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The effect of slurry processing on phosporus bioavailability and mobility

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THE EFFECT OF SLURRY PROCESSING ON PHOSPHORUS BIOAVAILABILITY AND MOBILITY

A thesis submitted to Bangor University by

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Dedication

A dedication to my friends and family who always support me

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Abstract

Livestock slurry is a valuable source of phosphorus (P) fertiliser for crops, but can also result in eutrophication following mobilisation and delivery to watercourses. The transfer of slurry-derived P to watercourses is likely to be strongly influenced by its particle size distribution and the chemical form of the P within the slurry. Advanced slurry processing (e.g. acidification, anaerobic digestion) is also likely to alter the forms and distribution of P within slurry particle size fractions. In response to this, this thesis explores the effects of slurry processing on P speciation within a range of slurry particle size fractions and on potential P bioavailability and movement through soil. The P characteristics of cattle slurry, acidified slurry, anaerobically digested slurry, in terms of P speciation of different particle size fractions, was studied in Chapter 3-5. The results showed that in the whole untreated cattle slurry, labile inorganic P (IP-H_2O + IP-$NaHCO_3$) and labile organic P (OP-H_2O + OP-$NaHCO_3$) dominated the total P (TP) of the whole slurry (45% and 24% of TP respectively). Acidification increased the labile inorganic P proportion to 55% of TP and decreased the stable P (IP-HCl + OP-HCl + TP-Residue) proportion from 21% to 15% of the TP of whole slurry. Anaerobic digestion increased the labile inorganic P and moderately labile P (IP-NaOH + OP-NaOH) proportions of whole slurry to 53% and 12%, but decreased the labile organic P proportion to 12% of TP. After physical separation, the proportion of TP in the liquid fractions (<2000, <500, <63 µm) represented by labile inorganic P were greater than that of the solid fractions (>2000, >500, >63 µm), while solid fractions tended to show greater proportions of moderately labile and stable P than liquid fractions. After acidification, the P speciation of the solid fractions showed little change. However, the labile inorganic P and the moderately labile P proportions increased in TP in the acidified liquid fractions, while stable P proportion in the liquid fractions decreased. After anaerobic digestion, in the TP of both solid and liquid fractions, the labile inorganic P and moderately labile P proportions increased, while the labile organic P proportion was reduced. The colloidal fraction (0.45-63 µm) accounted for 62% of the TP of whole untreated cattle slurry. Acidification reduced this proportion, while anaerobic digestion did not change it. In contrast, anaerobic digestion increased the labile inorganic P content of the colloids. P availability in soil after slurry application was explored in an incubation experiment monitored by sequential fractionation method (Chapter 7), and in a pot experiment involving P uptake by ryegrass (Chapter 8). The vertical mobility of P in soil was studied in laboratory microcosms (Chapter 6) and a pot experiment (Chapter 8). The results showed that untreated and treated cattle slurry increased the labile P content of soil and the P lability decreased gradually over time. Ryegrass P uptake was also increased by addition of different slurries. However, the differences between untreated and treated slurry amended treatments was not necessarily significant for labile P content in the soil and P uptake by ryegrass. In Chapter 6, the results implied that colloid-associated P in cattle slurry caused greater potential P leaching and that anaerobic digestion even increased this mode of P loss. Although in the pot experiment, slurry amended treatments showed no difference in cumulative P leaching compared to the control, the colloidal fraction in untreated and treated slurries was still a dominant fraction associated with a large portion of P. Further work is necessary to demonstrate the effect of slurry processing on P bioavailability and mobility at the plot- and field-scale, and on recovering colloidal P prior to land spreading.

Abbreviations

AD	Anaerobic digested slurry
ADL	Anaerobically digested liquid slurry
ADS	Anaerobically digested solid slurry
AD+	Inoculatedly anaerobically digested slurry
AL	Acidified liquid slurry
AS	Acidified solid slurry
C	Carbon
CL	Untreated liquid cattle slurry
CS	Untreated solid cattle slurry
Ca	Calcium
CH_4	Methane
CO_2	Carbon dioxide
DM	Dry matter
EC	Electrical conductivity
ha^{-1}	Per hectares(s)
HCl	Hydrochloric acid
H_2O	Water
H_2SO_4	Sulphuric acid
IP	Inorganic P
K	Potassium
KCl	Potassium chloride
kg	Kilogram(s)
L	Litre(s)
M	Molar(s)
mg	Milligram(s)
Mg	Magnesium
mL	Millilitre(s)
mM	Millimolar(s)
N	Nitrogen
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4^+	Ammonium

NON	Non-digested slurry without inoculum
NON+	Non-digested slurry with inoculum
OP	Organic Phosphorus
P	Phosphorus
PO_4^{3-}	Phosphate
VS	Volatile solids
μ	Micro
μL	Microliter(s)
μg	Microgram(s)
μm	Micrometer(s)
$\mu S\ cm^{-1}$	MicroSiemens per centimetre

Table of Contents

List of figures

List of tables

1 Introduction

Phosphorus (P) is an essential nutrient for crop production. The world phosphate rock reserves, the source of P fertiliser production, is estimated to become depleted in the the next <100 up to 400 years (Cordell et al., 2009; Van Vuuren et al., 2010; Desmidt et al., 2015; Withers et al. 2015). Livestock slurry represents a great substitute for inorganic fertiliser and is routinely used as an organic amendment to soil. This helps to recycle nutrients and enhance soil organic matter within a range of crop production systems (Waldrip-Dail et al., 2009; Liu et al., 2016). However, the risk of nutrient loss (e.g. P, N and K) from land can increase following livestock slurry application and can result in severe environmental pollution (Sharpley et al., 1999; King et al., 2015).

The characteristics of P in the livestock slurry can subsequently influence P availability and P mobility in soil after application (Bol et al., 2016; Mackay et al., 2017). Furthermore, manure processing strategies, such as mechanical separation, acidification and anaerobic digestion, may have an impact on the characteristics of livestock slurry, thereafter influencing P availability and mobility in soil after application. Therefore, this thesis aims to (1) determine the effects of different slurry processing strategies on the physicochemical properties of cattle slurry, and (2) explore how slurry processing affects P availability, P speciation and P loss in soil after application. The overall hypotheses are that a) small particle size fractions of cattle slurry dominate the total P content, and have great mobility in soil, and b) slurry acidification and anaerobic digestion modify the P speciation and distribution in cattle slurry, and thereafter affect P availability and mobility in soil.

The thesis comprises 9 chapters with Chapters 3-8 describing laboratory and pot experiments. Chapter 2 is a literature review describing the recent understanding of the effects of slurry processing strategies on livestock slurry properties, especially P forms, and subsequent implications for P cycling and losses from soil. From this literature review we identified weaknesses and/or gaps in knowledge of the effects of slurry processing on P cycling, that were addressed in subsequent experimental Chapters. Chapters 3-5 focussed on the characterization of differently processed cattle slurries, whilst Chapters 6-8 focussed on slurry-P availability and mobility in soil.

In Chapters 3 and 4, acidified and anaerobically digested slurry were sieved into >2000, 500-2000, 63-500 and <63 μm particle size fractions, and P speciation was analysed using the modified Hedley fractionation procedure. In Chapter 5, further detailed analysis was conducted on the smaller slurry size particle fractions. This specifically focused on particles <63 µm, of differently processed slurries, as these are believed to be more bioavailable and at greater risk of being vertically transported through the soil profile. These particles were operationally separated into 0.45-63 µm (colloids), 0.03-0.45 µm (nanoparticles), 0.001-0.03 µm (nanoparticles), and <0.001 µm fractions. A modified Hedley fractionation was conducted on the colloidal fraction, and reactive (inorganic) and unreactive (organic) P was differentiated within the fractions <0.45 µm. Based on the findings in Chapter 5, a small-scale experiment was performed to explore the effect of slurry processing on potential P leaching of colloidal and nanoparticulate slurry fractions (Chapter 6). Chapter 7 determined changes in P forms in soil following the applications of separated solid and liquid fractions of untreated, acidified and anaerobically digested cattle slurries (based on Chapters 3 & 4 results) in an 18 week-incubation experiment with a grassland soil. In Chapter 8, a pot experiment was used to evaluate P uptake by ryegrass following the application of differently processed cattle slurry fractions. Potential P leaching was also assessed in this experiment. The links between these chapters are shown below in Figure 1.1.

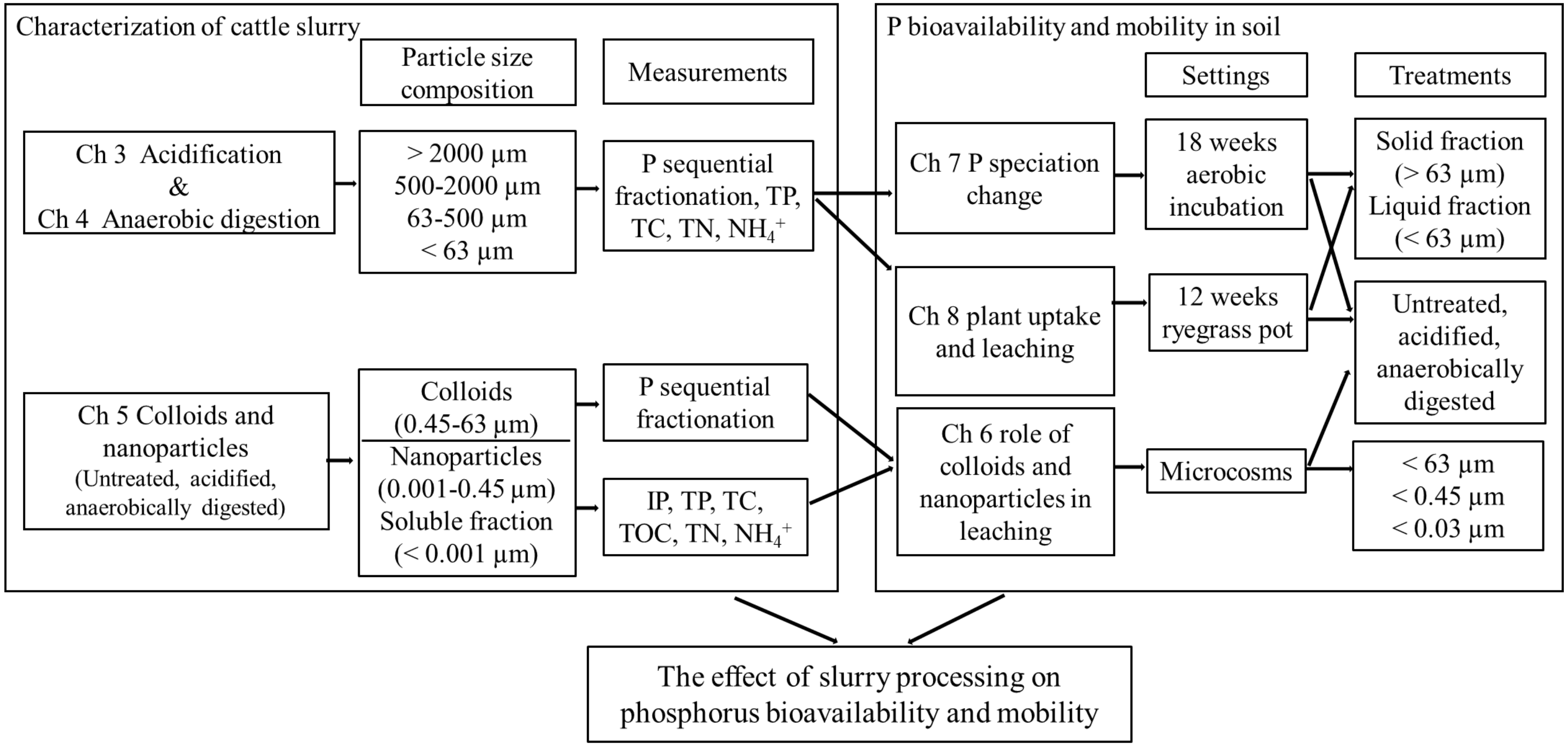

Figure 1.1 Summary of the thesis outline

The main content and hypotheses for each chapter are described in the following section:

Chapter 3 describes the effects of slurry acidification on N and P forms and their distribution in cattle slurry. The hypotheses were (1) in cattle slurry, the major proportion of dry matter and carbon would be distributed in the coarse particle size fractions, whilst the majority of the N and P would be in the small particle size fractions; (2) acidification would result in even greater N and P distributing in the small particle size fractions; and (3) acidification would increase the potential bioavailability of N and P in separated slurry size fractions.

Chapter 4 describes the effect of anaerobic digestion on N and P forms and their distribution in cattle slurry. The hypotheses were that (1) the dry matter and C distribution would be altered by anaerobic digestion, and the proportion of coarse particle fraction would be smaller in the anaerobically digested slurry; (2) bioavailability of N and P in livestock slurry, especially in small particle size fractions, would be increased by anaerobic digestion; and (3) anaerobic digestion would reduce the mechanical separation efficiency of the dry matter, C, N, and P contents.

Chapter 5 determines the P and N distribution in colloids and nanoparticles of untreated, acidified and anaerobically digested cattle slurry. The hypotheses were: (1) colloids and nanoparticles contain a significant proportion of the N and P content of the livestock slurry; and (2) acidification and anaerobic digestion both increase the potential bioavailability of N and P in the separated liquid slurry fraction (<63 µm), which is primarily associated with the colloidal fraction.

Chapter 6 explores colloidal P fraction transport (vertical movement) after the application of processed cattle slurry to soil. The hypothesis was that acidification and anaerobic digestion would reduce P mobility and potential P leaching by conversion of colloidal P to orthophosphate which readily sorbs to mineral surfaces, reducing its vertical movement in soil.

Chapter 7 evaluates P fractionation changes in soils applied with solid and liquid fractions of processed cattle slurry. The hypotheses were: (1) addition of livestock slurry, non-processed and processed, would immediately increase the labile P content of the soil; (2) the potential bioavailability of P forms would reduce over time; (3) both acidified and anaerobically digested slurry would result in a greater labile P content of soil than untreated slurry; and (4) organic P in the liquid fractions would mineralise to a greater degree, and at a faster rate than organic P in solid fractions.

Chapter 8 determines the effect of slurry processing on P availability for plant uptake and potential leaching. The hypotheses were: (1) livestock slurry, untreated and treated, could improve plant growth and P uptake, and increase potential P leaching; (2) acidified and anaerobically digested slurry would result in more P uptake and potential P leaching from soil than untreated slurry; and (3) the liquid fraction of separated slurry treatments would result in greater P uptake and potential P leaching in soil than from the solid fraction.

This thesis provides new insights into the mechanisms of P cycling from differently processed livestock slurry to soil. It does this by providing a better understanding of P speciation associated with particle distribution after slurry acidification, anaerobic digestion and slurry separation.

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2 Literature review: effect of processing strategies on phosphorus cycle from livestock slurry to soil

2.1 Abstract

Phosphorus (P) is a critical but finite element for our food production systems. To improve the use efficiency and reduce loss are the challenges in P management. Livestock slurry is an important nutrient source for crop production. This chapter reviews the P characteristics of livestock slurry, and its subsequent behaviour in the soil after application. Specifically, the chapter explores the impact of slurry processing strategies, i.e. separation, acidification and anaerobic digestion, on P solubility and bioavailability in livestock slurry and soil. P is present in livestock slurry in either inorganic or organic P. The solubility and availability of added P in soil is controlled by desorption/adsorption, dissolution/precipitation and mineralization/immobilization processes. Added soluble P tends to be adsorbed to clay and mineral oxides in soil. The sorption affinity of inorganic P to soil is generally greater than organic P, except for phytic acid. Colloids from slurry and soil can facilitate P transport in soil. Solid fractions produced from mechanical separation generally contain higher amounts of dry matter and have a higher C/P ratio, and lower quantities of nutrients and microbial activity than separated liquid fractions. The liquid fraction could result in greater organic P mineralisation than the solid fraction after application. Acidification lowers the pH and increases P solubility in livestock slurry. The acidified slurry could provide more available P to crop initially after application, but the pH may increase P sorption in soil. Degradation of organic matter during anaerobic digestion increases inorganic P concentration, but precipitation occurs during this process, which reduces the P solubility. In addition, decomposition during anaerobic digestion may impact on the mobility of colloid-associated P. The P characteristics in livestock slurry would partly influence P transformation and movement in soil after application. The particle sizes of differently treated livestock slurry, with which P associated, may determine the contact with microorganisms and clay and minerals in soil, affecting the P sorption/desorption, immobilisation/mineralisation, dissolve/precipitation. Moreover, colloid-associated P is likely to transport further. However, information on P in slurry associated with different sizes of particles and how they may affect P in soil is limited. Therefore, this thesis tried to address such questions.

2.2 Introduction

Annually >200 million tons of phosphate rock are mined globally (U.S. Geological Survey, 2017), most of which are used in agricultural applications, especially fertiliser production (Cisse & Mrabet, 2004; Desmidt et al., 2015). The world phosphate rock reserves are approximately 68 billion tons estimated in 2016 (U.S. Geological Survey, 2017). And it has been predicted to be depleted in the next <100 up to 400 years (Cordell et al., 2009; Van Vuuren et al., 2010; Desmidt et al., 2015; Withers et al. 2015). To avoid or slow down the depletion, efforts should be made on closing the global phosphorus (P) cycle by improving use efficiency, lowering P demand, and minimising P losses and generation of waste P that can no longer be re-used (Withers et al. 2015). P is an unsubstitutable and essential element for crop production in agriculture. Livestock slurry is a good resource for P, recycling waste from livestock industry. Moreover, P loss from farmland to surface water and groundwater is of serious concern (Schindler et al., 2008; McDowell et al., 2015). Livestock slurry use efficiency and control of P losses should be considered priorities in crop production.

However, P bioavailability and potential P loss from slurry amended soil are related not only to total P input from slurry, but also associated with slurry P species and their interactions with the soil matrix (He et al., 2004; Toth et al., 2006). There are some processing strategies for different purposes, which may alter the P characteristics in livestock slurry. It is therefore important to understand the original P characteristics in differently processed slurries and its implication on the interaction between slurry-derived P and soils over time.

Therefore, this chapter reviewed the P characteristics in livestock slurry and P dynamics in slurry amended soils (in terms of the change of P species and P loss from soil), and the effect of processing strategies of separation, acidification and anaerobic digestion.

2.3 P in livestock slurry

Livestock slurries contain considerable P. For example, cattle slurry contains 0.5-0.7g P kg^{-1}, and swine slurry contains 0.6-1.3 g P kg^{-1} on fresh weight basis (Møller et al., 2002; Fangueiro et al., 2012). P in manure generally includes two forms of inorganic and organic P. Studies have shown that inorganic P constitutes more than 50% of TP in swine and cattle manure (Dou et al., 2000; Sharpley & Moyer, 2000; Ajiboye et al., 2004; Pagliari & Laboski, 2012). Struvite ($MgNH_4PO_4 \cdot 6H_2O$) and dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) are the main phases controling P solubility in swine and cattle manure based on analysis of X-ray absorption near-edge structure (XANES) (Ajiboye et al., 2007; He & Zhang, 2014). Güngör et al. (2007) found 57% and 43% of P of 25-53μm size particle fraction of dairy manure was associated with dicalcium phosphate anhydrous ($CaHPO_4$) and struvite respectively. Such P solubility is controlled by pH. Most Ca-P minerals could be dissolved at pH 5.5-6.7 (Fordham & Schwertmann, 1977).

Monoesters phosphate and phytate have been reported to be the predominant forms of organic P in swine and cattle manure by ^{31}P NMR and enzyme hydrolysis methods (Leinweber et al., 1997; He & Honeycut, 2001; Turner & Leytem, 2004; McDowell & Stewart, 2005; He et al., 2009; Pagliari & Laboski, 2012). Turner & Leytem (2004) and Pagliari & Laboski (2012) combined sequential fractionation with ^{31}P NMR and enzyme hydrolysis, respectively, to speciate organic P in swine and cattle manure. Turner & Leytem (2004) found monoester-P made up the majority of organic P in the H_2O, $NaHCO_3$, and NaOH extracts, while phytate was the major form of organic P in the HCl and NaOH extracts in swine and cattle manure (Turner & Leytem, 2004). In the study of Pagliari & Laboski (2012), hydrolysable organic P accounted for about half of the total organic P in swine and cattle manure. And they claimed phytate was the dominant hydrolyzable organic P in the H_2O extract, representing over 30% of organic P in swine and cattle manure, while in the $NaHCO_3$ extract, monoester-P was the main organic P form. They also presented a difference between swine and cattle manure in phytate proportion in the NaOH and HCl extracts. Phytate accounted for a larger proportion of organic P in the NaOH and HCl extracts than monoester in swine manure, but represented a less or equivalent proportion to monoester in cattle manure, which is due to their different digesting systems (Taylor, 1965).

2.4 P dynamics in soil

2.4.1 P transformation derived by livestock slurry

Biological, biochemical and physicochemical processes regulate the P dynamics in soils (Bünemann, 2015). Inorganic orthophosphate (HPO_4^{2-}, H_2PO^{4-}) is the primary source of P for plants (and microorganisms) (Kruse et al., 2015). Livestock slurry contains a considerable amount of such available P, and can supply to soils after application. However, the solubility and availability of added P after slurry application gradually decrease over time, which is mainly controlled by physicochemical processes. The inorganic P can adsorb onto surfaces of clay and oxides minerals, and be taken up by plants and microbes when enters the soil matrix (Sato et al, 2005). At high soil pH, the precipitation to Ca-P dominates, while binding to Al and Fe oxides prevails at low soil pH (Kruse et al., 2015). In a calcareous soil, long-term manure applications result in the transformation of relatively soluble Ca-P, such as monetite and brushite, into more crystalline Ca-P, such as $^{\beta}$-tricalcium P (TCP) and octacalcium P (OCP) (Sato et al., 2005; Audette et al., 2016). In acid soil, bind with Fe and Al oxides is the main limiting factor for P solubility (Kruse et al., 2015). The sorption increases with decreased pH (Perassi & Borgnino, 2014). Factors that can influence solubility of Al- and Fe-oxides, such as redox, can also affect P solubility (Kruse et al., 2015). Some organic acids from livestock slurry inhibit crystallization of Fe and Al oxides in soils. Abdala et al. (2015) observed a sharp increase in amorphous Fe and Al amounts (55% and 80% increase) due to the consecutive application of manures, which may otherwise enhance P sorption through the creation of highly-reactive amorphous oxide minerals. Organic matter in manure could impact sorption properties of soils (He et al., 2004). Low molecular organic anions represent important competitors with P for sorption sites and help to improve P solubility and mobility in soil (Grossl and Inskeep, 1991; Violante and Pigna, 2002; Sato et al., 2005; Kruse et al., 2015). Organic P moieties may also be adsorbed and precipitate in a similar mechanism as inorganic P (He et al., 2006). For example, in soil with pH greater than 6.5, the formation of Ca-organic P complexes, such as Ca-phytates may occur (Audette et al., 2016).

The biological P dynamic process is controlled primarily by bacterial immobilization, and mineralization, (Bolan, 1991; Walbridge, 1991; Mackay et al., 2017). Organic P release is negatively correlated with the C/P ratio and positively correlated with initial P content of the organic amendments. Organic matter with a total P content of >2 g kg^{-1} (on a dry weight basis) generally results in positive net P mineralization (Floate, 1970; Nziguheba et al., 1998). Different chemical forms of the organic P from organic amendments may be different in mineralisation. Diester-P tends to be mineralized more rapidly than monoester-P (Turner et al., 2002; Condron et al., 2005). Phytic acid, a monoester, has a higher charge density than other monoesters, allowing it to form relatively stable complexes in soil that are protected from microbial degradation (Greaves & Webley, 1965; Celi et al., 1999).

Furthermore, livestock slurry also provides microorganisms, exophosphatases to soil (Dick & Tabatabai, 1984; Crouse et al., 2002). Microbial and exoenzyme activities derived by slurry may contribute to P dynamics. And the inorganic P from slurry could influence biological processes in soil by stimulating microbial growth (Martin, 1970).

2.4.2 P losses from livestock slurry amended soil

P losses from agricultural land is an important P source of eutrophication (Haygarth et al., 1998; Heathwaite and Dils, 2000; Withers & Lord, 2002). It has been estimated that about half of the annual P load to UK waters is from agriculture land (Defra, 2004). Manure addition influences the P runoff and leaching from the agricultural field. P losses sourced from manure is reported to be greater than from inorganic fertilizer (Frossard et al., 1989; Simard et al., 1995; McDowell et al., 2005). Vadas et al (2015) estimated that 30% of P runoff from pasture was derived from manure. In addition, it is also suggested that manure represents the main source of leachable P from soil (Gerritse, 1981; Toor, 2003).

Addition of livestock slurry negatively affects P sorption while positively affects P availability in soil as well as inorganic P fertilisers (Siddique & Robinson, 2003). When the P sorption capacity of the Al- and Fe-oxides is saturated, P leaching losses reach critical levels, and tend to increase (Kruse et al., 2015). Extraction of slurry can provide estimation of P which may saturating P sorption sites in soil. Water extractable P of manure has been reported to be correlated to potential P runoff and leaching from manure freshly amended soils (Sharpley and Moyer, 2000; Elliott et al., 2002; Miller et al., 2006; Kleinman et al., 2007; Roberts & Israel, 2017), although the relationship between water extractable P in manure and P runoff was poor in Volf et al. (2007). Typically, organic P leaching dominates P leaching, due to the weaker sorption affinity of organic P to binding sites than inorganic P (Anderson & Magdoff, 2005; Bol et al., 2016), with exceptions such as phytic acid (Lessa & Anderson, 1996; Leytem et al., 2002).

Colloids transport in soil is an important pathway for colloid-associated P loss, primarily through macropores during high flow events (Haygarth et al., 1997; de Jonge et al., 2004; Schelde et al., 2006; Withers et al., 2009; Glæsner et al., 2013; Regelink et al., 2013). In P-unsaturated soil, colloid-facilitated P loss is dominant in P loss from soil (Turner et al., 2003a; Heathwaite et al., 2005), although Rick et al. (2011) evidenced that nanoparticles of 10-200 nm in soil only contained a trace amount of P, and they did not necessarily contribute to P transport. Preedy et al. (2001) also suggested the potential of slurry particles to act as carriers for P transfer in soil. Makris et al. (2006) isolated soil colloids from manured soil and applied to soil column, and detected 30% of the added colloids in the effluent and observed particulate P vertical movement. However, we lack information on slurry particles and colloids, and P they associated with, and colloids in soil originated from slurry at present.

2.5 Effect of processing strategies on P in livestock slurry and soil

The characteristics of livestock slurry, such as P species, P-particle association, particle composition, pH, C, microorganisms, and etc., could impact the P biological, biochemical and physico-chemical process in soil after application. Processing strategies, e.g. separation, acidification, and anaerobic digestion, on livestock slurry may alter these characteristics, thereafter affect P performance in soil.

Separation

Solid-liquid separation is developed to reduce the volume of slurry requiring storage, and to save the cost of transporting water. The separated liquid fraction is intended for on-farm use, whilst the solid fraction can be exported to fields further more distant from the farmstead, and even off-farm (Møller et al., 2000; Sørensen et al., 2003). Separation may also benefit a reduction in odour emission (Zhang & Westerman, 1997).

Particle size composition of the slurry is an important factor for separation performance. For example, filtration will only retain particles above a certain size, and particles between 1 nm and 1 µm (i.e. colloids) are subject to Brownian motion and hardly settle (Hjorth et al., 2010). Slurry separation technologies vary in their features for separation of different particle sizes. Typically, solid fractions produced from mechanical separation technologies contain high amounts of dry matter and low amounts of nutrients due to the specific separation of larger particles (Møller et al., 2000; Møller et al., 2002). Peters et al. (2011) stated that particles >25µm represent the majority of C in solid fractions, while particles <25µm accounted for larger proportions of N and P. Meyer et al. (2007) also observed that >80% of N and P of cattle slurry was in the <125µm fraction. Some studies show that >70% of the undissolved N and P in cattle slurry is in particles between 0.45 and 250µm (Masse et al., 2005; Meyer et al., 2007). Some studies suggest separated liquid manure has greater microbial activity than solid manure (Ghonsikar & Miller, 1973; Magid et al., 1996; Turner et al., 2003b; Hansen et al. 2004).

Decomposition of manure in soils may be related to their particle size distribution. Smaller particles have a larger surface area to volume ratio, thereafter are more susceptible to microbial degradation (Magid et al., 2010). Further, smaller particles can be more mobile, transporting from the soil surface to the upper soil or even deeper (McGechan, 2002).

Acidification

Acidification is introduced to address the ammonia volatilisation problem from livestock production, which maintains manure N fertiliser value (Ndegwa et al., 2008; Aguerre et al., 2012). Acidification has been shown to reduce NH_3 emissions from pig houses (70%), storage (10%) and following application (67%) (Kai et al., 2008). As well as mitigation of ammonia emission, acidification can also reduce methane emissions by 87% from slurry stores (Petersen et al., 2012; Bastami et al., 2016).

Moreover, acidification has also been reported to improve the dissolved P content of slurry (Güngör et al., 2007; Fangueiro et al., 2015). Struvite and brushite are the main phases controling P solubility in swine and cattle manure. Most such P minerals and other occluded P can be dissolved at pH 5.5-6.7 (Fordham and Schwertmann, 1977; Güngör et al., 2007; He & Zhang, 2014; Fangueiro et al., 2015). Some papers also suggest that acidification can induce slurry particle aggregation and accelerated hydrolysis (Hjorth et al., 2015). Roboredo et al. (2012) observed that the total phosphorus (TP) content distributed in the solid fraction of separated (by centrifugation) slurry was reduced by 50% after acidification.

Addition of acidified slurry to soil causes greater labile P content of soil compared with non-acidified slurry (Roboredo et al., 2012; Petersen et al., 2012). The increase in labile P and other nutrients in soil after application of acidified slurry is found to improve crop production (Kai et al., 2008; Petersen et al., 2012; Birkmose & Vestergaard, 2013). Acidity could also alter microbial structure and function and P sorption in soil after application. It is reported that acidified slurry decreases soil respiration, nitrification and microbial biomass C (Fangueiro et al., 2013). Roboredo et al. (2012) claimed acidification may inhibit P immobilisation, but accelerate mineralisation in soil after application. However, there does not appear to be any studies on the effect of acidified manure on P loss in soil after application.

Anaerobic digestion

Anaerobic digestion is a green energy production technology of importance, producing biogas for heat and power generation, and reducing odour and pathogens in livestock slurry (Smet et al., 1999; Sahlström, 2003; Clemens et al., 2006). Anaerobic digestion is conservative in terms of slurry nutrients, unless N is lost via ammonia volatilisation from digestate storage, and hence can be applied as a source of nutrients for crop/grass production (Walsh et al., 2012).

Anaerobic digestion reduces the dry matter and C concentration of animal slurry, but maintain P concentration (Marcato et al., 2008). Organic P mineralisation is negatively correlated with the C/P ratio and positively correlated with initial P content of the organic materials (Floate, 1970; Mafongoya et al., 2000). Thus, the anaerobically digested slurry may result in greater mineralization in soil after application than untreated slurry. In addition, the particle size distribution changes during anaerobic digestion (Shon & Vigeswaran, 2006; Marcato et al., 2008; Hjorth et al., 2010). In anaerobic digesters, warm and stirred condition facilitates microbial degradation of large particles, leaving relatively small particles with slowly degradable material (Hjorth et al., 2010). In a cold unstirred slurry, the slow transformation of particles primarily degrades small particles, transforming only a minor fraction of the large particulate material (Hjorth et al., 2010). Bacterial flocs and filaments may form during anaerobic digestion (Marcato et al., 2008). Masse et al. (2005) observed that particles <10μm accounted for 64% of dry matter content in raw slurry, while it increases to 84% of dry matter content in anaerobically digested slurry. Researchers reported particles <1 or <1.6μm were most susceptible to biodegradation during anaerobic digestion (Elmitwalli et al., 2001; Møller et al., 2002; Shon & Vigeswaran, 2006). And Shon & Vigeswaran (2006) observed that particles >10 μm appeared more recalcitrant during anaerobic digestion. Marcato et al. (2008) found the 3-25μm particles contributed greatest (30%) to the specific surface area of the whole slurry than other particle size fractions, and their area increased (40%) after digestion.

During anaerobic digestion, degradation processes transforms organic P into inorganic P (Masse et al., 2005). Inorganic P especially dissolved orthophosphate is bioavailable (Rausch & Bucher, 2002). But anaerobic digestion tends to reduce P solubility, due to the formation of struvite and Ca-P (Suzuki et al., 2007; Marcato et al. 2008; Güngör & Karthikeyan, 2008; Rico et al. 2012). In the 25-53μm particle fraction of the anaerobically digested slurry, 78% of P presents as struvite and 22% of P is associated with hydroxylapatite (HAp) (Güngör et al., 2007). Stutter (2015) claimed that although precipitation reactions limit inorganic P solubility, no crystalline P minerals were observed in digestates.

After addition of digestates to soil, the bulk density is reported to decrease and saturated hydraulic conductivity and moisture retention capacity of soil increase (Garg et al., 2005). Microbial basal respiration and metabolic quotient in soil are higher following digestate amendment, indicating greater microbial activity, than with untreated slurry amendment (Bougnom et al., 2012; Hupfauf et al., 2016). Hupfauf et al. (2016) argues that P availability is reduced in digestate by the formation of recalcitrant P minerals during the fermentation process, triggering microbes to increase their metabolic activity, compared to undigested slurry, to obtain sufficient amounts of P for development. But in the study of Juárez et al. (2013), the basal respiration showed no difference between untreated and anaerobically digested slurry treatments. Sapp et al. (2015) indicates the bacterial diversity may be slightly greater in soils amended with digestate than inorganic nutrients. Bachmann et al. (2016) showed lower soil enzymes activities in digestates amended treatments than untreated slurry amended treatments.

Some studies report that digestates are found to positively influence plant growth and P uptake (Bachmann et al., 2011; Abubaker et al., 2012; Hupfauf et al., 2016). However, compared to untreated slurry, most available results from field experiments have indicated no additional effect of digestates on plant P uptake and P availability in soil (Loria & Sawyer, 2005; Möller & Stinner, 2010; Bachmann et al., 2011; Bachmann et al., 2014). Moreover, Stutter (2015) observed that digestates weakened P sorption in soil columns compared to control soil, suggesting short-term leaching risks for soil applied digestates. This may result from the competition of organic molecules in digestates. For P runoff, García-Albacete et al. (2016) found digestates resulted in lower P runoff loss than undigested slurry.

2.6 Conclusions

Livestock slurry is an important nutrient source for crops, but also represent a source of P loss, which is thought to be greater than from inorganic P fertilisers. Slurry provides inorganic and organic P forms for crop production. The availability and solubility of added P in soil is controlled by desorption/adsorption, dissolution/precipitation and mineralization/immobilization processes. Soluble P added from slurry tends to be adsorbed to clay and mineral oxides in soil. The sorption affinity of organic P to soil is generally lower than inorganic P, except phytic acid. Organic matter added from slurry could compete for sorption site with P and weaken P sorption. However, organic acid increases amorphous Fe and Al release, and may enhance P sorption. Colloid associated P has a great mobility and is an important source of P loss.

Processing strategies, such as separation, acidification and anaerobic digestion, may alter P species, particle size composition and other properties of livestock slurry, with impacts on soil processes after application. For example, solid fractions produced by mechanical separation technologies generally contain high amounts of dry matter and C/P ratios, and lower quantities of nutrients and microbial activity than liquid fractions. This may result in more labile P input from liquid fraction and greater organic P decomposition in soil after liquid fraction amendments. Acidification increases P solubility and availability in livestock slurry. Anaerobic digestion has a greater impact on particle composition of slurry than acidification, which may influence the mobility of colloid associated P. Degradation of organic P increases inorganic P content, but precipitation occurs during this process, which may affect the P solubility.

At present, the information on particle size composition, especially colloids in livestock slurry and P characteristics associated with these particles is limited. There are some researches revealing the effect of anaerobic digestion on slurry particles, but without detailed description or discussion on possible P transformation in soil after application related to these particle changes. Such information on acidified slurry is even less. To better understand the effect of addition of differently treated livestock slurry on P dynamics in soil and improve livestock slurry recommendation, further exploration on particle-associated P in livestock slurry and its transformation and movement in soil is needed. Therefore, this thesis tried to elucidate the effect of differently treated livestock slurry on P availability and mobility in soil with respect to P speciation of different particle sizes of slurry.

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3 The effect of acidification on N and P forms and their distribution in cattle slurry

3.1 Abstract

Acidification is known to reduce ammonia losses from livestock slurry management. However, this mitigation strategy may modify the forms and relative distribution of phosphorus (P) and nitrogen (N) in the acidified slurry with implications for nutrient availability for crops and the risk of losses to the environment. In this study, untreated and acidified (pH 5.5) slurry was separated into different particle size fractions and analysed for N and P content and forms. Sequential fractionation was conducted on each particle size fraction to determine the effects of slurry acidification on P speciation. In untreated cattle slurry, the <63 µm particle size fraction represented 35% of the dry matter content, and *ca.* 70% of the total N (TN) and total P (TP) content of the whole slurry. The NH_4^+-N and water extractable P contents of cattle slurry doubled after acidification and this change was mainly associated with the <63 µm fraction. The TP-H_2O, TP-$NaHCO_3$, TP-NaOH, TP-HCl and TP-residue forms accounted for 31%, 38, 10%, 10% and 11% of the TP in the untreated cattle slurry. Further, >55% of the TP-H_2O, TP-$NaHCO_3$ and TP-HCl forms was present as inorganic P (IP). Acidification increased the labile P (IP-H_2O+IP-$NaHCO_3$) content, dissolved the precipitated P (IP-HCl) and degraded the OP-$NaHCO_3$ form. After separation, different to the untreated slurry, the liquid fractions (<2000, <500 and <63 µm) separated from the acidified slurry had a higher proportion of labile P than the solid fractions (>2000, >500 and >63 µm). This study showed that acidification increases the bioavailability of N and P in livestock slurry.

