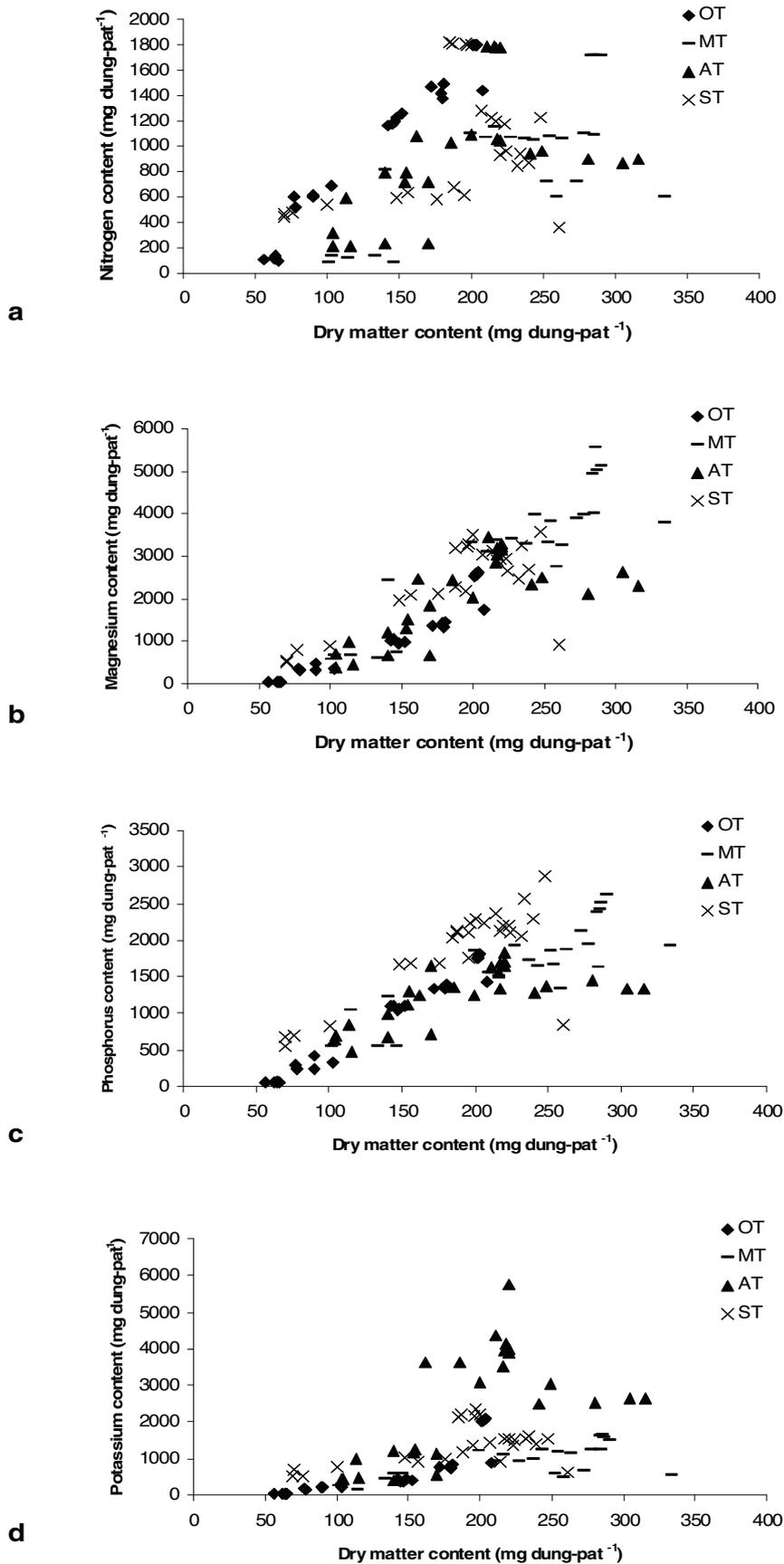
Figure 13: Relationships between nutrient (N [a], Mg [b], P [c] and K [d]) and DM contents in the dung-pats over the first 60 days of decomposition of the four trials.

4.4 Experiment 3: The Effect of the Dung-Pat on Soil P Dynamics

The grazing animal contributes significantly to nutrient cycling in grazed grassland by returning large amounts of P and other nutrients in dung and urine (Haynes and Williams, 1993). The process of dung decomposition leads to the release of these nutrients into the soil environment, with the mechanism of return of each nutrient dependent on its solubility and therefore its mobility (Underhay and Dickinson, 1978; Dickinson and Craig, 1990; Haynes and Williams, 1993; Aarons et al., 2004b).

Table 7 shows the impact of the dung-pats in the MT on soil Pt compared to the Pt in the baseline soil. No significant differences between the dung-pat affected and baseline soils were found in the first 28 days, even though there was a significant decrease in the Pt content of the dung-pats during this time. After 60 days there was a 22% increase in soil Pt associated with P moving from the dung-pats, which slowly declined between Days 60 and 90. The effects of sampling day ($P < 0.001$), treatment ($P < 0.01$) and the interaction of the two ($P < 0.05$) were all significant, showing that differences in soil Pt between the mean on each day of sampling followed a different trend depending on the treatment (presence or absence of dung-pats).

Table 7 also demonstrates the effect of the dung-pats in the MT on soil Pi compared to the Pi in the baseline soil. Again, no significant differences between the dung-pat affected and baseline soils were found in the first 28 days

of the decomposition trial. There was even a slight decrease (although not significant) in Pi concentration in the samples analysed on Days 14 and 28. By Day 60 there was a 48% increase in soil Pi in the dung-pat affected soil, and again – like soil Pt – this declined slowly between Days 60 and 90. Similar to Pt, the effects of sampling day ($P < 0.001$), treatment ($P < 0.01$) and the interaction of the two ($P < 0.001$) on soil Pi were all significant, showing that differences in Pi between the mean on each day of sampling followed a different trend, depending on the treatment (presence or absence of dung-pats).

Table 7 shows also the effect of the dung-pats in the MT on soil Po compared to the Po in the baseline soil. The effects of the dung-pats on soil Po were different to Pt and Pi, which had mostly behaved similarly. No significant differences at all were measured between the dung-pat affected and the baseline soil Po concentrations. This would suggest that very little Po was moving from the dung-pat to the soil, or that the Po, potentially in labile forms, was mineralised easily when entering the soil. The results of the analysis of variance confirm that although there was a significant sampling day effect on soil Po ($P < 0.001$), this effect was the same for both treatments and that therefore no interaction effect was possible.

Finally, Table 7 demonstrates the effect of the dung-pats in the MT on soil MBP compared to the MBP in the baseline soil. The effects of the dung-pat on changes in MBP concentrations were more variable that the other P fractions

Table 7: Effect of dung-pats on soil Pt, Pi, Po and MBP in decomposition trials, March 2003.

Sampling day	Pt	Pi	Po	MBP
	% difference[1] (significance level in parenthesis[2])			
1	2 (n.s.3)	1 (n.s.)	4 (n.s.)	4 (n.s.)
7	0 (n.s.)	0 (n.s.)	0 (n.s.)	38 (***)
14	2 (n.s.)	-7 (n.s.)	13 (n.s.)	-1 (n.s.)
28	3 (n.s.)	-5 (n.s.)	14 (n.s.)	28 (*)
60	22 (**)	48 (***)	-10 (n.s.)	42 (***)
90	18 (**)	34 (**)	-3 (n.s.)	86 (***)

[1]Difference between concentrations in dung-pat affected soils compared to baseline soils.
[2]Significance levels: * = $P << 0.05$, ** = $P << 0.01$, *** = $P << 0.001$. 3n.s. = non-significant.

described so far. After only seven days, a 38% increase in MBP was measured because of the presence of the dung-pat. This would indicate a high microbial population increase due to the input of carbon and nutrients into the soil or perhaps may result from the movement of microbes into the soil from the dung-pat itself. Between sampling Days 7 and 14, the MBP concentration under the dung-pats decreased again, returning to concentrations similar to that of the baseline soil. By Day 28 the MBP concentrations in the dung-pat-affected soil were significantly < higher ($P < 0.05$) than the baseline concentrations (28%). This trend continued on sampling Days 60 and 90, with significant increases of 42 and 86% measured ($P < 0.001$), respectively.

These results potentially reflect the much more labile and dynamic nature of this microbial-related fraction, reflecting not only the P inputs into the soil system, but other properties such as C and N concentrations, as well as environmental conditions such as soil moisture and temperature. The effects of sampling day ($P < 0.001$), treatment ($P < 0.001$) and the interaction of the two ($P < 0.001$) on soil MBP were all significant, showing that differences in MBP between the mean on each day of sampling followed a different trend depending on the treatment (presence or absence of dung-pats).

These results underline the important role microbial biomass plays the in P dynamics at this grassland site, with net accumulation in the microbial biomass of P moving from the dung-pat into the soil over the 90-day decomposition period.