3.2 Introduction

Ammonia (NH_3) emissions from livestock production represent a severe challenge for manure management, resulting in a significant reduction in the manure N fertiliser value (Ndegwa et al., 2008; Aguerre et al., 2012), as well as a significant environmental risk to natural and semi-natural ecosystems (Fangmeier et al., 1994). Acidification has been shown to reduce NH_3 emissions from animal houses, slurry stores and following application to land (Vandre & Clemens, 1996; Clemens et al., 2002; Kai et al., 2008). Acidification can also reduce methane emissions from slurry stores (Petersen et al., 2012).

To improve the management of livestock slurry, mechanical separation is commonly utilised, so that the solid fraction can be composted, and transported to, and spread on fields further from the farm steading, whilst liquid fractions may be pumped more easily and perhaps used for fertigation (Roboredo et al., 2012). The separated solid and liquid fractions have different characteristics and may display different N and P dynamics in the soil after application. Generally, in untreated cattle and pig slurry, although the dry matter and carbon content can be concentrated in the solid fraction after separation, a large proportion of the nutrients (N, P) tend to be present in the liquid fraction (Meyer et al., 2007; Hjorth et al., 2010). Peters et al. (2011) reported that the majority of C in the separated solid fraction of cattle and pig slurry was present in particles >25 µm, whereas the total N and P were present in larger proportions in particles <25 µm. Meyer et al. (2007) observed that 80% of the total N and P in cattle slurry occurs in particle size classes <125 µm. When applying the separated liquid slurry fraction to soil, it supplies more available N and P nutrients to plants (Hjorth et al., 2010; Sommer et al., 2015). Smaller slurry particle size fractions have larger surface area-to-volume ratios and may be more susceptible to bacterial colonisation and reaction, leading to greater hydrolysis (Levine et al., 1991; Masse et al., 2005). Additionally, smaller particles have greater potential to be transported through soil pores than larger particles (McGechan & Lewis, 2002).

However, acidification may alter the physical and chemical characteristics of livestock slurry, and influence the separation performance, and thereafter the N and P dynamics in soil. Acidification has been reported to markedly increase NH_4^+-N and dissolved P content in slurry (Güngör et al., 2007; Fangueiro et al., 2013; Sommer et al., 2015; Fangueiro et al., 2015). As a result, less N and P is recovered in the solid fraction of slurry (Roboredo et al., 2012; Sommer et al., 2015). Changes in slurry characteristics during acidification also include increased electrical conductivity, a greater content of free metals (Hjorth et al., 2015) and solubilisation of inorganic precipitates (Fangueiro et al., 2009; Hjorth et al., 2015). It has been also reported that acidification can induce slurry particle aggregation and accelerate hydrolysis (Hjorth, 2015). Soil application of acidified slurry is reported to decrease soil respiration, nitrification, and microbial biomass values, and increase slurry P bioavailability (Roboredo et al., 2012; Fangueiro et al.,2013).

The simple description of P forms as dissolved P, TP, and organic P does not present the more complex transformations of P and the range of P forms that exist in livestock slurry. Sequential fractionation offers an approach to quantify the forms of P that can be progressively extracted by increasingly strong extractants (i.e. in water, bicarbonate, hydroxide and acid), providing information on readily available P forms through to 'recalcitrant' (unavailable) residual P (Cross & Schlesinger, 1995). Sequential fractionation although does not directly identify P species, it does give indirect information about the P solubility (He & Zhang, 2014). Inorganic P (IP) extracted by these reagents in livestock manure is interpreted sequentially as: water-soluble; physically adsorbed on crystalline surfaces; associated with surface of Ca carbonates, Al and Fe oxides; and mineral or amorphous phases of Ca-P (He et al., 2006). Organic P forms can also be defined using the same approach (He et al., 2006). Acidification could improve the availability of P in manure by dissolving the dominant precipitates of struvite and Ca-P at low pH (Güngöret al., 2007).

Therefore, this study aimed to determine the N and P forms associated with different particle size fractions in cattle slurry, and the effect of acidification on these characteristics. Particle sizes were operationally defined by different mesh sizes, and we used the Hedley fractionation method (Dou et al., 2000) to determine the P forms in each particle size fraction. We hypothesized that: (1) in cattle slurry, a large proportion of dry matter and carbon would distribute in the coarse particle size fractions, while the majority of N and P would be in the smaller particle size fractions; (2) acidification would exacerbate this imbalance in the N and P distribution; and (3) acidification would increase the availability of N and P in separated slurry size fractions.

3.3 Materials and Methods

3.3.1 Sample Collection

Three 10 L samples of cattle slurry was collected independently from a commercial beef farm in Wales, UK. These slurry samples were stored in a cold room (4°C) in closed vessels until acidification could take place. From these samples, three replicate batches of 1.5 L of slurry were acidified to pH 5.5 by adding *ca.* 35 ml 15% H_2SO_4 L^{-1} to the slurry. Three untreated 1.5 L slurry samples remained non-acidified. All slurry samples were stored in a 4°C cold room for 7 d until slurry separation and subsequent Hedley fractionation.

3.3.2 Particle fractionation

Approximately 800 ml of untreated and acidified slurry were wet-sieved sequentially and separated into fractions of >2000, 500-2000, 63-500, and <63 μm. The fresh total weight of each fraction was recorded. Subsamples of particle fractions were taken for determination of the dry matter (DM) content and volatile solid (VS) content by drying at 105ºC and 550ºC respectively, whilst the remaining sample was air-dried and stored for further analysis. 4 g of fresh >2000, 500-2000, 63-500 and <63 μm fractions were also extracted by shaking with 20 ml 0.5 M K_2SO_4 for 0.5 h. Extracts were centrifuged at 18,500 g for 10 min and the suspension was kept for the determination of the NH_4^+-N and PO_4^{3-}-P concentrations by colorimetric methods (Mulvaney, 1996; Murphy & Riley, 1962). In a preliminary test we demonstrated no statistical difference in the extractable PO_4^{3-}-P concentration between slurry fraction shaken with water (1:200) or 0.5 M K_2SO_4 (1:5 w/v) (data not shown). Thus, the term of 'water extractable P' was used in this study. TP was also determined by combusting dried samples at 550°C overnight and dissolving the ash in 20% (v/v) HCl. The DM, TP, TN, and all N and P form contents for whole slurry were calculated by summing the contents of all the particle size fractions. The total carbon (C) and TN content of the different particle size fractions was determined by TruSpec® CN analyzer (Leco Corp., St Joseph, MO). Since NH_4^+ is the dominant inorganic N form in livestock slurry (Fangueiro et al., 2014), we assumed that the organic N content of the different particle size fractions was the difference between TN and NH_4^+-N.

3.3.3 P sequential fractionation

P sequential fractionation (Dou et al., 2000) was applied to the whole slurry and the >2000, 500-2000, 63-500, and <63 μm fractions of untreated and acidified slurry. 0.3 g air-dried samples were sequentially extracted by 30 ml of distilled water, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl, in triplicate. During each extraction, the mixture of sample and extractant was shaken for 16 h and then centrifuged at 13,600 g for 10 min. Finally, the supernatant was filtered through Whatman 42 filter papers, and residues on the filters were rinsed with the next extractant into centrifuge tubes in preparation for the next extraction.

Extracted inorganic P (IP) in each fraction was detected by the colorimetric method (Murphy & Riley, 1962). TP was determined after persulfate digestion (Rowland & Haygarth, 1997). Finally, the undissolved residue was combusted in a muffle furnace at 550˚C and then dissolved in 20% HCl for TP analysis. The difference between TP and IP was defined as organic P (OP).

3.3.4 Calculation

The quantity of a N or P form in a particle size fraction is expressed on the basis of one kg of fresh whole slurry as shown in Eqn (1).

$$C_i = CF_i \times WF_i / 1000 \qquad (1)$$

In the above equation, CF_i = concentration of a N or P form of the particle fraction *i* on this fraction's dry basis (g kg^{-1}); WF_i = dry weight of the particle fraction *i* in 1 kg of fresh whole slurry (g); C_i = total amount of a N or P form of the particle fraction *i* in 1 kg of fresh whole slurry (g kg^{-1}).

The distribution of a N or P form in the whole slurry is expressed as the proportion of a N or P form in a particle size fraction represents in the total quantity of such N or P form of the whole slurry, Eqn (2).

$$P_i = C_i \times 100\% / \sum_{i=1}^{n} C_i \qquad (2)$$

In the above equation, P_i = the proportion of a N or P form of the particle fraction *i* in the total quantity of such N or P form of whole slurry (%).

The P form distribution in the whole slurry is defined as the proportion (%) of the TP of the whole slurry represented by the different P forms of the particle fractions, Eqn (3).

$$P_{ij} = C_{ij} \times 100\% / \sum_{j=1}^{m} \sum_{i=1}^{n} C_{ij} \qquad (3)$$

C_{ij}= the quantity of a P form *j* of the particle fraction *i* in 1 kg of dry whole slurry (g kg^{-1}); P_{ij} = the proportion of the P form *j* of the particle fraction *i* in the TP of whole slurry (%).

The P form distribution in separated solid or liquid fraction is defined as the proportion (%) of the TP of separated fraction represented by each P form, Eqn (4).

$$PF_{ij} = C_{ij} \times 100\% / \sum_{j=1}^{m} C_{ij} \qquad (4)$$

In the above equation, PF_{ij} = the proportion of the P form j in the particle fraction i (%).

All statistics were performed with SPSS 22. Comparison between untreated and acidified slurry was performed by t-test with effects considered significant at $p<0.05$.

3.4 Results

3.4.1 Particle fractionation

Particle size composition

There were differences in the particle composition between the untreated and acidified cattle slurry based on the distribution of DM among different particle size fractions. In untreated cattle slurry, the >2000 µm and <63 µm fractions contained most of the DM, composing 40% and 35% of the DM of whole slurry, respectively (Figure 3.1). The remaining fractions, 500-2000 and 63-500 µm, contained 14% and 12% of the total DM content, respectively (Figure 3.1). In the acidified slurry, the proportion of the >2000 µm fraction increased to 51%, while 500-2000 and 63-500 µm fractions decreased to 11% and 10% ($p<0.05$) (Figure 3.1). In addition, the <63 µm fraction accounted for a similar proportion in the acidified slurry and the untreated slurry (Figure 3.1).

For the VS, the comparison between untreated and acidified treatments was consistent with the DM. 43% and 35% of VS was allocated in the >2000 µm and <63 µm fractions of the untreated slurry, and 47% and 35% of VS were in these fractions in the acidified slurry (Figure 3.1). In contrast to DM and VS, the distribution of C was not affected by acidification (Figure 3.1).

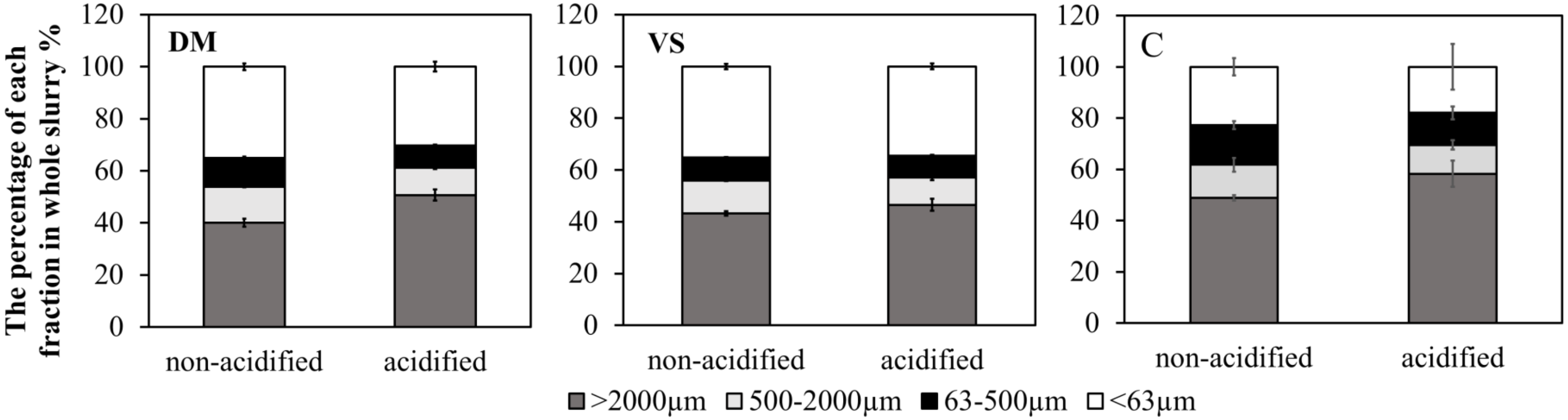

Figure 3.1 Distribution of DM, VS and C in different slurry particle size fractions. Values represent means ± SEM, n = 3.

N and P distribution in particle size fractions

In the untreated cattle slurry, the majority of the NH_4^+-N content (82%) was in particles <63 µm as expected, as it is present in a soluble form. A large proportion of the TN (69%) and organic N (69%) was also in the <63 µm fraction (Figure 3.2). This is supported by other studies (Meyer et al., 2007; Peters et al., 2011).

Acidification did not influence the TN and organic N content of the whole slurry (Table 3.1) and distribution in different particle size fractions (Figure 3.2). However, the NH_4^+-N content and distribution changed after acidification (Figure 3.2). The NH_4^+-N content of the whole slurry rose over 2 times from 0.17 g kg^{-1} to 0.42 g kg^{-1} after acidification. The increase in the NH_4^+-N content of the whole slurry after acidification was mainly associated with the >2000 and <63 µm fractions (Figure 3.2). In 1 kg of fresh whole slurry, the NH_4^+-N quantity of the >2000 µm fraction increased from 0.014 g kg^{-1} to 0.047 g kg^{-1}, and the quantity of the <63 µm fraction increased from 0.14 g kg^{-1} to 0.34 g kg^{-1} (Figure 3.2). The <63 µm fraction contained the largest proportion of NH_4^+-N (81%) in the whole slurry of acidified treatment (Figure 3.2).

Table 3.1 Characteristics of untreated and acidified cattle slurry (Data expressed on a fresh weight basis). Values represent means ± SEM, n = 3.

Index	Untreated cattle slurry	Acidified cattle slurry
DM g kg^{-1}	109.1(±9.0)	144.0(±15.9)*
VS g kg^{-1}	74.5(±3.4)	78.4(±1.4)
TP g kg^{-1}	0.60(±0.03)	0.75(±0.05)*
TN g kg^{-1}	3.8 (±0.6)	4.6(±0.3)
Organic N g kg^{-1}	3.6(±0.6)	4.2(±0.3)
C g kg^{-1}	21.0(±4.9)	36.7(±6.5)
NH_4^+-N g kg^{-1}	0.17(±0.01)	0.42(±0.1)*
Water extractable P g kg^{-1}	0.16(0.09)	0.34(0.01)*
EC mS cm^{-1}	6.4(±0.2)	7.0(±0.8)
pH	7.4(±0.1)	5.6(±0.0)*
ORP mv	-42.3(±3.2)	-64.0(±1.5)*

*indicates statistically significant difference between untreated and acidified treatments (p<0.05).

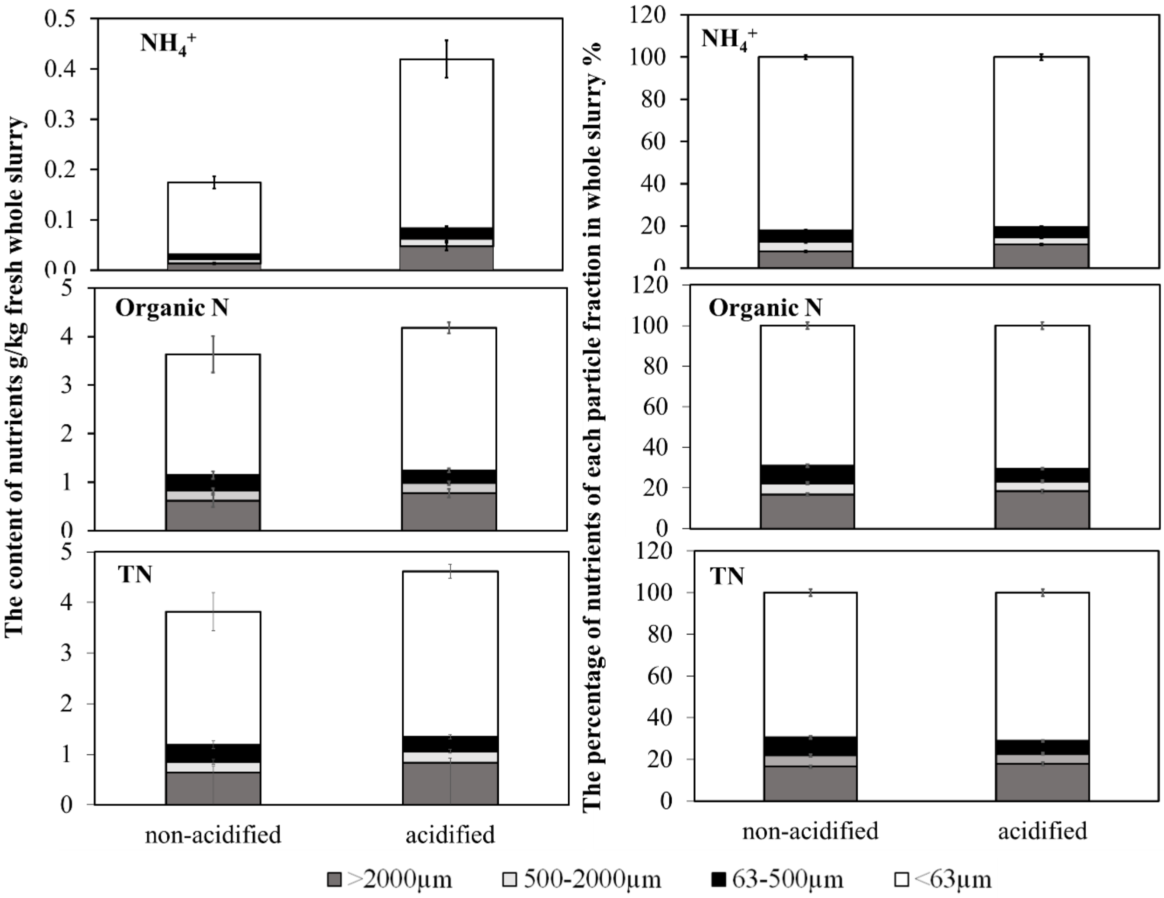

Figure 3.2 The contents and distribution of NH_4^+-N, organic N and total nitrogen (TN) in different slurry particle size fractions. Values represent means ± SEM, n = 3.

The water extractable P content of the whole slurry increased from 0.16 g kg^{-1} to 0.34 g kg^{-1} after acidification (Table 3.1). In addition, the water extractable P quantities of all fractions increased to different extents due to acidification. Further, the largest increase caused by acidification was seen in the water extractable P quantities of the >2000 and <63 µm fractions, almost doubling from 0.02 g kg^{-1} to 0.05 g kg^{-1} and from 0.14 g kg^{-1} to 0.28 g kg^{-1} respectively, resulting in the increase in the water extractable P content of the whole slurry (Figure 3.3). The distribution in the whole slurry experienced a slight change.
The >2000, 500-2000, 63-500 and <63 µm fractions accounted for 10%, 5%, 7% and 78% of the water extractable P content of whole slurry in the untreated treatment respectively, and represented 13%, 4%, 5% and 78% in the acidified slurry, respectively (Figure 3.3).

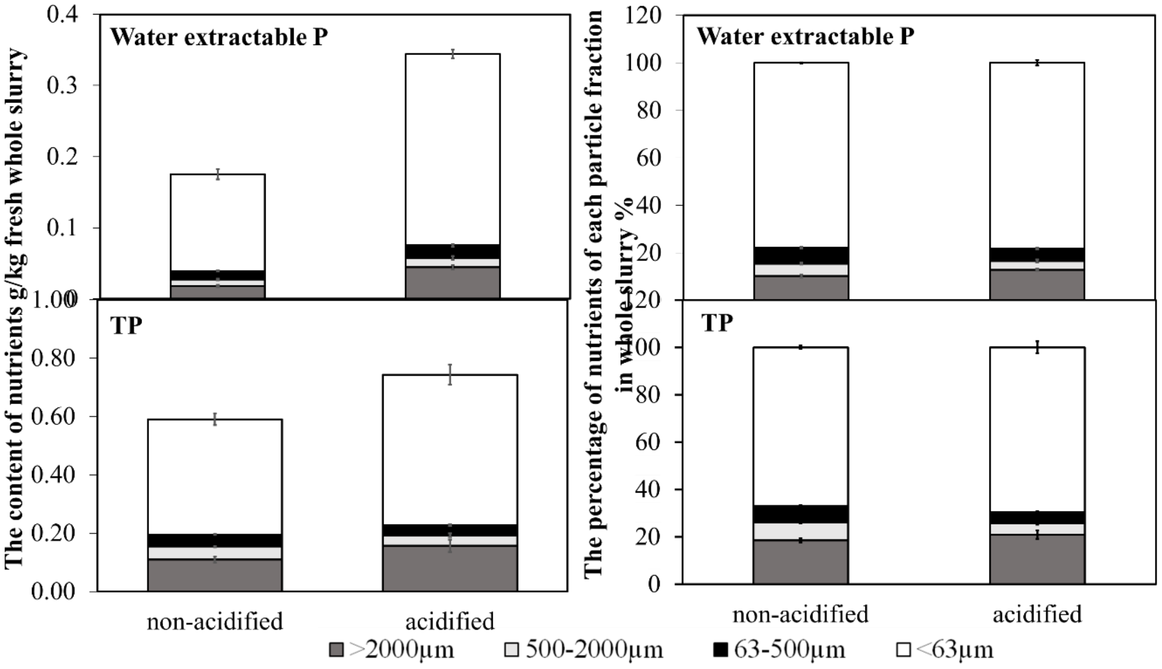

Figure 3.3 The water extractable P and total P (TP) in different slurry particle size fractions. Values represent means ± SEM, n = 3.

The change of the distribution for TP was consistent with the water extractable P, which included the increase in the >2000 µm fraction and a decrease in the 63-500 µm fraction. Specifically, the >2000, 500-2000, 63-500 and <63 µm fractions accounted for 19%, 7%, 7% and 67% of the TP in untreated slurry respectively, and 21%, 5%, 5% and 69% in acidified slurry respectively (Figure 3.3).

Acidification helped release P into water extractable forms and increased water extractable P/ TP ratios of whole slurry and most fractions. The water extractable P/TP ratio of whole slurry increased from 30% to 47% and this ratio also increased in each particle size fraction.

3.4.2 P form distribution in the whole slurry

The calculated TP contents, by adding up all P form fractions, of the untreated whole slurry were 0.57 g kg^{-1}, respectively. They seemed smaller than measured TP contents in Table 3.1, but there was no significant difference between the calculated and measured TP. The same was true for the acidified treatments. In untreated cattle slurry, the particle size fractions of >2000, 500-2000, 63-500 and <63 µm accounted for 20%, 7%, 8% and 65% of the TP of whole cattle slurry, respectively. This distribution was similar to the result in Figure 3.3 calculated from the measured TP contents of particle fractions. After acidification, the TP content of whole slurry of the acidified treatment was 0.65 g kg^{-1}, statistically the same as the untreated treatment. The distribution of TP between the different slurry particle size fractions was not affected much by acidification. In the acidified slurry 22%, 5%, 6% and 68% of the TP was in the fractions of >2000, 500-2000, 63-500 and <63 µm, respectively.

In the untreated cattle slurry, the TP-H_2O, TP-$NaHCO_3$, TP-NaOH, TP-HCl and TP-residue forms represented 31%, 38, 10%, 10% and 11% of the TP of the whole slurry. 79%, 55%, 27% and 62% of the TP-H_2O, TP-$NaHCO_3$, TP-NaOH and TP-HCl was inorganic P. In the composition of particle sizes, the <63 µm particles contributed the largest quantity of P in every form. The <63 µm fraction accounted for 14%, 17%, 2%, 4%, 5%, 11%, 4%, 3% and 6% of the TP of the whole slurry of the untreated treatment as IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl, OP-H_2O, OP-$NaHCO_3$, OP-NaOH, OP-HCl, and TP-residue, respectively. In addition, the second largest proportion was in the >2000 µm fraction (Figure 3.4).

Acidification transformed IP-$NaHCO_3$, IP-HCl, OP-$NaHCO_3$ and OP-HCl into IP-H_2O and OP-NaOH in the whole cattle slurry. *Ca.* 50% of the IP-$NaHCO_3$, IP-HCl and OP-HCl proportion and 25% of OP-$NaHCO_3$ proportion in the TP of whole slurry was reduced. The proportion of IP-H_2O and OP-NaOH increased by 85% and 24%, respectively. These transformations were more associated with smaller particle fractions. The >2000 µm fraction seldom contributed to the alteration of the whole slurry. The increase of IP-H_2O and OP-NaOH contents in the whole slurry was due to the increase in the amount of IP-H_2O and OP-NaOH of the <63 µm fraction (Figure 3.4), the quantities of which increased by 95% and 20%, respectively. The quantity of IP-$NaHCO_3$ decreased in the 500-2000, 63-500 and <63 µm fractions (Figure 3.4), resulting in *ca.* 50% reduction of the IP-$NaHCO_3$ content of the whole slurry. The reductions of the quantities of IP-HCl and OP-$NaHCO_3$ in the 63-500 fraction, were 48% and 52%, respectively, and their decrease in the <63 µm fraction was 68% and 29% after acidification, resulting in the decrease of the whole slurry. The quantity of OP-HCl in the 500-2000 µm fraction of the acidified slurry was 68% lower than in the untreated slurry. The quantities of IP-NaOH, OP-H_2O and TP-residue were relatively stable.

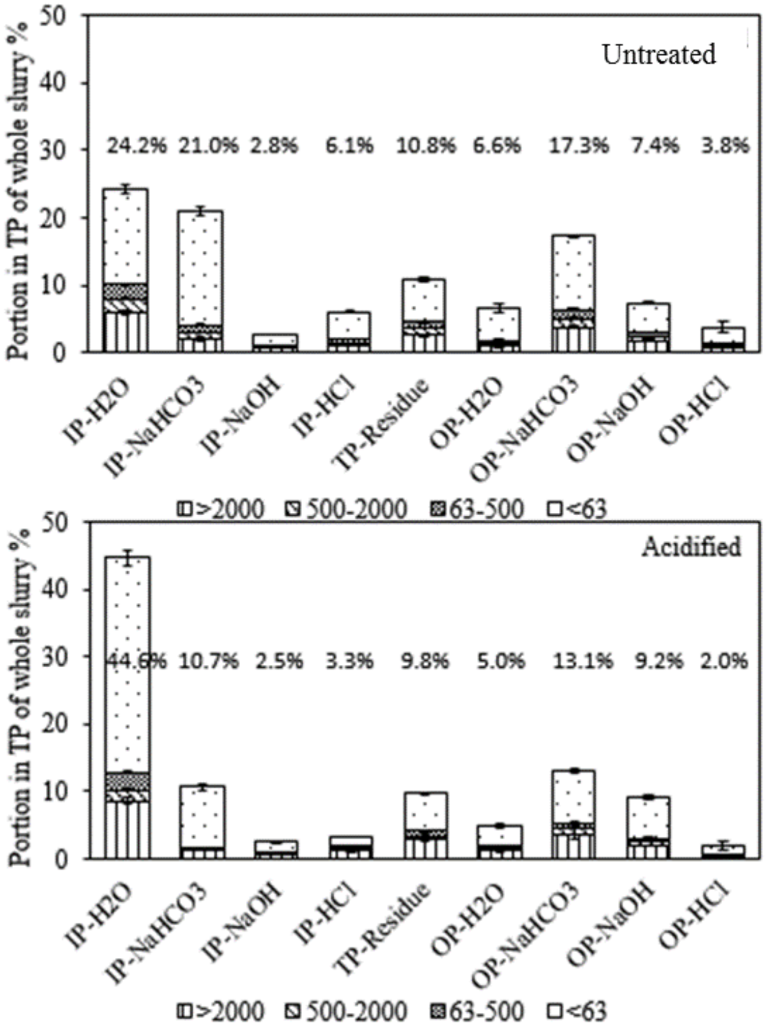

Figure 3.4 Distribution of P forms in untreated and acidified cattle slurry. Values represent means ± SEM, n = 3.

3.5 Discussion

3.5.1 DM, N and P in untreated and acidified slurry

The majority of the DM in the cattle slurry used in our study was present in the coarse fractions rather than in the small fractions <63 µm. However, this distribution is quite different to studies using pig slurry (Fernandes et al., 1988; Møller et al., 2002; Peters et al., 2011). More DM is in the small particle size fractions in pig slurry. For example, the <50 µm fraction represented between 53% and 70% of DM of pig slurry (Fernandes et al., 1988; Møller et al., 2002).

The acidified whole cattle slurry showed a greater DM content than untreated slurry (Table 3.1). This was not expected and was not seen in our preliminary experiment. This difference may be because in this experiment we acidified a greater volume of cattle slurry to ensure enough sample analysis. In addition, during acidification the foaming made the volume of cattle slurry expand in the container, so although we tried to mix the sample thoroughly, we may take more long straw residue and large particles out for particle fractionation, resulting in overestimation of the DM content of the whole acidified slurry and large particle fractions. For the same reason, the TP, VS, TN, and C contents of the acidified whole slurry also tended to be slightly greater than the untreated slurry, but only the difference in TP proved significant (Table 3.1). The observed increase of the proportion of the >2000 µm fraction in the DM of whole slurry after acidification may also be a result of this overestimation. However, Hjorth et al. (2015) observed an increase in the percentage of particles of 100-1000 µm in pig slurry, and a decrease in the percentage of particles <100 µm after acidification, and argued that this could be the result of particle aggregation and coagulation during acidification. Thus, this could also be a possible reason for the increase of the proportion of the >2000 µm fraction in our study of cattle slurry.

Hjorth et al. (2013) claimed acidification accelerated hydrolysis of organic matter, and they found reduced particles quantity <50 µm after acidification and observed more carbohydrate hydrolysis products in the acidified slurry. Although in our result, acidification tended to lower the DM quantity of the <63 µm fraction, this was not significant. This may because of the short processing period. In the study of Hjorth et al. (2013), the experiment was conducted for over 70 days with a daily addition of acid, while in our study the acidification period was only one week with a two-stage acid addition.

Mechanical separation is a common practice used in livestock farms (Hjorth et al., 2010). Generally, filtration retains particles above a certain size, e.g pore sizes of 700-3200 µm for pressurized filtration (Hjorth et al., 2010). In our study, we used three pore sizes of sieves, 2000, 500 and 63 µm for particle fractionation. Table 3.2 summarized the calculated separation efficiency for these three pore sizes, i.e. the ratios of >2000 µm /whole, >500 µm /whole and >63 µm /whole. The P form distribution in these solid fractions (>2000, >500, >63 µm) and liquid fractions (<2000, <500, <63 µm) separated from these three pore sizes was also calculated and shown in Table 3.3. Although solid fractions separated from these three pore sizes all hold a large proportion of DM, a large quantity of N and P tends to concentrate in the liquid fractions (Table 3.2), as stated in our hypothesis that in cattle slurry, large proportion of dry matter and carbon would distribute in the coarse particle size fractions, while majority of N and P would be in the small particle size fractions. Even to use the smallest pore size of 63 µm in our study, the separation efficiency of N and P was still lower than 35% (Table 3.2). Peters et al. (2011) also observed that the majority of TN and TP was in particles <25 µm. Further, Meyer et al. (2007) found over 80% TN and TP in cattle slurry was in particles <125 µm. In pig slurry, some reported that 85-88% of TN and 93-95% of TP in the fraction <500 µm (Møller et al., 2000), and 70% of TP was in the <10 µm fraction (Masse et al., 2005).

Table 3.2 Separation efficiency of DM, VS, C, NH_4^+-N, TN, water extractable P and TP by different filter pore sizes separating solid fractions (%). Values represent means, n = 3.

Measurements	Separation size µm	2000	500	63
DM	Untreated	40.0	53.9	65.0
	Acidified	50.7*	61.2	69.7
VS	Untreated	43.2	56.0	64.8
	Acidified	46.5	57.1	65.5
C	Untreated	48.8	61.8	77.3
	Acidified	58.3	69.6	82.1
NH_4^+-N	Untreated	7.9	12.5	17.9
	Acidified	11.2*	14.8	19.5
TN	Untreated	16.5	21.9	30.5
	Acidified	17.9	22.7	28.9
Water extractable P	Untreated	10.3	15.5	22.2
	Acidified	12.9	16.7	21.7
TP	Untreated	18.6	26.0	33.1
	Acidified	21.0	25.8	30.5

Note: * indicates the significant difference between untreated and acidified treatments ($p<0.05$).

Acidification increased NH_4^+-N and water extractable P contents of cattle slurry, especially in the <63 µm fraction, but the separation efficiency of NH_4^+-N and water extractable P, TN and TP was not changed significantly (Table 3.2). Therefore, our hypothesis that acidification would exacerbate this imbalance in the N and P distribution was not supported. But acidification did increase the quantities of the inorganic N and extractable P of both separated solid and liquid fractions, improving their N and P nutrient availability to crops. On the other hand, acidification could increase the risk of N and P leaching after application to soil. This is especially so for the liquid fractions separated from acidified slurry, where the available N and P is not only high because of the acidification, but N and P associated with the smaller particles is more reactive (because of the high surface area to volume ratio) and the smaller particle sizes can be transported through the soil (McGechan, 2002). A high water extractable P content of manure has been related to increased risk of P runoff (Kleinman et al., 2003; Roberts & Israel, 2017). Therefore, the potential P runoff was also increased by acidification.

3.5.2 P speciation and distribution

Our sequential fractionation presented the information on readily available P forms through to recalcitrant residual P for untreated and acidified different cattle slurry particle sizes (Cross & Schlesinger, 1995). The measurements of water extractable P (Figure 3.3) showed differences to the IP-H_2O from sequential fractionation. However, the two values for the whole slurry and most particle size fractions showed no significant difference, except for the <63 µm particle size fraction. The IP-H_2O content of the <63 µm fraction was less than water extractable P, resulting in a larger separation efficiency if using IP-H_2O. This may be because the sample for water extractable P was fresh, while for sequential fractionation it was air-dried. Also, the <63 µm fraction contained much higher water content before air dry than other fractions. During air drying, the water soluble P in the <63 µm fraction may have transformed to other P forms e.g. IP-$NaHCO_3$, while this effect on other fractions was minimal. Thus, when analysing the P result from sequential fractionation, we define both IP-H_2O and IP-$NaHCO_3$ as labile inorganic P. In untreated cattle slurry, the labile inorganic P represented a large proportion in both solid and liquid fractions separated from any of the three pore sizes (Table 3.3). In addition, the labile inorganic P proportion in the liquid fractions were significantly larger than in the solid fraction, except for separation with 2000 µm pore sizes. If applied to the crop field on a TP basis, the liquid slurry may show advantage in improving crop production than solid slurry due to the greater labile P input.

Acidification increased the IP-H_2O proportion and decreased the IP-$NaHCO_3$ and IP-HCl proportions in cattle slurry. The increase in the IP-H_2O quantity was mainly the result from dissolution and hydrolysis. The IP-$NaHCO_3$ pool represents the P that was weakly sorbed to Fe and Al oxides, and slightly soluble Ca-P precipitates (Cross & Schlesinger, 1995). IP-HCl mainly represents the compounds of struvite and Ca-P, which are the dominant precipitates in livestock manure, especially in poultry manure (Güngör et al., 2007). The low pH dissolved these compounds resulting in the increase in the IP-H_2O content. In addition, after separation, these alterations were reflected in the P speciation of both solid and liquid fractions (Table 3.3). The labile inorganic P proportion in the TP of the liquid fractions of slurry was significantly higher in acidified treatment than untreated treatment, which is supportive to our hypothesis that acidification would increase the availability of P in separated slurry size fractions. In the acidified treatment, this labile inorganic P proportion in the liquid fractions was larger than the solid fractions, while the proportion of IP-HCl and TP-residue in the liquid fractions was smaller than the solid fractions. Thus, the liquid fraction from acidified slurry would have better agronomic benefit than the solid fraction.

Table 3.3 The P form distribution in the separated solid and liquid fractions by different filter pore sizes (%). Values represent means, n = 3.

Separated fractions μm	Treatment	>2000	<2000	>500	<500	>63	<63
IP-H_2O	Untreated	34.6	25.3	34.4	26.6	33.7	26.8
	Acidified	45.4*	50.5*	44.1*	50.0*	45.3*	49.4*
IP-$NaHCO_3$	Untreated	12.0	26.4	12.4	24.3	13.8	24.0
	Acidified	6.2*	13.3*	6.1*	12.2*	6.1*	12.2*
IP-NaOH	Untreated	3.6	3.0	3.7	3.1	3.7	3.1
	Acidified	3.3	2.6	3.2	2.8	3.2	2.7
IP-HCl	Untreated	6.4	7.0	6.5	6.9	6.9	6.8
	Acidified	6.9	2.8*	7.2	3.4*	6.9	3.5*
OP-H_2O	Untreated	6.2	7.6	5.8	7.6	6.2	7.3
	Acidified	6.8	5.2	6.6	5.5	6.8	5.4
OP-$NaHCO_3$	Untreated	21.7	18.9	21.8	19.2	21.3	19.4
	Acidified	19.1	13.2*	20.3	13.9	19	14.6
OP-NaOH	Untreated	10.8	7.7	10.6	8.2	10.2	8.2
	Acidified	10.8	10.0*	10.9	10.1	11	10.1
OP-HCl	Untreated	4.8	4.2	4.9	4.3	4.3	4.4
	Acidified	1.4	2.4	1.6	2.2	1.8	2.1*
TP-residue	Untreated	14.9	11.5	15.2	11.8	15.0	12.0
	Acidified	14.8	9.8	14.6	10.7	14.5	10.6

Note: * indicates the significant difference between untreated and acidified treatments (p<0.05).

Hydrolysis is suggested to be accelerated under acidification (Hjorth et al., 2013), thus contributing to the increase in the quantity of IP. It was reported that *ca.* 50% of the OP-H_2O in cattle and pig slurry was hydrolysable (He & Honeycutt, 2001). The hydrolysable rate of other OP forms was also quite low (He & Honeycutt, 2001). However, in our study, there was no significant reduction in OP-H_2O. The OP-$NaHCO_3$ and OP-HCl forms were the two organic pools that decreased significantly after acidification. A small proportion of this decrease may contribute to the increase of OP-NaOH, but the larger proportion might be associated with mineralisation. These transformations in organic P were also shown in the proportions of TP of the separated solid and liquid fractions, although this was not always significant (Table 3.3). The difference in terms of the OP-$NaHCO_3$ proportion between the liquid and solid fractions tended to increase after acidification (Table 3.3). Hayes et al. (2000) and He & Honeycutt (2001) suggest that *ca.* 90% of OP-$NaHCO_3$ in livestock slurry is not enzymatically hydrolysable. In our acidification, *ca.* 25% of OP-$NaHCO_3$ of cattle slurry was hydrolysed, markedly improving the P availability and alleviating the difficulty of organic P decomposition in soil after application.

3.6 Conclusions

In cattle slurry, the <63 µm particle size fraction represented 35% of DM content, and *ca.* 70% of TN and TP of the whole slurry. The NH_4^+-N and water extractable P contents of cattle slurry doubled after acidification and this change was mainly associated with the <63 µm fraction. However, the proportion of NH_4^+-N and water extractable P, TN and TP in the <2000, <500 and <63 µm was not influenced by acidification.

The TP-H_2O, TP-$NaHCO_3$, TP-NaOH, TP-HCl and TP-residue forms accounted for 31%, 38%, 10%, 10% and 11% of the TP content of untreated cattle slurry. 79%, 55%, 27% and 62% of the TP-H_2O, TP-$NaHCO_3$, TP-NaOH and TP-HCl was inorganic P. Acidification increased the P bioavailability of cattle slurry by increasing the labile P (IP-H_2O+IP-$NaHCO_3$) content, dissolving the precipitated P (IP-HCl) and degrading the OP-$NaHCO_3$. After separation, the liquid fractions separated from the acidified slurry had a higher proportion of labile P than the solid fractions. Acidification increases the N and P availability of cattle slurry, especially in the liquid fraction.

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4 Anaerobic digestion alters the forms and distribution of N and P in cattle slurry

4.1 Abstract

Non-digested, non-inoculated and inoculated anaerobically digested cattle slurry were separated into different particle size fractions, and measurements of dry matter (DM), volatile solid (VS), carbon (C), NH_4^+-N, water extractable P, total N (TN) and total P (TP) followed. P speciation was conducted by sequential fractionation. Results showed that particles >63 µm dominated the DM, VS and C contents in the non-digested slurry. In contrast, the majority of the NH_4^+-N, water extractable P, TN and TP was in the <63 µm particle fraction. Anaerobic digestion increased NH_4^+-N, water extractable P and labile inorganic P contents, especially in the <63 µm fractions. These increases were not affected by the presence of an inoculum. 60% of the OP-$NaHCO_3$ content in the cattle slurry degraded during anaerobic digestion. After mechanical separation, the solid fraction contained more moderately labile P and recalcitrant P, while the liquid fraction had more labile inorganic P. Anaerobic digestion lowered the separation efficiency of DM, VS, water extractable P, labile inorganic P, and TN. Results show that anaerobic digestion increases the bioavailability of both N and P, particularly in the smaller particle size fraction with implications for both plant uptake and potential increased losses to the environment.

4.2 Introduction

Anaerobic digestion is a green energy production technology of importance, producing biogas for heat and power generation, and reducing odour and pathogens in livestock slurry (Smet et al., 1999; Sahlström, 2003; Clemens et al., 2006). Anaerobic digestion is conservative in terms of slurry nutrients, unless N is lost as ammonia from digestate stores, and hence can be applied as a source of nutrients for crop/grass production (Walsh et al., 2012). Although the process of anaerobic digestion has been utilised and studied for several decades (Yenigün & Demirel, 2013), information on the effects of anaerobic digestion on nutrient forms and distribution is largely lacking.

The particle size composition and chemical form of N and P in livestock slurry may be influenced by the anaerobic digestion process. During mechanical separation, particles above a certain size will be retained in the solid fraction while some smaller particles may end up in the liquid fraction (Hjorth et al., 2010). It has been reported that N and P are more difficult to recover in the solid fraction by some separation techniques (Hjorth et al., 2010). For example, the pore size of pressurized filtration ranges from 700-3200 µm, and the separation efficiency of dry matter (DM) is 13%-64%, while the separation efficiency of total N and total P is only 4%-13% and 7%-46% respectively (Hjorth et al., 2010).

Mineralisation during anaerobic digestion reduces the DM and volatile solid (VS) contents (Marcato et al., 2008). The total N (TN) content is conserved during the anaerobic process itself (Masse et al., 2005; Marcato et al., 2008). However, studies have showed the NH_3-N/TN ratio increased from 0.74 in non-digested manure to 0.85 in anaerobically digested manure, mostly due to the mineralization of organic N during anaerobic treatment (Masse et al., 2005.). The organic P fraction can also mineralize by anaerobic digestion (Hjorth et al., 2009). Small particle sizes have a greater surface area/volume ratio than large particles, and are more susceptible to bacterial colonisation and reaction (Hills and Nakano, 1984; Levine et al., 1991; Magid et al., 2010). Thus, the smaller slurry particulate organic matter is expected to degrade to a greater extent during anaerobic digestion (Marcato et al., 2008). Shon and Vigeswaran (2006) found the proportion of small slurry particles (i.e. <1 µm) was reduced via degradation during anaerobic digestion. On the other hand, bacterial flocs and filaments containing immobilised N and P are formed (Marcato et al., 2008). Some studies also suggest that large slurry particles break apart and reduce in size during anaerobic digestion (Masse et al., 2005). Hjorth et al. (2010) concluded that anaerobic digesters which are stirred and held at high temperatures facilitate the microbial degradation of large particles in comparison to smaller particles. In contrast, in digesters containing cold unstirred slurry, the microbial community primarily degrades the small particles. The mineralised N and P tends to be more soluble, resulting in a high nutrient content in the smaller particles or separated liquid fractions. In contrast, the formation of struvite and Ca-P and other precipitates (Marcato et al. 2008; Güngör & Karthikeyan, 2008; Rico et al. 2012) increases the P level in solid fractions.

Sequential fractionation is a practical approach to quantifying P speciation in livestock slurry. It was originally developed for soil samples, and has subsequently been modified and used in manure studies (Leinweber et al., 1997; Dou et al., 2000; He et al., 2006; Pagliari & Laboski, 2012). Briefly, P forms are sequentially extracted by increasingly stronger extractants, i.e. water, bicarbonate, hydroxide and acid, providing information on readily available P forms through to recalcitrant residual P (He et al., 2006). P extracted by these reagents in livestock manure is interpreted sequentially as: water-soluble; physically adsorbed on crystalline surfaces; associated with the surface of Ca carbonates, Al and Fe oxides; and mineral or amorphous phases of Ca-P (He et al., 2006).

Therefore, this study aimed to determine changes in particle size during the anaerobic digestion of cattle slurry, and the N and P speciation associated with these different slurry particle size fractions. We hypothesized:(1) DM and C distribution would be altered by anaerobic digestion and the proportion of coarse particles would be less in the anaerobically digested slurry; (2) bioavailability of N and P in livestock slurry, especially in small particle size fractions, would be increased by anaerobic digestion; and (3) anaerobic digestion would reduce the mechanical separation efficiency of the DM, C, N, and P contents.

4.3 Materials and Methods

4.3.1 Sample Collection

Three cattle slurry samples of 10 L were collected from a commercial beef farm in Wales, UK. The slurry was stored in a cold room (4ºC) in closed vessels until processing could take place. Three slurry samples of 1.5 L were anaerobically digested without inoculum (AD), and with inoculum (AD+) under laboratory conditions. The remaining slurry samples remained in the cold room until analysis. A digestate inoculum was collected from a commercial anaerobic digester in Wrexham, Wales, UK, that received cattle slurry and poultry manure. The inoculum added in the treatment of AD+ was at the rate of 30% by volume. The dry matter (DM) content of the cattle slurry with and without inoculum was both adjusted to 8% with distilled water before incubation, in order to maximise the methane production (Sun et al., 2012). The containers were then sealed and connected to foil bags for gas collection, and incubated at 35°C for 30 days. The containers were shaken gently for 1 h every day and the gas in the bags was released when full. The properties of untreated and treated cattle slurry are summarised in Table 4.1.

Table 4.1 Characteristics of untreated cattle slurry without (NON) and with inoculum (NON+), anaerobic digestate without (AD) and with inoculum (AD+) (on a fresh weight basis). Values represent means ± SEM, n = 3.

Index	NON	NON+	AD	AD+
DM g kg^{-1}	80.0 (±0.0)	80.0 (±0.0)	49.0 (±2.6)	48.6 (±1.7)
VS g kg^{-1}	55.0 (±2.0)	49.3 (±2.2)	31.5 (±1.9)	30.9 (±1.0)
TC g kg^{-1}	15.4 (±3.6)	13.2 (±2.0)	22.2 (±4.3)	27.7 (±10.6)
NH_4^+-N g kg^{-1}	0.1 (±0.0)	0.6 (±0.0)	0.5 (±0.1)	0.9 (±0.1)
TN g kg^{-1}	2.8 (±0.4)	3.5 (±0.4)	4.0 (±0.5)	4.3 (±0.5)
Water extractable P g kg^{-1}	0.1 (±0.0)	0.1 (±0.0)	0.2 (±0.0)	0.3 (±0.0)
TP g kg^{-1}	0.4 (±0.0)	0.6 (±0.0)	0.5 (±0.0)	0.5 (±0.0)
EC mS cm^{-1}	6.4 (±0.2)	-	9.7 (±0.6)	11.4 (±0.3)
pH	7.4 (±0.1)	-	7.8 (±0.0)	8.6 (±0.0)
ORP mV	-42.3 (±3.2)	-	-43.0 (±2.3)	-81.3 (±2.2)

4.3.2 Particle fractionation

Non-digested cattle slurry (NON), non-inoculated digested and inoculated digested slurry and the inoculum samples were wet-sieved sequentially and separated into fractions of >2000, 500-2000, 63-500, and <63 µm. The original fresh weight of each fraction (whole, >2000, 500-2000, 63-500, <63 µm) was recorded.

Subsamples of particle fractions were taken for determination of the dry matter content (DM) and volatile solid content (VS) by drying at 105ºC and 550ºC respectively, whilst remaining sample was air-dried and stored for further analysis. Fresh samples (4 g) of the >2000, 500-2000, 63-500 and <63 µm fractions were also extracted with 0.5 M K_2SO_4 (20 ml) by shaking for 0.5 h and centrifuging at 18,500 g for 5 min. Extracts were determined by colorimetric methods for NH_4^+-N and PO_4^{3-}-P (Mulvaney, 1996; Murphy & Riley, 1962). The K_2SO_4 extracts were interpreted as water extractable P, because in a preliminary experiment, this 0.5 M K_2SO_4 (1:5 w/v) extractable P showed no statistical difference to water (1:200 w/v) extractable P (data not shown). TP was also detected by combusting dried samples in 550°C overnight and dissolving the ash in 20% (v/v) HCl. The values of the whole slurry were calculated by summing the values of all particle fractions. Total carbon (C) and total nitrogen (TN) were measured for these particle size fractions using a TruSpec® CN analyzer (Leco Corp., St Joseph, MO). Since NH_4^+ is the dominant inorganic N form present in livestock slurry (Fangueiro et al., 2014), we assume the organic N content is the difference between TN and NH_4^+-N.

4.3.3 P sequential fractionation

P sequential fractionation was applied to the sieved particle fractions (described above) of the NON, AD and AD+ treatments and the inoculum. A modified protocol for sequential extraction was used (Dou et al., 2000). 0.3 g air-dried samples were sequentially extracted by 30 ml of distilled water, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl, in triplicate. During each extraction, the mixture of sample and extractant was shaken for 16 h. The suspension was then centrifuged at 13,600 g for 10 min. Finally, the supernatant was filtered with Whatman 42 and residues on filters were rinsed with the next extractant into centrifuge tubes for next extraction. Extracted inorganic P in each fraction was detected by a colorimetric method (Murphy & Riley, 1962). TP was determined after persulfate digestion. Finally, the undissolved residue was combusted in a muffle furnace under 550°C and then the ash was dissolved in 20% (v/v) HCl for TP analysis. The difference between TP and IP was defined as organic P (OP). The forms of inorganic and organic P extracted by H_2O, $NaHCO_3$, NaOH and HCl and P in the residue include IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl, OP-H_2O, OP-$NaHCO_3$, OP-NaOH, OP-HCl, TP-residue, in this study.

4.3.4 Calculation

The quantity of a N or P form in a particle size fraction is expressed on the basis of one kg of fresh whole slurry, Eqn (1).

$$C_i = CF_i \times WF_i / 1000 \qquad (1)$$

In the above equation, CF_i = Concentration of a N or P form of the particle fraction i on this fraction’s dry basis (g kg^{-1}); WF_i = dry weight of the particle fraction i in 1 kg of fresh whole slurry (g); C_i = total amount of a N or P form of the particle fraction i in 1 kg of fresh whole slurry (g kg^{-1}).

The distribution of a N or P form in the whole slurry is expressed as the proportion of a N or P form in a particle size fraction represents in the total quantity of such N or P form of the whole slurry, Eqn (2).

$$P_i = C_i \times 100\% / \sum_{i=1}^{n} C_i \tag{2}$$

In the above equation, P_i = the proportion of a N or P form of the particle fraction i in the total quantity of such N or P form of whole slurry (%).

The P form distribution in the whole slurry is defined as the proportion (%) of the TP of the whole slurry represented by the different P forms of the particle fractions, Eqn (3).

$$P_{ij} = C_{ij} \times 100\% / \sum_{j=1}^{m} \sum_{i=1}^{n} C_{ij} \tag{3}$$

C_{ij} = the quantity of a P form j of the particle fraction i in 1 kg of dry whole slurry (g kg^{-1}); P_{ij} =the proportion of the P form j of the particle fraction i in the TP of whole slurry (%).

The P form distribution in separated solid or liquid fraction is defined as the proportion (%) of the TP of separated fraction represented by each P form, Eqn (4).

$$PF_{ij} = C_{ij} \times 100\% / \sum_{j=1}^{m} C_{ij} \tag{4}$$

In the above equation, PF_{ij} = the proportion of the P form j in the particle fraction i (%).

All statistical analyses were performed with SPSS v22 (IBM Inc., Armonk, NY). Comparison between the slurry of NON and AD, NON+ and AD+ was performed using paired t-test with effects considered significant at $p<0.05$.

4.4 Results and Discussion

4.4.1 Particle fractionation

DM, VS, and C distribution in particle size fractions

Due to the degradation of organic matter during anaerobic digestion, the slurry DM and VS contents were significantly reduced by *ca.* 40% (Table 4.1). This degradation and reduction of the DM and VS contents is consistent with other research on pig slurry and wastewater sludge (Lake et al., 1985; Hsu & Lo, 2001; Marcato et al. 2008). In contrast, there was no significant difference in the carbon (C) content between the NON and AD, or between the NON+ and AD+ treatments, due to the high sample variability. There was no significant difference in the DM, VS and TC between the AD and AD+ treatments either.

According to Figure 4.1, coarse fractions >63 µm in cattle slurry (NON) dominated the DM, VS and C contents rather than the <63 µm fraction. In the DM of the whole slurry in the NON treatment, the >2000, 500-2000, 63-500 and <63 µm fractions accounted for 40%, 14%, 11% and 35%, respectively. In addition, they also represented 43%, 13%, 9% and 35% of the VS of the whole NON slurry, and 49%, 13%, 15%, 23% of the C of the whole NON slurry. The particle size distribution in cattle slurry is different to pig slurry presented in other papers. The DM content of cattle slurry tends to be distributed more in the coarser size fractions than pig slurry. In pig slurry, 38-41% of the DM content was in the >500 µm fraction (Møller et al., 2000) and 27% was in the >1000 µm fraction (Fernandes et al., 1988), which is much lower than the percentage of DM distributed in the coarse fraction in our cattle slurry. Further, 53% of DM was in the <50 µm fraction of pig slurry in the study of Fernandes et al. (1988) and 60% of DM was in the <10 µm fraction of pig slurry in the study of Masse et al. (2005), which is quite high compared with the small fraction present in our cattle slurry.

The DM and VS distribution in both NON and NON+ slurry was influenced by anaerobic digestion. All particle size fractions degraded during anaerobic digestion, showing a decline in DM quantity, except for the 63-500 µm fraction. Also, the degradation of coarse particles >500 µm was larger than for smaller size particles. As a result, the proportion of the particle size fractions of >2000 and 500-2000 µm in the DM of whole slurry reduced to 24% and 9% in the AD treatment, respectively (Figure 4.1). While the DM proportions represented by 63-500 and <63 µm fractions significantly increased from 11% to 17% and from 35% to 50% respectively, when comparing the slurry of NON and AD treatments (Figure 4.1). The same pattern was seen between the NON+ and AD+ treatments (Figure 4.1).

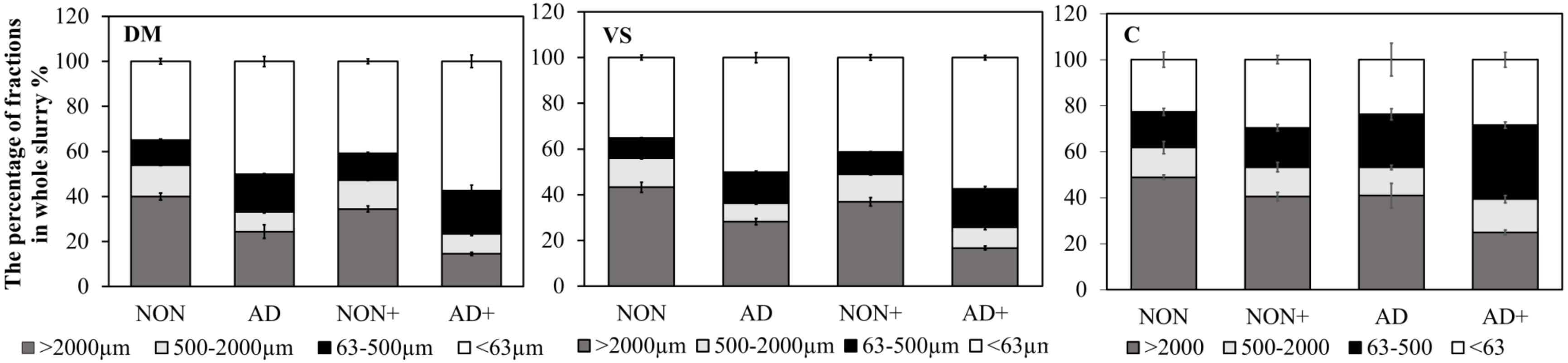

Figure 4.1 Distribution of dry matter (DM), volatile solid (VS) and carbon (C) in untreated cattle slurry without (NON) and with inoculum (NON+), anaerobic digestate without (AD) and with inoculum (AD+). Values represent means ± SEM, n = 3.

The change in the VS distribution as a result of anaerobic digestion was similar to the DM distribution. Such alteration was also seen in the distribution of C. The comparison between NON+ and AD+ in C distribution showed the proportion of >2000 µm fractions in the AD+ treatment was significantly lower than the NON+ treatment, while the 63-500 µm fraction proportion in the AD+ treatment was significantly higher than the NON+ treatment, but no difference was seen between the NON and AD treatments (Figure 4.1). Masse et al. (2005) found particles <10 µm increased from 64% to 84% of the DM of whole pig slurry after anaerobic digestion, which lends support to the hypothesis that the degradation rate of coarse particles is greater than for small particles. In contrast, Møller et al. (2002) observed the concentration of particles <1.6 µm was reduced most during anaerobic storage in slurry stores. Generally, in anaerobic digesters under the condition of stirring and high temperatures, microbial degradation of large particles is relatively fast compared to small particles (Hjorth et al., 2010). In the digesters with a cold unstirred slurry, the particles transformation primarily degrades small particles (Hjorth et al., 2010).

N distribution in particle size fractions

In the cattle slurry of the NON treatment, the majority of NH_4^+-N content (82%) was in particles <63 µm as expected, since it is a soluble form. A large proportion of TN (69%) was also in the <63 µm fraction (Figure 4.2). This is similar to other studies (Meyer et al., 2007; Peters et al., 2011).

Mineralisation during anaerobic digestion resulted in a greater proportion of the total N content as NH_4^+ in livestock slurry (Masse et al., 2005). The NH_4^+-N content of both whole NON and NON+ slurry significantly increased after anaerobic digestion. The NH_4^+-N content of the AD treatment was almost 5 times greater than the content of the NON treatment, and the AD+ treatment was about 1.5 times greater than the NON+ treatment. This indicates that the inoculation does not necessarily accelerate the degradation of organic N. The TN content of the whole slurry was not affected by the anaerobic digestion process, which agrees with Masse et al. (2005).

The NH_4^+-N quantity in >2000, 63-500 and <63 µm fractions of NON increased after anaerobic digestion (Figure 4.2), contributing to the significant increase of the NH_4^+-N quantity of the whole slurry. In addition, the increase in the quantity of NH_4^+-N in the <63 µm fraction was *ca.* 10 times greater than the other two fractions (Figure 4.2). The NH_4^+-N quantity in the NON+ treatment was affected by anaerobic digestion to a similar extent.

Therefore, the particle size fraction of <63 µm dominated the NH_4^+-N quantity change of the whole slurry. As for the NH_4^+-N distribution between particle size fractions, >2000, 500-2000, 63-500 and <63 µm fractions accounted for 8.0%, 4.6%, 5.4% and 82% in the NON treatment, respectively, and 2.4%, 2.4%, 6.9% and 88% in the NON+ treatment, respectively (Figure 4.2). Anaerobic digestion did not change this distribution. In addition, the inoculum did not influence the NH_4^+-N content and distribution change, since no difference was seen between AD and AD+ treatments.

The increase in the NH_4^+-N content of whole slurry can be ascribed to the mineralisation of organic matter present in the slurry. However, no statistically significant reduction was seen in the organic N content of whole slurry after anaerobic digestion, maybe because of the large variability. Only some changes in organic N distribution was observed. Specifically, the proportion of the 500-2000 µm fraction was reduced, while the proportion of the <63 µm fraction increased, in the comparison between NON and AD ($p<0.05$).

As for TN, the proportion of small particle fractions in the digested slurry tended to be greater than in the non-digested slurry. The TN proportion of whole slurry represented by the >2000 µm fraction in the AD+ treatment was significantly smaller than the NON+ treatment, while the proportion of the <63 µm fraction in the AD+ treatment was significantly greater (Figure 4.2). The same trend was seen for the NON and AD treatments, but was not statistically significant. The TN proportion of the 500-2000 µm fraction in the AD slurry was significantly smaller than in the NON slurry (Figure 4.2). The increased TN and NH_4^+-N quantity of the <63 µm fraction of the digested slurry may indicate a greater risk of potential N leaching following application to the land, although the greater availability of N in this fraction would also benefit crop growth if applied at appropriate rates and times.

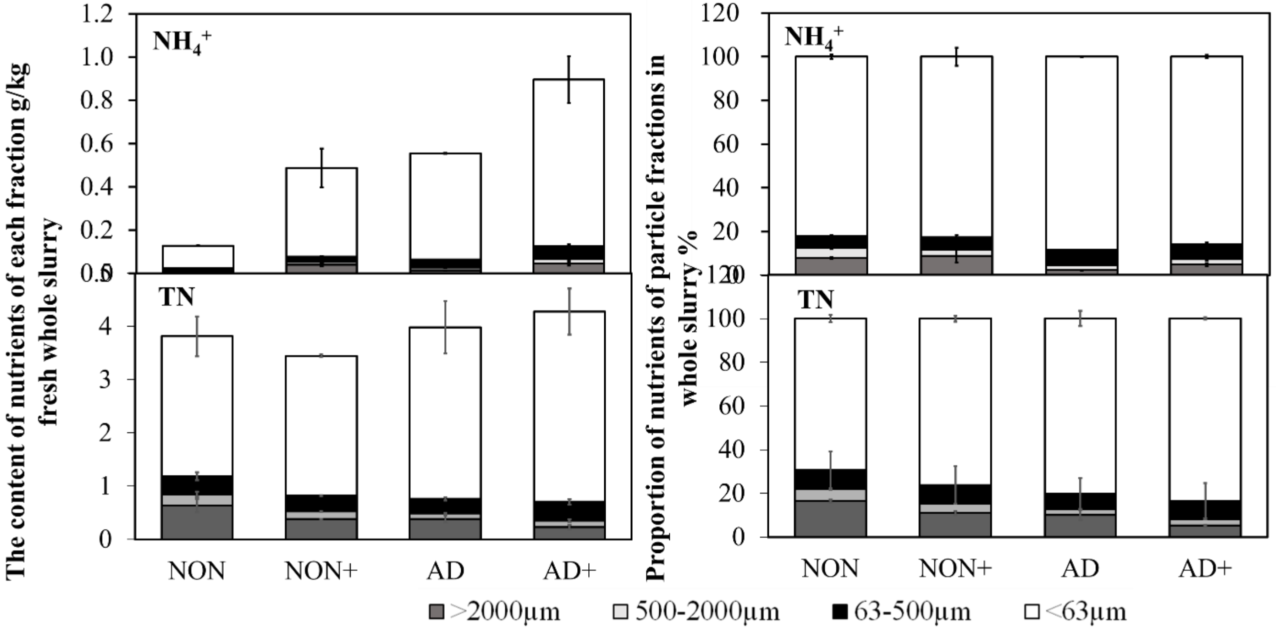

Figure 4.2 Distribution of NH_4^+-N and total N (TN) in different particle fractions in the NON, AD, NON+ and AD+ treatments. Values represent means ± SEM, n = 3.

P distribution in particle size fractions

In the NON treatment, the TP content of the whole slurry was 0.4 g kg^{-1}, of which 67% was present in the <63 µm fraction, which is similar to other studies (Meyer et al., 2007; Peters et al., 2011). The other particle size fractions of >2000, 500-2000 and 63-500 µm accounted for 19%, 7% and 7% of the TP of whole cattle slurry, respectively (Figure 4.3). During anaerobic digestion, the TP content of the whole slurry did not show any change on a fresh weight basis, but due to the decline of the DM content from 8% to 5% during anaerobic digestion, on a dry weight basis the TP content increased about 1.6 times. With respect to the distribution of TP in the particle size fractions, only the TP quantity of the 500-2000 µm fraction decreased, while the 63-500 µm fraction increased after anaerobic digestion (p<0.05), so were the proportions represented by these two fractions (Figure 4.3). The TP proportions of >2000, 500-2000, 63-500 and <63 µm fractions changed to 19%, 5%, 10%, 66% after anaerobic digestion (Figure 4.3), respectively. Comparison between NON+ and AD+ treatments showed the same trend.

The water extractable P content of the whole slurry of the NON and NON+ treatments both significantly increased on a fresh weight basis after anaerobic digestion, due to organic matter decomposition. Water extractable P contents of the whole slurry of the AD and AD+ treatments were over 1.6 times greater than the content of the NON and NON+ treatments, respectively. Similar to TP, the <63 µm fraction represented the largest proportion of water extractable P in NON and NON+ treatments (both 79%) (Figure 4.3). In the NON slurry, the water extractable P of >2000, 500-2000, and 63-500 µm fractions accounted for 10%, 5%, and 7% of the water extractable P of whole slurry, respectively (Figure 4.3). The water extractable P quantity of the 63-500 and <63 µm fractions of the NON slurry increased by 0.008 g kg^{-1} and 0.075 g kg^{-1} of fresh whole slurry respectively, while the 500-2000 µm fraction decreased by 0.002 g kg^{-1}, resulting in the increase of water extractable P content of the whole slurry after anaerobic digestion (Figure 4.3). Therefore, the increase in the <63 µm fraction dominated the change of the water extractable P. In addition, after anaerobic digestion the water extractable P in the <63 µm fraction increased to 83% of the AD slurry. The trend was similar comparing NON+ and AD+ treatments. AD and AD+ treatments did not have differences in terms of water extractable P content and distribution among particle fractions.

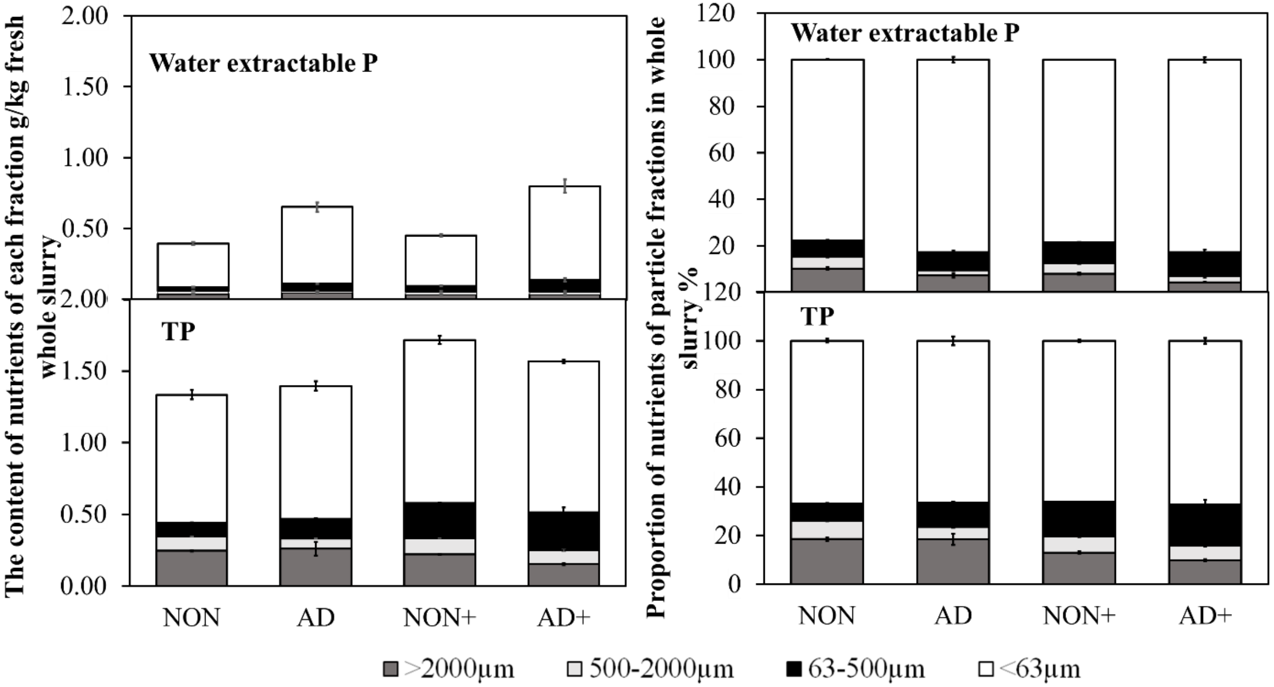

Figure 4.3 Distribution of the water extractable P and total P (TP) in different particle fractions in the NON, AD, NON+ and AD+ treatments. Values represent means ± SEM, n = 3.

4.4.2 P speciation and distribution in particle size fractions

The P speciation in the different particle size fractions quantified by sequential fractionation is shown in Figure 4.4 as the percentage of the TP of the whole slurry. The summed TP content of whole slurry, by adding up all P form fractions, were 0.42, 0.43, 0.55 and 0.52 g kg^{-1} for NON, AD, NON+ and AD+ treatments, respectively. Overall, they showed no difference to the above measured TP in Table 4.1. TP-H_2O, TP-$NaHCO_3$, TP-NaOH, TP-HCl and TP-residue forms represented 31%, 38, 10%, 10% and 11% of the TP of the whole slurry of the NON treatment. A similar distribution was measured in the NON+ treatment. This distribution of P forms is within the range presented by Sharpley & Moyer (2000). However, the proportion of TP-H_2O is smaller than the 70% from Dou et al. (2000). In our study, 79%, 55%, 27% and 62% of the TP-H_2O, TP-$NaHCO_3$, TP-NaOH and TP-HCl was present as inorganic P. The IP content (sum of IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl) increased ($p<0.05$) due to organic matter degradation during anaerobic digestion. The IP content of the whole slurry of AD was 1.3 times greater than in the slurry of the NON treatment, and the IP content of the AD+ slurry was 1.1 times greater than in the NON+ slurry. Further, after anaerobic digestion, the content of IP-H_2O, TP-residue, and OP-$NaHCO_3$ of the whole NON slurry decreased by 71% (0.071 g kg^{-1}), 18% (0.008 g kg^{-1}), and 58% (0.042 g kg^{-1}), while the content of IP-$NaHCO_3$, IP-HCl and OP-NaOH increased by 127% (0.111 g kg^{-1}), 67% (0.017 g kg^{-1}) and 35% (0.011 g kg^{-1}). Similarly, comparing the slurry of NON+ and AD+, the content of IP-H_2O, IP-NaOH, TP-residue, and OP-$NaHCO_3$ decreased, and the content of IP-$NaHCO_3$ increased significantly.

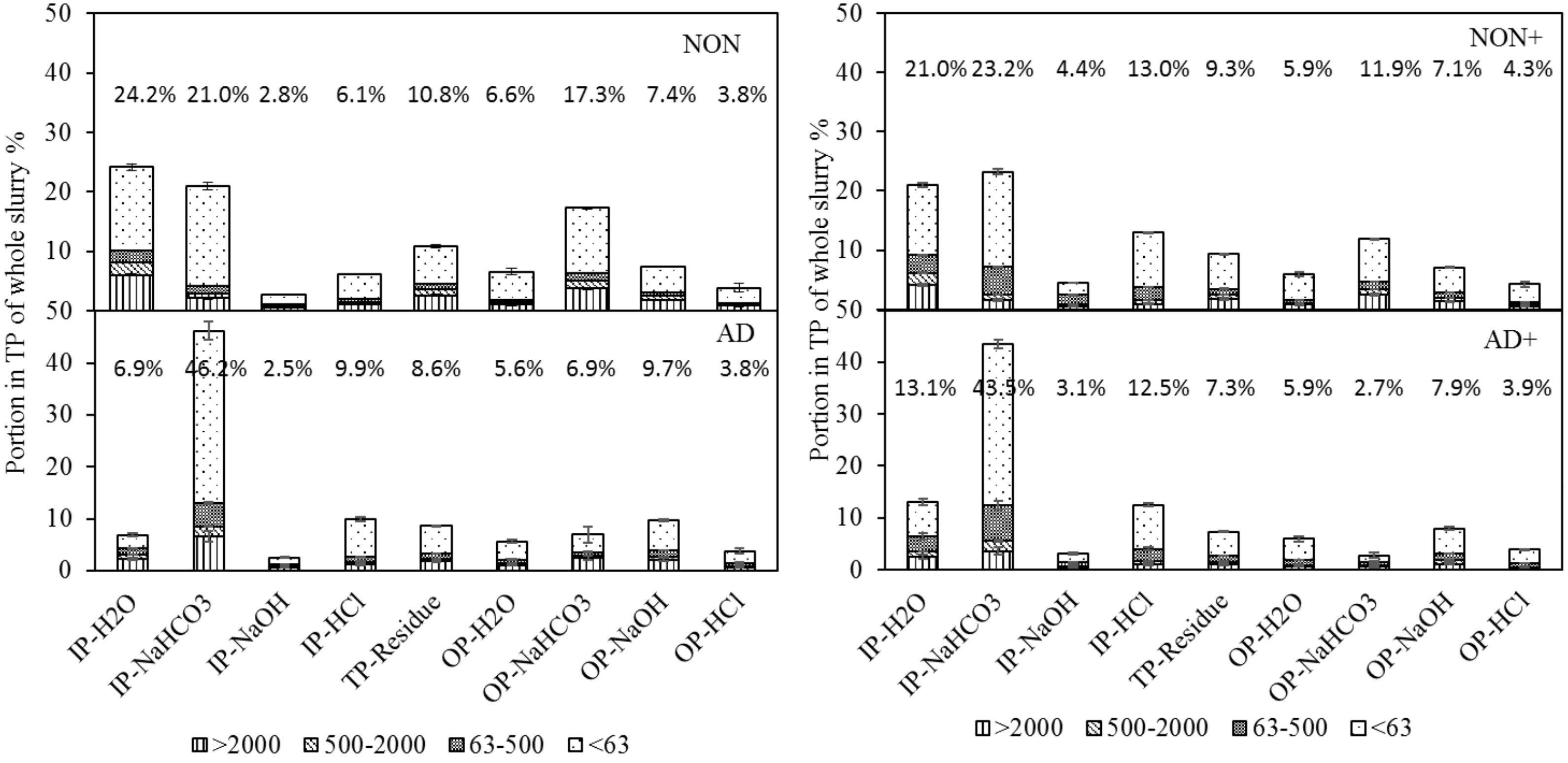

Figure 4.4 Distribution of P forms in each particle size fraction in the NON, AD, NON+ and AD+ treatments. Values represent means ± SEM, n = 3.

All particle size fractions contributed to the increase in the IP-$NaHCO_3$ content and the decrease in the IP-H_2O content of the whole NON slurry during anaerobic digestion. Further, the decrease in the OP-$NaHCO_3$ content of whole NON slurry caused by anaerobic digestion was associated with all particle size fractions, except for the >2000 μm fraction. The increase of the IP-HCl and OP-NaOH contents of the whole NON slurry resulted from the increase in the 63-500 and <63 μm fractions. As for the TP-residue content, only the 500-2000 μm fraction showed a statistically significant decrease, although the >2000 μm fraction had decreased markedly. Similar changes in the IP-$NaHCO_3$ and OP-$NaHCO_3$ content were also seen in the NON+ treatment. The decrease of the IP-H_2O content of NON+ after anaerobic digestion was only associated with 500-2000 and <63 μm particles. Less TP-residue quantity was found in the >2000, 500-2000 and <63 μm fractions, and less IP-NaOH quantity was measured in the 500-2000 and 63-500 μm fractions in the AD+ slurry than the NON+ slurry.