Figure 14 shows the effect of the dung-pats in the MT, AT and ST on Pm compared to Pm in the baseline soil. In the MT, no significant differences were found between the dung-pat affected and baseline soils up until Day 28, whereas significant increases were measured after seven days in the AT and ST, 39 and 74%, respectively ($P < 0.001$). The increases were much faster in the AT and ST up until Day 28 compared to the MT, but between Days 28 and 60 an increase in Pm concentration of over 400% was measured in the dung-pat affected soil in MT, significantly larger than in the other two trials (Table 8; Figure 14). In all three trials, the maximum difference between the dung-affected and baseline soil Pm concentrations was found in the Day 60 samples, 452, 235 and 196%, for the MT, AT and ST, respectively ($P < 0.001$) (Table 8; Figure 15). There was a decrease in all three trials in the Day 90 Pm concentrations in dung-pat affected soils. The effects of sampling day ($P < 0.001$), treatment ($P < 0.001$) and the interaction of the two ($P < 0.001$) on soil Pm, were all significant, showing that differences in Pm between the mean on each day of sampling followed a different trend depending on the treatment (presence or absence of dung-pats). The trial period was also shown to have a significant effect on the Pm concentration ($P < 0.001$), and its interaction with both sampling day and treatment was also significant ($P < 0.001$).
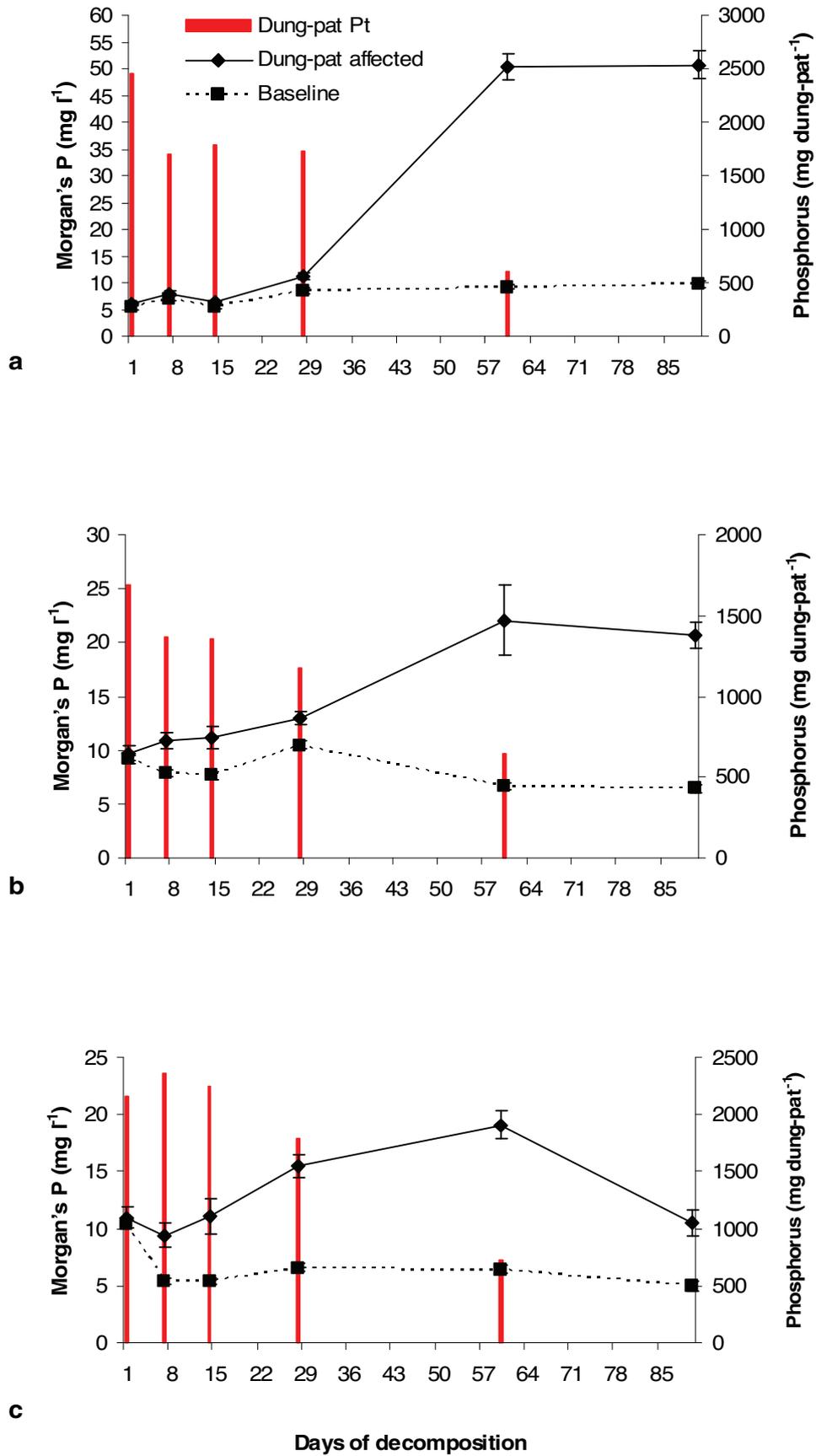
Figure 14: Effect of decomposing dung-pats on soil available phosphorus (Pm) in March trial (a), August trial (b), and September trial (c) (changes in dung-pat Pt shown on secondary y-axis).
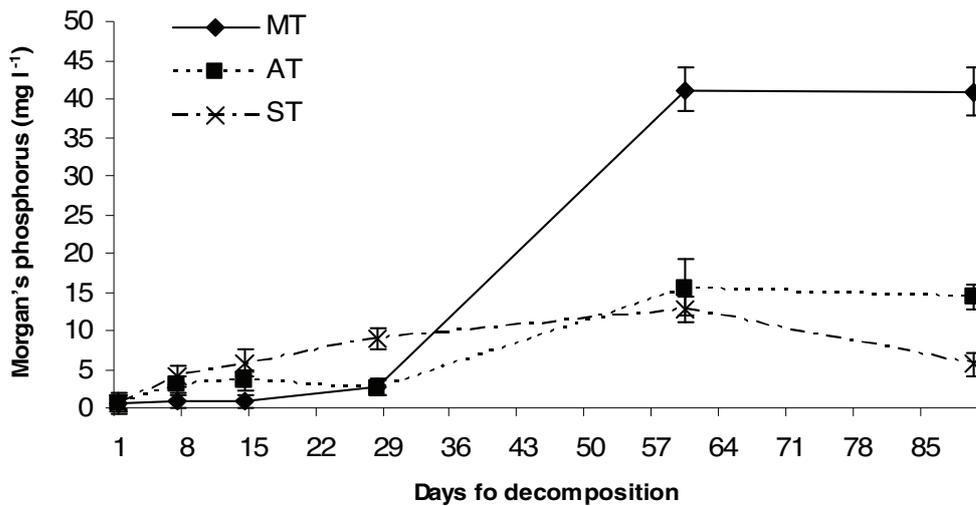
Figure 15: Comparison of changes in soil Pm for three dung-pat decomposition trials. Values presented are based on difference between dung-pat affected and baseline soil Pm.

The three-way interaction of trial period, sampling day and treatment suggests that the differences in Pm concentration measured between the mean on each sampling day followed a different trend depending on the treatment (presence or absence or dung-pats) and also the trial (MT, AT or ST). Aarons et al. (2004b) showed similar highly significant effects on available P (bicarbonate-extractable Pi, Colwell Pi and Olsen Pi). Aarons et al. (2004a) examined the possibility of dung affecting the sorption capacity of the soil and thus influencing the availability of P. They concluded that the measured increase in soil Olsen P was not due to decreased P sorption, and that the dung therefore was not responsible for a reduction in the sorption capacity of the soil. Most of the nutrients had been lost from the dung-pats over the 90-

day decomposition periods. Dickinson and Craig (1990) found that the loss of nutrients from dung-pats was not mirrored by an equivalent gain in nutrients in the underlying soil. They found that there was significant lateral as well as downward movement of the nutrients. This may result in the nutrients moving outside of the immediate sampling area and diluting the overall impact of the dung-pat. No soil samples were taken outside the area immediately affected by the dung-pat during this study. The destructive nature of the sampling, i.e. taking a core underneath the dung-pat, may aid the downward movement of the nutrients. The high earthworm biomass recorded during these experiments, whose activity is assumed to have incorporated the organic matter rich dung into the soil, may also influence the final destination and overall impact of the dung (Holter, 1979).

Table 8: Effect of dung-pats on Pm (percentage change) in decomposition trials, March, August and September 2003.

Sampling day	MT	AT	ST
	% difference[1] (significance level in parenthesis)[2]		
1	10 (n.s.)	6 (n.s.)	5 (n.s.)
7	12 (n.s.)	39 (***)	74 (***)
14	14 (n.s.)	46 (**)	108 (***)
28	33 (**)	24 (**)	134 (***)
60	452 (***)	235 (***)	196 (***)
90	417 (***)	221 (***)	111 (***)

[1]Difference between concentrations in dung-pat affected soils compared to baseline soils.
[2]Significance levels: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Significance levels based on t-test for independent samples (two-tailed).

4.5 Experiment 4: Characterisation of Po in Grassland Soils

A large proportion of soil Pt is present in organic forms – typically between 29 and 65% but up to 90% in some organic soils (Dalal, 1977; Harrison, 1987). Most of the identifiable soil Po exists as orthophosphate monoesters, which constitute between 60 and 90% of the total Po in alkaline soil extracts (Condron et al., 1985; 1990; Condron and Goh, 1990; Cade-Menun and Preston, 1996). With so much Po potentially found in Irish grassland soils, it is surprising how little is known about the chemical (structural) nature and quantities of the Po forms present, their bioavailability, and what these might mean for P losses from Irish grassland soils to the receiving water bodies.

Table 9 shows the results of the wet chemical analysis using NaOH-EDTA as the soil P extractant. The alkaline solution was shown to be effective at removing up to 88% of Pt in the soil with more than 60% of this in an organic form. Little variation in the concentrations was observed over the 90-day period from September to December 2003.

Table 10 and Figure 16 present the results obtained using ^{31}P NMR spectroscopy to characterise the Po in the soil samples. Because of the expense and time required to analyse the samples, only one sample per sampling day was analysed. Inorganic orthophosphate is shown to account for approximately 52% of the P in the NaOH-EDTA extracted soils over the 90 days. The majority of the Po identified belongs to the orthophosphate monoesters,

accounting for approximately 42% of the soil Pt and more than 90% of soil Po. This falls in line with most soil Po studies (Condron et al., 1985; Turner et al., 2002). This dominance of orthophosphate monoesters (e.g. inositol phosphates and mononcleotides) over other forms of soil Po is not reflected in the composition of Po inputs into the soil (Magid et al., 1996). For example, orthophosphate monoesters constitute only 10–25% of the Po inputs to the soil from microbial and plant sources, of which inositol phosphates are a minor fraction. By contrast, orthophosphate diesters (e.g. nucleic acids [RNA, DNA], aromatic diesters and phospholipids) constitute most of the Po in inputs of fresh microbial and plant matter to the soil (Turner et al., 2002). In these samples, the orthophosphate diesters accounted for a little over 3% of the soil Pt in the NaOH-EDTA extracts. The concentrations of orthophosphate monoesters are, however, likely to be overestimated in studies involving alkaline extraction, due to degradation of some orthophosphate diesters, specifically RNA and phosphatidyl choline, during extraction and analysis (Turner et al., 2003b).