The <63 μm particles contributed the largest quantity of P in every form of whole slurry in NON and NON+ treatments (Figure 4.4), and dominated the change of each P form content of whole slurry during anaerobic digestion. During anaerobic digestion, over 60% of the change in the content of almost every P form was associated with the <63 μm fraction. For example, *ca.* 66% and 72% of the decrease of IP-H_2O and OP-$NaHCO_3$ of the whole NON slurry, 82% and 64% of the increase of IP-HCl and OP-NaOH content of the whole NON slurry (respectively) was associated with the <63 μm fraction.

Water-extractable P content of fresh manure is reported to be well correlated with P runoff, and has been suggested as a good indicator of potential P runoff (Kleinman et al., 2002). However, the IP-H_2O quantified by sequential fractionation in this section showed differences to the water extractable P measured (section 4.4.1.3). This may be because of the differences in methods used, e.g. the extraction time for sequential fractionation (16 h) was much longer than the 0.5 M K_2SO_4 extraction (0.5 h), which is supposed to extract more P in the solution. The water extractable P quantities were significantly smaller than the IP-H_2O quantities in the >2000 and 500-2000 μm fractions of the NON and NON+ treatments. However, the water extractable P of whole slurry of the AD and AD+ were all significantly greater than the IP-H_2O content. Also, the water extractable P quantities were greater than the IP-H_2O quantity in the <63 μm fractions of the NON and NON+ treatments and 63-500 and <63 μm fractions of the AD and AD+ treatments. This may be because the extraction of sequential fractionation was conducted on air-dried samples, while the 0.5 M K_2SO_4

extraction used fresh samples. During air drying, under alkaline condition, water soluble P may be adsorbed onto the surface of particles, contributing to the IP-$NaHCO_3$ form. Especially in the <63 µm fraction, where the water content was quite high, and digested slurry contained higher pH, which may be two factors resulting in greater alteration. However, the water extractable P had a good linear relationship with the sum of IP-H_2O and IP-$NaHCO_3$ (n = 60) ($R^2 = 0.92$). The influence of anaerobic digestion on the water extractable P and the sum of IP-H_2O and IP-$NaHCO_3$ showed the same pattern. The sum of IP-H_2O and IP-$NaHCO_3$ represented the labile inorganic P form, provided a better indicator of bioavailability and lability of P, and may be a better indicator of potential P runoff.

The labile P (sum of TP-H_2O and TP-$NaHCO_3$) was mainly present as inorganic P (65% and 71% in the NON and NON+ treatments, respectively), representing high bioavailability. While in the moderate labile P group (TP-NaOH), organic P was dominant (73%). 62% of occluded P (TP-HCl) was present as inorganic P. Cattle as a ruminant animal is better at utilising phytate, so the OP-HCl content, which is mainly composed of phytate (Baxter et al., 2003; He et al., 2004), represented a minor portion of the total. The labile inorganic P content, significantly increased after anaerobic digestion, implying an improved bioavailability of P in digestates, and a higher potential P runoff if applied to the land.

Crystal formation of struvite and Ca-P during anaerobic digestion (Güngör et al., 2007; Marcato et al., 2008), which is composed of the IP-HCl, occurred mainly in particles <500 µm. OP-$NaHCO_3$ is reported to be a hardly hydrolysable source (Hayes et al., 2000; He & Honeycutt, 2001). It is the largest proportion in organic P (Figure 4.4). The considerable reduction of 60% of the OP-$NaHCO_3$ content during anaerobic digestion was mainly transferred to inorganic P by microbial breakdown. This could be a positive effect in terms of crop availability. There may also be a slight transformation of OP-$NaHCO_3$ to OP-NaOH according to the minor increase in OP-NaOH in comparison between NON and AD treatments ($p<0.05$). In addition, smaller particles are more likely to leach through soil (McGechan, 2002). In addition, more IP proportion was seen in the <63 µm fraction of digested slurry. Thus, this may indicate higher IP leaching risk from digestate amended soil.

The addition of a microbial inoculant is expected to accelerate and improve organic matter degradation during the digestion process. In comparing the AD and AD+ treatments, the labile inorganic P, IP and TP contents of the whole slurry were significantly greater in AD+ treatment compared to the AD treatment. However, the comparison between the ratios of AD/NON and AD+/NON+ (representing the increase extent) in the labile inorganic P and IP contents of whole slurry showed a contrasting result ($p<0.05$). Again, this ratio of the particle size fractions did not display any difference between non-inoculated and inoculated treatments, except in the 63-500 µm fraction that the AD/NON ratio of the IP quantity was higher than the AD+/NON+ ratio. This implies that the inoculation does not necessarily accelerate the transformation of organic P to inorganic P.

4.4.3 Separation efficiency

Slurry particle size distribution is an important factor for recovering the solid and liquid fractions by mechanical separation technology. Generally, filtration retains particles above a certain size (Hjorth et al., 2010). For example, the filter pore size for drainage technologies is *ca.* 100-3000 µm, and filter pore size of pressurized filtration is *ca.* 700-3200 µm (Hjorth et al., 2010). The separation efficiency of filter pore size of 2000, 500 and 63 µm of our study is listed in Table 4.2. Separation methods simply based on filtration could relatively efficiently retrieve DM, VS and C. Pore size of 500 µm could separate out about half of the DM (Table 4.2). However, for N and P, the separation efficiency was quite low. Even the smallest size of 63 µm only obtained *ca.* 30% of TN and TP in the solid product (Table 4.2). Therefore, to improve the N and P recovery rates, other treatments, e.g. chemical additions (flocculants) and centrifuges would be needed, singly or in combination as shown in other studies (Hjorth et al., 2008; Hjorth et al., 2010; Karunanithi et al., 2015).

Anaerobic digestion degraded large size particles more than small size particles, and more nutrients tended to concentrate in the small size fractions. As a result, using the same pore sizes of filters, the separation efficiency of DM, VS and water extractable P of the AD slurry was significantly lower than the NON slurry (Table 4.2). Besides, the separation efficiency of C and TN of the AD+ treatment was also lower than the NON+ treatment (Table 4.2).

The solid and liquid products showed different P form distribution (Table 4.3). Generally, solid products for both non-digested and digested treatments always contained higher proportion of IP-H_2O, IP-NaOH, OP-NaOH and TP-residue than liquid products, while IP-$NaHCO_3$ represented larger proportion in the liquid products than the solid products. The labile inorganic P tended to be more in liquid portion of non-digested slurry than in the solid product, although not statistically significant. If applied on the same total P basis, the solid fraction may provide more moderately labile P and recalcitrant P, while the liquid fraction has more labile inorganic P.

Table 4.2 Separation efficiency of DM, VS, C, NH_4^+-N, TN, water extractable P and TP by different filter pore sizes separating solid fractions (%). Values represent means, n = 3.

Filter pore size µm		2000	500	63		2000	500	63
DM	NON	40.0	53.9	65.0	NON+	34.5	47.3	59.2
	AD	24.4*	33.1*	49.8*	AD+	14.6*	23.5*	42.6*
VS	NON	43.2	56.0	64.8	NON+	36.9	48.9	58.8
	AD	28.2*	36.2*	49.9*	AD+	16.7*	25.7*	42.5*
C	NON	48.8	61.8	77.3	NON+	40.5	53.3	70.4
	AD	40.9	53.1	76.2	AD+	24.9*	39.3*	71.6
NH_4^+-N	NON	7.9	12.5	17.9	NON+	2.35	4.71	11.59
	AD	8.95	11.88	17.32	AD+	5.0	7.41	14.27
TN	NON	16.5	21.9	30.5	NON+	11.1	15.3	23.8
	AD	10.1	12.8*	19.8	AD+	5.3*	8.2*	16.4*
Water extractable P	NON	10.3	15.5	22.2	NON+	8.0	12.7	21.4
	AD	7.4	9.5*	17.4*	AD+	4.4*	7.0*	17.4
TP	NON	18.6	26.0	33.1	NON+	13.0	19.6	33.8
	AD	18.5	23.6	33.6	AD+	9.8*	16.0	32.9

Note: * indicates the differences between the NON and AD treatments, or between the NON+ and AD+ treatments are statistically significant ($p<0.05$).

In the digested slurry, IP-$NaHCO_3$ and OP-NaOH represented a larger proportion of the TP of both liquid and solid products than the non-digested slurry, while IP-H_2O and TP-residue accounted for a lesser proportion (Table 4.2). Further, the proportion of the labile inorganic P was also greater in the liquid and solid products from digested slurry, indicating better agronomic benefit than undigested slurry. In contrast to the non-digested slurry, in the digested treatments, no difference in the labile inorganic P proportion was seen between the solid and liquid products. Thus, if applied to land on a TP basis, the solid and liquid products from digested slurry would provide similar amount of bioavailable P to plants.

Table 4.3 The P form distribution in the separated solid and liquid products by different filter pore sizes (%). Values represent means, n = 3.

Particle size µm		>2000	<2000	>500	<500	>63	<63
IP-H_2O	NON	30.1	22.7	29.9	23.8	29.3	23.9
	AD	12.1*	5.7*	12.6*	6.5*	11.9*	6.4*
IP-$NaHCO_3$	NON	10.4	23.7	10.7	21.7	12.0	21.4
	AD	34.7*	48.9*	35.1*	46.8*	36.8*	47.0*
IP-NaOH	NON	3.1	2.7	3.2	2.7	3.2	2.7
	AD	3.6	2.2	3.5	2.4	3.3	2.4
IP-HCl	NON	5.5	6.3	5.6	6.1	6.0	6.0
	AD	6.7	10.6*	7.0	10.0*	7.6	10.0*
OP-H_2O	NON	5.4	6.8	5.0	6.8	5.4	6.6
	AD	6.0	5.6	6.0	5.6	6.0	5.6
OP-$NaHCO_3$	NON	18.9	16.9	18.9	17.2	18.5	17.3
	AD	12.8	5.5*	11.6*	6.8*	10.1*	6.9*
OP-NaOH	NON	9.4	6.9	9.2	7.3	8.9	7.3
	AD	11.1*	9.3*	11.3*	9.6*	11.0*	9.6*
OP-HCl	NON	4.2	3.7	4.2	3.8	3.8	4.0
	AD	3.3	3.8	3.3	3.8	4.1	3.5
TP-residue	NON	12.9	10.3	13.2	10.6	13.1	10.7
	AD	9.7*	8.3*	9.6*	8.5*	9.1*	8.6*

Particle size µm		>2000	<2000	>500	<500	>63	<63
IP-H_2O	NON+	29.9	19.6	30.1	20.3	25.4	21.4
	AD+	21.5	12.0*	20.6	12.7*	18.6	12.3*
IP-$NaHCO_3$	NON+	11.0	25.2	11.8	23.9	19.9	21.9
	AD+	32.2*	44.9*	32.8*	44.1*	35.8*	44.5*
IP-NaOH	NON+	3.5	4.6	4.1	4.4	6.6	3.4
	AD+	4.1	2.9*	4.1	3.0*	4.0*	2.9*
IP-HCl	NON+	6.5	14.0	7.5	13.2	10.3	12.8
	AD+	8.8	13.0	9.5	12.6	11.6	12.3

OP-H_2O	NON+	5.3	6.0	4.8	6.1	4.3	6.3
	AD+	5.2	6.0	5.4	5.9	5.2	6.1
OP-$NaHCO_3$	NON+	17.9	10.9	16.6	11.7	12.9	12.6
	AD+	5.8*	2.4*	5.4*	2.6*	4.3*	2.6*
OP-NaOH	NON+	9.3	6.7	9.0	7.0	7.9	7.2
	AD+	10.7	7.6	10.5	7.8	9.2	8.0
OP-HCl	NON+	4.1	4.3	4.0	4.3	3.2	4.7
	AD+	2.6	4.1	2.8	4.0	3.5	3.9
TP-residue	NON+	12.4	8.8	12.1	9.1	9.6	9.9
	AD+	9.1*	7.1*	8.9*	7.3*	7.9*	7.4*

Note: * indicates the differences between the NON and AD treatments, or between the NON+ and AD+ treatments are statistically significant (p<0.05).

4.5 Conclusions

Particles >63 µm dominated the DM, VS and C contents in non-digested cattle slurry, while the majority of N and P was present in particles <63 µm. Anaerobic digestion increased NH_4^+-N, water extractable P and labile inorganic P contents, especially in the <63 µm fractions. The inoculation did not necessarily accelerate the degradation of organic N and P. After mechanical separation, the solid fraction tended to have more moderately labile and recalcitrant P, while the liquid fraction has more labile IP. Anaerobic digestion lowered the separation efficiency of DM, VS, water extractable P, labile inorganic P, and TN.

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5 Phosphorus and nitrogen distribution in colloids and nanoparticles of untreated, acidified and anaerobically digested cattle slurry

5.1 Abstract

Livestock slurry applications supply colloids and nanoparticles to soil, which are important carriers of phosphorus (P) and other nutrients that can be transported through and over the soil to watercourses. However, advanced slurry processing, such as acidification and anaerobic digestion could alter the proportions of different slurry particles (including nanoparticles) as well as the P forms associated with these smaller slurry particle sizes. In this study, we separated colloidal and nanoparticulate fractions from the liquid fraction (<63 μm) of untreated, acidified and anaerobically digested cattle slurry using vacuum filtration (through a 0.45 μm filter) and ultrafiltration (through 0.03 μm (100 kDa) and 0.001 μm (3 kDa) filters). Phosphorus forms that passed through a 0.45 μm filter were operationally defined as dissolved. Thus, in this study, the 0.45-63 μm particles were categorised as the colloidal fraction, and the <0.45 μm fraction was the dissolved fraction. Nanoparticles were therefore within the dissolved fraction. The quantity of total carbon (TC), total nitrogen (TN) and total P (TP) were analysed in the colloidal and nanoparticulate fractions, and P sequential fractionation was applied to the colloidal fraction only. Results showed that the colloidal fraction of the untreated liquid cattle slurry accounted for >80% of the dry matter, TC, and TP contents, and all P forms contents, while >90% of NH_4^+ and TN was in the dissolved fraction. Nanoparticles of the untreated cattle slurry existed in the 0.03-0.45 μm range and contained only a small amount of P. Acidification increased NH_4^+, organic N and TN quantity of the liquid cattle slurry, which was mainly associated with the colloidal fraction. While anaerobic digestion increased the NH_4^+ quantity of the dissolved fraction and the organic N quantity of the colloidal fraction. There was a smaller quantity of P associated with nanoparticles after anaerobic digestion, while there was almost no P associated with nanoparticles after acidification. Whilst the TP content of the colloidal fraction was not influenced by acidification and anaerobic digestion, acidification did reduce the quantity of IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl, TP-residue and OP-$NaHCO_3$ forms of the colloidal fraction significantly. Anaerobic digestion decreased the quantity of IP-H_2O, TP-residue and OP-$NaHCO_3$ forms of the colloidal fraction, and increased the quantity of IP-$NaHCO_3$ and IP-HCl forms. In summary, acidification increased inorganic and organic N content and

reduced the labile P content of the colloidal fraction, anaerobic digestion increased organic N and labile P content of the colloidal fraction, with implications for crop utilisation and losses to watercourses. The nanoparticulate fractions of untreated and treated slurry may have little impact on potential P leaching.

5.2 Introduction

Colloid- and nanoparticle-facilitated vertical transport is important in nutrient movement through soils (Hens and Merckx, 2002; McGechan & Lewis, 2002; Heathwaite et al., 2005). Makris et al. (2006) isolated colloids from long-term manured soil and added them to silt loam columns and found that 30% of the added colloids could be recovered in leachate from the base of the columns. de Jonge et al. (2004) and Hens & Merckx (2002) both found nanoparticles in leachates from the soil. However, Rick & Arai (2011) questioned the role of nanoparticle in P transport, based on the unreactive results from their P desorption and P retention and release experiments. Moreover, recently colloidal P from some soil has been reported to contribute to plant P uptake (Montalvo et al., 2015). Livestock slurry as a source of particulate carriers of nutrients, its particle composition is an important factor that influences nutrient (N and P) mobilization and even loss from the soil after application. Moreover, decomposition of organic matter in soil tends to be accelerated with decreasing particle size due to the higher surface area/volume ratio (Levine et al., 1991). However, to date there have been few studies on the colloid and nanoparticle characterization of livestock slurry. Furthermore, advanced slurry processing strategies, e.g. acidification and anaerobic digestion, may alter not only the slurry particle size characteristics, but also the N and P forms associated with these slurry particles.

Acidification is an approach to reduce NH_3 emissions from manure management (Kai et al., 2008), retaining N and the fertiliser value of livestock slurry (Eriksen et al., 2008; Sutaryo et al., 2013). Indeed, the available P content of slurry also increases during acidification (Güngör et al., 2007; Fangueiro et al., 2015). Roboredo et al. (2012) separated untreated and acidified pig slurry by centrifugation at 1509 *g* for 15 min, and found the TP distributed in the solid fraction decreased by >50% after acidification compared to untreated slurry. Anaerobic digestion is a green energy production technique, providing biogas generation and odour and pathogen reduction (Clemens et al., 2006; Lee & Han, 2012). Anaerobic digestion degrades organic matter and reduces the dry matter and volatile solids content of livestock slurry (Marcato et al., 2008), but increases NH_3 or NH_4^+ content

(Angelidaki & Ahring, 1993; Masse et al., 2005). Hjorth et al. (2010) claimed microbial degradation during anaerobic digestion proceeds faster on large particles relative to small particles, under the condition of stirring and high temperatures. In contrast, under non-stirred and cold conditions, the degradation is mainly associated with small particles. Masse et al. (2005) found the NH_3-N/TN ratio increased from 74% in non-digested manure to 85% in anaerobically digested manure, and due to the increase in NH_4^+-N concentration, the pH value of livestock slurry is generally found to be increased (Strik et al., 2006; Pang, 2008). As the pH increases, the solubilisation of P is reduced and P associated with solid phases is stabilised after anaerobic digestion (Güngör & Karthikeyan, 2008). However, more information on the effect of acidification and anaerobic digestion on both colloidal and nanoparticulate size characterization, and the N and P forms associated with these smaller slurry particle size fractions is still needed.

Thus, this study aimed to improve our understanding of the colloidal (0.45-63 µm) and nanoparticulate (0.001-0.45 µm) composition and their N and P distribution in untreated, acidified and anaerobically digested livestock slurries. We hypothesised that: 1) colloids and nanoparticles contain a significant proportion of the N and P content of the livestock slurry; and 2) acidification and anaerobic digestion both increase the availability of N and P in the separated liquid slurry fraction (<63 µm), which is primarily associated with the colloidal fraction.

5.3 Materials and Methods

5.3.1 Sample Collection

Cattle slurry was collected from a commercial beef farm in Gwynedd, Wales, UK. Cattle slurry samples (2 L) were taken on 3 separate occasions as three replicates, and then treated by acidification or anaerobic digestion. Cattle slurry samples were acidified to pH 5.5 by adding *ca.* 35 ml 15% (v/v) H_2SO_4 per L of slurry. Anaerobic digestion was conducted in the laboratory using two approaches (with three replicates of each), i) with a 30% (v/v) inoculum (collected from a nearby commercial anaerobic digester receiving cattle slurry and food waste feedstocks), and ii) without an inoculum. The dry matter (DM) content of the two slurry treatments was adjusted to 8% with distilled water prior to incubation, in order to maximise methane production (Sun et al., 2012), and then sealed in 2 L containers connected to foil bags for gas collection, and incubated at 35°C for 30 d. The containers were shaken gently for 1 h every day and the gas in the bags was released when full. The properties of untreated and treated cattle slurry are summarised in Table 5.1.

5.3.2 Particle Fractionation

Replicate batches of untreated cattle slurry, acidified cattle slurry, the inoculum, inoculated anaerobically digested slurry (AD+) and non-inoculated anaerobically digested slurry (AD) were wet-sieved through a 63 µm mesh. Then the <63 µm fraction was vacuum filtered through a 0.45 µm membrane to separate out particles of 0.45-63 µm. Colloids are generally defined as particles between 1 µm and 1 nm in size (Kretzschmar et al., 1999). Here we operationally defined particles of 0.45-63 µm as the colloidal fraction. The <0.45 µm regarded as the dissolved fraction (Haygarth & Sharpley, 2000), was subsequently ultrafiltered into <0.03 and <0.001 µm fractions with 100 and 3 kDa filters (Millipore Ultracel® 100 kDa Ultrafiltration Discs, Millipore Ultracel® 3 kDa Ultrafiltration Discs), respectively (Turner et al., 2004). Particles of 0.001-0.45 µm were operationally defined as nanoparticles (Henderson et al., 2012). The classification of particles and measured and calculated characteristics are summarized in Table 5.2.

The DM content and volatile solid content (VS) of whole slurry and the liquid fraction were determined by drying at 105°C and 550° C, respectively. Whole slurry and the liquid fraction was also extracted with 0.5 M K_2SO_4 (1:5 w/v), shaking for 0.5 h and centrifuging at 18,500 *g* for 5 min. The NH_4^+ and P in the extracts was determined colorimetrically using the methods of Mulvaney (1996) and Murphy & Riley (1962), respectively. An aliquot of the extract of the liquid slurry was also used to determine the total organic carbon (TOC) content using a N/C 2100 analyzer (Analytik Jena, Jena, Germany). The P extracted by 0.5 M K_2SO_4 was interpreted as water extractable P, because a preliminary experiment showed this 0.5 M K_2SO_4 (1:5 w/v) extractable P had no statistical difference to water (1:200 w/v) extractable P (data not shown).

Table 5.1 Characteristics of whole non-acidified and acidified cattle slurry, non-digested slurry without inoculum (NON) and non-digested slurry with inoculum (NON+), anaerobic digestate without inoculum (AD) and anaerobic digestate with inoculum (AD+) (on a fresh weight basis). Values represent means ± SEM, n = 3.

	Non-acidified cattle slurry	Acidified cattle slurry	NON	NON+	AD	AD+
DM g kg^{-1}	109.1(±9.0)	144.0(±15.9)	80.0(±0.0)	80.0(±0.0)	49.0(±2.6)	48.6(±1.7)
VS g kg^{-1}	74.5(±3.4)	78.4(±1.4)	55.0(±2.0)	49.3(±2.2)	31.5(±1.9)	30.9(±1.0)
NH_4^+ g kg^{-1}	0.2(±0.01)	0.4(±0.1)	0.1(±0.0)	0.6(±0.0)	0.5(±0.1)	0.9(±0.1)
TP g kg^{-1}	0.6(±0.03)	0.75(±0.05)	0.4(±0.0)	0.6(±0.0)	0.5(±0.0)	0.5(±0.0)
TN g kg^{-1}	3.8 (±0.6)	4.6(±0.3)	2.8 (±0.4)	3.5(±0.4)	4.0(±0.5)	4.3(±0.5)
TC g kg^{-1}	21.0(±4.9)	36.7(±6.5)	15.4(±3.6)	13.2(±2.0)	22.2(±4.3)	27.7(±10.6)
EC mS cm^{-1}	6.4(±0.2)	7.0(±0.8)	-	-	9.66(±0.6)	11.37(±0.3)
pH	7.4(±0.1)	5.6(±0.0)	-	-	7.79(±0.0)	8.62(±0.0)
ORP mV	-42.3(±3.2)	-64.0(±1.5)	-	-	-43.0(±2.3)	-81.33(±2.2)

Table 5.2 Classification of particles and the measured and calculated characteristics of different particle size fractions.

Particle size µm	Fraction categories	TN	TP	TC	TOC	DM	NH_4^+	Extractable P	Sequential fractionation
<63	Liquid fraction	Measured	Measured	Measured	Measured	Measured	Measured	Measured	Measured
<0.45	Dissolved fraction	Measured	Measured	Measured	Measured	Calculated difference	Measured	Measured	-
<0.03		Measured	Measured	Measured	Measured	-	Measured	Measured	-
<0.001	Soluble fraction	Measured	Measured	Measured	Measured	-	Measured	Measured	-
0.45-63	Colloidal fraction	Calculated difference	Calculated difference	Calculated difference	Calculated difference	Measured	Calculated difference	Calculated difference	Measured
0.03-0.45	Nanoparticles	ANOVA	ANOVA	ANOVA	ANOVA	-	ANOVA	ANOVA	-
0.001-0.03	Nanoparticles					-			-

The total P (TP) content of the whole slurry and the liquid fraction was also detected by combusting dried samples at 550ºC overnight and dissolving the ash in 2.4 M HCl. The total carbon (TC) and total nitrogen (TN) content of the whole slurry and the liquid fraction were determined by TruSpec® CN analyzer (Leco Corp., St Joseph, MO). The difference between TN and NH_4^+ was assumed to be organic N, since in livestock slurry the content of NO_3^- is minimal. The total P content of the dissolved fraction, and the <0.03 and <0.001 µm fractions was determined after persulfate acid digestion (1 mL slurry + 2 mL 0.9 M H_2SO_4 + 0.1 g ammonium persulfate, 120ºC, 5 h). The dissolved fraction, and the <0.03 and <0.001 µm fractions were also measured for NH_4^+, inorganic P (reactive P), TC, TN, and TOC content. For the colloidal fraction (0.45-63 µm), due to the small quantity available, only the DM content was measured, and the rest was kept for P sequential fractionation. The N and P quantity of the colloidal fraction was calculated by subtracting the values in the dissolved fraction from the liquid fraction. The water extractable P content of the colloidal fraction was calculated by subtracting the inorganic P content of the dissolved fraction from the water extractable P of the liquid fraction. The DM content of the dissolved fraction was the difference between colloidal and liquid fractions. Organic P of the dissolved fraction, and the <0.03 and <0.001 µm fractions were calculated by the difference between TP and inorganic P.

5.3.3 P sequential fractionation

P sequential fractionation was applied to the colloidal fractions of the untreated and treated slurry treatments and the inoculum. A modified protocol for sequential extraction was used (Dou et al., 2000). 0.3 g air-dried samples were sequentially extracted by 30 ml of distilled water, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl, in triplicate. During each extraction, the mixture of sample and extractant was shaken for 16 h. The suspension was then centrifuged at 13,600 *g* for 10 min. Finally, the supernatant was filtered with membrane (MilliporeSigma HVLP04700 Durapore® PVDF Membrane Filter, 0.45 µm), and residues on filters were rinsed with the next extractant into centrifuge tubes in preparation for the next extraction. Extracted inorganic P in each fraction was detected colorimetrically (Murphy & Riley, 1962). TP was determined after persulfate digestion (as described earlier). Finally, the undissolved residue was combusted in a muffle furnace at 550ºC and then the ash was dissolved in 20% HCl prior to TP analysis. The difference between TP and IP was organic P (OP). Then P forms of inorganic and organic P extractable by H_2O, $NaHCO_3$, NaOH and

HCl and P in the residue represent the following forms; IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl, OP-H_2O, OP-$NaHCO_3$, OP-NaOH, OP-HCl, TP-residue in this study.

5.3.4 Calculation

Nutrient content/quantity in a particle size fraction is expressed on the basis of one kg of the fresh whole slurry, Eqn (1).

$$M_i = C_i \times WF_i / 1000 \qquad (1)$$

Where, C_i=Concentration of nutrient of the particle size fraction 'i' on fresh basis (g kg^{-1}); WF_i= fresh weight of the particle size fraction 'i' in 1 kg of fresh whole slurry (g); M_i= total nutrient amount of the particle size fraction 'i' in 1 kg of fresh whole slurry (g kg^{-1}).

The nutrient distribution in the whole slurry/the liquid slurry was defined as the proportion (%) of the total nutrient quantity of the whole slurry/the liquid slurry represented by the nutrient quantity of the particle size fractions, Eqn (2).

$$P_i = C_i \times 100\% / \sum_{i=1}^{n} C_i \qquad (2)$$

C_i= total nutrient quantity of the particle size fraction 'i' in 1 kg of fresh whole slurry/ liquid slurry (g kg^{-1}); P_i=the proportion of the nutrient quantity of the particle size fraction 'i' in the total nutrient quantity of whole slurry/liquid slurry (%). The values for non-acidified slurry were the values measured for the untreated slurry. But the values for DM adjusted non-digested slurry (NON) was calculated using values of untreated slurry and DM adjust ratio (DM was adjusted to 8% as described above). The values for inoculated non-digested slurry (NON+) was calculated by values of the inoculum, untreated slurry and their mixture ratio.

All statistical analyses were performed with SPSS v22 (IBM Inc., Armonk, NY). Comparison between the slurry of non-acidified and acidified, NON and AD, NON+ and AD+ were analysed using paired t-tests, with effects considered significant at $p<0.05$. Comparisons of nutrient content between different particle size fractions within the same slurry were conducted by ANOVA with Tukey pairwise post-hoc testing, with effects considered significant at $p<0.05$.

5.4 Results and discussion

5.4.1 Colloids in livestock slurry

Comparison between the untreated and acidified slurry

Separated liquid slurry (<63 μm) represented the majority of the TN and TP, and half the DM content of whole cattle slurry (Chapter 3). In the separated liquid fraction of the non-acidified treatment, 79% of particles were in the colloidal fraction (0.45-63 μm) (Table 5.3). Moller et al. (2002) and Masse et al. (2005) also reported that colloidal particles of 1.6-50 μm and 0.45-10 μm represented a considerable proportion of the DM content of the whole slurry, respectively. Correspondingly, the colloidal fraction accounted for the majority of the TOC, TC, water extractable P, and TP content in the separated liquid slurry fraction, while the dissolved fraction (<0.45 μm) contained the largest proportion of NH_4^+ and TN (Table 5.3). Masse et al. (2005) also found 50% of the TP of whole pig slurry was in the 0.45-10 μm fraction, and 20% was in the <0.45 μm fraction.

Acidification did not influence the distribution of DM and TC in the separated liquid slurry fraction, but did result in a change in the NH_4^+, TN, water extractable P and TP quantity and distribution. Slurry acidification is promoted for the mitigation of ammonia emission (Ottosen et al., 2009; Sorensen & Eriksen, 2009; Petersen et al., 2012; Wang et al., 2014). In our study, the NH_4^+ and TN contents of the separated liquid slurry fraction increased markedly by the acid as expected ($p<0.05$) (Table 5.3). This increase was more associated with the colloidal fraction rather than the dissolved fraction. The NH_4^+ quantity of the colloidal fraction increased substantially by the addition of acid, by almost 18 times ($p<0.05$) (Table 5.3). In contrast, the NH_4^+ quantity in the dissolved fraction did not change. Only 7% of the NH_4^+ quantity and 3% of the TN quantity of the separated liquid slurry fraction was in the colloidal fraction in the non-acidified treatment, while it was 53% of the NH_4^+ quantity and 25% of the TN quantity in the colloidal fraction in the acidified treatment (Table 5.3). Acidification protonated NH_3 and NH_4^+ can become electrically absorbed on colloidal surfaces. The TN quantity of the colloidal fraction increased by 1.02 g kg^{-1} fresh whole slurry, and the increase in the NH_4^+ quantity of the colloidal fraction was 0.17 g kg^{-1} fresh whole slurry, indicating the increase in the TN quantity mainly resulted from the increase of organic N, which may be from the break-down of coarse particles.

Table 5.3 The nutrient content (g kg^{-1} based on fresh weight of whole slurry) and percentage of colloidal and dissolved fractions in the whole slurry and the liquid fraction of non-acidified and acidified treatments (%). Values represent means, n = 3.

Treatment	Particle size fraction	Quantity g kg^{-1}		Percentage in whole %		Percentage in the liquid fraction %	
		Non-acidified	Acidified	Non-acidified	Acidified	Non-acidified	Acidified
DM	Liquid slurry	38.07	43.05 *	35.0	30.3	100	100
	Colloids	30.24	31.80	27.8	22.2	79.4	73.9
	Dissolved	7.84	11.25	7.2	8.1	20.6	26.1
TC	Liquid slurry	4.65	6.30	22.7	18.0	100	100
	Colloids	3.84	5.28	18.3	12.1	80.6	67.1
	Dissolved	0.81	1.02	4.4	5.9	19.4	32.9
TOC	Liquid slurry	22.07	21.54	-	-	100	100
	Colloids	20.81	19.49	-	-	94.4	90.5
	Dissolved	1.26	2.05 *	-	-	5.6	9.6
NH_4^+	Liquid slurry	0.14	0.34 *	82.1	80.5	100	100
	Colloids	0.01	0.18 *	8.3	42.2 *	6.7	52.5 *
	Dissolved	0.13	0.15	76.6	38.3 *	93.4	47.5 *
TN	Liquid slurry	3.80	4.55 *	69.5	71.1	100	100
	Colloids	0.13	1.15 *	2.3	17.7	3.3	24.9 *
	Dissolved	3.67	3.40	67.2	53.4	96.7	75.1 *
Water extractable P	Liquid slurry	0.14	0.27 *	77.8	78.3	100	100
	Colloids	0.12	0.07 *	66.7	18.8 *	85.7	24.0 *
	Dissolved	0.02	0.21 *	11.1	59.5 *	14.3	76.0 *
TP	Liquid slurry	0.39	0.52	66.9	69.5	100	100
	Colloids	0.37	0.27	62.1	36.5 *	92.9	52.5 *
	Dissolved	0.03	0.24 *	4.8	33.0 *	7.1	47.5 *

Note: * indicates the differences between non-acidified and acidified treatments are statistically significant ($p<0.05$).

The water extractable P content in the separated liquid slurry fraction also increased significantly as a result of acidification (Table 5.3). In contrast to NH_4^+ above, this increase of the water extractable P content was mainly associated with the dissolved fraction, of which the quantity of water extractable P was increased by *ca.* 10 times after acidification (Table 5.3). This is in agreement with other research (Christensen et al., 2009; Hjorth et al., 2010). In contrast, the quantity of water extractable P of the colloidal fraction decreased (Table 5.3). Therefore, the proportion in the whole slurry occupied by the dissolved fraction increased. The increased water extractable P resulted mainly from desorption and solubilisation of precipitates. The acidification process was conducted on the whole slurry before separation. The dissolution of dominant precipitates, i.e. struvite and calcium phosphate and other occluded P, and P desorption occurs at low pH in whole slurry (Güngör et al., 2007; Güngör & Karthikeyan, 2008). After separation, this dissolved and desorbed P from both solid and liquid fractions entered the dissolved fraction. Hjorth et al. (2015) suggest accelerated hydrolysis of organic matter during acidification, so this may also be a source of the increased P content in in the dissolved fraction.

Similarly, the TP quantity of the dissolved fraction increased *ca.* 8 times after acidification, while the TP quantity of the colloidal fraction did not change. Thereafter, the proportion of TP of the dissolved fraction was increased in both the separated liquid slurry fraction and whole slurry.

Colloid-facilitated transport is thought to be an important pathway for P leaching, and slurry is one potential sources for organic matter colloids (McGechan & Lewis, 2002; Siemens et al., 2004). In an unsaturated P soil, if separated liquid slurry was applied on a TP basis, P in the soluble fraction is more likely to be bound by soil minerals, while P in the colloidal and nanoparticulate fractions may dictate the vertical movement of P in soil. Since the proportion of the colloidal fraction in the TP of the acidified liquid slurry was lower than in the non-acidified liquid slurry, it should follow that potential TP leaching associated with colloids from the liquid acidified slurry would be less than the non-acidified liquid slurry. Likewise, the risk of inorganic P (water extractable P) leaching would also be less in the acidified liquid treatment. On the other hand, when a separated liquid slurry fraction is applied to cropping land on a TP basis, the available P in the soil would be increased immediately, and acidified slurry treatment would result in greater available P to plants than from a non-acidified slurry treatment, as other studies have reported (Roboredo et al., 2012).

In our study, the NH_4^+/TP ratio was 0.36 and 0.65 for non-acidified and acidified separated liquid slurry fraction, respectively, thus the N benefit for crops would also be higher in acidified slurry treatment. If applied on a TN basis, the TN/TP ratio of the acidified separated liquid slurry fraction would be 8.7, less than the 9.7 for the non-acidified treatment. Thus, more N and P input with a greater potential agronomic benefit would be expected from an acidified separated liquid slurry fraction. P leaching associated with the colloidal fraction from acidified liquid slurry would still be less than non-acidified liquid slurry. The NH_4^+ and organic N proportion of the colloidal fraction in the acidified liquid slurry was greater than in the non-acidified liquid slurry, so the colloid-facilitate N leaching would be greater in acidified slurry treatment.

Comparison between the untreated and anaerobically digested slurry

Anaerobic digestion reduces the DM content of livestock slurry (Lake et al., 1985; Hsu & Lo, 2001; Marcato et al., 2008; Chapter 3) and after separation the liquid slurry fraction contained less DM. The DM of the whole slurry was reduced by 40%, while for the separated liquid slurry fraction the reduction in DM content was only 13% (NON) or 15% (NON+). The DM quantity of the colloidal fraction of the NON and NON+ treatments decreased by 18% and 7% after anaerobic digestion, respectively ($p<0.05$). Hjorth et al. (2010) suggested that in anaerobic digesters, stirring and high temperatures facilitate microbial degradation of large particles, leaving relatively small particles which are more recalcitrant. This supports our observation of the low DM reduction in the separated liquid slurry fraction and colloidal fraction relative to whole slurry. The breakdown of large particles may also supplement the colloid loss during anaerobic digestion. The TC quantity of the colloidal fraction of the NON and NON+ treatments tended to decrease after anaerobic digestion, but this was not statistically significant.

Table 5.4 The nutrient content (g kg^{-1} based on fresh weight of whole slurry) and percentage of colloidal and dissolved fractions in the whole slurry and the liquid fraction in untreated and anaerobic digestion treatments (%). Values represent means, n = 3.