A decrease of approximately 50% of the orthophosphate diesters was measured between 20 October (Julian Day 293) and 17 November (Julian Day 321) (Table 10). This coincides with a significant drop in temperature during that period, and may be explained by the mineralisation (hydrolysis) of Po forms into orthophosphate. The orthophosphate diesters represent one of the more labile Po forms, having a low sorption capacity and, therefore, a

Table 9: Concentrations of total, available and NaOH-EDTA extractable soil P of a grassland soil determined on five sampling dates between 23 September and 15 December 2003 at Red House Field site.

Date	Total P (ICP)	Water extractable P[a]	Soil test P (Morgan's P)[a]	NaOH-EDTA				Pt/Pt[b]
				Pt	Pi	Po	Po/Pt	
	mg kg⁻¹		mg l⁻¹	mg kg⁻¹			%	
23/09/03	759	13.37 (1.1)	10.4 (0.38)	666	251	416	62	88
30/09/03	819	11.72 (0.08)	5.4 (0.31)	615	238	377	61	75
20/10/03	803	3.55 (0.15)	6.6 (0.30)	606	231	375	62	75
17/11/03	764	3.49 (0.07)	6.4 (0.39)	582	200	381	66	76
13/12/03	723	3.53 (0.07)	5.0 (0.40)	559	203	356	64	77

[a]Standard errors of the mean are shown in parenthesis. [b]Percentage Pt (NaOH-EDTA) of Pt (ICP).

Table 10: Percentage of functional P classes in NaOH-EDTA extracts of a grassland soil determined on five sampling dates between 23 September and 15 December 2003 at Red House Field site as determined by ^{31}P NMR spectroscopy.

Date	^{31}P NMR (%)				Monoester-to-diester ratio
	Inorganic orthophosphate	Orthophosphate monoesters	Orthophosphate diesters	Pyrophosphates, etc.	
23/09/03	56.18	39.33	2.25	2.25	17.48
30/09/03	52.63	42.63	3.16	1.58	13.49
20/10/03	48.78	43.41	4.39	3.41	9.89
17/11/03	54.35	41.30	2.17	2.17	19.03
13/12/03	50.51	42.42	3.54	3.54	11.98

high potential for mineralisation. Although the amount detected (< 3%) may seem small in terms of overall Pt, it may be quite significant in terms of available P, as measured in Ireland as Pm. Few other changes with time were noted except perhaps around 20 October when decreases in orthophosphate were mirrored by increases in orthophosphate monoesters, orthophosphate diesters and other P forms, including pyrophosphates. Significant variation in the monoesters to diesters ratio was found with the lowest ratio of approximately 10 found on 20 October and the highest ration of approximately 19 found on 17 November.

Other forms of P identified accounted for just under 3% on average of soil Pt extracted with the NaOH-EDTA solution, with pyrophosphates making up most of this P. Pyrophosphate is believed to be involved in biological P cycling in the soil, and some may be present as an organic ester which is hydrolysed during alkali extraction (Anderson and Russell, 1969). The presence of pyrophosphate is linked strongly to high levels of microbial activity (Turner et al., 2003a) as is evident in the Red House Field soil. The relative proportions of Pi and Po

determined using the wet chemical analysis and ^{31}P NMR spectroscopy varied, with more inorganic orthophosphate measured using the ^{31}P NMR spectroscopy than using the wet chemical analysis. This may be due to hydrolysis of the Po forms during the analysis procedure or to inaccurate distinction between the adjacent inorganic and orthophosphate monoester peaks (Condron et al., 1985).

The high proportions of Po reflect the fact that significant quantities of Po are continually added to the soil in plant, animal, and microbial detritus. For example, Po constitutes 30 to 60% of the Pt in most plant material (Harrison, 1987) and more than 90% of Pt in microorganisms, mainly in the form of nucleic acids (Stewart and Tiessen, 1987; Magid et al., 1996). On an annual basis, between 60 and 95% of the P taken up by plants in grazed pasture systems is returned to the soil in litter, root residues and/or animal excreta (Haynes and Williams, 1993). Although ^{31}P NMR spectroscopy has the potential to give great detail in terms of the chemical nature of the soil Po, further studies are required to understand the bioavailability of the Po forms present in different soil types and under various environmental conditions.
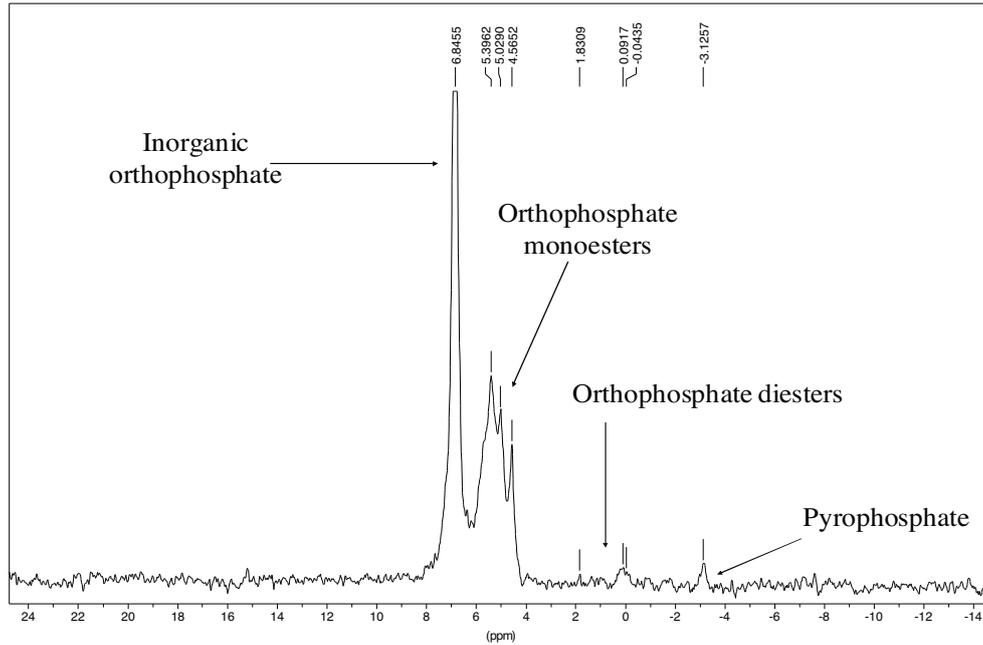
Figure 16: ^{31}P NMR spectra of soil sample collected on Day 28 (20 October 2003) in Red House Field site.

4.6 Experiment 5: Characterisation of P in Fresh Dung

4.6.1 Dung Pt, Pi and Po

Table 11 outlines the summary statistics of all dung properties measured in the current study based on all the dung samples collected from the six P fertiliser treatments.

Significant differences were found in Pt concentrations measured in the dung samples collected from the six P fertiliser treatments ($P < 0.001$) (Table 12). In particular, there were notable differences between the 0 to 0 treatment and all other treatments that have received P fertiliser at some time in the recent past. The Pt ranged from 0.23 to 1.26% (expressed as a percentage of dry weight) with a mean value of 0.78% of the dung sample for all P fertiliser treatments. The Pt concentration expressed as wet weight ranged from 293 to 1730 mg kg^{-1} for all P fertiliser treatments.

A significant positive linear relationship between the P concentration of the diet (grassland herbage) of the grazing animal and the total amount of P excreted in the dung was found. The plots receiving the most fertiliser P had the highest P concentrations in the herbage (diet) which resulted in the highest P concentrations in the excreted dung.

Significant differences in Pi and Po fractions were also found between the six P fertiliser treatments ($P < 0.001$) (Table 12). The 0 to 0 and 30 to 0 plots (no P fertiliser) had a mean of 67% of Pt found in the Po fraction (Table 12). The opposite trend was shown for all other plots receiving P fertiliser, with most of the Pt found in the inorganic fraction (mean of 64%).

These results are consistent with the results of Barrow and Lambourne (1962) who proposed a simple model to describe the changes in the proportions of Pi to Po depending on the concentration of P in the diet. In the current study the lowest dung Pt results corresponded with the lowest herbage P concentrations (r = 0.83). The higher the Pt concentration measured, the higher the proportion of Pi present in the dung. This trend is seen very clearly in the six P fertiliser treatments examined. The herbage in the plots receiving fertiliser P had significantly higher P concentrations and this resulted in most of the P being excreted in the dung in an inorganic form. Those animals grazing unfertilised grassland (plots 0 to 0 and 30 to 0) excreted dung dominated by Po (72 and 61%, respectively).

Table 11: Summary statistics for all dung samples taken from the six P fertiliser treatments in Cowlands, July 2004.

Dung property[1]	Mean	Standard error	Median	Min.	Max.	n
Pt (mg kg^{-1} wet weight)	968	71	920	296	1730	30
Pt (mg kg^{-1})	7789	512	7987	2349	12647	30
Pi (mg kg^{-1})	4089	349	4208	1004	7961	30
Po (mg kg^{-1})	3701	431	3274	790	10028	30
% Po	46.40	3.64	44.87	15.3	79.29	30
Pt (ICP) (mg kg^{-1})	7467	971	7827	3912	9978	6
Pw (TDP) (mg kg^{-1})	1485	115	1309	574	2828	30
Pw (DRP) (mg kg^{-1})	1270	101	1173	437	2440	30
Pw (DUP) (mg kg^{-1})	215	25	147	50	520	30
% DUP	14.80	1.36	13.94	3.83	30.85	30
% Pw (TDP) of Pt	22.12	1.74	19.70	8.84	45.24	30
% DRP of TDP	85.20	1.36	86.06	69.15	96.17	30
NaOH-EDTA Pt (mg kg^{-1})	5397	694	5937	2642	6989	6
NaOH-EDTA Pi (mg kg^{-1})	3600	659	4149	1042	4992	6
NaOH-EDTA Po (mg kg^{-1})	1797	111	1811	1402	2137	6
% NaOH-EDTA Po	36.6	5.6	33.7	21.9	60.6	6
% Recovery	74.9	8.7	72.2	41.6	98.9	6
TN (%)	2.44	0.05	2.41	1.94	3.10	30
OC (%)	39.07	0.49	39.24	31.54	42.82	30
OM (%)	81.49	0.80	81.61	72.28	92.88	30
K (mg kg^{-1} wet weight)	1931	86	1856	1170	2667	30
Mg (mg kg^{-1} wet weight)	921	48	914	387	1531	30
DM (%)	12.37	0.39	12.15	8.90	17.90	30

[1]All results with units indicated as mg kg^{-1} are calculated based on the dung dry weight except where otherwise indicated.

Table 12: Mean concentrations of P fractions (total, total inorganic, total organic and percentage organic) in dung samples from six P fertiliser treatments in Cowlands (standard errors in parenthesis, n = 5).

Treatment (P fertiliser)	Pt[1]	Pt[1]	Pi[1]	Po[1]	% Po[1]
	mg kg^{-1} (wet weight)	mg kg^{-1} (dry weight)			
0 to 0	465 (89)[a]	3912 (607)[a]	1384 (150)[a]	2528 (616)[a]	61 (6)[a]
0 to 30	825 (27)[b]	6533 (556)[b]	4142 (195)[b,c]	2391 (529)[a]	35 (6)[b]
15 to 15	918 (179)[b]	7070 (1011)[b]	5190 (1083)[c]	1880 (515)[a]	29 (8)[b]
15 to 5	1035 (75)[b,c]	9626 (528)[b]	5577 (529)[c]	4049 (817)[a]	41 (7)[b]
30 to 30	1156 (172)[b,c]	9617 (1302)[b]	5526 (320)[c]	4091 (1011)[a]	39 (6)[b]
30 to 0	1410 (122)[c]	9978 (764)[b]	2712 (116)[a,b]	7266 (777)[b]	72 (2)[a]
Significance[2]	***	***	***	***	***

[1]Phosphorus fertiliser treatments within columns with different superscripts (a, b, c, d) for each dung phosphorus fraction are significantly different at $P < 0.05$.
[2]Significance levels of differences found between the six P fertiliser treatments using one-way ANOVA: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Importantly, these results show how quickly the P cycle changes with changes in fertiliser management, with the fertiliser applications changing in half the grassland plots in the Cowlands in 1998. The 0 to 30 plots also demonstrate this change in P fractions as the dung samples here were dominated by Pi (65%), having only received fertiliser P since 1998 after 30 years without.

4.6.2 Water-Extractable Dung P

Two P fractions of the dung samples extracted in water were measured: total dissolved P (TDP) and dissolved reactive P (DRP), and the difference between them is dissolved unreactive P (DUP). DRP is predominantly made up of inorganic orthophosphate while DUP is mostly composed of

organic fractions but may also contain condensed P forms (Shand et al., 1994) and is treated as such in this study.

Table 13 shows that there was a significant difference in the Pw fractions between the P fertiliser treatments. A mean value for all treatments of 1485 mg kg^{-1} was found, ranging between 574 and 2828 mg kg^{-1} (Table 11). The percentage of Pt made up of TDP ranged between 9 and 45%, with the highest percentages (*c*. 30% each) found in the 0 to 0 and 0 to 30 treatments, the plots which had received least P fertiliser over the previous 36 years. Most of the water soluble P was contained in the DPR fraction (77 to 92%) (Table 13). DUP appeared to have a very similar concentration regardless of TDP concentration (Table 13).

Table 13: Mean concentrations of Pw fractions (TDP, DRP, DUP, %DUP and %TDP of Pt) in dung samples from six P fertiliser treatments in Cowlands (standard errors in parenthesis, n = 5).

Treatment (P fertiliser)	Water extractable phosphorus[1]				
	TDP	DRP	DUP	DUP[2]	TDP of Pt[3]
	mg kg^{-1} (dry weight)			%	
0 to 0	1079 (173)[a]	821 (128)[a]	258 (57)[a,b]	23 (3)[b]	29 (5)[b]
0 to 30	1667 (182)[a]	1402 (152)[a,b,c]	266 (52)[a,b]	16 (2)[a,b]	30 (4)[b]
15 to 15	1248 (175)[a]	1120 (180)[a,b]	128 (23)[a]	11 (3)[a]	18 (1)[a,b]
15 to 5	2153 (434)[a]	1933 (507)[c]	220 (73)[a,b]	11 (6)[a]	22 (2)[a,b]
30 to 30	1986 (389)[a]	1657 (326)[b,c]	328 (74)[b]	17 (2)[a,b]	22 (5)[a,b]
30 to 0	1179 (80)[a]	1083 (83)[a,b]	96 (14)[a]	8 (1)[a]	12 (1)[a]
Significance[4]	*	**	*	***	**

[1]Phosphorus fertiliser treatments within columns with different superscripts (a, b, c, d) for each dung phosphorus fraction are significantly different at $P < 0.05$.
[2]The percentage of TDP made up of DUP.
[3]The percentage of Pt (total P in the dung) made up of TDP. [4]Significance levels of differences found between the six P fertiliser treatments using one-way ANOVA: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 14: Mean concentrations of NaOH-EDTA extractable Pt, Pi and Po, and percentage Po, and percentage recovery (Pt) by NaOH-EDTA extraction of Pt (ICP) in dung samples from the six P fertiliser treatments in Cowlands (n = 1).

Treatment	Pt (ICP)	NaOH-EDTA			% Po[1]	% recovery[2]
		Pt	Pi	Po		
		mg kg^{-1}				
0 to 0	3912	2642	1042	1600	61	68
0 to 30	6533	5479	3456	2023	37	97
15 to 15	7070	6989	4852	2137	31	99
15 to 5	9626	6394	4992	1402	22	74
30 to 30	9617	6725	4842	1883	28	70
30 to 0	9978	4152	2414	1738	42	42

[1]Percentage of Pt (extracted with NaOH-EDTA) made up of Po (extracted with NaOH-EDTA).
[2]Percentage of Pt (measured using ICP) made up of (recovered by) Pt (extracted with NaOH-EDTA).

4.6.3   *NaOH-EDTA Extractable Dung P*

The mean percent recovery of dung Pt by NaOH-EDTA extraction in the dung samples from the six P fertiliser treatments was 75% (Table 14). This is comparable with recovery rates of 78 and 59% found in other manures extracted (Hansen et al., 2004). Concentrations of NaOH-EDTA Pt in the dung ranged from 2642 to 6989 mg kg^{-1}. The percentage Po of Pt in the NaOH-EDTA extracts followed similar patterns to those described in Tables 12 and 13, with the highest proportions found in the unfertilised plots, in treatments 0 to 0 and 30 to 0, 61 and 42%, respectively. When a certain concentration of Po was reached, any excess P in the dung was composed of an inorganic P form or fraction. The percentage recovery of Pt made up of NaOH-EDTA extracted Pt was notably lower (42%) in the dung samples from the 30 to 0 plot compared to the other treatments (mean of 82%) (Table 14).

Distribution of P forms in NaOH-EDTA extracts was calculated from the ^{31}P NMR spectra by integration of the peaks (Figures 17 and 18). Four distinct P compounds were detected: (i) inorganic orthophosphate (δ = 6.64 to 6.69 ppm) with a mean of 6.68 ppm; (ii) orthophosphate monoesters (δ = 5.26 ppm); (iii) orthophosphate diesters (δ = -0.85 to 2.33 ppm), and (iv) pyrophosphates (δ = -3.65 to -3.29 ppm) with a mean of -3.52 ppm. The spectral assignments were based on a number of key studies that compared spectra of model P compounds with spectra of soils extracted with NaOH-EDTA (Turner et al., 2003a; 2003b; Cade-Menun, 2005). Phosphonates (normally occurring at δ = 20 ppm) were not detected in the dung samples analysed.
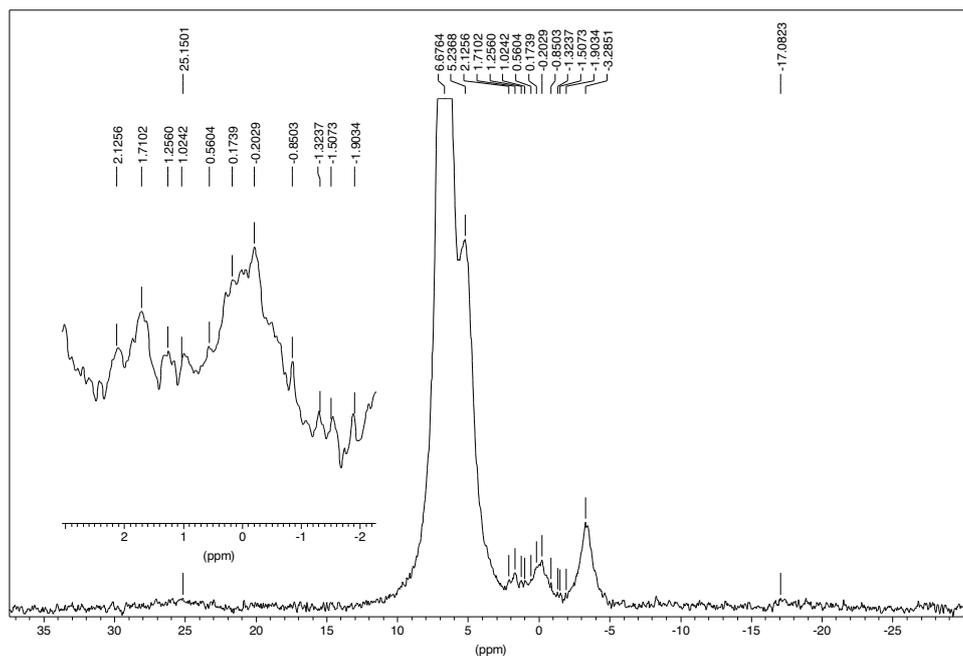