Treatment	Particle size fraction	Quantity g kg^{-1}				Percentage in whole %				Percentage in the liquid fraction %			
		NON	AD	NON+	AD+	NON	AD	NON+	AD+	NON	AD	NON+	AD+
DM	Liquid slurry	28.02	24.46 *	32.62	27.80 *	35.00	50.2 *	40.8	57.4 *	100	100	100	100
	Colloids	22.28	18.30 *	22.39	20.78 *	27.80	37.4	31.9	42.9	79.4	74.8	68.6	74.8
	Dissolved	5.74	6.16	10.23	7.02	7.20	12.8	8.9	14.5 *	20.6	25.2	31.4	25.3 *
TC	Liquid slurry	3.41	2.10	3.90	2.52	22.68	23.8	29.6	28.4	100	100	100	100
	Colloids	2.82	1.29	3.22	1.81	15.22	13.0	24.5	20.4	80.6	54.5	82.6	71.8
	Dissolved	0.59	0.81	0.68	0.71	3.06	10.8	5.2	8.0	19.4	45.5	17.4	28.2
TOC	Liquid slurry	16.20	21.18	16.65	15.54	-	-	-	-	100	100	100	100
	Colloids	15.27	19.90	15.53	14.35	-	-	-	-	94.4	93.9	93.3	92.4
	Dissolved	0.92	1.28	1.12	1.19	-	-	-	-	5.6	6.1	6.7	7.6
NH_4^+	Liquid slurry	0.11	0.41	0.49	0.77	82.10	82.7	88.4	85.7	100	100	100	100
	Colloids	0.01	0.08	0.26	0.26	5.50	14.7	47.0	28.5	6.7	17.1	53.2	33.2
	Dissolved	0.10	0.33	0.23	0.51 *	76.60	68.0	41.4	57.2	93.3	83.0	46.8	66.8

Treatment	Particle size fraction	Quantity g kg^{-1}				Percentage in whole %				Percentage in the liquid fraction %			
		NON	AD	NON+	AD+	NON	AD	NON+	AD+	NON	AD	NON+	AD+
TN	Liquid slurry	2.79	3.57	2.63	5.14 *	2.31	80.2	76.2	83.6 *	100	100	100	100
	Colloids	0.10	1.43	0.72	3.08 *	-57.70	28.9	20.8	50.1 *	3.3	36.0	27.3	59.9 *
	Dissolved	2.69	2.14	1.91	2.06	60.01	51.3	55.3	33.5 *	96.7	64.0	72.7	40.1 *
Water extractable P	Liquid slurry	0.10	0.18 *	0.12	0.22 *	77.80	82.6 *	78.6	82.6	100	100	100	100
	Colloids	0.09	0.17 *	0.11	0.22 *	66.70	80.0 *	69.0	81.0 *	85.7	96.9 *	87.7	98.1 *
	Dissolved	0.01	0.00 *	0.01	0.00 *	11.10	2.6 *	9.6	1.6 *	14.3	3.1 *	12.3	1.9 *
TP	Liquid slurry	0.29	0.30	0.37	0.34	66.90	66.4	66.2	67.1	100	100	100	100
	Colloids	0.27	0.28	0.35	0.33	62.10	62.0	62.4	64.8	92.9	93.4	94.2	96.6
	Dissolved	0.02	0.02	0.02	0.01	4.80	4.4	3.8	2.3	7.1	6.6	5.8	3.5

Note: * indicates the differences between NON and AD, or between NON+ and AD+ treatments are statistically significant ($p<0.05$).

Anaerobic digestion increased the quantity of NH_4^+, organic N and TN of the separated liquid slurry fraction, although the differences between NON and AD treatments were not statistically significant. The difference in the TN quantity of the separated liquid slurry fraction was significant between NON+ and AD+ treatments. In contrast to acidification, the increase in the NH_4^+ quantity of the separated liquid slurry fraction caused by digestion was associated with the dissolved fraction rather the colloidal fraction (Table 5.4). But the increase of organic N and TN quantity of the liquid fraction was more associated with the colloidal fraction (Table 5.4). Decomposition of organic matter during anaerobic digestion results in mineralisation of organic N to NH_3, solubilisation of which resulted in the increase of NH_4^+ and OH^- content in the dissolved fraction. On the other hand, although the organic N in the liquid fraction was mineralised, the breakdown of coarse particles supplemented the organic N quantity of the liquid fraction, and tended to concentrate on colloids rather than in a dissolved form. Thus, the organic N quantity of the liquid fraction was not reduced, in contrast, it increased.

There was no impact on TP quantities of these fractions after anaerobic digestion. But the water extractable P content of the separated liquid slurry fraction increased after anaerobic digestion (Table 5.4). In contrast to acidification, the water extractable P quantity of the dissolved fraction decreased in both NON and NON+ treatments by 0.01 g kg^{-1} fresh whole slurry after anaerobic digestion, due to precipitation of dissolved inorganic P with Ca and Mg (Güngör & Karthikeyan, 2008; Marcato et al., 2008; Rico et al., 2012). But the water extractable P quantity of the colloidal fraction increased by 0.08-0.11 g kg^{-1} fresh whole slurry (Table 5.4), contributing to the increase in the separated liquid slurry fraction. This increase in P content in the colloidal fraction may be related to decomposition of organic P during the anaerobic digestion process. As a result, in the separated liquid slurry fraction, the percentage of water extractable P represented by the colloidal fraction increased, and the percentage of the dissolved fraction decreased. This water extractable result is different from the IP-H_2O from sequential fractionation, which is discussed in more detail in section 5.4.3.2. The added inoculant treatment did not make a significant difference in P distribution and speciation in our study.

Since the water extractable P/TP ratio for the separated liquid slurry fraction increased, and the water extractable P in the colloidal fraction increased after anaerobic digestion, the risk of colloid-facilitated P leaching could be greater from the anaerobic digestion treatment, because more bioavailable P may transfer into water. This study also suggests that colloid-facilitated organic N leaching from digested slurry may also be of concern.

5.4.2 Nanoparticles in livestock slurry

Within the dissolved fraction, nanoparticles existed and comprised inorganic and organic compounds. In non-acidified slurry, significant differences were seen between the <0.03 and <0.45 µm fractions in the inorganic P, organic P, TP, and TC contents, indicating that nanoparticles between 0.03-0.45 µm contained inorganic and organic P compounds, and carbonate crystal or organic carbon. However, no NH_4^+ or TN was found in this nanoparticulate range. The TOC content of the nanoparticle size fractions (Table 5.5) appeared to be greater than the TC content. This may be because of dilution errors associated with analysis of the TOC, which often required diluting by >200 times to fit within the detection range of the instrument and protect the needles from being damaged by the high salinity. Such dilutions may amplify errors during calculations. However, we still have confidence in the use of these data to explore the relationship between TOC contents of different nanoparticle size fractions. There is a trend that the TOC quantity decreases with the reduction of the ultrafilter size in both the non-acidified and acidified treatment, but differences between the two successive fractions were not significant (Table 5.5). This may indicate that organic carbon exists within the 0.03-0.45 µm or within 0.001-0.03 µm size fractions as nanoparticles, but they were not detected because of the large variability. Through calculating the quantities of inorganic P, organic P, TP, TOC and TC in the nanoparticles by differences between two successive fractions, we found that over half of inorganic P, organic P and TP, 40% of TOC and 26% of TC of the dissolved fraction was seen in the nanoparticulate fraction of 0.03-0.45 µm.

Table 5.5 The distribution of NH_4^+, TN, TC, inorganic P and TP within the dissolved fractions of non-acidified and acidified treatments (mg kg^{-1} based on fresh weight of whole slurry). Values represent means, n = 3.

Treatment	Separated fraction µm	NH_4^+	TN	Inorganic P	TP	TOC	TC
Non-acidified	<0.45	133	3667	20 a^{Ɨ}	28 a^{Ɨ}	1258 a^{Ɨ}	807 a
	<0.03	140	3279	9 b^{Ɨ}	13 b^{Ɨ}	733 ab^{Ɨ}	599 b
	<0.001	134	3279	9 b^{Ɨ}	12 b^{Ɨ}	617 b^{Ɨ}	469 bƗ
		ns	ns	*	*	*	*
Acidified	<0.45	154	3405	205	244 a	2048 a	1019
	<0.03	158	3664	203	238 a	1754 ab	717
	<0.001	147	3291	196	225 b	1535 b	821
		ns	ns	ns	*	*	ns

Note: ns indicates no difference, and * indicates significant difference between three fractions and the letter a,b,c indicates the significant difference between three fractions of <0.45, <0.03 and <0.001 in the same slurry ($p<0.05$).^{Ɨ} indicates the significant difference between the non-acidification and acidification treatments ($p<0.05$).

Acidification increased the inorganic P, organic P, TP and TOC contents of the <0.45, <0.03 and <0.001µm fractions markedly ($p<0.05$) (Table 5.5). But the NH_4^+ and TN quantities of these fractions were not influenced by the acidification treatment. In the acidified slurry, not only was there no difference between <0.03 and <0.45 µm fractions in NH_4^+ and TN, but such difference in the TC content, inorganic P, organic P and TP also disappeared. This change might because that P bound in nanoparticles of 0.03-0.45µm were dissolved by acid, or nanoparticles of 0.03-0.45 µm were degraded releasing P during acidification, transforming P to the <0.03 µm fraction. The difference observed in the untreated slurry, in TC content between the <0.45 and <0.03 µm fractions was not apparent in the acidified slurry, perhaps due to the large variability. The TOC content of the 0.03-0.45 µm and 0.001-0.03 µm and 0.001-0.45 µm nanoparticle ranges, showed no significant differences between non-acidified and acidified treatments. So the increased TOC content caused by acidification was generally found in the <0.001 µm fraction. That is also the reason for the significant increase in the TC content of that fraction.

Anaerobic digestion tended to increase the NH_4^+ concentration of the <0.45, <0.03 and <0.001 µm fractions, although the differences between the NON and AD treatments were not significant (Table 5.6). The TN quantity of these fractions was stable during anaerobic digestion. In contrast to the acidification treatment, anaerobically digested slurry still contained nanoparticles associated with inorganic P, organic P and TP in the fraction of 0.03-0.45 µm, based on the significant difference between <0.03 and <0.45 µm fractions. The formation of struvite and Ca-P may occur (Marcato et al., 2008; Güngör & Karthikeyan, 2008) and influence the inorganic P content in these fractions. The inorganic P content of the <0.45, <0.03 and <0.001 µm fractions tended to decrease after anaerobic digestion, although not significantly in the <0.03 and <0.001 µm fractions. Also, the inorganic P content of the nanoparticles in the 0.03-0.45 µm fraction of anaerobically digested slurry seemed lower than in non-digested slurry, although this was only significant between the NON+ and AD+ treatments. The organic P and TP quantities of nanoparticles between 0.03-0.45 µm were not affected by anaerobic digestion. But the organic P and TP quantities of the <0.001 µm were significantly reduced by anaerobic digestion, comparing both the NON and AD, and NON+ and AD+ treatments. In contrast, the impact of anaerobic digestions on TOC content was rarely seen in these fractions. The smallest organic P compound in the <0.001 µm fraction may be the easiest to be decomposed, but the magnitudes of organic P and TOC of the <0.001 µm fraction were not the same, so the decomposition of organic P could not be reflected in the TOC change.

The DM, TOC, TC, and the P quantity associated with nanoparticles (0.001-0.03, 0.03-0.45µm) were very small compared to the colloidal fractions. Therefore, the impact of nanoparticles on potential P leaching in soil may be small. The reported importance of nanoparticles in P mobility and pollution in other studies may be more associated with the native nanoparticles developed within soils, rather than organic matter nanoparticles associated with slurry amendments. Relatively, the colloidal fractions of slurry may have a larger influence on P leaching than the nanoparticles of slurry.

Table 5.6 The distribution of NH_4^+, TN, TC, inorganic P and TP within the dissolved fractions of untreated and anaerobic digestion treatments (mg kg^{-1} based on fresh weight of whole slurry). Values represent means, n = 3.

Treatment	Separated fraction µm	NH_4^+	TN	Inorganic P	TP	TOC	TC
NON	<0.45	98	2691	14 a^{ƚ}	21 a	923 a	592 a
	<0.03	102	2406	6 b	9 b	538 ab^{ƚ}	440 b
	<0.001	98	2406	6 b	9 b	453 b	344 b
		ns	ns	*	*	*	*
AD	<0.45	334	2143	5 a	20 a	1282 a	806 a
	<0.03	306	2280	4 b	6 b	866 ab	576 ab
	<0.001	306	1985	4 b	5 b	551 b	406 b
		ns	ns	*	*	*	*
NON+	<0.45	229 ^{ƚ}	1907 a	14 a^{ƚ}	21 a	1119	679 a
	<0.03	230	1736 ab	7 b	10 b	834	584 a
	<0.001	229 ^{ƚ}	1554 b	6 b	9 b^{ƚ}	612	363 b
		ns	*	*	*	ns	*
AD+	<0.45	506	2061	4 a	12 a	1187 a	706 a
	<0.03	502	2234	4 b	6 ab	1085 a	553 ab
	<0.001	490	1805	3 b	4 b	522 b	350 b
		ns	ns	*	*	*	*

Note: ns indicates no difference, and * indicates significant difference between three fractions and the letter a,b,c indicates the significant difference between three fractions of <0.45, <0.03 and <0.001 in the same slurry ($p<0.05$).^{ƚ} indicates the significant difference between the NON and AD, or between NON+ and AD+ treatments ($p<0.05$).

5.4.3 P fractionation in the colloidal fraction

Comparison between the untreated and acidified slurry

The colloidal fraction of untreated and treated slurry is important in storing P and facilitating P movement through and from soil as described above. To better understand this, further analysis was conducted on the P speciation on this colloidal fraction by sequential fractionation.

According to our results (Table 5.3), the majority of P in each form in the separated liquid slurry fraction was allocated to the colloidal fraction in the non-acidified slurry. Specifically, particles of the colloidal fraction represented almost all of the IP-H_2O, IP-NaOH, IP-HCl, and OP-NaOH forms, and TP-residue of the separated liquid slurry fraction. Also, 60% of the IP-$NaHCO_3$, 32% of OP-H_2O, 77% of OP-$NaHCO_3$ and 64% of OP-HCl content of the separated liquid slurry fraction was seen in the colloidal fraction. However, in the acidified slurry this situation changed significantly. After acidification, the quantity of the four IP forms and OP-$NaHCO_3$ decreased significantly (Figure 5.1). P-$NaHCO_3$, P-NaOH and P-HCl represent P that is physically adsorbed on crystalline surfaces, associated with the surface of Ca carbonates, Al and Fe oxides, and mineral or amorphous phases of Ca-P, respectively (He et al., 2006). The reduction caused by acidification was mainly associated with dissolution and desorption, transforming P from colloids to the dissolved fraction. As a result, in the acidified separated liquid slurry fraction, only 24% of IP-H_2O, 15% of IP-$NaHCO_3$, 50% of IP-NaOH and 62% of IP-HCl forms were in the colloidal fraction, respectively.

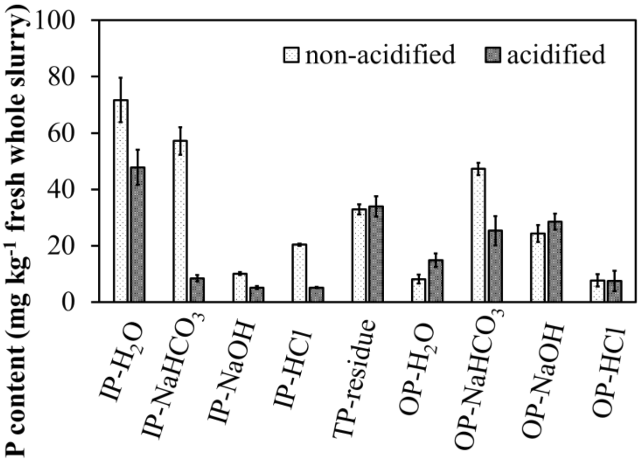

Figure 5.1 The contents of P forms of the colloidal fractions of non-acidified and acidified cattle slurry. Values represent means ± SEM, n = 3.

The water extractable P quantity of the non-acidified and acidified colloidal fraction in Table 5.3 was lower than the value of IP-H_2O by sequential fractionation. This may be because the extraction time of sequential fractionation was longer than the 0.5 M K_2SO_4 extraction, leading to more P solubilised in the extractant. But the difference between non-acidified and acidified colloidal fractions of these two extractions showed the same trend.

As for the P distribution in the colloidal fraction, in the non-acidified fraction, the IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl, OP-H_2O, OP-$NaHCO_3$, OP-NaOH, OP-HCl and TP-residue forms accounted for 25%, 20%, 4%, 7%, 3%, 17%, 9%, 3% and 12% of the TP, respectively. After acidification, the percentage represented by IP-$NaHCO_3$, IP-NaOH and IP-HCl forms decreased to 5%, 3% and 3%, respectively, while the proportion of TP-residue, OP-H_2O and OP-NaOH forms increased to 19%, 8% and 16%, respectively. We speculate that colloid-facilitated P leaching from acidified slurry may be dominated by organic P.

Comparison between the untreated and anaerobic digestion

Anaerobic digestion decreased the quantity of IP-H_2O, TP-residue and OP-$NaHCO_3$ forms of the colloidal fraction, and increased the quantity of IP-$NaHCO_3$ and IP-HCl forms (Figure 5.2). Specifically, comparing the colloidal fraction of the NON and AD treatments, 52% of the quantity of IP-H_2O, 17% of TP-residue and 57% of OP-$NaHCO_3$ form of the colloidal fraction was reduced after anaerobic digestion. And the IP-$NaHCO_3$ and IP-HCl quantities of the colloidal fraction increased by 165% and 79%, respectively. Comparing the NON+ and AD+ treatments, the quantity of IP-H_2O, TP-residue and OP-$NaHCO_3$ forms in the colloidal fraction was reduced by 41%, 21% and 74%, while the quantity of $NaHCO_3$ and IP-HCl forms increased by 149% and 13%, respectively. The IP-H_2O quantity of the colloidal fraction may be reduced mainly by adsorption and formation of struvite and Ca-P (Cooperband & Good, 2002; He et al., 2004; Marcato et al., 2008; Güngör & Karthikeyan, 2008), resulting in the increase of IP-$NaHCO_3$ and IP-HCl forms. Another explanation for the marked increase in the IP-$NaHCO_3$ quantity of the colloidal fraction was the break-down of larger particle size fractions. Anaerobic digestion was performed on the whole cattle slurry and biodegradation processes occurred in each particle size fraction, producing more labile IP. After separation, most of this released labile IP was concentrated in the liquid fraction. And because of the adsorption capacity of colloidal particles, much labile IP remained in this particle fraction as IP-$NaHCO_3$.

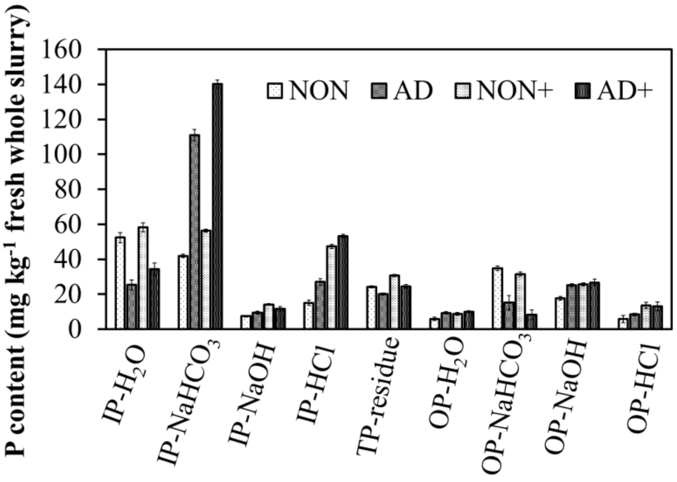

Figure 5.2 The quantities of P forms in the colloidal fraction on a fresh basis of whole slurry. Values represent means ± SEM, n = 3.

Comparing the AD and AD+ treatments, no differences were seen in the quantities of OP forms of the colloidal fraction. But the quantity of almost all IP forms except for IP-NaOH and TP of the AD+ slurry of the colloidal fraction was higher than the AD slurry. Based on our results of the NON and NON+ treatments, this difference in IP forms was mainly because of the original high amount of IP in the inoculum used in the AD+ treatment, rather than accelerated decomposition caused by inoculation.

The quantity of water extractable P in the colloidal fraction of the NON and NON+ treatments (Table 5.4) was lower than the value for IP-H_2O measured by sequential fractionation. The reason for this is the same as that explained earlier, but for AD and AD+ the opposite was true. This may be because the extractions for sequential fractionation were conducted on air dried samples, while the 0.5 M K_2SO_4 extraction used fresh samples. In the AD and AD+ treatments, perhaps the high pH condition made the water soluble P more susceptible to being adsorbed on the surface of particles during air drying, contributing to the IP-$NaHCO_3$ form. Therefore, although the quantity of IP-H_2O of non-digested slurry was higher than anaerobically digested slurry, which is in contrast to the conclusion of water extractable P, the sum of the IP-H_2O and IP-$NaHCO_3$ forms in the non-digested slurry was smaller than anaerobically digested slurry.

The colloidal fractions in both non-digested and digested liquid slurry accounted for the same proportion of TP. Thus, when applying slurry based on a TP loading, colloids from digested liquid slurry may transport more bioavailable P into the subsurface, potentially leading to greater P transfer to watercourses than non-digested slurry. Therefore, recovering the colloidal fraction and P could be a direction to improve the separation strategy in livestock slurry management.

5.5 Conclusions

The colloidal fraction accounted for over 80% of the DM, TC, and TP content, and all other measured P forms in untreated liquid fraction of cattle slurry. While over 90% of the NH_4^+ and TN in untreated liquid fraction of cattle slurry was present in the dissolved fraction. Nanoparticles existed in the 0.03-0.45µm range and contained only a small amount of P (4% of the TP of the liquid fraction, 8% of the water extractable P of the liquid fraction).

Acidification increased the quantity of NH_4^+ and TN in the separated liquid cattle slurry fraction, which was associated with colloidal fraction, while the increase in NH_4^+ quantity of liquid cattle slurry caused by anaerobic digestion was mainly related to the dissolved fraction. In the dissolved fraction, the inorganic P and TP quantity increased by *ca.* 10 times after acidification, but the content of these P forms was reduced in the colloidal fraction. In contrast, after anaerobic digestion, the water extractable P quantity of the colloidal fraction increased, while in the dissolved fraction it declined. There was a smaller quantity of P on nanoparticles after anaerobic digestion, while there was almost no P left on nanoparticles after acidification. The TP quantity of the colloidal fraction was not affected by acidification or anaerobic digestion. In the P fractionation, acidification decreased the quantity of IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl, TP-residue and OP-$NaHCO_3$ forms of the colloidal fraction significantly. While anaerobic digestion decreased the quantity of IP-H_2O, TP-residue and OP-$NaHCO_3$ forms of the colloidal fraction, and increased the quantity of IP-$NaHCO_3$ and IP-HCl forms.

The characteristics of colloids may regulate N and P potential losses from soil. Both acidification and anaerobic digestion improved the agronomic benefit of liquid livestock slurry, but may increase the risk of N leaching. Moreover, the anaerobically digested liquid slurry may increase the risk of potential P leaching because of its effect on available P content of the colloidal fraction.

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6 The colloidal phosphorus fraction regulates the vertical movement of P after the application of treated cattle slurry to soil

6.1 Abstract

Slurry processing such as acidification and anaerobic digestion are being used to retain nitrogen within the manure management chain, and generate renewable energy, respectively. However, these processes may modify the chemical form and molecular weight of organic and inorganic phosphorus (P) in slurry, altering their availability to plants, as well as their potential for transfer to watercourses. To better understand the impact of applying treated cattle slurry on soil P movement, three particle size fractions (<63, <0.45 and <0.03 µm) of untreated, acidified and anaerobically digested cattle slurry were applied to the surface of an agricultural sand-textured soil in laboratory microcosms and subjected to leaching. After application of simulated rainfall, the location of the slurry-derived P in the soil was determined by performing Olsen-P and total-P (TP) extracts at different depths (0-16cm). Inorganic P (IP) and TP contents of leachates were also measured. The results showed that P leaching from the livestock slurry was largely associated with the colloidal slurry fraction (0.45-63 µm). Slurry acidification and anaerobic digestion both increased the proportion of IP in the <63 µm fraction. However, whilst acidification resulted in no measurable increase in P leaching, anaerobic digestion increased potential P leaching in comparison with untreated slurry. We ascribe both to the importance of the colloidal fraction. We conclude that the colloidal fraction is a key regulator of movement of slurry-derived P in soil and that pre-treating slurry has the potential to significantly alter this process.

6.2 Introduction

Livestock slurry contains large amounts of phosphorus (P) and is frequently applied to soil to recycle essential nutrients required for crop growth (Dao & Schwartz, 2010). However, if livestock slurry is repeatedly applied to fields, P gradually accumulates and is at greater risk of transfer to water bodies via runoff and leaching (McDowell & Sharpley, 2001; Volf et al., 2007; Hahn et al., 2012; Liu et al., 2012). It is thought that the progressive saturation of P sorption sites in soil, and the input of colloidal slurry particles (which act as P 'carriers') are two main factors that contribute to the loss of livestock slurry-derived P (Sharpley et al., 1994; Siemens et al., 2004).

After application to soil, P contained within livestock slurry can be sorped to Al and Fe oxyhydroxide, Ca and clay surfaces until the mineral surfaces become saturated, at which point P becomes susceptible to desorption and leaching (Hinsinger, 2001; McGechan & Lewis, 2002a; Guppy et al., 2005). The sorption affinity of organic P (OP) to soil has been shown to be lower than for inorganic P (IP) (Anderson & Magdoff, 2005), except phytate (Leytem et al., 2002). Toor et al. (2003) found organic P leaching prevailed in grassland receiving dairy slurry. Further, organic matter competes with P for sorption sites and decreases P sorption (Perassi & Borgnino, 2014; Kruse et al., 2015).

Colloid- and nanoparticle- facilitated P transport in soil has been reported to dominate P loss from P unsaturated soils (Turner et al., 2003; de Jonge et al., 2004; Heathwaite et al., 2005; Makris et al., 2006; Schelde et al., 2006; Regelink et al., 2013). However, Rick & Arai (2011) questioned the role of nanoparticles in P transport, based on the unreactive results from their P desorption and P retention and release experiments. Slurry is an important source of colloids and nanoparticles that are already carrying considerable sorbed P when applied to the soil (McGechan & Lewis, 2002b).

Different slurry management regimes are increasingly being utilised within the livestock industry to achieve greater sustainability. For example, acidification of slurry can greatly reduce ammonia volatilisation leading to increased N retention (Kai et al. 2008), while anaerobic digestion can be used to generate CH_4 for renewable energy production (Clemens et al., 2006; Lee & Han, 2012). These processes, however, are also expected to lead to shifts in a range of chemical, biological and physical properties of the slurry that may affect their subsequent behaviour after addition to soil. For example, studies have shown that acidification can increase the dissolved P content of slurry (Güngör et al., 2007; Fangueiro et al., 2015). It implies soil amended with acidified slurry could be more subjected to P leaching from saturated soil in comparison with untreated slurry. However, P sorption in soil increases with decreased pH (Gustafsson et al., 2012). Acidified slurry may cause greater P sorption. It is difficult to know if the greater dissolved P content and greater P sorption in acidified treatment would result in more or less P leaching. No information on the effect of acidified slurry on P leaching has been revealed yet, as far as we aware.

Anaerobic digestion with stirred and high temperature conditions are more likely to degrade large particles in comparison to smaller particles (Hjorth et al. 2010). Marcato et al. (2008) observed that <10% of particles in a pig slurry and a digested slurry were <3 µm, and particles of 3-25 µm made a strong contribution to the total specific surface area (30% and 40% for untreated and digested slurry). They also suggested that anaerobic digestion resulted in greater biodegradation of smaller sized organic matter particles <3 µm. This is supported by Elmitwalli et al. (2001) and Shon et al. (2006) who observed that particles <1 µm were most susceptible to biodegradation, while particles >10 µm appeared more recalcitrant during anaerobic digestion. Stutter (2015) observed that anaerobic digestate reduced P sorption in soil columns, suggesting an increased short-term leaching risk after digestate application. However, information on the effect of anaerobically digested slurries on P loss are still limited.

The aim of this study was to explore the effect of the liquid (<63 µm) fractions of differently treated livestock slurry on the potential P leaching from soil, especially the role of colloid- and nanoparticle-associated P in potential P vertical movement. We hypothesized that, (1) both acidification and anaerobic digestion would increase solubility of P contained in liquid livestock slurry, (2) addition of liquid livestock slurry, untreated and treated, to soil would increase potential P leaching from the soil, and this would be particularly associated to the colloidal fraction, and (3) compared with untreated liquid slurry, acidification and anaerobic digestion would reduce mobility of P and potential P leaching by conversion of colloidal P to orthophosphate which readily sorbs to mineral surfaces, reducing its vertical movement in soil.

6.3 Materials and methods

6.3.1 Livestock slurry collection and treatment

Four replicates of cattle slurry samples were collected on four independent occasions from a commercial beef farm in Gwynedd, Wales, UK. Slurry samples were then treated by acidification or anaerobic digestion. Four replicates of cattle slurry were acidified to pH 5.5 by adding 15% H_2SO_4 to achieve a final sulfuric acid concentration of *ca.* 100 mM in the slurry. Anaerobic digestion with three replicates (due to the space limitation in the laboratory incubator used for the anaerobic digestion of the slurry) was conducted in the laboratory using two approaches, i) with a 30% (v/v) inoculum (collected from a nearby commercial anaerobic digester receiving cattle slurry and food waste feedstocks), and ii) without an inoculum. The dry matter (DM) contents of both slurries was adjusted to 8% with distilled water prior to incubation and then sealed in containers connected to foil bags for gas collection, and incubated at 35°C for 30 d. The containers were shaken gently for 1 h every day and the gas in the bags was released when full.

Replicate batches of untreated cattle slurry, acidified cattle slurry, anaerobically digested slurry inoculated (AD+) and non-inoculated (AD) slurry were wet-sieved with 63 µm for the liquid fraction of slurry (<63 µm). The liquid fraction was vacuum filtered through a 0.45 µm membrane for the <0.45 µm fraction. The <0.45 µm fraction was subsequently ultrafiltered into <0.03 µm fractions with 100 kDa filters (Millipore Ultracel® 100 kDa Ultrafiltration Discs) (Turner et al., 2004). The weight ratio of slurry before and after 0.45 µm filtration was recorded. It was assumed the volume and weight of slurry before and after 100 kDa ultrafiltration was same. The TP content of the <63 µm fraction was measured after combusting samples at 550°C and dissolving the ash in 2.4 M HCl. The TP content of the <0.45 and <0.03 µm fractions were measured after digestion with ammonium persulfate in an autoclave (Rowland & Haygarth, 1997). TP and IP concentrations in digests and slurries were subsequently determined using the molybdate blue method of Murphy & Riley (1962). The difference between the IP and TP content of a fraction was assumed to be organic P (OP). The values for the 0.03-0.45 µm and 0.45-63 µm fraction were calculated by difference.

6.3.2 Soil collection

The sand textured Eutric Cambisol (80% sand, 12% silt, 8% clay) was collected from the upper soil layer (0-15 cm; Ahp horizon) of a grassland field located in Abergwyngregyn, Gwynedd, Wales, UK (53° 14' 33" N, 4° 1' 15" W). The site has a temperate oceanic climate with mean annual temperature of 10.6°C and annual rainfall of 1060 mm. After collection, the soil was sieved to pass 2 mm to remove roots and stones. The soil had a gravimetric moisture content of 19.2 %, a pH of 5.84 (1:2.5 w/v distilled water extract), electrical conductivity of 30 µS cm^{-1} (1:5 w/v distilled water extract), Olsen-P content of 9.2 mg kg^{-1} and a total P content of 0.4 g kg^{-1}.

6.3.3 Phosphorus sorption isotherms

To determine the relative affinity of inorganic P for soil, an adsorption isotherm was performed. Briefly, soil (1 g) was shaken (200 rev min^{-1}, 24 h, 20°C) with solutions (25 ml) containing different levels of P (0 to 80 mg l^{-1} in 0.01 M $CaCl_2$). Chloroform (25 µl) was added to the tubes as an inhibitor of microbial activity. After centrifugation (3000 *g*, 15 min), the equilibrium P concentration in the supernatant was determined using the molybdate blue method of Murphy & Riley (1962). The Langmuir Equation

$$C/S = 1/(k \times S_{\max}) + C/S_{\max} \qquad \text{(Eqn. 1)}$$

was then fitted to the experimental data, where S is the amount of P adsorbed to the solid phase (mg kg^{-1}), k is affinity constant (L mg^{-1}), $S_{\max}$ is sorption maxima (mg kg^{-1}), and C is the equilibrium solution P concentration (mg L^{-1}). A similar sorption isotherm was also carried out for the <63 µm cattle slurry fraction as described above.

6.3.4 Phosphorus leaching experiment

Following the method described in Abaas et al. (2012), polyethylene tubes (0.8 cm diameter, 16 cm long) were packed with 9.60 g (fresh weight) of field-moist soil and suspended over a leachate collection vial. The 4 slurry types (acidified, digested + inoculum, digested – inoculum, untreated) each with 3 size fractions (<63 µm, <0.45 µm, <0.03 µm) and a control of distilled water were added to the surface of individual soil columns. There were 8 columns for each treatment, including 4 replicates for Olsen-P determination and 4 replicates for TP determination, except for AD and AD+ treatments with 6 columns. The slurry application rate for all treatments were based on the same weight of the <63 µm fraction, which was applied at an equivalent of 10 kg TP ha^{-1} (49 µg TP $column^{-1}$). The summary of the application rates is shown in Table 1.

After slurry application, the surfaces of the columns were sealed with Parafilm M® (Bemis Inc., Oshkosh, WI) to reduce evaporative water loss and the columns placed in a climate-control room at 10 °C. After 24 h, 2 ml of distilled water (equivalent to a 1 h rainfall storm event of 40 mm h^{-1}) was applied to the soil surface of each column. After 48 h, the leachate was recovered, its volume recorded, and the columns sectioned using a scalpel into the following depths; 0-1, 1-2, 2-3, 3-4, 4-6, 6-8, 8-10 and 10-16 cm. Soil samples recovered from each depth were analysed for Olsen P (0.5 M $NaHCO_3$), TP (aqua regia digest) and gravimetric moisture content (105°C, 24 h). The TP concentrations of leachates were measured after digestion with ammonium persulfate in an autoclave (Rowland & Haygarth, 1997) and determined using the molybdate blue method (Murphy & Riley, 1962). Aliquots of leachates were directly measured by the molybdate blue method for IP concentrations. OP concentration of leachates was calculated by the difference between the IP and TP concentration.

6.3.5 Statistical analysis

All statistical analysis was performed with SPSS v22 (IBM Inc., Armonk, NY). Differences in IP, OP and TP leachate concentrations and Olsen-P and TP contents of soil between any slurry amended treatment and control were analysed using unpaired t-tests, and between untreated and treated slurry treatments were analysed by paired t-tests, both with effects considered significant at $p<0.05$. Comparisons of Olsen-P and TP among different soil depths within the same column were conducted by ANOVA with Tukey pairwise post-hoc testing with effects considered significant at $p<0.05$.

6.4 Results and Discussion

6.4.1 Phosphorus distribution in untreated and treated slurry

Within the cattle slurry (<63 µm), 28% of the TP was present as IP (Table 6.1). After size fractionation, we found that the majority of P was present in the 0.45-63 µm particle fraction, representing *ca.* 80% of the total IP and 95% of the total OP present in the slurry (<63 µm). The nanoparticulate fraction (0.03-0.45 µm) contained a small amount of P according to the significant difference between the <0.45 and <0.03 µm fractions. Further, the majority of TP of the <0.45 and <0.03 µm fraction was present as IP (64% and 63% respectively).

Acidification was conducted on the whole cattle slurry before separation and released IP from coarse particles, resulting in a large increase in the IP content of the <63 µm slurry (from 28% to 49% of the TP) while the IP content of the <0.45 and <0.03 µm slurry also rose 6-fold (Table 6.1). Concomitantly, around 38% of the IP, 31% of the OP and 35% of TP of the 0.45-63 µm fraction was lost during acidification. The nanoparticulate fraction (0.03-0.45 µm) contained no detectable amount of IP or TP after acidification.

Table 6.1 Application rates for different slurry treatments. Values represent means, n = 4 for untreated and acidified treatments, n = 3 for AD and AD+ treatments.

	Particle fraction µm	TP µg column^{-1}	IP µg column^{-1}	OP µg column^{-1}	IP/TP of <63 µm %	OP/TP of <63 µm %	TP/TP of <63 µm %
Untreated	<63	48.9	13.7	35.2	28.3	71.7	100
	0.45-63	44.3	10.8	33.5	22.5	68.4	90.9
	<0.45	4.6	2.9	1.7	5.8	3.3	9.1
	0.03-0.45	1.0	0.6	0.4	1.4	0.7	2.1
	<0.03	3.6	2.3	1.3	4.4	2.6	7.0
Acidified	<63	48.9	23.8	25.1	48.8	51.2	100
	0.45-63	28.7	6.2	22.5	12.7	46.3	59.0
	<0.45	20.2	17.6	2.6	36.1	4.9	41.0
	0.03-0.45	1.0	-0.3	1.3	-0.4	2.3	1.9
	<0.03	19.2	17.9	1.3	36.5	2.6	39.1
AD	<63	48.9	28.4	20.5	58.2	41.8	100
	0.45-63	45.6	27.4	18.2	56.4	37.0	93.4
	<0.45	3.3	1.0	2.3	1.8	4.8	6.6
	0.03-0.45	2.3	0.3	2.0	0.7	3.9	4.6
	<0.03	1.0	0.7	0.3	1.1	0.9	2.0
AD+	<63	48.9	30.7	18.2	62.9	37.1	100
	0.45-63	47.3	30.0	17.3	61.7	34.8	96.5
	<0.45	1.6	0.7	0.9	1.2	2.3	3.5
	0.03-0.45	0.6	0.0	0.6	0.2	1.6	1.8
	<0.03	1.0	0.7	0.3	1.0	0.7	1.7

Anaerobic digestion significantly increased the proportion of IP and decreased OP in the TP of <63 µm cattle slurry irrespective of inoculation status (Table 6.1). This was most evident in the 0.45-63 µm particle fraction, in which IP proportion increased from 28% to 60% (AD) or 64% (AD+) and OP proportion decreased from 72% to 40% (AD) or 36% (AD+). Further, more TP was observed in the 0.45-63 µm fraction (p<0.05). On the contrary, less TP and IP was seen in the <0.45 and <0.03 µm fractions (Table 6.1), although the decrease of TP of the <0.45 µm fraction was not statistically significant. In contrast to the acidified slurry, the nanoparticulate fraction (0.03-0.45 µm) was found to contain P. No significant difference in IP, OP and TP was found between the AD and AD+ treatments.