Figure 17: ^{31}P NMR of fresh dung collected from grazed plots with the 30 to 0 fertiliser treatment in Cowlands. Inset shows close-up of orthophosphate diester peaks.
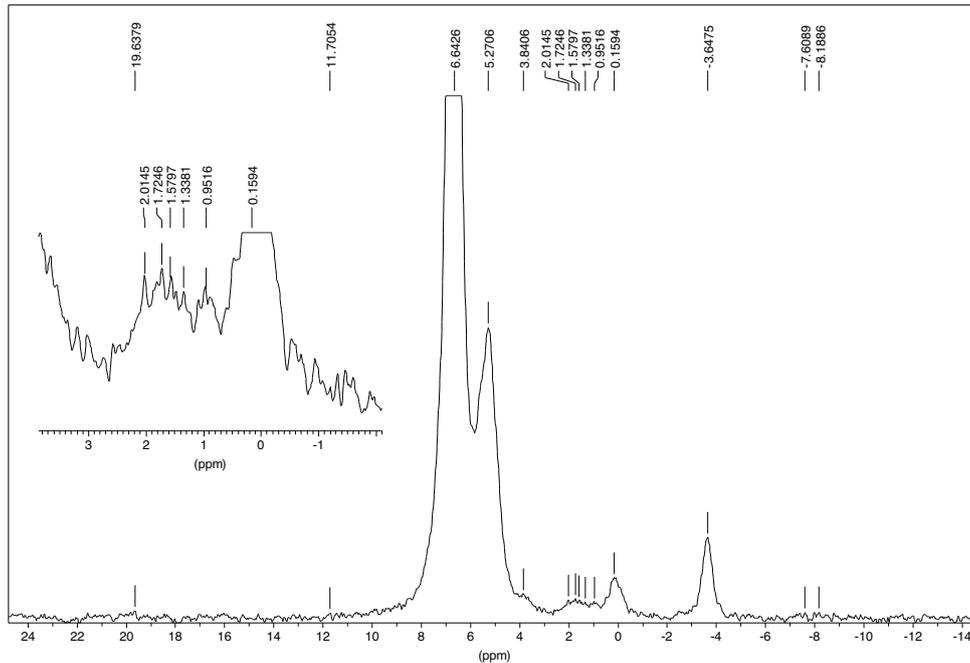
Figure 18: ³¹P NMR spectra of fresh dung collected from grazed plots with the 30 to 30 fertiliser treatment in Cowlands. Inset shows close-up of orthophosphate diester peaks.

Table 15: Functional classes (spectral assignment in brackets) of P determined in NaOH-EDTA extracts of the dung samples from the six phosphorus fertiliser treatments in the Cowlands by solution ³¹P MNR spectroscopy (n = 1).

Treatment	³¹P NMR				
	Pi {6.64 to 6.69}	P monoester {3 to 6}	P diester {2 to -2}	P other {-3 to -6, 20}	Monoester: diester ratio
	% of total P extracted with NaOH-EDTA				
0–0	54.1	35.7	5.4	4.9	6.6
0–30	77.5	18.6	1.6	2.3	12.0
15–15	74.6	18.7	3.0	3.7	6.3
15–5	77.5	17.1	2.3	3.1	7.3
30–30	74.6	17.9	3.7	3.7	4.8
30–0	62.9	26.4	4.4	6.3	6.0

For all treatments, the principal form of extracted P was orthophosphate (Table 15). Orthophosphate monoesters, pyrophosphate and orthophosphate diesters were also present, in decreasing order of concentration (Table 15). Higher proportions of orthophosphate monoesters, orthophosphate diesters and pyrophosphates were found in the 0 to 0 and 30 to 0 plots, reflecting the higher proportion of Po in these treatments compared to the others. In these no-fertiliser treatments, 36% on average of the P as quantified using NMR spectroscopy was organic. No obvious trends were revealed in the ratios between orthophosphate monoesters and orthophosphate diesters (Table 15). The quality of the ³¹P NMR spectra was good using the Bruker Avance DPX 400 NMR spectrometer. Most studies in recent times use more powerful NMR spectrometers obtaining better separation of the orthophosphate monoester and diester peaks (Koopmans et al., 2003; Turner et al., 2003a; 2003b; McDowell and Stewart, 2005; Turner, 2006). It proved difficult to sub-classify the orthophosphate monoester and orthophosphate diester regions of the spectra for these dung samples.

4.7 Experiment 6: Characterisation of Po in Overland Flow

4.7.1 Wet Chemistry

Figure 19 shows the concentrations of various P fractions measured in the overland flow samples collected during a rainfall simulation experiment on 5 May 2004. This is a single set of data, which was part of the larger rainfall simulation experiment described in more detail in the Final Report on Section B (LS-2.1.2b) of the Grazed Pastures Project (Kurz and O'Reilly, 2006).

The presence of the grazing animal is shown there to have a highly significant effect on all P fractions measured. It shows that, for all fractions of P, the concentrations from grazed plots are larger than the non-grazed plots. A comparison of each fraction from grazed and non-grazed plots as a percentage of TP shows that a higher proportion of DRP was measured in

the overland flow from the non-grazed plots than in that from the grazed plots. A similar difference is seen for TDP with a higher overall proportion measured on non-grazed plots. The opposite position holds for all the other fractions (particulate P [PP], total unreactive P [TUP], dissolved unreactive P [DUP], and particulate unreactive P [PUP]) which represent higher proportions of Pt in overland flow from the grazed plots.

Of particular significance is that all of the unreactive (predominantly organic) fractions make up a much larger proportion of Pt as shown in the overall concentrations of these fractions. The biggest differences between the P fractions in the overland flow collected from the grazed and non-grazed plots during the rainfall simulation experiments overall, as well as in that of 5 May, are seen in the unreactive fractions or the Po fractions (Kurz and O'Reilly, 2006).

It was decided to further characterise the P fractions, specifically the organic P using ^{31}P NMR spectroscopy.
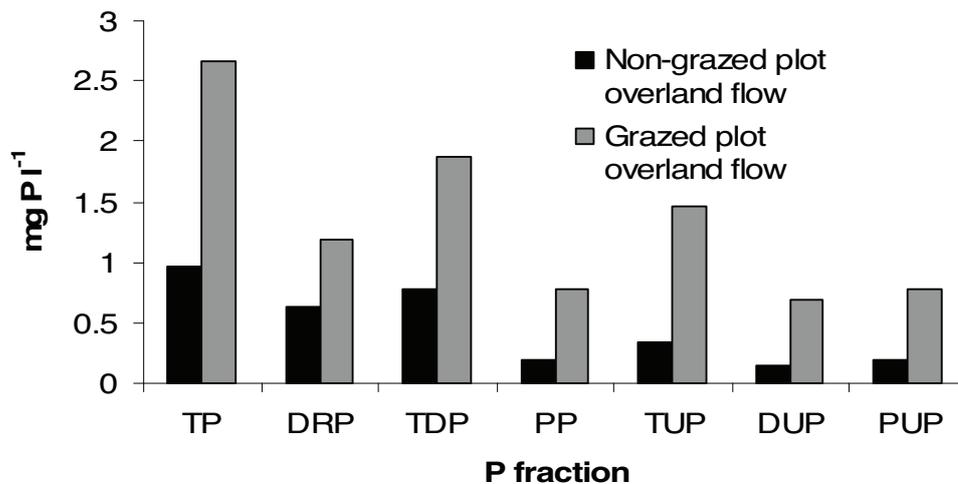

Figure 19: Concentration of various P fractions in overland flow samples collected from grazed and non-grazed grassland small plots using rainfall simulator in run 9 on 5 May 2004 (Kurz and O'Reilly, 2006).
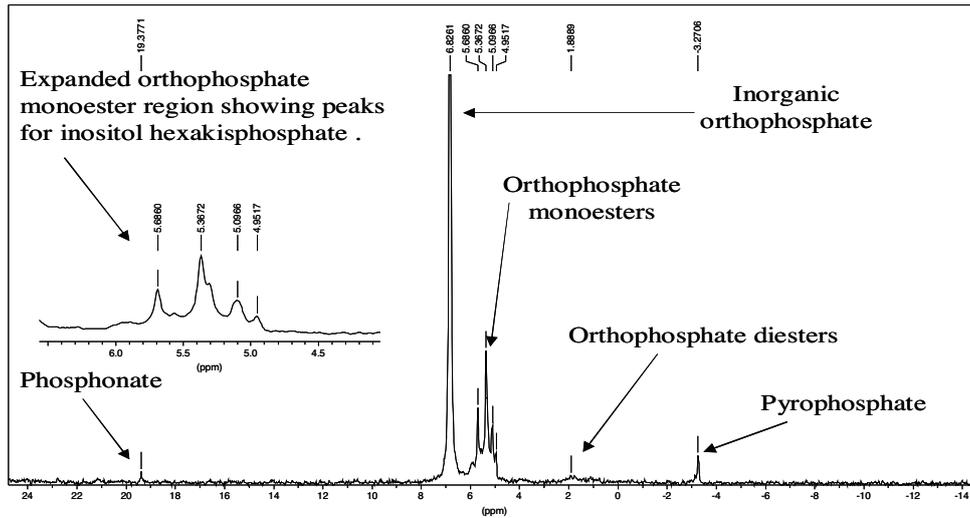
Figure 20: ^{31}P NMR spectra of overland flow sample collected from a grazed plot using rainfall simulator in run 9 on 5 May 2004. Insets show close-ups of orthophosphate monoester peaks.
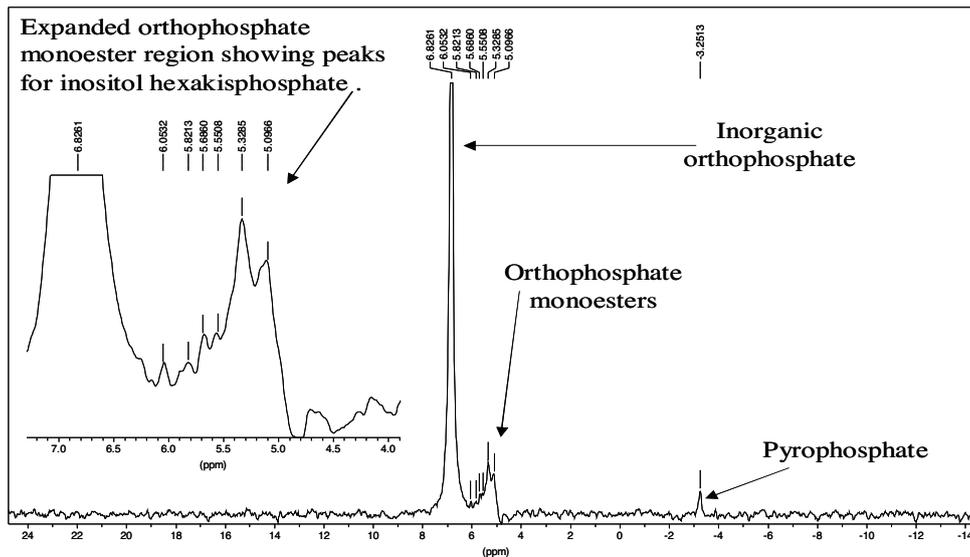

Figure 21: ^{31}P NMR spectra of overland flow sample collected from a non-grazed plot using a rainfall simulator on in run 9 on 5 May 2004. Insets show close-ups of orthophosphate monoester peaks.

Table 16: Distribution of Po forms in NaOH-EDTA extracts of overland flow samples from small plots using rainfall simulator in run 9 on 6 May 2004, calculated from NMR spectra by peak integration.

Plot	^{31}P NMR (%)				Monoester-to-diester ratio
	Inorganic orthophosphate	Orthophosphate monoesters	Orthophosphate diesters	Phosphonates, pyrophosphates, etc.	
Grazed	72.5	24	1.5	2	16.5
Non-grazed	84	13.5	n.d.*	2.5	n.d.*

*n.d. = not detectable in sample.

4.7.2   *NaOH-EDTA Extractable Overland Flow P*

Figures 20 and 21 illustrate the spectra, obtained using ^{31}P NMR spectroscopy, for overland flow samples collected from the grazed and non-grazed plots. Characterisation of the overland flow P in NaOH-EDTA extracts revealed that most of the P was present as inorganic orthophosphate, measuring 72.5 and 84% respectively on the grazed and non-grazed plots (Table 16). This indicates that the percentage of organic P in the overland flow from grazed plots (25.5%) was twice that for the non-grazed plots (13%) (Table 16). The Po was dominated by orthophosphate monoesters for both the grazed (24%) and non-grazed (13%) plots. Orthophosphate diesters and phosphonates were found only in the overland flow samples collected from the grazed plot. Similar proportions of other P forms (mostly pyrophosphate) were found in the samples from the grazed and non-grazed plots (Table 16).

The dominance of orthophosphate monoesters in the Po was expected because of the dominance of orthophosphate monoesters in most soil types. It can be hypothesised that the higher proportion of orthophosphate monoesters found in the grazed samples had an association with larger particles. Stewart and Tiessen (1987) demonstrated that inositol hexakisphosphate (IHP) (known to make up a large proportion of orthophosphate monoesters) strongly sorbs onto clays and sesquioxides. These unreactive P forms are very mobile in the soil system having weak fixation properties. This would strengthen the argument that the monoesters in the overland flow from the grazed plot are associated with the elevated PUP levels and increased suspended sediments as caused by the grazing animal. In a study of Po in leachate, Toor et al. (2003), using various phosphatase enzymes, showed that the orthophosphate monoesters in their samples were mostly comprised of labile monoester P (LMP) and IHP. They suggested that this finding had direct implications for water quality as both LMP and IHP can be utilised directly by aquatic microorganisms (Whitton et al., 1991).

Orthophosphate diesters (e.g. phospholipids, DNA) and phosphonates were only found in the overland flow samples collected from the grazed plots. Pyrophosphates (the only other form of Pi identified) were found in almost equal quantities in overland flow from the grazed and ungrazed plots. Turner et al. (2003a, 2003b) described how relationships between functional P groups and soil properties suggest that different processes control the presence of P forms in the soil. In particular, DNA and pyrophosphate are strongly correlated with the microbial biomass, suggesting that these are active compounds linked to biological nutrient turnover. Toor et al. (2005) accounted for the presence of phospholipids, and in particular DNA in faeces, to microbial debris that is excreted by the grazing animal. Under certain agricultural management practices and intensities, Po losses to natural waters via overland flow and subsurface drainage may be an important component of the overall phosphorus input; thus, assessment of the bioavailability (e.g. using phosphatase enzymes) of these Po forms in receiving waters must be a priority in the future (McDowell and Koopmans, 2005).

5   Overall Conclusions

- Significant seasonal variation in P fractions (and the other soil properties), particularly Pm (ranging from 5 to 10.5 mg P l^{-1}) was observed in the grassland soil studied. Soil temperature, soil moisture and biological demand appear to be the key controls in this variation. The soil microbial biomass plays an important role in P cycling in Irish grassland soils. An annual turnover through the microbial biomass of approximately 50 kg P ha^{-1} yr^{-1} in this soil type was calculated.

- The grazing animal and associated dung deposition play a major role in increasing the rate at which P is recycled in the grazed grassland ecosystem. Dung-pat decomposition was affected by seasonality and the amount of rainfall over the decomposition period. There was a strong relationship between DM content and changes in dung-pat Pt over the decomposition period. The principal P mechanism of entry into the soil from the dung-pat was through physical incorporation, relying on soil fauna and physical degradation. Phosphorus leaching from the dung-pat into the soil was important during periods of high rainfall.

- Dung-pats significantly affected soil P. Pm concentrations were shown to increase three- to four-fold over the 90-day decomposition period. Owing to the uneven distribution of nutrients within grazed grassland (e.g. at drinking troughs, ring feeders, shelter areas) dung-pat increased soil P may create critical source areas (partial source areas combined with P sources) of greater potential risk of P loss to water.

- Fertiliser P treatment (affecting soil and herbage P) affected Pt excreted in dung significantly. Dung collected from grassland not receiving P fertiliser had a higher proportion of total P found in organic forms (67%) than dung collected from plots receiving fertiliser P where most (64%) of the P was in the inorganic form. The amount of water soluble P in Pt ranged from 12 to 30% of dung Pt. The proportion of TDP in dung Pt was shown to have a significant positive relationship with %DRP, while being inversely related to %DUP. ^{31}P NMR spectroscopy revealed that the Po concentration and forms present in dung depended on fertiliser treatment. Orthophosphate monoesters, pyrophosphate and orthophosphate diesters were shown to be present, in decreasing order of concentration. Significantly higher concentrations of orthophosphate monoesters, orthophosphate diesters and pyrophosphates were found in plots receiving no fertiliser P compared to the plots receiving fertiliser P.

- A comparison of P forms (using wet chemistry and ^{31}P NMR spectroscopy) in overland flow revealed that inorganic orthophosphate was the principal form of P found in overland flow samples collected from grazed and non-grazed plots. The main differences found between the two treatments were in the organic P forms identified. A higher percentage and a more diverse range of orthophosphate monoesters were found in grazed plot overland flow compared to non-grazed plot overland flow. Orthophosphate diesters were found only in grazed plot overland flow. The higher percentages of orthophosphate diesters and pyrophosphate suggest that there was more microbial activity associated with the grazed plot overland flow, as these P forms are commonly associated with microbes.

6 Action Recommendations

- Farm according to good farming practice with minimum soil P for optimum grassland production, i.e., not exceeding crop requirements (Teagasc Soil P Index 2).

- Elevated soil test P (Pm) concentrations and high fertiliser P application rates will lead to higher than required herbage P concentrations, thus increasing dung P concentrations. Therefore, under intensive animal production systems, maintain sustainable best-management practices, which will help decrease the risk of P loss to water from high P soils and high P dung.

- When developing nutrient-management plans and assessing areas of risk on a field-by-field basis, consider areas where high dung-pat deposition occurs, unevenly redistributing and elevating soil P levels, such as at drinking troughs, ring feeders and shelter areas.

- Consider measures for reducing P concentrations in animal diets, decreasing P in excreta and the overall reduction of P surpluses at the field-, farm- and catchment-scale. The enzyme phytase has become widely used to increase P use efficiency in animal concentrates. The use of low phytate grains in concentrate mixes may also help reduce surplus P in the animal's diet.

7   Research Recommendations

- This study showed significant seasonal changes in soil test P in a recently unfertilised grassland soil. This indicates the need to investigate seasonal changes in P (available P or soil test P such as Morgan's) on different soil types, over a range of total P concentrations, with various P fertiliser regimes. It is noted, however, that Teagasc currently recommends soil sampling at the same time of year to allow for the effects of seasonality.

- Undertake an investigation into the link between biologically mediated processes (mineralisation, immobilisation and solubilisation) associated with soil microbial biomass and soil enzyme activity and the release of P (from various soil types and P concentrations) to water.