6.4.2 Effect of untreated cattle slurry on P movement in soils

In this study, the Langmuir Equation for the P sorption isotherm of this sandy soil was $C/S = 0.0045 + 0.0038C$. The measured soil P sorption maxima ($S_{\max}$) was 86 mg kg^{-1}, and ‘k’ was 58480 L mg^{-1}. To avoid saturation-driven P leaching, the application rate selected in this study was 49 µg column^{-1} of TP of <63 µm slurry (=10 kg TP ha^{-1}), containing 14 µg column^{-1} of IP (Table 6.1), and the adsorption capacity of the first 1 cm soil already exceeded the IP amount from the <63 µm cattle slurry.

The Olsen-P contents at the different soil depths is shown in Figure 6.1. Due to the short period of this experiment (48 h) and low temperature (10 °C), OP had little chance to mineralize into IP. Thus, the Olsen-P content of different depths of soil was more associated with the movement of IP. In the control treatment, no change in the Olsen-P content of the soil was seen at the different depths (8-9 mg kg^{-1}), except a slight decrease in the last 10-16 cm layer (6 mg kg^{-1}) ($p<0.05$) (Figure 6.1). However, in the <63 µm treatment there was a significant decreasing trend down the column ($p<0.05$). The 2-3 cm layer of soil in the <63 µm treatment contained significantly higher Olsen-P (11 mg kg^{-1}) content than the control (Figure 6.1). It is implied that P from the <63 µm slurry was transported over 1 cm in the soil profile and some was gradually retained.

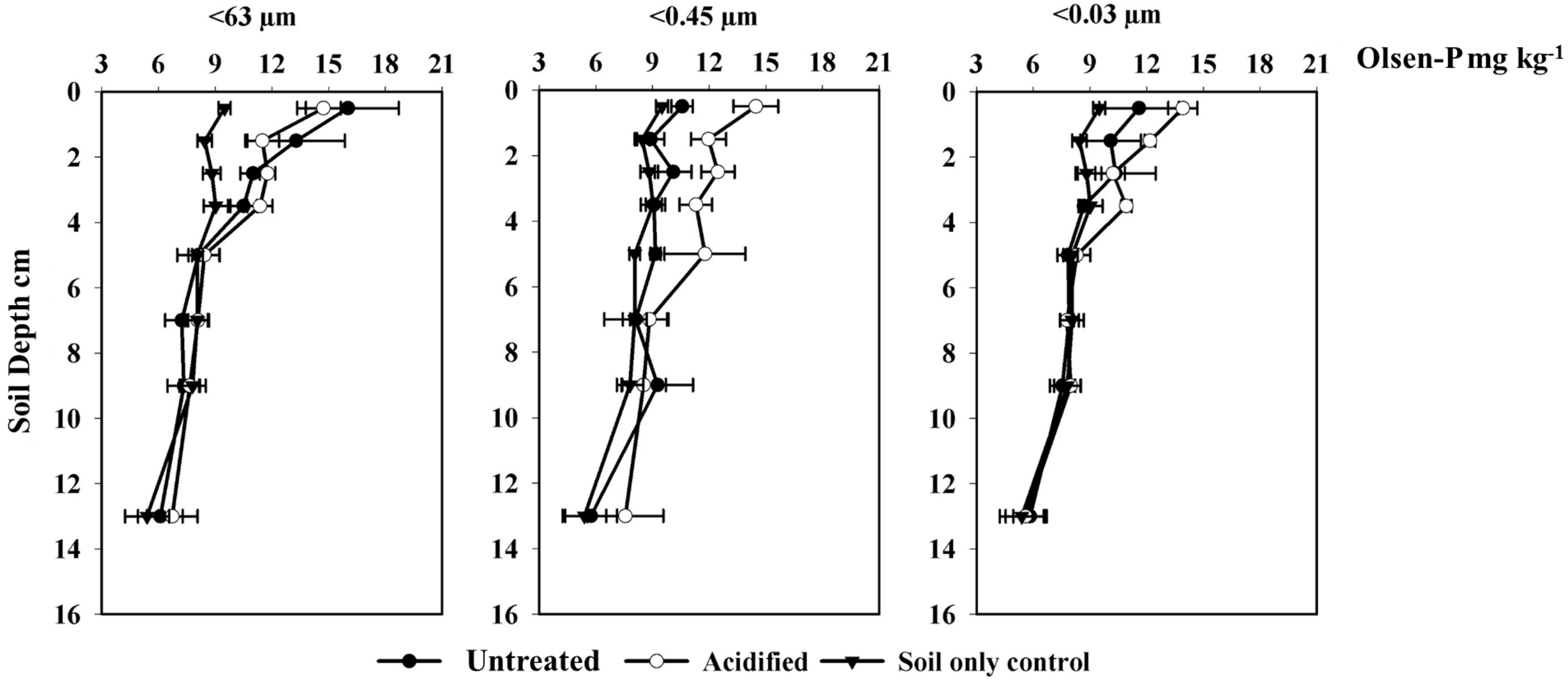

Figure 6.1 The Olsen-P content of soil from untreated and acidified slurry treatments. Values represent means ± SEM, n = 4.

In the leachate, the <63 µm treatment resulted in significantly more IP, OP and TP than the control (Figure 6.2). The amount leached equated to 0.15 µg IP column^{-1} and 0.24 µg OP column^{-1} in the leachate of control, while in the <63 µm treatment it was 0.61 µg IP column^{-1} and 0.84 µg OP column^{-1}. The quantity of additional P leached relative to the control was equivalent to *ca*. 3.3% of the added IP and 1.7% of OP of the <63 µm slurry.

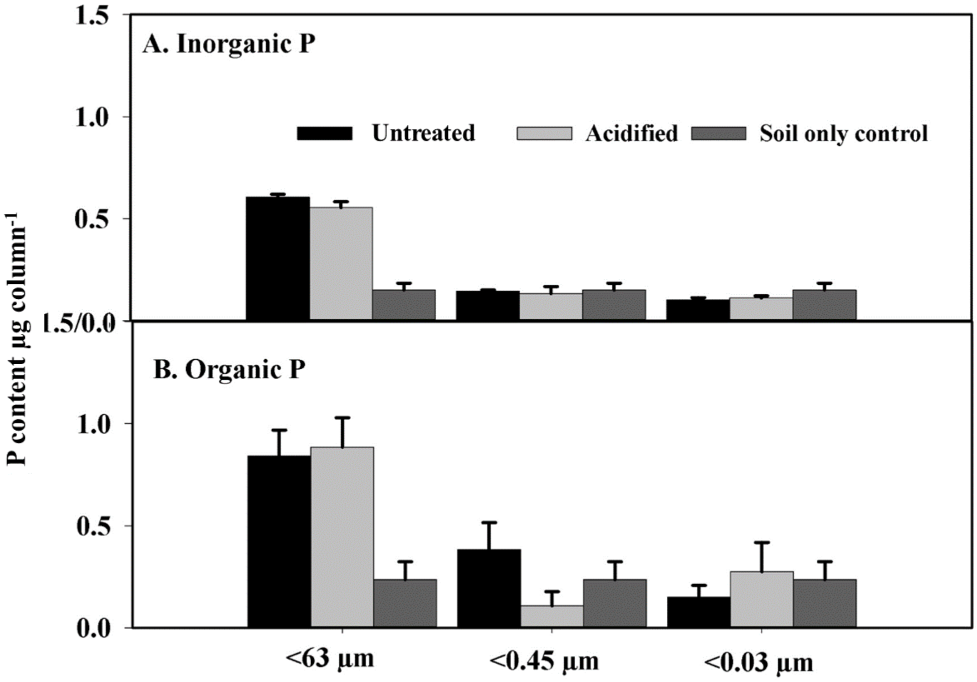

Figure 6.2 The inorganic P (IP) and organic P (OP) content of leachates from untreated and acidified slurry treatments. Values represent means ± SEM, n = 4.

Soil TP content of the control was consistent at the different depths from 0.36 to 0.46 g kg^{-1}. Because the quantity of TP applied in the slurry was relatively low compared with the original TP content of soil, there was no apparent change in the TP content of the soil after the <63 µm application.

The <0.45 and <0.03 µm treatments did not result in any differences in terms of Olsen-P and TP content of soil, nor with TP and IP contents of leachates compared with the control, because of the low TP and IP content in the <0.45 and <0.03 µm fractions. In this study the TP and IP contents of <0.45 and <0.03 µm fractions were statistically different (Table 6.1), indicating a certain amount of P contained in the nanoparticles of 0.03-0.45µm. Nanoparticles are supposed to facilitate P mobility (Hens & Merckx, 2002; Regelink et al., 2013). However, maybe because the number of nanoparticles in cattle slurry was very small, no differences in the soil and leachate were made in the comparison in the <0.45 and <0.03 µm treatments.

In comparison, the 0.45-63 µm particles had a much greater influence on P leaching than the nanoparticulate fraction. Small or colloidal particles have small volumes and large surface area to volume ratios indicating the high mobility and the high P adsorption capacity (Marcato et al., 2008). Particles of 0.45-63 µm contained the majority IP and OP in the <63 µm slurry. The high mobility of these particles enabled the IP and OP they carried to be collected in leachate. In addition, the sorption isotherm test for slurry failed to show any measurable sorption, indicating that the slurry particles were already P saturated. Thus, no P from soil, adsorbed and carried by slurry particles, contributed to the extra P leaching caused by cattle slurry.

6.4.3 Effect of acidification on slurry-derived P movement

At the same application rate of TP of the fraction <63 µm in both untreated and acidified treatments, the acidified treatment significantly input more IP than the untreated treatment (Table 6.1). As a result, the 3-4 cm and 10-16 cm soils in acidified treatment possessed significantly higher Olsen-P content (11 and 7 mg kg^{-1}, respectively) than the untreated treatment (10 and 6 mg kg^{-1}, respectively) (Figure 6.1). The acidified <0.45 µm slurry fraction contained >6 times more IP than the corresponding untreated treatment (Table 6.1). This increase was also reflected in the Olsen-P content. The 0-4cm soil in the acidified <0.45 µm treatment contained significantly higher Olsen-P (11-14 mg kg^{-1}) than the untreated treatment (9-10 mg kg^{-1}) (Figure 6.1). Although the acidified slurry was adjusted to pH 5.5, the fractions in the acidified treatments were diluted with distilled water before application for the identical TP and water volume application rate. Consequently, the pH was not as low as present in the bulk acidified slurry. Because the quantity of TP applied was relatively low compared with the original TP content of soil, no statistical differences were seen in the TP content of soil between untreated and acidified treatments and control.

In comparison with the control, the acidified <63 µm treatments caused significantly higher IP, OP and TP leaching (0.55, 0.89 and 1.44 µg column^{-1}, respectively), while the acidified <0.45 and <0.03 µm treatments did not show any difference (Figure 6.2). Thus, the 0.45-63 µm again strongly contributed to the IP, OP and TP leaching in the acidified treatment. The IP, OP and TP content of acidified 0.45-63 µm slurry were only 62%, 69% and 65% of the amount found in the untreated slurry, respectively. However, compared with the untreated treatments, no significant difference was seen in the leachates, which indicates that the amount of P (IP and OP) transported by colloidal particles to the leachates was the same between untreated and acidified <63 µm treatments. It also implies that the particle characteristics (such as mobility and P content) of 0.45-63 µm particles are similar between untreated and acidified slurry. Hjorth et al. (2015) suggested a long-term treatment of acidification could accelerate hydrolysis. However, in this study, the acidification process was relatively short (over a period of 7 d), and the particle size distribution may not have been altered greatly. To reflect long slurry storage periods, further work is therefore required to look at the impact of long term acidification on P leaching.

6.4.4 Effect of anaerobic digestion on slurry-derived P movement

AD and AD+ treatments of the <63 µm slurry fractions both resulted in a significantly higher Olsen-P content than the control in the 1-2 cm and 0-3 cm layers of soil, respectively (Figure 6.3). However, no treatments affected the TP content of the soil. Although the IP contents of the <63 µm slurry of both AD and AD+ were almost 2-times greater than untreated slurry (Table 6.1), unlike the acidified slurry, no significant augmentation in the Olsen-P content of the different soil depths was found compared with the untreated treatment (Figure 6.3).

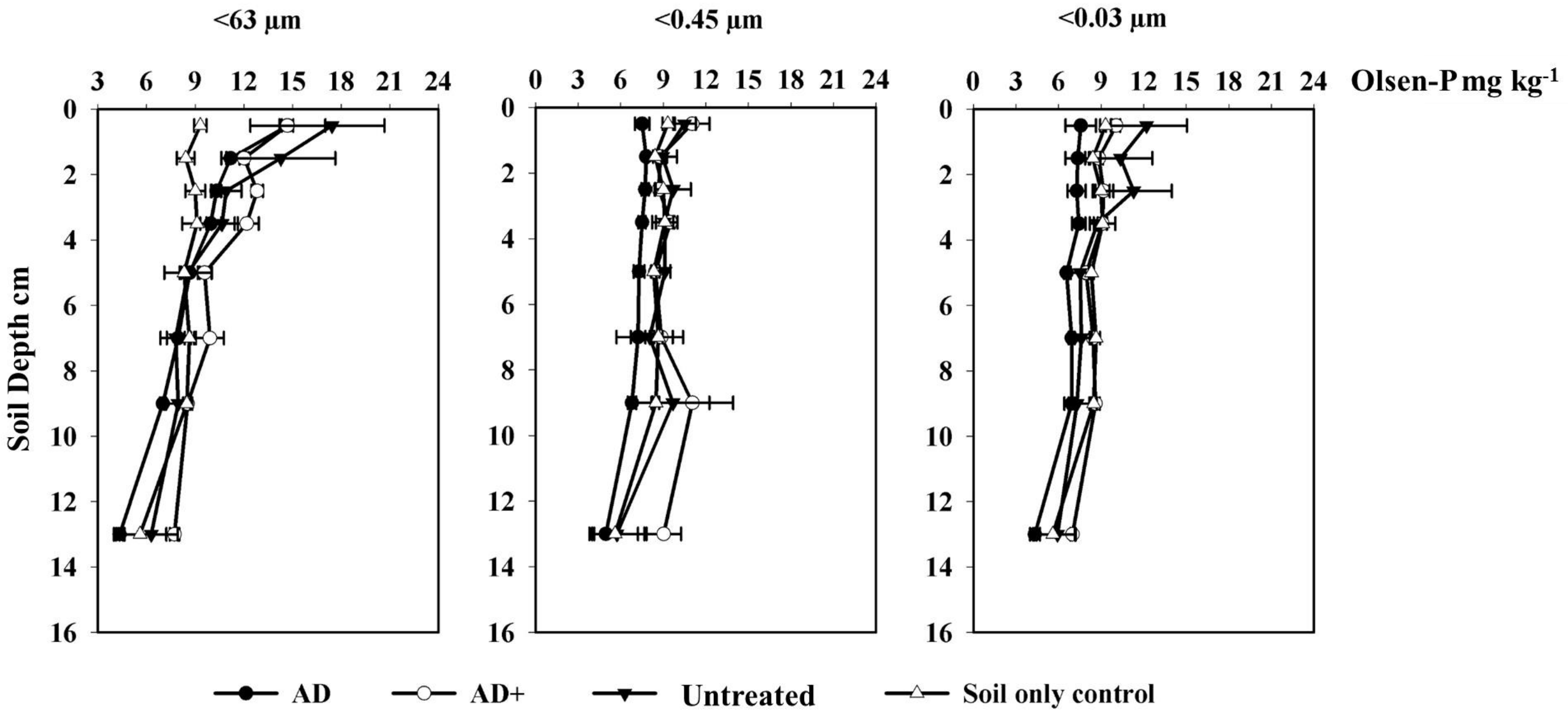

Figure 6.3 The Olsen-P content of soil from the untreated slurry, the non-inoculated anaerobically digested slurry (AD) and the inoculated anaerobically digested slurry (AD+) treatments. Values represent means ± SEM, n = 3.

The AD and AD+ <63 µm treatments resulted in significantly higher IP, OP and TP contents of leachates than the control as expected (Figure 6.4). Additionally, the AD <63 µm treatment tended to results in greater IP and OP leaching (1.01 and 1.92 µg column^{-1} respectively) than the untreated treatment (0.61 and 0.84 µg column^{-1} respectively) (although the rise in the IP content of the leachates was not statistically significant). Again, the 0.45-63 µm particle size fraction dominated P leaching. The difference in the IP and OP content of the leachates between the AD and untreated <63 µm treatments should be associated with the 0.45-63 µm particles. The percentage of the added IP from the <63 µm slurry transported into the leachate was similar (*ca.* 3.0%) in both untreated and AD treatments. The IP associated with the slurry colloids interacts with the soil matrix via sorption and desorption during the vertical movement, and a certain amount of IP was retained in the soil. But due to the higher content of IP of the 0.45-63 µm slurry in the AD treatments, more IP leaching was seen in the AD <63 µm treatments than untreated treatment. In contrast, a greater percentage (8.2%) of the added OP from the <63 µm slurry of AD treatment was transported to leachate than the untreated treatment (1.7%). Thus, although the OP content of the <63 µm slurry in the AD treatment was significantly smaller than untreated slurry, greater OP leaching was observed. This difference in OP leaching may be because biodegradation during the AD process changed the characteristics of the OP associated colloids, either in terms of increased colloid content or reduced colloidal size, or alteration of OP species. Karr and Keinath (1978) found the concentration of 1-100 µm colloidal particles increased in municipal wastewater after anaerobic digestion. Marcato et al. (2008) found particles of 3-25 µm made a strong contribution to the total specific surface area of both untreated and digested slurry, and the contribution was even larger in digested slurry. On the other hand, different species of OP show different sorption affinity with soil particles. For example, phytic acid (myo-inositol hexakisphosphate) is a strong competitor for P sorption sites. Hansen et al. (2004) found phytic acid added from manure tended to be retained on the upper soil, while other orthophosphate monoesters were found to be higher in the deeper layer than in the upper layer of soil. Therefore, in the AD liquid slurry, we speculate that the proportion of bindable OP was lower, and the mobility of OP colloids was greater than untreated slurry.

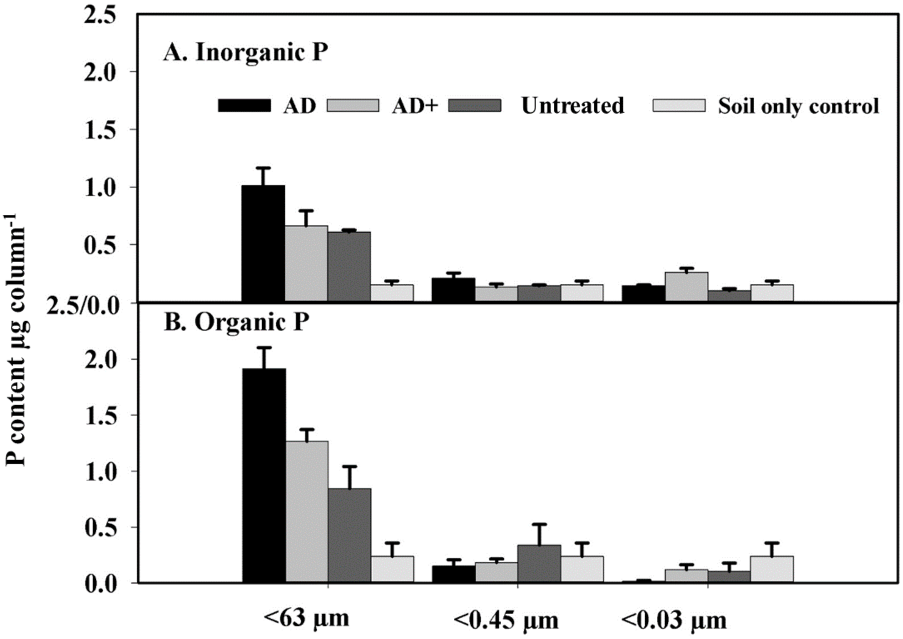

Figure 6.4 The IP and OP content of leachates from the untreated slurry, the non-inoculated anaerobically digested slurry (AD) and the inoculated anaerobically digested slurry (AD+) treatments. Values represent means ± SEM, n = 3.

Comparing AD and AD+ showed no significant difference in the P contents of the slurry fractions (Table 6.1), but the Olsen-P contents of the 8-10 and 10-16 cm layers was significantly greater in the AD+ <63 µm treatment (Figure 6.3), coupled with the lower IP content of leachate (Figure 6.4). This may indicate that more P in the AD+ treatment was adsorbed by the soil, while more P in the AD treatment was transferred to leachate. This implies that particles of 0.45-63 µm in AD slurry tend to be more mobile than AD+ slurry.

6.5 Conclusions

The majority of the P in the <63 µm slurry was contained in the 0.45-63 µm particle size fraction. Acidification increased the overall IP content of the cattle slurry and specifically reduced TP in the 0.45-63 µm particle fraction. Anaerobic digestion tended to increase the proportion of IP in the <63 µm slurry and distributed more IP and less OP into 0.45-63 µm particles. The colloidal fraction (0.45-63 µm) in cattle slurry had a substantial influence on P mobility through the soil. Despite the increase in the proportion of TP as IP in acidified slurry, the potential for P leaching was not increased by acidification, which may be because the colloidal particles characteristics were not altered. Anaerobic digestion may promote the transformation of the colloidal particles characteristics and could cause a higher risk of P leaching, but would need verifying in field trials.

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7 Phosphorus fractionation changes in soils affected by solid and liquid fractions of processed cattle slurry

7.1 Abstract

Livestock slurry is an important nutrient source for crops. A range of slurry processing strategies (e.g. acidification, anaerobic digestion and mechanical separation) are now being practiced to reduce their environmental footprint and improve handling. However, these processes can alter their chemical, biological and physical properties which may result in a difference in P dynamics and speciation when applied to soil. To elucidate the potential impact following land application, slurry was treated by acidification (A) or anaerobic digestion (AD) and then separated into solid (S) and liquid (L) fractions. The separated untreated and treated slurry fractions were incorporated into an agricultural sandy loam soil and incubated for 4 months. Soil samples were taken on days 1, 11, 21, 42, 84 and 126, and sequential fractionation was used to analyse the P forms present. The results showed that application of livestock slurry instantly increased the contents of most P forms in soil, especially the labile P form. At day 1, the slurry-derived labile P fractions in all slurry amended treatments were 16-30% of IP-H_2O, 12-19% of OP-H_2O, 10-14% of IP-$NaHCO_3$ and 12-21% of OP-$NaHCO_3$. The content of IP-H_2O form decreased following first order decay kinetics in all treatments and the proportion of slurry-derived moderately labile and stable P tended to increase during the incubation. The value of the IP-H_2O first order decay rate constant tended to be larger for the acidified slurry amended treatments but smaller for the anaerobically digested slurry amended treatments. We also conclude that acidification may help improve lability of occluded P in soil. Anaerobically digested slurry may represent a better slow-release P supply for plants than untreated slurry. Liquid fraction of slurry with smaller C/P ratio may induce faster P degradation in soil than the solid fraction.

7.2 Introduction

Phosphorus (P) is a primary element for plant growth (Waldrip-Dail et al., 2009). Its dynamics in soil are complex and controlled by physico-chemical factors (sorption-desorption) and by biological reactions (immobilization-mineralization) (Cross & Schlesinger, 1995; Buehler et al., 2002). Some studies claimed that exchangeable P during soil incubation can partly or even predominantly (20-90%) result from biological and biochemical rather than physico-chemical processes (Bünemann, 2015). But He et al. (2004) and Griffin et al. (2003) indicated IP-$NaHCO_3$ and IP-NaOH forms are controlled by the sorption/immobilization capacity of Al and Fe oxides in soil. Indeed, the relative contribution of biological and biochemical processes tends to be lower in arable soils than under grassland and forest soils, and is negatively related to the availability of inorganic P and positively to concentrations of soil organic carbon (Bünemann, 2015). P hydrolysis in soil is also related to soil clay content, where soils with a high clay content result in greater phytate adsorption, preventing its hydrolysis (Celi et al., 2001; Giaveno et al., 2010; Pagliari & Laboski, 2014). Pagliari & Laboski (2014) suggest that soils with a clay content <71 g/kg provides conditions that are suitable for hydrolysis of enzymatic hydrolysable P. In a silt loam, IP-$NaHCO_3$ and IP-NaOH forms fluctuated less than in a sandy loam, indicating the silt loam had a higher capacity to regulate changes in inorganic P (He et al., 2004). IP-NaOH is reported to be important in buffering available P supply while significant depletion of organic forms are rarely measured (Buehler et al., 2002). Waldrip-Dail et al. (2009) also monitored a transformation from IP-$NaHCO_3$ to IP-NaOH over an 84 d incubation after poultry manure application to a sandy loam soil.

Livestock slurry addition affects the P dynamics by altering the physico-chemical, biological, and biochemical soil properties (He et al., 2004). First, livestock slurry contains considerable amounts of P, especially labile P forms such as H_2O, bicarbonate and NaOH extractable inorganic P (Guo & Yost, 1998; Pagliari & Laboski, 2012). Repeated applications of manures increase soil P content, with a predominance of inorganic P over organic P (Hountin et al., 2000; Abdala et al., 2015). In addition, livestock slurry also provides microorganisms and exophosphatases to soil (Crouse et al., 2002). He et al. (2004) suggest that in the first 14 d after application to soil, manure-borne microbial and exoenzyme activities might contribute to more variable P dynamics, and that P amendments stimulate microbial organic P utilization. Cooperband & Good (2002) also argue that the organic carbon (C) content of manure could alter soil structure (e.g. through a lower bulk density and greater aggregate stability), leading to changes in the sorptive properties. They found both inorganic P fertiliser and dairy manure increased the soil IP-H_2O content initially. But eventually the IP-H_2O level of inorganic P fertiliser treatment decreased to the same level as in the unamended soil, while in dairy manure treated soil the IP-H_2O content remained at a higher level. Some researchers have claimed that the transformation of stable organic P forms (e.g. OP-HCl) could be an important mechanism for maintaining a long-term balance between labile and immobile P in soils (He et al., 2008; Waldrip-Dail et al., 2009).

Slurry processing strategies change the properties of slurry, so may affect the interaction of slurry nutrients with soil after incorporation. Acidification generally improves P bioavailability within slurry (Fangueiro et al., 2015; Sommer et al., 2015; Chapter 3), and anaerobic digestion induces the biodegradation of organic P (Hjorth et al., 2009; Chapter 4), but the differences in pH and other aspects may result in additional effects on soil processes. Acidified slurry contains more labile P and the lower pH could alter microbial structure and function and soil P sorption after application. On the other hand, digestates have greater proportions of less labile or recalcitrant P (Marcato et al., 2008; Güngör & Karthikeyan, 2008; Rico et al. 2012; Chapter 4), and the high pH may induce P precipitation or sorption with calcium in soil (Hinsinger, 2001). Digestates tend to cause greater microbial basal respiration in soil than untreated slurry (Hupfauf et al., 2016). Separated solid and liquid slurry fractions contain different amounts of organic C, and hence have different C/P ratios. Cumulative P release by mineralisation was negatively correlated with the C/P ratio and positively correlated with initial P content of the organic amendments (Floate, 1970; Mafongoya et al., 2000). It is also reported that separated liquid slurry has greater microbial activity than the solid slurry (Hansen et al., 2004), which also applied to digestates (Nkoa, 2014).

Therefore, this study explored the changes in inorganic and organic P forms in soil after incorporation with solid and liquid fractions of untreated, acidified, and anaerobically digested cattle slurry. The aim was to elucidate the influences of differently processed slurry addition on P forms in soil. The hypotheses were, i) addition of livestock slurry, non-processed and processed, would increase labile P content of soil instantly, ii) the availability of P forms would reduce over time, iii) both acidified and anaerobically digested slurry would cause greater labile P content of soil than untreated slurry, and iv) organic P in liquid fractions would mineralise to a greater degree, and at a faster rate than organic P in solid fractions.

7.3 Materials and methods

7.3.1 Cattle slurry collection and separation

Cattle slurry was collected from a commercial beef farm on four independent occasions as four replicates in Gwynedd, Wales, UK. Slurry samples were kept at 4ºC in the laboratory before processing by acidification or anaerobic digestion. Cattle slurry was acidified to pH 5.5 by adding *ca.* 35 ml 15% H_2SO_4 L^{-1} slurry per week. Anaerobic digestion (AD) was simulated and conducted in the laboratory. The dry matter (DM) content of the cattle slurry was adjusted to 8% with distilled water prior to incubation to maximise the methane production (Sun et al., 2012), and then sealed in containers connected to foil bags for gas collection, and incubated at 35°C for 35 d. The containers were shaken gently for 1 h every day and the gas in the bags was released when full. The untreated and acidified samples were both stored under 10°C for these 35 d. Replicate batches of untreated and treated samples were wet-sieved into solid (>63 µm) and liquid (<63 µm) fractions This resulted in 6 slurry treatments: solid fraction of untreated cattle slurry (CS), liquid fraction of untreated cattle slurry (CL), solid fraction of acidified slurry (AS), liquid fraction of acidified slurry (AL), solid fraction of AD slurry (ADS), and liquid fraction of AD slurry (ADL). Sub-samples of each slurry treatment were combusted at 550°C and then dissolved by 2.4 M HCl and the TP content finally determined by the molybdate blue method of Murphy& Riley (1962). The total C of separated fractions was also measured on fresh subsamples by TruSpec® CN analyzer (Leco Corp., St Joseph, MO). The characteristics of separated slurry are shown in Table 7.1.

Table 7.1 Characteristics of solid and liquid fractions of cattle slurry, acidified slurry, anaerobically digested slurry (on a dry weight basis). Values represent means ± SEM, n = 4.

Treatment	CS	CL	AS	AL	ADS	ADL
TC content g kg^{-1}	365 (±25)	322 (±39)	388 (±11)	281 (±2)	447 (±8)	341 (±12)
TP content g kg^{-1}	4.8 (±0.3)	11.5 (±0.5)	3.9 (±0.4)	9.6 (±0.6)	5.8 (±0.2)	11.5 (±0.2)
TN content g kg^{-1}	24.8(±1.5)	113.1(±10.4)	31.8(±2.0)	107.0(±13.5)	46.5(±3.3)	116.4(±4.3)
C/P	76.6 (±6.7)	28.5 (±4.7)	99.4 (±4.9)	29.2 (±1.1)	77.8 (±3.9)	29.7 (±0.6)
C/N	14.8(±1.0)	2.9(±0.3)	12.3(±0.8)	2.8(±0.4)	9.7(±0.7)	2.9(±0.1)

7.3.2 Incubation and sampling

250 g fresh soil was packed in plastic containers of 9 cm diameter and 3 cm height. All separated slurries were adjusted to the same water content and incorporated into the soil. Due to the water content of the liquid slurry fractions (and to avoid excess water application), the application rate for all treatments was set at 60 mg TP kg^{-1} dry soil (equivalent to 22 kg TP ha^{-1}). Then the soil was kept at field capacity during the incubation. Containers were incubated at 20°C in the dark for 126 days, and covered with perforated polyethylene film to maintain aeration and to limit evaporative losses. Approximately 1 g of soil was sampled from each container at days 1, 11, 21, 42, 84 and 126 for modified Hedley fractionation of P forms.

7.3.3 Modified Hedley fractionation

1 g soil was sequentially extracted by 20 ml of distilled water (H_2O), 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl. During each extraction, sample and extractant were shaken for 16 h. The suspension was then centrifuged at 18,500 *g* for 10 min. Inorganic P in each suspension was detected by colorimetry after Murphy & Riley (1962). TP was determined after persulfate digestion. The undissolved residue was digested with aqua regia for 4 h at 120°C for TP analysis. The deference between TP and IP was taken as organic P (OP). The P forms of inorganic and organic P extractable by H_2O, $NaHCO_3$, NaOH and HCl and P in the residue represent as IP-H_2O, IP-$NaHCO_3$, IP-NaOH, IP-HCl, OP-H_2O, OP-$NaHCO_3$, OP-NaOH, OP-HCl, TP-residue in this study. We define IP-H_2O, OP-H_2O, IP-$NaHCO_3$, OP-$NaHCO_3$ as labile P, IP-NaOH and OP-NaOH as moderately labile P, and IP-HCl and OP-HCl and residual-P as stable P forms (Negassa & Leinweber, 2009).

7.3.4 Statistical analysis

The concentration of P in this study is expressed as mg kg^{-1} on a dry weight basis. The proportions of slurry-derived total P from livestock slurry represented by the different P forms was calculated by subtracting the respective concentration of P in the control and then dividing by the total P difference to control. The distribution of P forms in the soil during the incubation are presented as proportions of total P of soil.

Temporal changes in the IP-H_2O concentration of the soil after the different slurry applied was fitted to a first order kinetics equation (1) (Petrucci, 2007).

$$\ln(P_t/P_0) = -kt \tag{1}$$

Where P_t was the IP-H_2O proportion in the soil after application at time t. P_0 was the IP-H_2O in the soil after application at time zero while k is a decay rate constant (day^{-1}). The half-lives of IP-H_2O in the soil after slurry application is $t_{1/2}$ (Petrucci, 2007).

$$t_{1/2} = \ln 2/k \tag{2}$$

Statistical analysis was conducted with SPSS v22 (IBM Inc., Armonk, NY). The differences between treatments and between sampling dates were analysed by ANOVA with Tukey pairwise post-hoc testing with effects considered significant for $p<0.05$.

7.4 Results and discussion

7.4.1 Livestock slurry derived P in soil

On day 1, the addition of the six treatments resulted in greater TP concentration of soil (530-539 mg kg^{-1}) than the control (462 mg kg^{-1}) (Table 7.2). In addition, the differences in TP concentrations on day 1 between amended treatments of CS, AS, ADS, CL, AL, ADL and control were 77±9 mg kg^{-1}, 74±5mg kg^{-1}, 68±4 mg kg^{-1}, 72±5 mg kg^{-1}, 75±5 mg kg^{-1}, and 76±8 mg kg^{-1}, respectively, which are all close to the added TP rate of 60 mg kg^{-1}. Generally, the moderately labile and stable P forms are the dominant P pools in soil (Cross & Schlesinger, 1995). In our experiment, the moderately labile and stable P forms represented 90% of TP in the control on day 1. Slurry contains a large portion of labile P (He et al., 2006; Pagliari & Laboski, 2012; Chapter 3&4). On day 1, there were greater concentrations of IP-H_2O, OP-H_2O and IP-$NaHCO_3$ forms in the slurry amended treatments than the control. Table 7.3 summarises the proportion of P forms in the slurry-derived TP. On day 1, a large portion of this slurry-derived TP in all slurry amended treatments was in the labile P forms of IP-H_2O (16-30%), OP-H_2O (12-19%), IP-$NaHCO_3$ (10-14%) and OP-$NaHCO_3$ (12-21%) (Table 7.3), which is consistent with other studies (Sui et al., 1999; Contreras et al., 2007; Roboredo et al., 2012). In the study of Roboredo et al. (2012), slurry-derived P-resin represented an even greater proportion (over 70%) of slurry-derived TP in the whole untreated, whole acidified, solid untreated and solid acidified slurry treatments following an application rate of 120 mg TP kg^{-1}, which was twice that of our application rate. The slurry-derived OP-HCl proportion of all slurry treatments and the slurry-derived OP-NaOH proportion of the CS, ADS and ADL treatments showed negative values on day 1, indicating transformation of OP-NaOH and OP-HCl to other P forms. Untreated and digested slurry with high pH may increase the soil pH, reducing P sorption to Al and Fe oxides in soil. Gustafsson et al. (2012) showed that in soils with <10% clay, P sorption increased with decreasing pH. In addition, organic matter from slurry may associate with Al and Fe, decreasing P sorption and increasing P availability (Iyamuremye et al., 1996), resulting in the low or negative slurry-derived OP-NaOH proportion in those treatments. In contrast, in the AS and AL treatments, the slurry-derived OP-NaOH proportion was positive. The low pH enhanced P sorption with Fe and Al oxides in soil (Holmén & Casey, 1996; Waldrip et al., 2011). Plus the competitive organic matter, in the AS and AL treatments the increase and decrease of P sorption may counterbalance.

Table 7.2 The concentration of P forms in soil amended with different livestock slurry at day 1 and day 126 (mg kg^{-1} on a dry weight basis). Values represent means, n = 4.

Day	Treatment	Concentrations of P forms in soil mg kg^{-1}									
		IP-H_2O	IP-$NaHCO_3$	IP-NaOH	IP-HCl	OP-H_2O	OP-$NaHCO_3$	OP-NaOH	OP-HCl	TP-residue	TP
1	CS	21.7ab	17.8a	41.2ab	65.8	12.2a	43.6	187.7ab	25.1	123.5a	538.6a
	AS	18.8abc	17.6a	38.1ab	70.5	11.0a	49.0	195.9ab	23.2	111.2ab	535.4a
	ADS	21.2ab	17.5a	39.6ab	81.1	9.2a	49.4	183.4b	22.2	105.9bc	529.5a
	CL	13.6c	17.1a	42.9a	61.0	11.1a	47.3	193.9ab	27.8	119.6ab	534.3a
	AL	16.7bc	19.0a	40.4ab	64.2	9.9a	45.8	203.6a	27.1	110.4abc	537.2a
	ADL	24.4a	19.7a	43.5a	81.8	14.5a	48.2	161.2c	22.5	122.1a	537.9a
	control	2.3d	9.2b	35.5b	63.5	0.7b	35.3	190.3ab	30.2	94.8c	461.8b
126	CS	4.8abc*	15.5c	40.1a*	67.7	16.2b	50.4	171.3c	45.4a	118.7a	530.1a
	AS	4.6abc*	18.9ab	40.5a	71.2	12.9b	55.7*	173.8c*	36.3abc*	120.0a*	533.9a
	ADS	6.2ab*	19.3ab	41.5a	72.7	16.3b	56.0	175.7bc	29.4c	105.0ab	522.2a
	CL	3.4bcd*	16.9bc	41.9a	64.8	14.9b	50.6	197.3ab	41.9ab*	106.9ab*	538.6a
	AL	3.0cd*	21.5a	46.1a	65.2	11.5b*	50.6	200.2a	39.2abc*	99.1b*	536.4a
	ADL	7.1a*	19.4ab	43.6a	69.5	31.3a*	67.0	165.8c	29.6bc	108.4ab	541.8a
	control	0.6d*	7.3d*	28.9b*	56.6*	12.5b*	41.1	168.7c*	44.2a	99.4b	459.2b

Note: the different letters in the same column indicate the significant difference between different treatments in the same P form ($p<0.05$). The column with no letters indicates no significant difference between treatments. * indicates significant differences between day 1 and day 126 ($p<0.05$). Same in Table 7.3.