- Upscale small-scale research results on the impact of dung-pats on soil nutrients to the field-, farm- and catchment-scale using spatial analysis and modeling techniques. Combined with data on the effects of grazing density/stocking density, grazing timing, grazing season duration and soil type, models could be developed to identify areas most at risk of P loss from soil to water.

- Estimate the impact of grazing animals in terms of direct deposition of dung and urine into streams, rivers and lakes, and the subsequent effects on water quality.

- Expand the dung P characterisation and dung-pat affected soil studies and link these results to P forms, fractions (bioavailability) and quantities that may be lost to water. It is hypothesised that the amount of fertiliser P applied to the soil will influence the amount and forms of P moving through the soil-plant-animal system, specifically in the dung deposited by the grazing animal. Conduct management-sensitive experimental techniques such as rainfall simulation studies in order to achieve the desired information.

- Obtain more information on the implications for decreased P in animal diets, the effect on P in animal excreta and P recycling, and the overall implications on P loss to water.

- Little information is available on P species, especially organic P, lost from grassland soils to water. Use techniques such as ^{31}P nuclear magnetic resonance spectroscopy to obtain detailed information on organic P species being lost to water in different management scenarios, which could assist in the identification of effective measures to counteract the risk to water quality from diffuse agriculture sources of P. An assessment of the bioavailability of these organic P forms (e.g. using phosphatase enzymes) will be very important.

8 References

Aarons, S.R., Hosseini, H.M., Dorling, L. and Gourley, C.J.P. (2004a). Dung decomposition in temperate dairy pastures. II. Contribution to plant-available soil phosphorus. Australian Journal of Soil Research, 42: 115–23.

Aarons, S.R., O'Connor, C.R. and Gourley, C.J.P. (2004b). Dung decomposition in temperate dairy pastures. I. Changes in soil chemical properties. Australian Journal of Soil Research, 42: 107–14.

Anderson, G. and Russell, J.D. (1969). Identification of inorganic pyrophosphate in alkali extracts of soil. Journal of Science of Food and Agriculture, 20: 78–81.

Barrow, N.J. (1987). Return of nutrients by animals. In: Snaydon, R.W. (ed.). Managed Grasslands – Analytical Studies. Ecosystems of the World 17B. Elsevier.

Barrow, N.J. and Lambourne, L.J. (1962). Partition of excreted nitrogen, sulfur and phosphorus between faeces and urine of sheep being fed pasture. Australian Journal of Agricultural Research, 13: 461–71.

Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C. (1999). Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry, 31: 1021–30.

Brookes, P.C., Powlson, D.S. and Jenkinson, D.S. (1984). Phosphorus in the soil microbial biomass. Soil Biology and Biochemistry, 16: 169–75.

Cade-Menun, B.J. (2005) Using phosphorus-31 nuclear magnetic resonance spectroscopy to characterise organic phosphorus in environmental samples. In: Turner, B.L., Frossard, E. and Baldwin, D.S. (eds), Organic phosphorus in the environment, 21–44. CABI, Wallingford, UK.

Cade-Menun, B.J. and Preston, C.M. (1996). A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. Soil Science, 161: 770–85.

Castle, M.E. and MacDaid, E. (1972). The decomposition of cattle dung and its effect on pasture. Journal of the British Grassland Society, 27: 133–7.

Chapman, H.D. (1965). Cation-exchange capacity. In: Black, C.A. et al., (eds) Methods of Soil Analysis: chemical and microbiological properties. Agronomy No. 9. Part 2, CABI, Wallingford, UK, pp. 140–57.

Chen, C.R., Condron, L.M., Davis, M.R. and Sherlock, R. R. (2003). Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. Forest Ecology and Management, 177: 539–57.

Condron, L.M. (2002). Phosphorus – surplus and deficiency. In: Schjonning, P., Christensen, B.T., and Elmholt, S. (eds). Managing Soil Quality – challenges for modern agriculture. CABI, Wallingford, UK.

Condron, L.M., Goh, K.M. and Newman, R. H. (1985). Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. Journal of Soil Science, 36: 199–207.

Condron, L.M., Frossard, E., Tiessen, H., Newman, R.H. and Stewart, J.W.B. (1990). Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. Journal of Soil Science, 41: 41–50.

Condron, L.M. and Goh, K.M. (1990). Nature and availability of residual phosphorus in long-term fertilised pasture soils in New Zealand. Journal of Agricultural Science, Cambridge, 114: 1–9.

Culleton, N., Murphy, J. and Murphy, W.E. (1999). The evaluation of environmental, agronomic and economic implications of high and low input dairy farms. Teagasc, Johnstown Castle, Wexford, Ireland.

Culleton, N., Liebhardt, W.C., Murphy, W.E., Cullen, J. and Cuddihy, A. (2000). Thirty years of phosphorus fertiliser on Irish pastures: animal–soil–water relationships. Teagasc, Johnstown Castle, Wexford, Ireland.

Culleton, E.B. and Diamond, J. (1972). Soils and land use. Teagasc, Johnstown Castle, Wexford, Ireland.

Culleton, E.B., Byrne, E. and Coulter, B. (1996). Soil sampling. O.T. Carton, M. Ryan and W.L. Magette (eds) Phophorous Recommendations for Grassland. Teagasc, Wexford, 65–75.

Dalal, R.C. (1977). Soil organic phosphorus. Advances in Agronomy, 29: 83–117.

Daly, K., Jeffrey, D.W., and Tunney, H. (2001). The effect of soil type on phosphorus sorption capacity and desorption dynamics in Irish grassland soils. Soil Use and Management, 17: 12–20.

Daly, K., Mills, P., Coulter, B., and McGarrigle, M. (2002). Modeling phosphorus concentrations in Irish rivers using land use, soil type, and soil phosphorus data. Journal Environmental Quality, 31: 590–9.

Dickinson, C.H. and Craig, G. (1990). Effects of water on the decomposition and release of nutrients from cow pats. New Phytologist, 115: 139–47.

Doody, D., Moles, R., Tunney, H., Kurz, I., Bourke, D., Daly, K. and O'Regan, B. (2006) Impact of flow path length and flow rate on phosphorus loss in simulated overland flow from a humic gleysol grassland soil. Science of the Total Environment, 372, 247–55.

Dormaar, J.F. (1972). Seasonal pattern of soil organic phosphorus. Canadian Journal of Soil Science 52: 107–12.

Drewry, J.J., Lowe, J.A.H. and Paton, R.J. (1999). Effect of sheep stocking intensity on soil physical properties and dry matter production on a Pallic Soil in Southland. New Zealand Journal of Agricultural Research, 42: 493–9.

Ebina, J., Tsutsui, T. and Shirai, T. (1983). Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. Water Research, 17: 1721–6.

FAO–UNESCO. (1974). Soil map of the world. UNESCO, Paris 1974.

Frossard, E., Condron, L.M., Oberson, A., Sinajm, S. and Fardeau, J. C. (2000). Processes governing phosphorus availability in temperate soils. Journal of Environmental Quality, 29: 15–23.

Hansen, J.C., Cade-Menun, B.J. and Strawn, D.G. (2004). Phosphorus speciation in manure-amended alkaline soils. Journal of Environmental Quality, 33: 1521–7.

Harrison, A.F. (1982). Labile organic phosphorus mineralisation in relationship to soil properties. Soil Biology and Biochemistry, 14: 343–51.

Harrison, A.F. (1987). Soil organic phosphorus. CABI, Wallingford, UK.

Haygarth, P.M. and Jarvis, S.C. (1997). Soil derived phosphorus in surface runoff from grazed grassland lysimeters. Water Research, 31: 140–8.

Haynes, R.J. and Williams, P.H. (1993). Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy, 49: 119–99.

Heathwaite, A.L. and Dils, R.M. (2000). Characterising phosphorus loss in surface and subsurface hydrological pathways. The Science of the Total Environment, 251/252: 523–38.

Holter, P. (1979). Effect of dung-beetles (Aphodius spp.) and earthworms on the disappearance of cattle dung. Oikos, 32: 393–402.

Hughes, K.J., Magette, W.L., and Kurz, I. (2005). Identifying critical source areas for phosphorus loss in Ireland using field and catchment scale ranking schemes. Journal of Hydrology, 304: 430–45.

Jeffrey, D.W. (1970). A note on the use of ignition loss as a means for the approximate estimation of soil bulk density. Journal of Ecology, 58: 297–9.

Jordan, P., Menary, W., Daly, K., Kiely, G., Morgan, G., Byrne, P., Moles, R. (2005). Patterns and processes of phosphorus transfer from Irish grassland soils to rivers – integration of laboratory and catchment studies. Journal of Hydrology, 304: 20–34.

Keane, T. and Collins, J.F. (eds) (2004). Climate, Weather and Irish Agriculture. 2nd edn, AGMET. ColourBooks Ltd, Dublin.

Koopmans, G.F., Chardon, W.J., Dolfing, J., Oenema, O., van der Meer, P. and van Riemsdijk, W.H. (2003). Wet chemical and phosphorus-31 nuclear magnetic resonance analysis of phosphorus speciation in a sandy soil receiving long-term fertiliser or animal manure applications. Journal of Environmental Quality, 32: 287–95.

Korte. C.J. and Harris, W. (1987). Effects of grazing and cutting. In: Snaydon, R. (ed.) Ecosystems of the world (17B): Managed grasslands and analytical studies. Elsevier Science Publishers, BV, Amsterdam, The Netherlands.

Kramer, S. and Green, D.M. (2000). Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland. Soil Biology and Biochemistry, 32: 179–88.

Kurz, I. (2002). Phosphorus exports from agricultural grassland in overland flow and subsurface drainage water. PhD thesis, Department of Geology, Trinity College, Dublin.

Kurz, I., Tunney, H., and Coxon, C.E. (2005a). The impact of agricultural management practices on nutrient losses to water: data on the effects of soil drainage characteristics. Water Science & Technology, 51: 73–81.