Table 7.3 The proportion of slurry-derived different P forms (as a% of slurry-derived TP) in the soil of each treatment at day 1 and day 126 (%). Values represent means, n = 4.

Day	Treatment	IP-H_2O	IP-$NaHCO_3$	IP-NaOH	IP-HCl	OP-H_2O	OP-$NaHCO_3$	OP-NaOH	OP-HCl	TP-residue
1	CS	27.9	12.1	7.7	0.6	16.4	12.2	-5.9a	-10.5	39.4a
	AS	22.9	11.7	3.5	9.2	14.4	18.9	7.0a	-9.7	22.2ab
	ADS	28.0	12.2	6.2	25.1	12.5	20.7	-9.3ab	-11.9	16.6b
	CL	15.9	11.0	10.4	-4.2	14.8	16.6	4.8a	-3.4	34.1ab
	AL	19.3	13.0	6.4	1.2	12.3	14.3	17.2a	-4.3	20.5ab
	ADL	30.4	14.2	10.8	21.3	18.5	17.5	-38.3b	-10	35.7ab
126	CS	6.0ab*	11.8	16.2*	16.1	5.3b	13.1	3.2c	0.6a	27.8a
	AS	5.6ab*	16.2	16.1*	20.1	0.5b*	20.4	4.6c	-11.0abc	27.4a
	ADS	9.5a*	20.2	21.1*	22.9	6.1b	25.0	12.6bc*	-23.8c*	6.3b
	CL	3.7b*	12.4	15.9	10.7*	3.0b*	11.9*	35.4ab*	-2.4ab	9.4ab*
	AL	3.4b*	19.2	22.8*	12.0	-1.3b*	9.3	41.2a*	-6.8ab	0.2b*
	ADL	7.7ab*	15.0	18.4	17.1	24.1a	29.5	-4.4c*	-18.5bc	11.1ab

Acidification increases IP-H_2O concentration and decreases IP-$NaHCO_3$ of both solid and liquid slurry (Chapter 3), while AD decreased IP-H_2O concentration and increased IP-$NaHCO_3$ concentration of slurry (Chapter 4). Acidification decreased IP-HCl concentration of liquid slurry (Chapter 3), while AD increased IP-HCl concentration of liquid slurry (Chapter 4). Both acidification and AD decreased OP-$NaHCO_3$ concentration, and increased OP-NaOH concentration of solid and liquid slurry (Chapter 3 & 4). Roboredo et al. (2012) found at the beginning of their incubation that the application of acidified pig slurry increased the IP-resin content in soil to a larger extent than untreated pig slurry. However, in our results, these concentration differences in different processed slurries were not reflected in the slurry-derived P form proportions of soil on day 1 (Table 7.3). This may be because the differences between different slurries were not big enough and variability between replicates was large. In addition, the separated solid fractions contained greater IP-H_2O concentrations than the liquid fractions in untreated, acidified and anaerobic digested slurry (Chapter 3&4). After application, the slurry-derived IP-H_2O proportion of the CL and AL treatments tended to be less than the CS and AS treatments respectively, although this was not statistically significant on day 1 (Table 7.3).

Over the 126 days of incubation, the slurry-derived IP-H_2O, IP-NaOH, OP-NaOH, OP-HCl, TP-residue proportions consistently presented differences between slurry treatments (Appendix 7.1). Finally, after 126 d of incubation, the labile IP form of the IP-H_2O concentration of all treatments had decreased compared with day 1, by 71%-82% (Table 7.2). Also, the proportion of slurry-derived IP-H_2O of all slurry treatments was significantly reduced when comparing the amount present at day 1 and day 126. The slurry-derived OP-H_2O proportion of the AS, CL, AL treatments also decreased significantly from day 1 to day 126, and in the CS and ADS treatments this proportion also tended to decrease, although not statistically. The other labile P forms, IP-$NaHCO_3$ and OP-$NaHCO_3$, did not show much change in concentration or in the slurry-derived proportion in each slurry treatment when comparing day 1 and day 126. In contrast, the proportion of slurry-derived moderately labile and stable P tended to increase after 126 d. Further, this increase in the slurry-derived IP-NaOH of the CS, AS, ADS and AL treatments, and the slurry-derived OP-NaOH of the ADS, CL, AL and ADL treatments was significant (Table 7.3). Therefore, we suggest that of the slurry-derived TP, the availability of P reduced during incubation, and that the transformation of P from IP-H_2O and OP-H_2O to IP-NaOH and OP-NaOH dominated. It is believed that organic materials with >2 mg kg^{-1} of TP show net P mineralization (Floate, 1970). Roboredo et al. (2012) monitored a significant increase in slurry-derived total IP proportion over 184 days incubation in whole untreated, whole acidified, solid untreated and solid acidified slurry treatments. However, although in our study, the TP concentration of all slurry applied was over 2 mg kg^{-1}, the slurry-derived total IP proportion only showed a significant increase in the CL, AS and AL treatments between day 1 to day 126, indicating rates of mineralization dominated those for immobilization. Anaerobically digested slurry may contain less degradable organic matter, and mineralization may be counterbalanced by immobilization. The extent of change in slurry-derived P forms from day 1 to day 126 was also calculated by dividing the value at day 126 by the value at day 1 (data not shown), but no differences were found between different slurry amended treatments in this ratio.

7.4.2 Patterns of P forms during incubation

Technically, the TP concentration of soil should not change during incubation. To avoid the impact of variability of concentration, the changes of P forms during incubation for each treatment were presented as the change in proportions of TP (Figure 7.1). The transformation of P forms are controlled both by sorption-desorption and immobilization-mineralization (Daroub et al., 2000; Buehler et al., 2002; He et al., 2004).

Over the incubation period, the IP-H_2O proportion of all treatments gradually decreased and shifted to the IP-$NaHCO_3$ and IP-NaOH forms. In the first 21 d, there was a significant decrease in the IP-H_2O proportion and a significant increase in the IP-$NaHCO_3$ and IP-NaOH proportions in all treatments (Figure 7.1), which is in agreement with Daroub et al. (2000), who found a movement of the isotope label from IP-resin to IP-NaOH over their 34 d incubation. In our study, after reaching the first peak at day 21, the proportion of IP-$NaHCO_3$ in each treatment gradually decreased until it became lower than on day 1 (Figure 7.1). He et al. (2004) found the IP-$NaHCO_3$ concentration of soil incubated with dairy manure fluctuated in the opposite manner to the IP-NaOH concentration, implying a transformation from IP-$NaHCO_3$ to IP-NaOH during the incubation. Other researchers also suggest IP-NaOH acts as a sink for added IP, and a source for IP-$NaHCO_3$ in the soil (Beck & Sanchez, 1994; Buehler et al., 2002; Zheng et al., 2002). However, the IP-$NaHCO_3$ and IP-NaOH proportion in our results did not show such counterbalanced pattern.

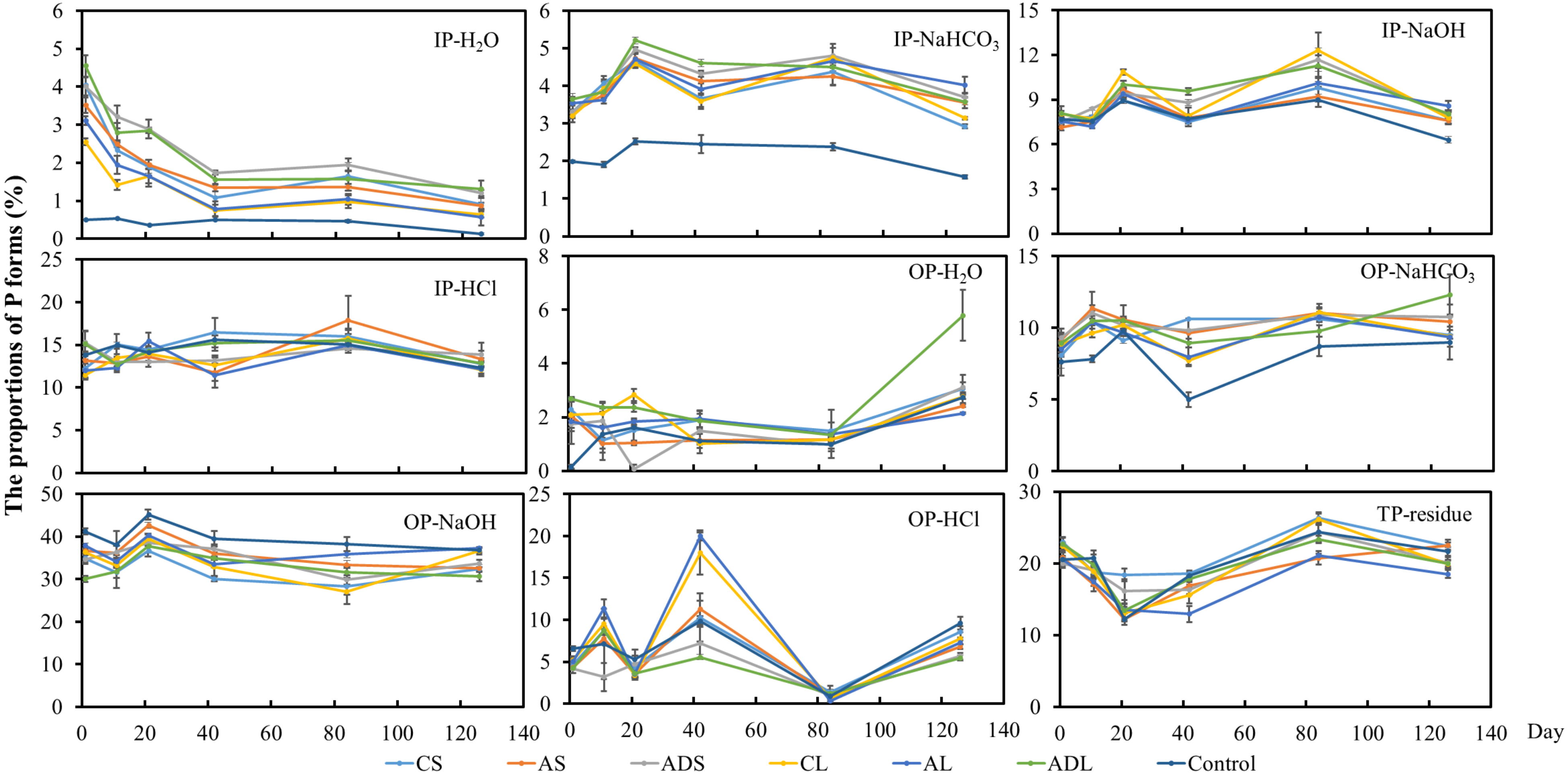

Figure 7.1 The change of the proportion of P forms in TP of different treatments. Values represent means ± SEM, n = 4.

The IP-H_2O concentration was mainly controlled by sorption and desorption kinetics, and the change of the IP-H_2O concentration of all treatments in our result fitted well to a first order decay kinetics (Table 7.4). The value of the decay rate constant, '*k*', of treatments showed no significant differences between six slurry amended treatments. The six slurry amended treatments all presented significantly greater '*k*' values than the control. However, there was a trend that AL > CS > CL > AS > ADL > ADS > control, indicating the decrease in the IP-H_2O concentration in the AL treatment was the fastest, and in the ADS treatment was the slowest between all slurry amended treatments. Comparing solid and liquid fractions, we found that the IP-H_2O concentration in the AL treatment decayed at a faster rate than the AS treatment, perhaps because the AL treatment resulted in the exposure of more sorption sites, accelerating P sorption to a greater extent than for the AS treatment.

Table 7.4 Index of first order kinetics of the decrease in the IP-H_2O concentration in soils of different treatments. Values represent means ± SEM, n = 4.

Treatment	k days^{-1}	R^2	$t_{1/2}$ days
CS	0.0141(±0.0026)	0.27(±0.11)	56(±13)
AS	0.0125(±0.0011)	0.70(±0.03)	57(±5)
ADS	0.0102(±0.0010)	0.75(±0.04)	70(±8)
CL	0.0132(±0.0017)	0.42(±0.16)	56(±8)
AL	0.0172(±0.0033)	0.52(±0.21)	45(±8)
ADL	0.0124(±0.0009)	0.40(±0.30)	57(±4)
Control	0.0074(±0.0007)	0.55(±0.02)	96(±8)

Mineralization and immobilization of P occur during incubations in soil (Roboredo et al., 2012). However, we could not quantify such changes based on our data. The study of Buehler et al. (2002) suggested inorganic P immobilization, because they found in soils with no P addition, >14% of the added inorganic ^{33}P was recovered in the OP-NaOH and OP-HCl forms after 14 d of incubation. Although our study did not present a relationship between the IP proportion and the OP-NaOH and OP-HCl proportions, the OP-NaOH and OP-HCl proportions were two quite changeable forms in all treatments (Figure 7.1).

The AS treatment had less TP-residue proportion at day 21 and day 84, compared with the CS treatment, and the AL treatment had a smaller TP-residue proportion at day 84 than the CL treatment, implying the release of occluded P in the acidified slurry amended soil. The ADL treatment was found to have a greater proportion of IP-$NaHCO_3$ at day 21 and 42, and less OP-HCl proportion at day 42 and day 126 than the CL treatment. Therefore, it indicates that anaerobically digested slurry, especially the liquid fraction, could be a slower but longer-term P supply product than untreated slurry.

Some researchers have argued that liquid manure has more microbial activity than solid manure (Ghonsikar & Miller, 1973; Magid et al., 1996; Turner et al., 2003). Mafongoya et al. (2000) also suggested cumulative P released in soil was negatively correlated with the C/P ratio. In our study, the solid fraction of slurry had a higher C/P than liquid fraction (Table 7.1). Thus, liquid treatments were expected to show higher or faster degradation of OP (Hansen et al., 2004). There was a significant reduction in the OP-H_2O form proportion in the first day 11 or day 21 for the three solid treatments, while in the three liquid treatments the proportion of OP-H_2O form remained stable. This may be related to the C/P ratio and microbial activity.

7.5 Conclusions

Application of livestock slurry instantly increased the soil content of most P forms, especially the labile P form. The soil concentration of IP-H_2O decreased via first order kinetics. The AL treatment tended to show a quicker reduction of IP-H_2O, while the ADL and ADS treatments tended to show a slower reduction of IP-H_2O.. The proportion of slurry-derived moderately labile and stable P tended to increase over the incubation period. Addition of acidified slurry tended to increase lability of the occluded P in soil. Anaerobically digested slurry may represent a better slow-release P supply for plants than untreated slurry. The liquid fraction of slurry with smaller C/P ratio may induce faster P turnover in soil than the solid fraction.

7.6 References

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Appendix 7.1 The proportion of slurry-derived different P forms (as a percentage of slurry-derived TP) in the soil of each treatment for each sampling date of the incubation. Values represent means, n = 4.

Treatment	Day	The proportion of slurry-derived P forms %								
		IP-H_2O	IP-$NaHCO_3$	IP-NaOH	IP-HCl	OP-H_2O	OP-$NaHCO_3$	OP-NaOH	OP-HCl	TP-residue
CS	1	27.9	12.1	7.7	0.6	16.4	12.2	-5.9a	-10.5	39.4a
AS		22.9	11.7	3.5	9.2	14.4	18.9	7.0a	-9.7	22.2ab
ADS		28.0	12.2	6.2	25.1	12.5	20.7	-9.3ab	-11.9	16.6b
CL		15.9	11.0	10.4	-4.2	14.8	16.6	4.8a	-3.4	34.1ab
AL		19.3	13.0	6.4	1.2	12.3	14.3	17.2a	-4.3	20.5ab
ADL		30.4	14.2	10.8	21.3	18.5	17.5	-38.3b	-10.0	35.7ab
CS	11	14.0abc	18.1	9.7ab	13.7	-0.6	28.4	-9.2b	18.7a	7.3
AS		13.9abc	14.6	7.5ab	-0.6	-1.1	31.5	25.7a	11.4ab	-3.0
ADS		21.7a	18.2	14.3a	-0.3	4.4	33.2	24.1a	-24.1b	8.4
CL		6.8c	16.1	8.9ab	3.8	6.5	20.8	5.4ab	22.5a	9.2
AL		9.9bc	13.4	5.3b	-2.5	2.8	24.9	12.2ab	34.6a	-0.5
ADL		15.8ab	15.1	7.9ab	-0.9	8.2	25.8	-4.4ab	18.2ab	14.4
CS	21	11.1b	17.5	9.5c	15.9	0.6ab	6.2	-11.4c	-1.5	52.1a
AS		12.9ab	19.8	14.7bc	10.9	-3.1b	16.5	25.7a	-8.9	11.5b
ADS		16.3ab	17.8	11.7bc	6.8	-3.5b	12.1	1.6bc	1.7	35.4ab
CL		10.0b	18.0	23.1a	13.9	10.8a	13.4	3.7bc	-10.8	18.0b

AL		9.7b	19.0	12.4bc	23.8	3.2ab	9.3	8.5ab	-8.0	22.1ab
ADL		19.2a	23.0	17.4ab	15.0	7.5ab	15.1	-10.8c	-7.6	21.1ab
CS	42	5.6abc	13.1	6.9c	26.7	7.4	42.6a	-43.3	14.4ab	26.5a
AS		6.5abc	14.4	8.6bc	-11.7	1.4	37.1ab	16.2	18.2ab	9.3ab
ADS		10.7a	18.1	16.8ab	-2.7	3.5	43.7a	22.3	-13.6b	1.2ab
CL		2.1c	10.6	9.6bc	-3.4	0.4	23.0b	-6.2	63.9a	0.0ab
AL		2.5bc	13.5	9.2bc	-9.5	3.0	27.3b	9.0	58.8a	-13.8b
ADL		8.0ab	18.0	21.0a	13.2	6.0	33.7ab	7.5	-22.7b	15.2ab
CS	84	10.4	19.3	16.6	23.1	5.4	25.0	-45.9ab	5.1	40.9a
AS		7.5	17.3	11.0	31.9	2.5	28.3	2.2ab	1.4	-2.1b
ADS		11.9	21.1	28.4	11.2	0.5	25.2	-25.2ab	1.6	25.3ab
CL		4.8	22.2	37.0	19.9	2.6	29.0	-55.0b	-0.9	40.5a
AL		5.0	20.4	17.7	13.9	3.1	25.2	18.3a	-1.7	-1.9b
ADL		8.8	18.4	26	19.0	3.6	16.7	-12.3ab	3.2	16.6ab
CS	126	6.0ab	11.8	16.2	16.1	5.3b	13.1	3.2c	0.6a	27.8a
AS		5.6ab	16.2	16.1	20.1	0.5b	20.4	4.6c	-11.0abc	27.4a
ADS		9.5a	20.2	21.1	22.9	6.1b	25.0	12.6bc	-23.8c	6.3b
CL		3.7b	12.4	15.9	10.7	3.0b	11.9	35.4ab	-2.4ab	9.4ab
AL		3.4b	19.2	22.8	12.0	-1.3b	9.3	41.2a	-6.8ab	0.2b
ADL		7.7ab	15.0	18.4	17.1	24.1a	29.5	-4.4c	-18.5bc	11.1ab

Note: the different letters in the same column indicate the significant difference between different treatments ($p<0.05$).

8 The effect of slurry processing on phosphorus availability for plant uptake and potential leaching

8.1 Abstract

Slurry processing approaches, such as separation, acidification and anaerobic digestion, have been applied in livestock slurry management to achieve greater sustainability. However, these processes may alter the phosphorus (P) bioavailability to plants and mobility in the soil after field application. To better understand the impact of applying processed cattle slurry on P plant uptake and potential P leaching from soil, separated solid and liquid fractions of untreated, acidified and anaerobically digested cattle slurries were applied to the surface of a ryegrass sward established on sand textured Eutric Cambisol in a pot experiment. Slurry treatments were applied on an equal total P loading basis, of 10 kg P ha^{-1} equivalent. Leachates were collected weekly after simulated water events, and ryegrass was harvested every three weeks for 12 weeks. Inorganic P (IP) and total P (TP) contents of leachates, and TP content of ryegrass were measured throughout the experiment. The Olsen-P content of the different depths of soil at the end of the experiment was also determined. Results showed that addition of the untreated, acidified and anaerobic digested solid and liquid slurry tended to increase offtake compared to the control treatment, especially the difference between the total P offtake of the 4 harvests of the acidified liquid slurry and anaerobic digested liquid slurry treatments and the control was significant. The untreated and anaerobic digested slurry treatments tended to increase the pH of soil, while the acidified slurry treatment decreased soil pH compared to the control. In addition, untreated, acidified, anaerobic digested slurry treatments all showed greater soil electrical conductivity (EC) than the control. Compared to the untreated slurry treatments, acidified slurry resulted in lower pH in the surface soil (0-3 cm), and greater EC in the whole soil column, but anaerobically digested slurry treatment showed no difference for soil pH and EC compared to the untreated slurry treatment. Application of the untreated, acidified, and anaerobically digested solid and liquid slurry significantly increased the Olsen-P content of the top 0-1 cm of soil. However, there was no difference in the Olsen-P content between different slurry-amended treatments. The inorganic and organic P concentration in leachates over the 12 weeks was not influenced much by addition of the slurry treatments, and there was no effect of the different treatments on the cumulative inorganic and organic P loss during the experiment in comparison with the control. The difference between separated solid and liquid slurry treatments was only found

in P offtake and P content of leachate between the anaerobic digested solid and liquid slurry treatments. P offtake of the third harvest, and the P lost from the fourth leaching event were significantly greater from the anaerobic digested liquid slurry treatment than the anaerobic digested solid fraction treatment. In summary, addition of untreated livestock slurry increased Olsen-P content in the surface soil and increased P offtake. Acidified and anaerobically digested slurry were both represented similar P sources for plants in comparison to untreated slurry, increasing the Olsen-P content of soil and P offtake of ryegrass to a similar extent. The liquid fraction of anaerobically digested slurry was better in improving P availability in soil than the solid fraction. The different slurry types did not greatly impact upon the potential for P leaching on one application.

8.2 Introduction

Phosphorus (P) is an essential nutrient for plant growth. Livestock slurry is an important source of P and other nutrients in agriculture (Waldrip-Dail et al., 2009). As well as the untreated slurry supplied directly from the animal house or reception pit, some slurries are treated by different approaches for the benefit of greater sustainability, e.g. acidification to reduce ammonia emission (Kai et al., 2008), and anaerobic digestion (AD) to produce green energy and reduce odour and viable pathogens (Smet et al., 1999; Clemens et al., 2006), and mechanical separation to concentrate dry matter in the solid fraction. The agronomic value may be different between these differently processed livestock slurries.

Acidification has been reported to improve the bioavailability of P in livestock slurry (Güngör et al., 2007; Hjorth et al., 2015). Low pH dissolves the dominant precipitates of struvite and Ca-P in livestock slurry (Güngör et al., 2007; Christensen et al., 2009). In Chapter 3, we also found acidification increased the labile P (IP-H_2O+IP-$NaHCO_3$) content, dissolving the precipitated P (IP-HCl) and degrading the OP-$NaHCO_3$ form. In addition, the acidity may release P from Ca-P complexes (Hinsinger, 2001), but may also release amorphous Fe and Al, increasing P sorption to soil (Holmén & Casey, 1996; Waldrip et al., 2011). During anaerobic digestion, degradation processes are thought to improve P plant availability of livestock slurry (Masse et al., 2011; Chapter 4). On the other hand, recalcitrant P minerals, i.e. struvite and calcium bound phosphates, are also formed during anaerobic digestion (Güngör et al., 2004; Marcato et al., 2008; Chapter 4). Digestates have been shown to cause higher microbial basal respiration in soils after being applied than untreated slurry (Hupfauf et al., 2016). However, Bachmann et al. (2014) detected, in comparison to untreated slurry, lower activities of soil enzymes after application of digestates. In addition, most results from field experiments have indicated no effects of anaerobic digestion on P availability in soil (Loria & Sawyer, 2005; Moller & Stinner, 2010; Bachmann et al., 2011).

Apart from the agronomic benefit of livestock slurry, whether treated or not, P loss via leaching from soils receiving livestock slurry can result in eutrophication of watercourses (Sharpley et al., 1999). In livestock slurry, P distributed in different sizes of particles may affect its mobility (McGechan, 2002). Hansen et al. (2004) noticed lagoon manure resulted in higher inorganic P concentration in subsurface soil than solid manure, but it was the opposite for organic P. Different P forms have different sorption affinities to soil minerals. The sorption affinity of organic P to soils was lower in comparison with inorganic P (IP) in the study of Anderson & Magdoff (2005), but higher in the results of Lessa & Anderson (1996), Leytem et al. (2002) and Berg & Joern (2006). Phytate, an important organic P form in livestock slurry, is the most competitive for P sorbing sites (Berg & Joern, 2006). Some research has reported organic P to be the main source of leaching after pig slurry application (Gerritse, 1981; Toor, 2003).

Acidification results in the transfer of some P from the solid fractions to liquid fractions in livestock slurry (Roboredo et al., 2012). In Chapter 3, we showed that a greater proportion of the labile P (IP-H_2O+IP-$NaHCO_3$) was distributed in the liquid fraction in the acidified slurry than in the untreated slurry. Other effects of acidifying slurry include a lower pH, higher EC, and greater content of free metals (Fangueiro et al., 2009; Hjorth et al., 2015). The degradation processes during anaerobic digestion are thought to work to a greater extent on smaller particles, leading to larger proportion of coarse particles in digestates (Shon et al., 2006; Marcato et al., 2008). Stutter (2015) observed that anaerobic digestion reduced P sorption in soil columns, suggesting an increased short-term leaching risk after digestate application. However, most previous studies did not consider plant growth and P uptake when evaluating P leaching risk. Ryegrass is P-efficient due to a high root/shoot ratio (Föhse et al., 1988), and its yield and P uptake increase with increasing P application rate (Tunney & Pmmel, 1987; Szogi et al., 2010). Hence, we used ryegrass in the following experiment.

Therefore, the aims of this study were to i) determine P movement and its potential to leach from soil after application of separated solid and liquid fractions from untreated, acidified and anaerobically digested slurry, and ii) determine the relative plant growth and P uptake following application of solid and liquid fractions from untreated, acidified and anaerobically treated slurries. Specifically, the experiment was designed to test the hypotheses that: i) livestock slurry, untreated and treated, could improve plant growth and P uptake, and increase potential P leaching; ii) acidified and anaerobically digested slurry would cause more P uptake and potential P leaching from soil than untreated slurry; and iii) the liquid fraction of separated slurry treatments would result in greater P uptake and potential P leaching in soil than from the solid fraction.

8.3 Materials and methods

8.3.1 Soil collection

A sandy loam textured Eutric Cambisol (80% sand, 12% silt, 8% clay) was collected from the Ahp horizon (0-15 cm) of a grassland field located in Abergwyngregyn, Gwynedd, Wales, UK (53° 14' 33" N, 4° 1' 15" W). The site has a temperate oceanic climate with mean annual temperature of 10.6ºC and annual rainfall of 1060 mm. After collection, the soil was sieved to pass a 5.6 mm mesh to remove roots and stones. The soil was dried at 105ºC for 24 h to determine moisture content. Fresh soil sample was mixed with distilled water to determine pH (1:2.5 w/v) and electrical conductivity (EC) (1:5 w/v). The Olsen-P content was determined by extracting 1 g (fresh weight) of soil with 20 ml 0.5 M $NaHCO_3$. The extraction comprised shaking at 200 rev min^{-1} (SM30 Edmund Bühler GmbH) for 0.5 h and centrifuging (Eppendorf centrifuge 5810, UK) at 18,500 g for 5 min, and the extractable P content of the suspension was determined by Murphy and Riley (1962). The total P (TP) measurement was conducted by digesting 1 g fresh soil with 10 ml of aqua regia at 120ºC for 5 h, and determining the P concentration by Murphy & Riley (1962) after cooling and making up to 20 ml volume. Exchangeable K was determined by extracting 2 g fresh soil with 20 ml 1 M NH_4OAc, shaking for 0.5 h and centrifuging at 18,500 g for 5 min, and determining the K concentration using flame emission spectroscopy (Sherwood 410 flame photometer, Sherwood Scientific, Cambridge, UK) (Sparks, 1996). Soil samples (4 g fresh weight) were also extracted with 20 ml 0.5 M K_2SO_4 (1:5 w/v) and shaken for 0.5 h and centrifuged at 18,500 g for 5 min, for the determination of NO_3^- and NH_4^+ (Miranda et al., 2001; Mulvaney, 1996). The soil had a gravimetric moisture content of 13.2 %, a pH of 5.9, EC of 55.5 µS cm^{-1}, Olsen-P content of 7 mg kg^{-1}, a TP content of 0.5 g kg^{-1}, NO_3^- content of 9 mg kg^{-1}, NH_4^+ content of 2 mg kg^{-1}, and exchangeable K content of 71 mg kg^{-1}.

8.3.2 Cattle slurry collection and treatments

Cattle slurry samples were collected on four independent occasions as four replicates from a commercial beef farm in Gwynedd, Wales, UK. Slurry samples were kept at 4ºC before processing by acidification or anaerobic digestion. Four replicates of cattle slurry were acidified to pH 5.5 by adding 15% H_2SO_4, using *ca.* 35 ml L^{-1} slurry. Anaerobic digestion was conducted in the laboratory. The dry matter (DM) content of the cattle slurry was adjusted to 8% with distilled water prior to incubation, in order to maximise the methane production (Sun et al., 2012), and then sealed in containers connected to foil bags for gas collection, and incubated at 35ºC for 35 d. The containers were shaken gently for 1 h every day and the gas in the bags was released when full. The untreated and acidified samples were both stored under 10ºC for these 35 d. The characteristics of cattle slurry before and after processing are shown in Table 8.1.

Replicate batches of untreated and treated samples were wet-sieved into solid fraction (>63 µm) and liquid fraction (<63 µm). Approximately 1 g of the separated fractions were combusted at 550ºC, then the ash was dissolved with 2 ml 2.4 M HCl and the volume made up to 20 ml, and finally the total P concentration determined by the molybdate blue method (Murphy & Riley, 1962). The total K content was also determined from this HCl dissolved solution by flame emission spectroscopy (Sherwood 410 flame photometer, Sherwood Scientific, Cambridge, UK). 4 g of slurry fraction was extracted with 20 ml 0.5 M K_2SO_4, to determine the extractable NH_4^+ content (Mulvaney, 1996). Water extractable P was conducted at 1:200 w/v ratio (Wolf et al., 2005). Total C and total N was also measured with fresh slurry fraction samples by TruSpec® CN analyzer (Leco Corp., St Joseph, MO).

8.3.3 Experimental treatments

There were 7 treatments (with four replicates of each) as follows:

- solid fraction of untreated cattle slurry (CS)
- liquid fraction of untreated cattle slurry (CL)
- solid fraction of acidified slurry (AS)
- liquid fraction of acidified slurry (AL)
- solid fraction of AD slurry (ADS)
- liquid fraction of AD slurry (ADL)
- control.

Table 8.1 Characteristics of untreated, acidified and anaerobically digested cattle slurry (dry weight basis, except DM and VS). Values represent means ± SEM, n = 4.

Measurements	Untreated cattle slurry	Acidified cattle slurry	Anaerobically digested cattle slurry
DM content %	14.6 (±0.1)	15.6 (±0.1)	6.3(±0.4)
VS content %	11.1(±0.1)	11.7(±0.1)	4.6(±0.3)
pH	7.5(±0.0)	5.5(±0.0)	7.4(±0.1)
EC mS cm^{-1}	14.6(±0.3)	14.2(±1.4)	16.1(±0.5)
ORP mv	-28.3(±1.6)	-80.0(±0.9)	-24.8(±1.6)
TC g kg^{-1}	426.8(±8.6)	386.0(±3.7)	392.4(±23.0)
TN g kg^{-1}	46.2(±2.3)	39.2(±4.3)	75.9(±8.7)
TP g kg^{-1}	8.0(±0.2)	6.6(±0.3)	9.5(±0.3)
TK g kg^{-1}	36.3(±2.3)	35.0(±1.3)	29.8(±3.9)
NH_4^+ g kg^{-1}	12.3(±1.2)	11.1(±0.7)	21.6(±1.7)
Water extractable P g kg^{-1}	4.0(±0.1)	4.4(±0.2)	4.1(±0.2)
C/N	9.3(±0.5)	8.9(±0.3)	5.6(±0.7)
C/P	53.3(±1.1)	59.5(±3.4)	41.7(±3.6)

4 kg (fresh weight) of sieved soil was packed into 28 pots, 16.5 cm diameter and 16 cm deep, at a bulk density of 1.24 g cm^{-3} on dry weight basis. A mixed perennial ryegrass (*Lolium perenne* L.) sward (Abergrain, Aberplentiful, Aberchoice; 1:1:1) was sown at a rate equivalent to 34 kg ha^{-1}. Nitrogen and potassium fertilizers (NH_4NO_3 and K_2SO_4) were applied to the soil at the equivalent rate of 100 kg N ha^{-1} and 12.4 kg K ha^{-1}, to ensure they were non-limiting. The pots were randomly placed on a bench in a greenhouse and regularly re-randomized. Air temperature was recorded every 4 h during the experiment by an iButton.

Slurry application and leachate collection

The soil was kept at 60% water holding capacity for the first 6 weeks before slurry application. At the end of 6th week, 885 ml water (equivalent to a 1 h rainfall storm event of 40 mm h^{-1}) was applied to the soil surface and the leachate was collected the following day in bottles as the background sample.

The ryegrass was cut to 2 cm above the soil surface before slurry addition. Differently processed slurries, adjusted to same water content, were then applied to the soil surface at the equivalent rate of 10 kg TP ha^{-1}. Additional N and K fertilizers were added to all treatments, including the control, at 50 kg N ha^{-1} and 65 kg K ha^{-1}, to ensure they were not limiting (Defra, 2010).

Water was added at a rate of 885 ml pot^{-1} and leachates were collected weekly after slurry application. Leachate volumes of 12 leaching events were recorded by weight. The inorganic P (reactive P) concentration of leachates was determined according to Murphy & Riley (1962). The leachates were also digested with ammonium persulfate (1 ml leachate + 0.1 g ammonium persulfate + 2 ml 0.9 M H_2SO_4) in the autoclave for the TP concentration. Organic P concentration was calculated by the difference (Total P – inorganic P). The P mass of the leachates (mg pot^{-1}) were calculated by multiplying the volume by the P concentration for each leaching event.

Ryegrass was harvested every 3 weeks (to a height of 2 cm above soil surface) after slurry application and there were four harvests. Total fresh ryegrass harvested was weighed and then grass was dried at 80ºC to determine total dry weight. After being ground, 0.5 g of dried ryegrass sample was combusted in a muffle at 550ºC, then dissolved in 2 ml 2.4 M HCl and made volume to 20 ml for TP concentration determination. The P offtake (mg pot^{-1}) was calculated by multiplying the TP concentration of the ryegrass by the total dry weight for each harvest. After the last ryegrass harvest, the soil was sectioned by depth; 0-1, 1-2, 2-3, 3-5, 5-10, 10-16 cm, and analysed for soil moisture content, pH, EC and Olsen-P.

8.3.4 Statistical analyses

The differences in leachate chemistry between different dates of leaching events were analysed by ANOVA with Tukey pairwise post-hoc testing with effects considered significant for $p<0.05$. The same statistical approach was used for the ryegrass harvest data. The comparisons between acidified and untreated treatments, between digested and untreated treatments, between solid and liquid treatments within each slurry processing approach were conducted by paired t-test, and between each amended treatment and control were analysed by unpaired t-test, both with effects considered significant at $p<0.05$. The statistical analysis was conducted with SPSS v22 (IBM Inc., Armonk, NY).

8.4 Results

8.4.1 Separated untreated cattle slurry, acidified cattle slurry, anaerobically digested cattle slurry

The information on properties of the amended materials are summarised in Table 8.2. After slurry separation, more water was allocated in the liquid fraction, resulting in the low DM and volatile solid (VS) contents in this fraction. Anaerobic digestion increased the total P (TP) and water extractable P contents of the solid fraction of cattle slurry, due to substantial loss of DM during biodegradation. Acidification only increased the water extractable P content of the liquid fraction of cattle slurry. Since the application rate was based on the same TP loading, the water extractable P/TP ratio may reflect the labile P input from each slurry-amended treatment. There was no significant difference in this ratio between untreated slurry and digested slurry treatments. The AL treatment showed a significant greater ratio than the CL treatment. Further, there was no difference in this ratio between solid and liquid treatments.

8.4.2 Potential P leaching

There was little effect of the different slurry treatments on the concentrations of inorganic and organic P in the leachate compared to the control treatment. Figure 8.1 shows that the only occasions there were significant effects of the slurry treatments on P leaching were; i) the 7th leaching event when the inorganic P concentration of the ADL treatment was greater than the control, ii) the organic P concentration of the 1st and 8th leaching events of the CL treatment were greater than the control, and iii) the 5th leaching event, when the organic P concentration of the ADS treatment were significantly greater than the control treatment. Acidification and anaerobic digestion did not significantly affect the inorganic and organic P concentration of leachates. Slurry separation tended to cause higher inorganic and organic P concentration in the leachates from the liquid slurry fraction treatments than the solid fraction treatments. Specifically, the inorganic P concentration of the 5th and 6th leachates of the ADL treatment was significantly greater than for the ADS treatment (Figure 8.1). The inorganic P concentration of the 9th and 10th leachates of the AL treatment was also higher than the AS treatment (Figure 8.1). The organic P concentration of the 1st leachate of the CL treatment was larger than the CS treatment. The organic P concentration of the 10th leachate of the AL treatment was larger than the AS treatment (Figure 8.1).