Kurz, I., Coxon, C., Tunney, H. and Ryan, D. (2005b). Effects of grassland management practices and environmental conditions on nutrient concentrations in overland flow. Journal of Hydrology, 304: 35–50.

Kurz, I., and O'Reilly, C. (2006). Small plot study on the impact of grazing animals on nutrient losses to water. Environmental Protection Agency (EPA), Wexford.

Lovell, R.D. and Jarvis, S.C. (1996). Effect of cattle dung on soil microbial biomass C and N in a permanent pasture soil. Soil Biology and Biochemistry, 28: 291–9.

MacDiarmid, B.N. and Watkin, B.R. (1972). The cattle dung patch. 2. Effect of a dung patch on the chemical status of the soil, and ammonia nitrogen losses from the patch. Journal of the British Grassland Society, 27: 43–54.

Magid, J. and Nielsen, N.E. (1992). Seasonal variation in organic and inorganic phosphorus fractions of temperate-climate sandy soils. Plant and Soil, 144: 155–65.

Magid, J., Tiessen, H., and Condron, L.M. (1996). Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. In: Piccolo, A. (ed.) Humic Substances in Terrestrial Ecosystems. Elsevier Science, Oxford, 429–66.

Marsh, R. and Campling, R.C. (1970). Fouling of pastures by dung. Herbage Abstracts, 40: 123–30.

McDowell, R.W. and Koopmans, G.F. (2005). Assessing the bioavailability of dissolved organic phosphorus in pasture and cultivated soils treated with different rates of nitrogen fertiliser. Soil Biology and Biochemistry, 38: 61–70.

McDowell, R.W., and Stewart, I. (2005). Phosphorus in fresh and dry dung of grazing dairy cattle, deer, and sheep: sequential fraction and phosphorus-31 nuclear magnetic resonance analyses. Journal of Environmental Quality, 34: 598–607.

McGill, W.B., Cannon, K.R., Robertson, J.A., and Cook, F.D. (1986). Dynamics of soil microbial biomass and water soluble organic C in Breton L after 50 years of cropping to two rotations. Canadian Journal of Soil Science, 66: 1–19.

Murphy, J. and Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27: 31–6.

Nguyen, M.L. and Goh, K.M. (1994). Sulphur cycling and its implications on sulphur fertiliser requirements of grazed grassland ecosystems. Agriculture, Ecosystems and the Environment, 49: 173–206.

O'Reilly, C.D. (2006). Influence of soil hydrology on phosphorus transport from soil. Unpublished PhD Thesis, National University of Ireland.

Peech, M. and English, L. (1944). Rapid microchemical soil tests. Soil Science, 57: 167–94.

Perrott, K. W., Sarathchandra, S. U. and Waller, J. E. (1990). Seasonal storage and release of phosphorus and potassium by organic matter and the microbial biomass in a high-producing pastoral soil. Australian Journal of Soil Research, 28: 593–608.

Pierzynski, G. M., Sims, J. T. and Vance, G. F. (2000). Soils and Environmental Quality, 2nd edn. CRC Press.

Pierzynski, G.M. (2000). Methods of phosphorus analysis for soils, sediments, residuals, and waters. Southern Cooperative Series, Bulletin 396.

Ritter, W.F. and Shirmohammadi, A. (eds). (2001). Agricultural Nonpoint Source Pollution: watershed management and hydrology. Lewis Publishers, Washington, DC, USA.

Roberts, A.M., Hudson, J.A. and Roberts, G. (1989). A comparison of nutrient losses following grassland improvement using two different techniques in an upland area of mid-Wales. Soil use and Management, 5: 174–9.

Ryden, J.C., Syers, J.K. and Harris, R.F. (1973). Phosphorus in runoff and streams. Advances in Agronomy, 25: 1–45.

Saunders, W.H.M. and Metson, A.J. (1971). Seasonal variation of phosphorus in soil and pasture. New Zealand Journal of Agricultural Research, 14: 307–28.

Schulte, R.P.O., Diamond, J., Finkele, K., Holden, N.M. and Brereton, A.J. (2005) Predicting the soil moisture conditions of Irish grasslands. Irish Journal of Agricultural and Food Research, 44: 95–110.

Shand, C.A., Macklon, A.E.S., Edwards, A.C. and Smith, S. (1994). Inorganic and organic P in soil solution from three upland soils. I. Effect of soil solution extraction conditions, soil type and season. Plant and Soil, 159: 255–64.

Sharpley, A.N. (1985). Phosphorus cycling in unfertilised and fertilised agricultural soils. Soil Science Society of America Journal, 49: 905–11.

Sparling, G.P., Hart, P.B.S., August, J.A. and Leslie, D.M. (1994). A comparison of soil and microbial carbon, nitrogen, and phosphorus contents, and macro-aggregate stability of a soil under native forest and after clearance for pastures and plantation forest. Biology and Fertility of Soils, 17: 91–100.

Stevenson, F.J. and Cole, M.A. (1999). Cycles of soil: carbon, nitrogen, phosphorus, sulphur, micronutrients, 2nd edn. John Wiley & Sons, Inc., New York.

Stewart, J. W. B. and Tiessen, H. (1987). Dynamics of soil organic phosphorus. Biogeochemistry, 4: 41–60.

Styles, D., Donohue, I., Coxon, C., Irvine, K., (2005). Linking soil phosphorus to water quality in the Mask catchment of western Ireland through the analysis of moist soil samples. Agriculture, Ecosystems and Environment, 112: 300–12.

Tate, K.R., Speir, T.W., Ross, D.J., Parfitt, R.L., Whale, K.N. and Cowling, J.C. (1991). Temporal variations in some plant and soil P pools in two pasture soils of widely different P fertility status. Plant and Soil, 132: 219–32.

Toor, G.S., Condron, L.M., Di, H.J., Cameron, K.C. and Cade-Menun, B.J. (2003). Characterisation of organic phosphorus in leachate from a grassland soil. Soil Biology and Biochemistry, 35: 1317–23.

Toor, G.S., Condron, L.M., Cade-Menun, B.J., Hi, H.J. and Cameron, K.C. (2005). Preferential phosphorus leaching from an irrigated grassland soil. European Journal of Soil Science, 56: 155–67.

Tunney, H. (2002). Phosphorus needs of grassland soils and loss to water. In: Steenvorden, J., Claessen, F. and Willems, J. (eds). Agricultural Effects on Ground and Surface Waters: research at the edge of science and society. International Association of Hydrological Sciences, 273: 63–9.

Turner, B.L. (2006). Organic phosphorus in Madagascan rice soils. Geoderma, 136: 279–88.

Turner, B.L., Bristow, A.W., and Haygarth, P.M. (2001). Rapid extraction of microbial biomass in grassland soils by ultra-violet absorbance. Soil Biology & Biochemistry, 33: 913–19.

Turner, B.J., Paphazy, M.J., Haygarth, P.M. and McKelvie, I.D. (2002). Inositol phosphates in the environment. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 357: 449–69.

Turner, B.J., Mahieu, N. and Condron, L.M. (2003a). Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. Soil Science Society of America Journal, 67: 497–510.

Turner, B.J., Mahieu, N. and Condron, L.M. (2003b). The phosphorus composition of temperate pasture soils determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy. Organic Geochemistry, 34: 1199–210.

Underhay, V.H.S. and Dickinson, C.H. (1978). Water, mineral and energy fluctuations in decomposing cattle dung pats. Journal of the British Grassland Society, 33: 189–96.

Walker, T.W. and Adams, A.F.R. (1958). Studies on soil organic matter. 1. Influence of phosphorus content of parent material on accumulation of carbon, nitrogen, sulphur and organic phosphorus in grassland soils. Soil Science, 85: 307–18.

Whitehead, D.C. (2000). Nutrient elements in grassland: Soil–plant–animal relationships. CABI, Wallingford, UK.

Whitton, B.A., Grainger, S.L.J., Hawley, G.R.W. and Simon, J.W. (1991). Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microbial Ecology, 21: 85–98.

Wright, A.F. and Bailey, J.S. (2001). Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a LECO CN-2000 dry combustion analyser. Communications in Soil Science and Plant Analysis, 32: 3243–58.

Yokoyama, K., Kai, H., Koga, T. and Aibe, T. (1991). Nitrogen mineralisation and microbial populations in cow dung, dung balls and underlying soil affected by paracoprid dung beetles. Soil Biology and Biochemistry, 23: 649–53.

Acronyms

^{31}P NMR Spectroscopy	phosphorus 31 nuclear magnetic resonance spectroscopy
C	carbon
CAN	calcium ammonium nitrate
CEC	cation-exchange-capacity
C:Po	carbon to organic phosphorus ratio
D_2O	deuterium
DRP	dissolved reactive phosphorus
DM	dry matter
DUP	dissolved unreactive phosphorus
EPA	Environmental Protection Agency (Ireland)
H_2O_2	hydrogen peroxide
H_2SO_4	sulphuric acid
K	potassium
LSD	least square difference
M	molar
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
MBP	microbial biomass phosphorus
Mg	magnesium
Ml	millilitre
N	nitrogen
NaOH-EDTA	sodium hydroxide-ethylenediaminetetra-acetic acid
NMR	nuclear magnetic ressonance
OC	organic carbon
OM	organic matter

P	phosphorus
pH	negative logarithm of hydrogen ion activity
Pi	inorganic phosphorus
Pm	Morgan's phosphorus
Po	organic phosphorus
%Po	percentage organic phosphorus
PP	particulate phosphorus
PRP	particulate reactive P
Pt	total phosphorus
PUP	particulate unreactive phosphorus
Pw	water-extractable phosphorus
r	correlation coefficient
SMC	soil moisture content
STP	soil test phosphorus (Morgan's P)
TDP	total dissolved phosphorus
TP	total phosphorus (water extractable)
TPP	total particulate P
TRP	total reactive P
TUP	total unreactive phosphorus
UP	unreactive phosphorus
UV	ultra-violet