Table 8.2 Characteristics of solid and liquid fractions of untreated, acidified and anaerobically digested cattle slurry (on dry weight basis except dry matter and volatile solid contents). Values represent means ± SEM, n = 4.

Treatments	Untreated cattle slurry		Acidified cattle slurry		Anaerobically digested cattle slurry	
Particle size fraction	Solid	Liquid	Solid	Liquid	Solid	Liquid
Dry matter content %	14.2(±0.4)	3.8(±0.4)	14.8(±0.5)	4.1(±0.1)	11.4(±0.4)	3.5(±0.2)
Volatile solid content %	11.3(±0.2)	2.6(±0.3)	12(±0.3)	2.7(±0.1)	8.9(±0.3)	2.4(±0.2)
C g kg^{-1}	365(±25)	321(±39)	388(±11)	280(±2)	446(±9)	341.2(±12)
TN g kg^{-1}	24.8(±1.5)	113.1(±10.4)	31.8(±2.0)	107.0(±13.5)	46.5(±3.3)	116.4(±4.3)
TK g kg^{-1}	23.4(±1.2)	29.2(±2.4)	20.2(±2.2)	55.2(±3.6)	31.8(±1.1)	34.1(±3.0)
Water extractable P g kg^{-1}	2.4(±0.3)	5.2(±0.6)	2.4(±0.1)	7.5(±0.1)	3.4(±0.1)	5.8(±0.7)
TP g kg^{-1}	4.9(±0.4)	10.5(±0.8)	3.9(±0.2)	11.0(±0.5)	6.5(±0.2)	12.3(±0.2)
Water extractable P/TP ratio	0.5(±0.1)	0.5(±0.1)	0.6(±0.0)	0.7(±0.0)	0.5(±0.0)	0.5(±0.1)
C/P	76.0(±8.8)	30.0(±1.7)	99.5(±5.7)	25.5(±1.1)	68.8(±2.1)	28.8(±0.9)

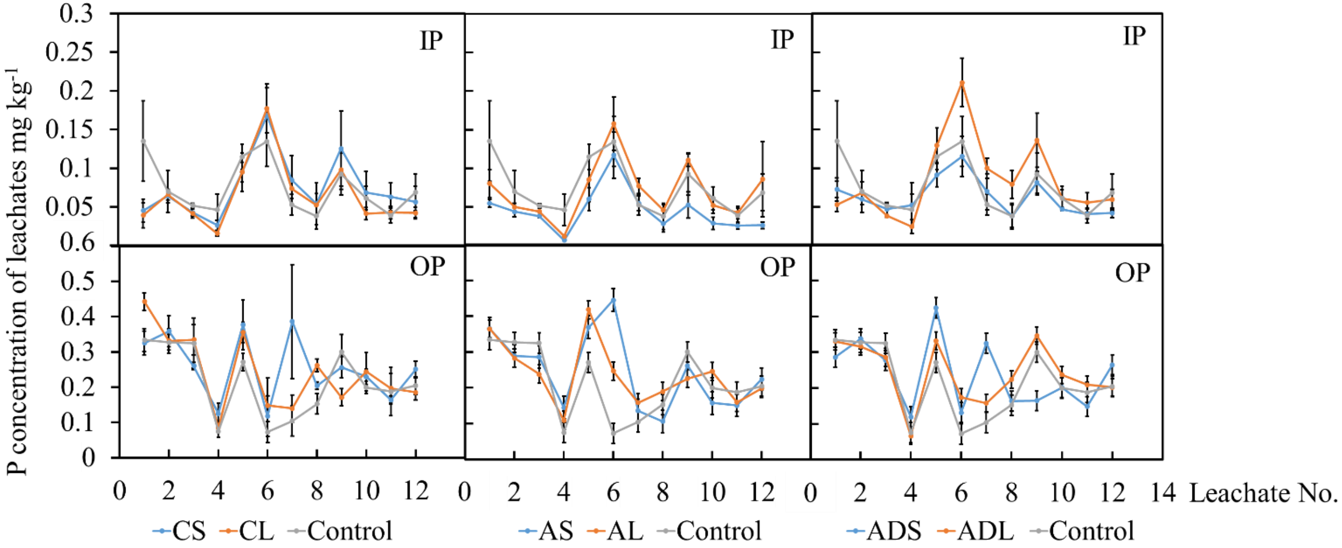

Figure 8.1 Inorganic and organic P concentrations of leachates over the incubation period. Values represent means ± SEM, n = 4.

There were no significant differences in inorganic P loss in each leachate from the different treatments compared to the control. Acidified and anaerobically digested treatments did not show much difference in the inorganic P lost in each leachate compared with untreated cattle slurry, irrespective of the solid or liquid fractions. Only for the 1st leachate of the AL treatment and the 5th leachate of the ADL treatment was there a significantly greater inorganic P loss than the CL treatment. Comparing the solid and liquid slurry treatments, there was a significant difference in the 4th and 5th leachates between the ADS and ADL treatments. Specifically, there was a greater inorganic P loss in the 4th and 5th leaching events from the ADL treatment than the ADS treatment ($p<0.05$).

Comparing the organic P loss of each leaching event, the 2nd leachate of the AL treatment and the 8th leachate of the CL treatment contained more than the control treatment. Within the solid treatments, the AS and ADS treatment caused less organic P leaching than CS in the 8th leachate ($p<0.05$). Within the liquid treatments, both AL and ADL treatments showed less organic P loss in the 8th leachate than the CL treatment ($p<0.05$). No difference was seen between the solid and liquid treatments of any of the slurry treatments.

A similar pattern in the cumulative loss of inorganic and organic P from the different treatments was observed over time (Figure 8.2). Comparing the cumulative inorganic and organic P mass of leachates between different treatments, no significant difference was seen over time. The total inorganic and organic P loss of all treatments over the 12 weeks was -0.53% - 0.12% and 0.11% - 0.66% of applied TP amount, respectively, without significant differences between these treatments.

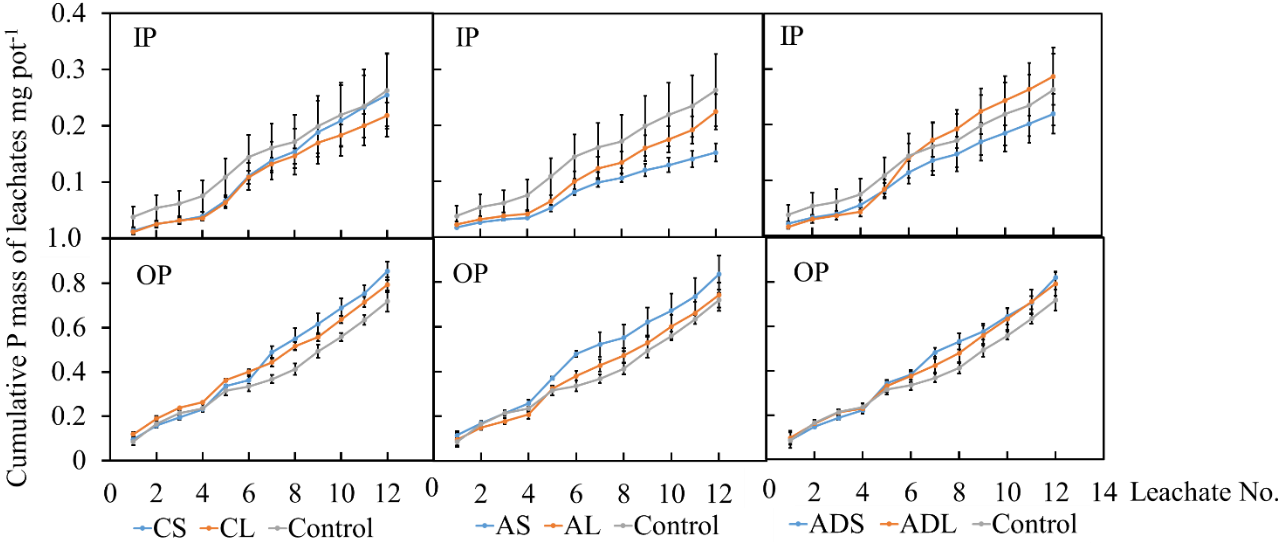

Figure 8.2 Cumulative inorganic and organic P mass of leachates. Values represent means ± SEM, n = 4.

8.4.3 Harvested ryegrass

There were no significant differences in the P offtake and dry matter yields of ryegrass for the solid fractions of the different slurries treatments on any of the harvests compared to the control treatment (Figure 8.3). In contrast, for the liquid fractions, the P offtake of the ADL treatment was consistently significantly greater than the control in the 1st, 2nd and 3rd harvests, and for the 3rd harvest also had a greater dry matter yield than the control (Figure 8.3). The CL and AL treatments resulted in a greater P offtake for the 2nd harvest than the control (Figure 8.3). In comparison to the untreated slurry amended treatments, acidified and anaerobically digested treatments showed no significant difference in the P offtake and dry matter of ryegrass of each harvest (Figure 8.3). Comparing solid and liquid treatments, there was only a significant difference for the anaerobically digested slurry, with a larger P offtake for the 3rd harvest for the liquid fraction treatment than the solid fraction treatment (Figure 8.3).

The cumulative P offtake after slurry application (i.e. sum of harvests 1-4) was 19-23 mg pot^{-1} in these treatments. The slurry-amended treatments tended to result in greater P offtake than the control treatment. The cumulative P offtake of the AL (23 mg pot^{-1}) and ADL (23 mg pot^{-1}) treatments were significant greater than the control (19 mg pot^{-1}), but differences between other slurry-amended treatments with the control were not statistically significant. There was also no difference in cumulative P offtake between untreated and acidified treatments, and between untreated and digested treatments. But there was an effect of separation on cumulative P offtake, with the cumulative P offtake of the ADL treatment being significantly greater than the ADS treatment.

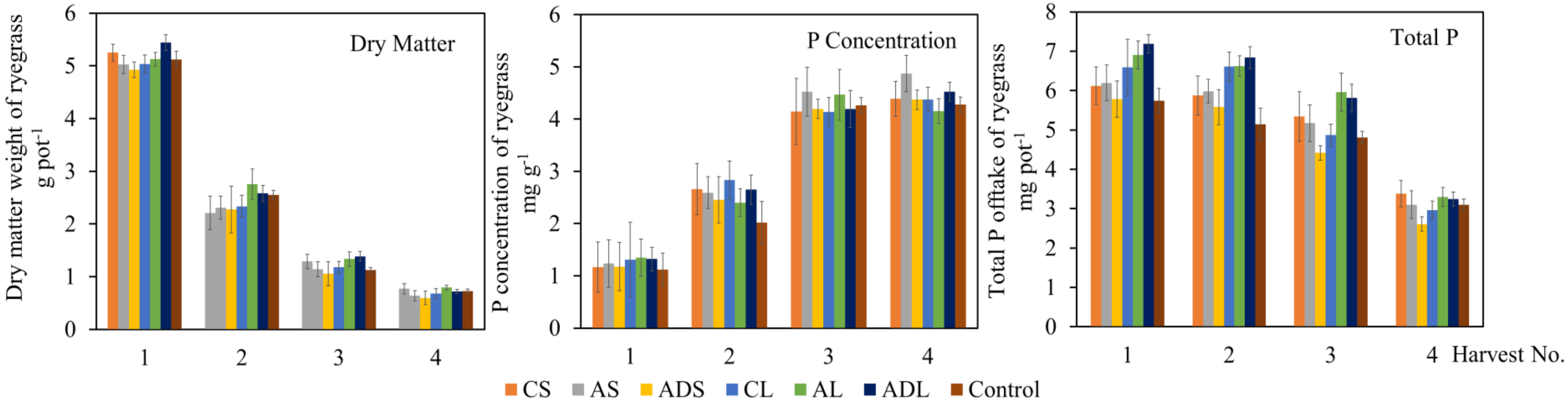

Figure 8.3 Dry matter weight, P concentration, and total P mass of harvest ryegrass. Values represent means ± SEM, n = 4.

8.4.4 Chemistry of different soil depths

Soil pH

The untreated slurry and digested slurry tended to increase the soil pH compared to the control, while the acidified slurry tending to decrease the pH (Figure 8.4). Specifically, the 0-1 cm and 3-5 cm soil of the CS treatment, 0-1 cm 1-2 cm, and 3-5 cm soil of the ADS and CL treatments, 1-2 cm and 3-5 cm soil of the ADL treatment showed significantly higher pH than the control (Figure 8.4). The pH of the 0-1 cm soil of the AS treatment and the pH of the 0-1 cm and 1-2 cm soil of the AL treatment was significantly lower than the control (Figure 8.4). Compared to the untreated slurry treatments, the digested treatments had no effect on soil pH. While the pH of the AS treatment was significant lower than the CS treatment in the 0-1 cm soil, and the pH of the AL treatment was lower than the CL treatment in the 0-3 cm soil (Figure 8.4). There was no difference between the CS and CL treatments, and between the ADS and ADL treatments. But the pH of the 1-2 cm soil of the AL treatment was significantly lower than the AS treatment (Figure 8.4). There was little change in soil pH with depth through the soil column, except in the AS and AL columns, which displayed a trend of increasing soil pH with depth (*p*<0.05).

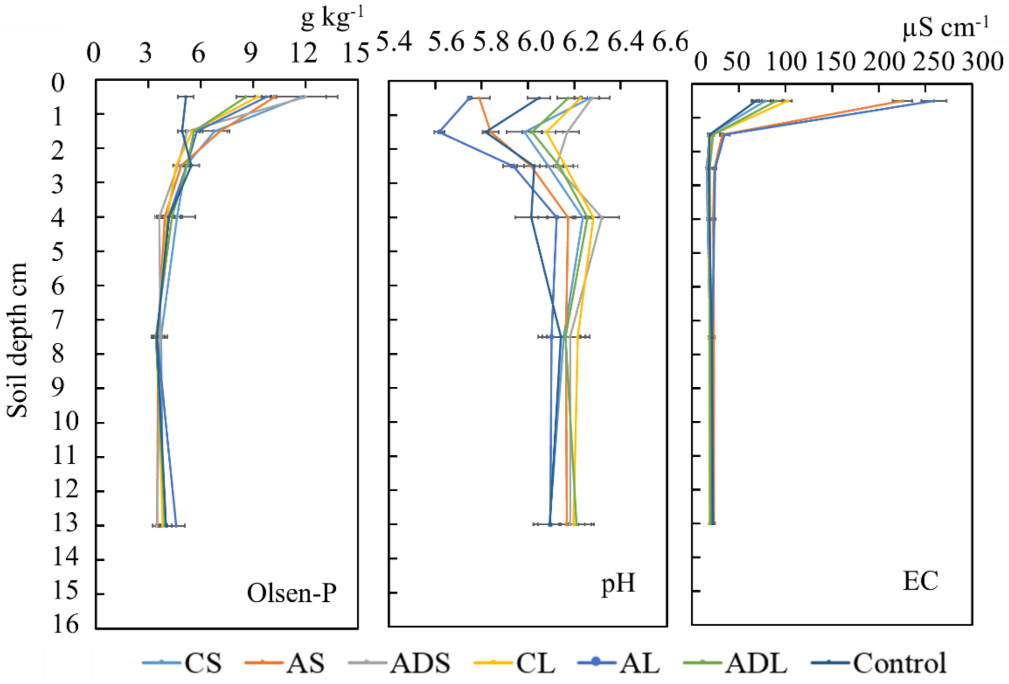

Figure 8.4 pH, EC and Olsen-P content of different soil depths at the end of the incubation. Values represent means ± SEM, n = 4.

Soil EC

Slurry addition tended to increase the EC of soil. Compared with the control treatment, the EC of the 0-1, 1-2, 2-3, 3-5 cm soil of the AS treatment, the 0-1 cm soil of CL, the 0-1 and 2-3 cm of AL, the 0-1 and 1-2 cm soil of the ADL treatment were significantly higher. The soil EC values in the slurry-amended soil cores decreased with depth (Figure 8.4), especially between the top two cm layers. Both AS and AL treatments had significantly greater EC values than the other two types of slurry treatments throughout the whole soil column, except the 5-10 cm soil of the AS treatment and the 10-16 cm soil of the AL treatment. Comparison between solid and liquid treatments did not show any difference in EC.

Olsen P content

All slurry-amended treatments had significantly greater Olsen-P contents than the control in the 0-1 cm layer (Figure 8.4). The Olsen-P contents of all treatments except the control showed a significant decrease with depth, comparing the 0-1 and 1-2 cm layers. The AS, AL and CL treatments also showed a trend that the Olsen-P content gradually decreased with greater depth (p<0.05), while the remaining treatments showed no difference between the different depths below upper 2 cm. There was no significant difference in the Olsen-P content of the 0-1 cm soil between the different slurry-amended treatments. Furthermore, there was seldom any significant differences seen between any treatments, including the control, in the Olsen-P content below the top 1 cm. Only the AS and AL treatments attained greater Olsen-P content in the 1-2 cm soil depth than the control. In addition, the 2-3 cm soil of the CL treatments was significantly lower than the AL and ADL treatments. No difference in the Olsen-P content was found between the solid and liquid fractions of any of the slurry treatments.

8.5 Discussion

8.5.1 Effect of untreated livestock slurry on P dynamics in soil

Addition of untreated livestock slurry improves the P offtake by ryegrass, because, the available P direct from livestock slurry acts as an inorganic P fertilizer. Also, addition of livestock slurry is known to accelerate organic P mineralisation in the soil, especially in long-term consecutive application experiments (Whalen et al., 2001; Leytem et al., 2002; Pagliari & Laboski, 2014). Moreover, some studies suggest that some organic P forms (e.g.

phytate) from livestock slurry might preferentially sorb onto soil particles, and exchange for indigenous soil inorganic P (Celi et al., 1999; Marshall & Laboski, 2006; Karathanasis & Shumaker, 2009).

As expected, our CS and CL treatments resulted in greater total P offtake than the control, which agrees with the hypothesis that livestock slurry improves P offtake of plants, although this was not necessarily significant. The lack of significance may because ryegrass roots located all over the soil column, absorbing P from both slurry and soil. The applied amount of P of slurry was small compared to the total and available P reserves in the soil profile. Further, most of the applied P remained in the upper soil layer where only some of the roots were located. Thus, the uptake of native P from soil may have dominated over than present in the added slurry. In addition, our results did demonstrate that ryegrass took P from soil reserves, based on the Olsen-P content reduction (28%-51%) of all depths of the control column compared to original sampled soil. If the slurry had been incorporated with the soil thoroughly rather than spread on the surface, this problem of interference from soil P reserves may have been avoided. But in that case, the slurry had to be applied before sown, while in this experiment we expected to conduct on mature root system simulating the field condition.

Our application rate may be conservative, resulting in larger portion of P uptake from soil than slurry. Föhse et al. (1988) found when the P fertilizer rate is lower than 0.10 mg g^{-1}, the shoot P content and dry weight of ryegrass increases with increasing P fertilizer rate in a linear manner. Therefore, we used a low application rate of livestock slurry in order to ensure it fell within this linear range and reflect the role of P input from livestock slurry, and avoid P site saturation in soil disturbing colloid-facilitate P leaching which is consistent with the Chapter 6.

In the study of Haque et al. (2015), their treatment of 5 g kg^{-1} of dry cattle slurry (which is close to the situation in our study if assuming that the 10 kg ha^{-1} of slurry is incorporated with upper 2 cm of soil) resulted in 6-12 mg kg^{-1} extra Olsen-P than the control over their monitoring incubation period, and P mineralization (the difference in Olsen-P content to the control) reached a peak within the 4^{th}-6^{th} week of incubation after slurry addition. In our study, we did not monitor the P of soils during the incubation, but the Olsen-P content at the end of the incubation in the untreated slurry treatments resulted in 4-7 mg kg^{-1} extra Olsen-P in the 0-1 cm soil layer compared with the control. Haque et al. (2015) did not include any plants, while we grew ryegrass. Consequently, the Olsen-P content in our experiment was lower than theirs because of the ryegrass uptake.

It is already known that in the soil with saturated P sorption sites added P could move deeper with irrigation (Sharpley et al., 1994; Menzies et al., 1999). However, in the short-term small-scale study of Chapter 6, we demonstrate that liquid slurry could cause greater potential P leaching under unsaturated conditions due to the transport of the colloidal slurry fraction. Therefore, in this experiment, we expected a similar behaviour for colloidal P in a soil sown with ryegrass over a longer period. The same soil type and slurry application rate was used. For organic P loss, we did find significantly greater organic P loss in the 8th leachates in the CL treatment than the control. However, an expected significantly greater inorganic P loss from untreated slurry treatments than the control was not shown. In Chapter 6, the interval between the slurry application and leachate collection was only one day, but in this chapter, there was a week interval between the slurry application and the 1st leachate collection, which was sufficient for upper soil to bind the P from slurry and for ryegrass to absorb the labile P, preventing inorganic P leaching. Therefore, the hypothesis that addition of livestock slurry increases potential P leaching was rejected.

8.5.2 Effect of acidification, anaerobic digestion and separation on P dynamics

Acidified slurry

Opposite to the hypothesis, in comparison to the untreated slurry treatments, the acidified treatments did not result in greater plant P offtake and potential P leaching. Acidification is known to dissolve the dominant precipitates of struvite and Ca-P in livestock slurry (Güngör et al., 2007; Christensen et al., 2009), and improve the bioavailability, as indicated by the water extractable P/TP ratio in our results. However, the absolute value difference of the water extractable P content between acidified and untreated slurry was not large enough to make a difference in plant P offtake and potential inorganic P leaching. However, the Olsen-P content of the 2-3 cm soil layer showed a greater value in the AL treatment than the CL treatment, which may be associated with the increased water extractable P content of the acidified slurry. The main impact of applying acidified slurry to soil is more likely to be associated with the reduction in soil pH and increase in soil EC. The AS and AL treatments resulted in significantly lower pH in the 0-1 cm and the 0-3 cm soil respectively than the CS and CL treatments. Low soil pH enhances the adsorption of P to Fe or Al oxides, reducing P mobility (Gahoonia et al., 1992; Waldrip-Dail et al., 2009). Moreover, the low pH dissolved and released salt ions (Hjorth et al., 2015), so we detected significantly greater EC values at almost all depths within the soil columns than the untreated slurry treatments. Since there was no difference in EC between untreated and acidified slurry, the increase EC in soil should be ascribed to low pH.

Anaerobically digested slurry

Anaerobic digestion is expected to improve P plant availability through the degradation processes (Masse et al., 2005), but our result showed the water extractable P/TP ratio of solid and liquid fractions was not significantly increased by anaerobic digestion. Anaerobic digestion could increase the microbial biomass and activity and basal respiration (Bougnom et al., 2012; Hupfauf et al., 2016). Organic P mineralisation is negatively correlated with the C/P ratio (Mafongoya et al., 2000). In our results, the ADS and ADL slurry tended to have less C/P than the CS and CL slurry, although not significantly so. Thus, the digested slurry treatments are supposed to cause higher organic P mineralization than untreated slurry treatments. However, neither our results of the Olsen-P content of soil nor P offtake in the ryegrass presented significant differences between the digested and the untreated slurry treatments. Many other field experiments also did not find significant effects of anaerobic digestion on P availability (Loria & Sawyer, 2005; Moller & Stinner, 2010; Bachmann et al., 2011). So despite measuring differences in the available P content of anaerobically digested slurry (compared to untreated slurry), these do not always result in agronomic benefit, which is opposite to our hypothesis.

Separated liquid and solid slurry

The liquid slurry fraction had a significantly lower C/P than the solid slurry fraction in the untreated, acidified and anaerobic digestion treatments in our results. Some researchers have argued that liquid manure has a greater microbial activity than the solid manure (Ghonsikar & Miller, 1973; Magid et al., 1996; Turner et al., 2003). Hence, the liquid manure-amended soil could have greater degradation of organic matter, including P associated with organic materials, than the solid manure-amended soil. Moreover, small size particles in slurry liquid fraction have greater mobility in soil than large sized particles in the slurry solid fraction (McGechan, 2002). Therefore, the liquid slurry treatments were expected to show more inorganic P release and mobility in terms of Olsen-P, or P offtake, or inorganic P leaching, compared to slurry solid treatments, as we hypothesised. For example, Pagliari & Laboski (2013) showed that the application of the liquid fraction of separated slurry increased soil test P (Bray-1) more than the application of the solid fraction. Hao et al. (2015) reported a greater yield and total P offtake in corn following a liquid swine manure treatment than a solid swine manure treatment, although this was not seen in soybean. The study of Hansen et al. (2004) with long-term (>10 years) application of liquid manure (lagoon manure) and solid manure suggested that the liquid manure treatment resulted in greater inorganic P content in subsurface soil (45-65 cm). However, in our study, such expected results were not seen for the Olsen-P content of soil layers, total P offtake or total P leaching loss over the experiment. Only for P offtake at the 3rd harvest and the total P loss in the 4th leachate, were there greater P values for the ADL treatment than the ADS treatment. This lack of significance may be because (1) the labile P content was similar in the solid and liquid fractions in our slurry samples, based on the water extractable P/ TP ratio, (2) the ryegrass roots took up P from the whole soil column rather than only the upper layer where most slurry remained, and (3) our slurry was only applied once and the rate may have been too low to have observed such differences. If the liquid and solid slurry had been applied for a longer period for several times or with a greater rate, the difference in C/P and organic P degradation, and particle mobility may have resulted in significant difference in soil test P, plant P uptake and potential P leaching.

8.6 Conclusions

The untreated, acidified and anaerobic digested solid and liquid slurry-amended treatments tended to result in a greater total P offtake than the control treatment, with the difference between the total P offtake of the AL and ADL treatments and the control being significant. Addition of untreated and anaerobic digested slurry tended to increase the pH of soil compared to the control, while addition of acidified slurry decreased soil pH. The untreated, acidified, and anaerobic digested slurry all increased soil EC. Comparing different slurry treatments, the acidified slurry caused lower pH than the untreated slurry in the top 3 cm of soil, and greater EC over the whole soil column. However, the anaerobically digested slurry resulted in no difference in soil pH and EC compared to the untreated slurry treatment. Application of untreated, acidified, anaerobic digested solid and liquid slurry significantly increased the Olsen-P content of 0-1 cm soil layer, but no difference was found between different slurry-amended treatments in the Olsen-P content. The cumulative inorganic and organic P loss in leachates over the 12 weeks was not influenced much by addition of untreated, acidified, or anaerobically digested slurry compared to the control, and there was no difference between slurry treatments. The difference between solid and liquid slurry was only found between the ADS and ADL treatments, where the P offtake of the 3rd harvest and the P lost in the 4th leachate of the ADL treatment were significantly greater than the ADS treatment.

In summary, our results from this pot experiment suggested that addition of untreated livestock slurry increased Olsen-P in the surface soil and tended to increase plant P offtake. Acidified and anaerobically digested slurry were both comparable P sources for plants to untreated slurry, increasing the Olsen-P content of soil and P offtake of ryegrass to a similar extent. The liquid fraction of anaerobically digested slurry was better in improving P availability in soil than the solid fraction. Potential P leaching was largely unaffected by addition of differently processed slurries.

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9 Summary and discussion

9.1 Objectives and hypothesis

Livestock slurry can be used to provide P for crops. Orthophosphate is the most bioavailable form, and livestock slurry does contain considerable amount of this P form (Guo & Yost, 1998; Pagliari & Laboski, 2012). However, different P species in livestock slurry are subject to a series of biological, chemical and physical processes in soil after application (Bünemann, 2015), with implication for P bioavailability in soil, and potential loss to watercourses (Simard et al., 1995; Vadas et al., 2015). In livestock slurry, particles of different sizes have different surface area/volume ratios, relating to bacterial colonisation and reaction, and interaction with soil clay minerals (Magid et al., 2010), and have different C/P ratios, with impacts on microbial immobilisation/mineralisation (Takeda et al., 2009; Malik et al., 2013). Furthermore, small particles, i.e. colloids, can facilitate P mobility in soil (Haygarth et al., 1997; de Jonge et al., 2004; Schelde et al., 2006; Withers et al., 2009; Glæsner et al., 2013).

Acidification and anaerobic digestion have the potential to affect slurry composition (Marcato et al., 2008; Fangueiro et al., 2015; Hjorth et al., 2015). However, we lack a clear understanding of the effects of these slurry processes on P speciation associated with particles of different sizes in differently treated livestock slurry and their effect on P bioavailability and P loss in soil after application.

Therefore, this thesis focused on both P speciation and particle size composition and distribution in livestock slurry to study the P cycle from livestock slurry through to the soil. This thesis aimed to characterize P in the differently processed cattle slurries with respect to P speciation of different sizes of particles, and to elucidate P bioavailability and mobility in soil after application. The overall hypotheses were a) small particle size fractions of cattle slurry dominate the total P content, and have great mobility in soil, and b) slurry acidification and anaerobic digestion modify the P speciation and distribution in cattle slurry, and thereafter affect P availability and mobility in soil. This Chapter synthesises the effects of slurry processing on P speciation and distribution within the different slurry particle sizes and on P availability and mobility in soil after application before returning to these specific hypotheses in the conclusions.

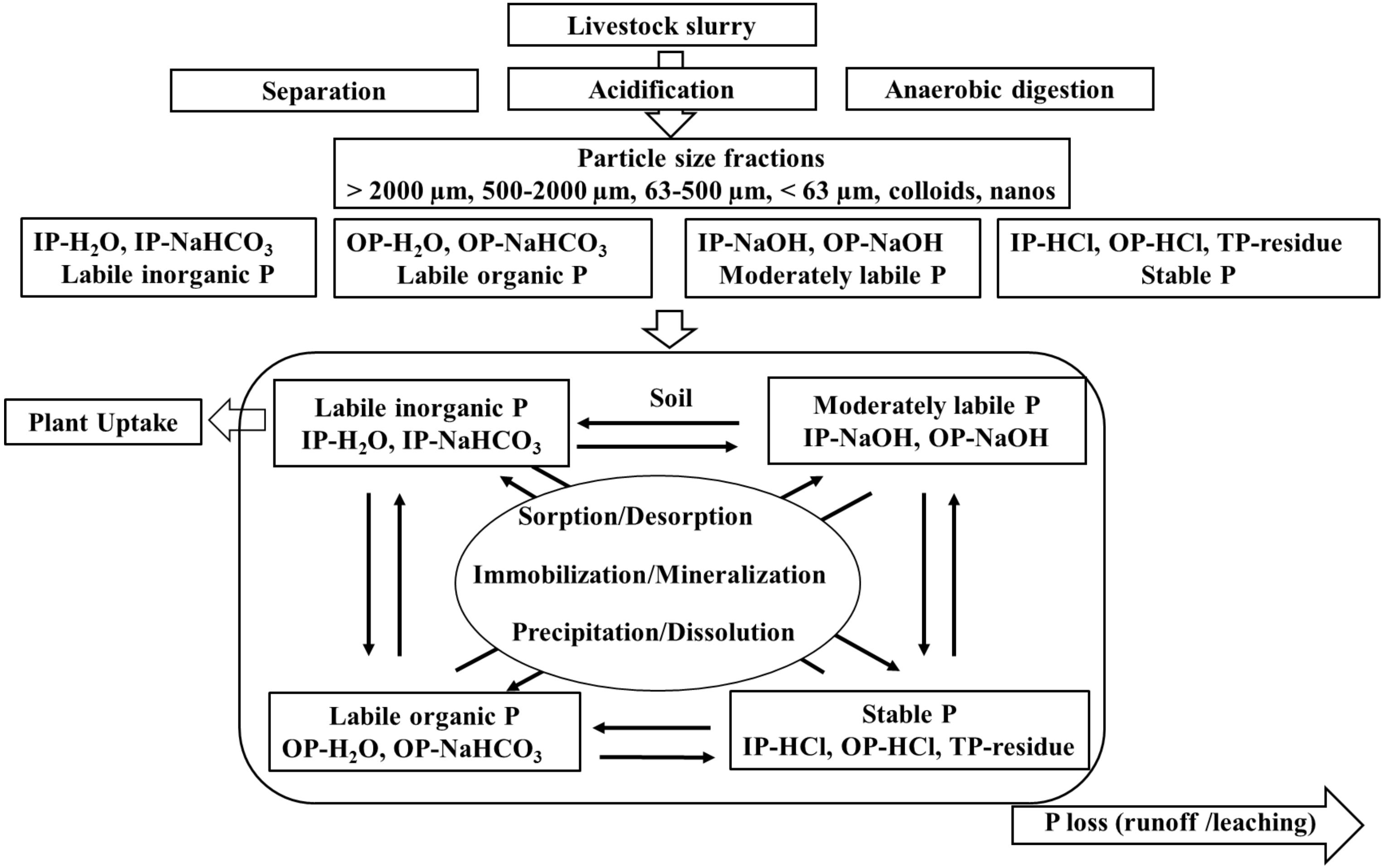

Figure 9.1 P cycle from livestock slurry to soil

9.2 P cycling from livestock slurry to soil

In this section, we discuss the P cycle from cattle slurry to soil (Figure 9.1) mainly based on our results from previous chapters. Slurry is composed of different particle size fractions which are associated with P species with different solubility and bioavailability. Advanced slurry processing, separation, acidification and anaerobic digestion, alter slurry P nutrient characteristics, which is largely related to the P speciation of different particle size fractions. Once applied to the soil, livestock slurry-derived P whether treated or untreated, experiences a series of biogeochemical processes, including sorption/desorption, immobilisation/mineralisation, precipitation/dissolution. As a result, transformation between different P pools occur over time, affecting plant P uptake and potential P loss.

9.2.1 Untreated Cattle Slurry

In untreated cattle slurry (DM of 11% and TP of 0.6 g kg^{-1} (on fresh basis)), the labile inorganic P (IP-H_2O + IP-$NaHCO_3$) and labile organic P (OP-H_2O + OP-$NaHCO_3$) represented 45% and 24% of TP of the whole slurry, respectively (Chapter 3). The moderately labile P (IP-NaOH + OP-NaOH) and stable P (IP-HCl + OP-HCl + TP-residue) fractions represented 10% and 21% of TP content of the whole slurry, respectively (Chapter 3). These results are consistent with other studies concluding labile P dominates in cattle slurry (Dou et al., 2000; He et al., 2006; Pagliari & Laboski, 2012) . In contrast, in soil, moderately labile and stable P forms are the dominant P pools (Cross & Schlesinger, 1995). Addition of slurry provides labile P to soil which is bioavailable for plants.

Dry matter (DM) distribution in the four (operationally defined) particle size fractions of >2000, 500-2000, 63-500 and <63 µm were 40%, 14%, 12% and 35%, and the TP distribution were 19%, 7%, 7% and 67%, respectively (Chapter 3), which is similar to other studies (Meyer et al., 2007; Peters et al., 2011). Other livestock slurry may show different characteristics. For example, in contrast to cattle slurry, in pig slurry, more DM is distributed in the smaller particle size fractions than larger particle size fractions (Møller et al., 2000; Masse et al., 2005). In poultry manure, struvite and Ca-P are the major P forms (Pagliari & Laboski, 2012).

To separate further, the small particle sizes of 0.45-63 µm, categorized as colloids in our study, accounted for 62% of TP of the whole untreated cattle slurry. The proportions of labile inorganic P, labile organic P, moderately labile P, and stable P in the colloidal fraction were 46%, 20%, 12%, and 22%, respectively. While the <0.45 µm fraction only accounted for 5% of TP of the whole cattle slurry. Similarly, Masse et al. (2005) also found 50% of the TP of whole pig slurry was in the 0.45-10 μm fraction, but greater proportion (20%) was in the <0.45 μm fraction. The nanoparticulate fraction of 0.03-0.45 μm represented only 3% of TP of whole cattle slurry (Chapter 5). Almost all the rest 2% of TP was in the most soluble fraction of <0.001 μm. Small particles are more likely to leach through soil, and colloid- and nanoparticle-facilitated transport is thought to be an important pathway for P leaching (Hens & Merckx, 2002; McGechan & Lewis, 2002; Siemens et al., 2004; Regelink et al., 2013).

Therefore, it was expected that colloid-associated P would have great mobility in soil and increase potential P leaching after slurry application, whist the nanoparticulate fraction would not contribute much to potential P leaching because of its low content. In Chapter 6, we confirmed this. In this leaching study, we demonstrated that the colloidal fraction predominantly contributed to potential P leaching after applying the liquid fraction of separated slurry to a low P status soil under unsaturated conditions, and 2.4% of the TP of the added colloidal fraction was transported to the leachate. The nanoparticulates in the separated cattle slurry made no difference to potential P leaching.

9.2.2 Separation

We estimated the characteristics of solid and liquid fractions from slurry separation, using results of the particle size composition and P distribution analysis following sieving through different sieve sizes. For the untreated cattle slurry, the separation efficiencies (solid/whole ratio) of DM for separating through sieve sizes of 2000, 500, 63 μm were 40%, 54% and 65%, respectively. The separation efficiencies of TP for these three sizes were 19%, 26% and 33%, respectively (Chapter 3).

Generally, in the liquid fractions of untreated cattle slurry from three separating sizes (<2000, <500, <63 μm), the proportion of TP represented by labile inorganic P (46%, 46%, 45%) were greater than that in solid fractions (41%, 41%, 41%), while solid fractions tended to show greater proportion of moderately labile (13%, 12%, 12%) in TP than liquid fractions (10%, 10%, 10%) (Chapter 3). Labile organic P proportions were *ca.* 24% in both solid and liquid fractions (Chapter 3).

Some researchers have argued that liquid manure has a greater microbial activity than the solid manure (Magid et al., 1996; Turner et al., 2003). Also, since liquid fractions have lower C/P ratio and small particle sizes (Chapter 3), it was expected that application of liquid slurry fractions would result in a greater labile inorganic P content in soil than solid fractions;

and that organic P in liquid fractions would mineralise to a greater degree, and at a faster rate than in solid fractions; and that the liquid fraction would result in greater plant P uptake and potential P leaching from soil than from the solid fraction.

In Chapters 7 and 8, cattle slurry was separated with a 63 µm sieve to generate solid and liquid fractions. These separated fractions were applied on the same TP and water volume basis. When incorporated with the soil, on the day 1, in the solid fraction (<63 µm) of the untreated cattle slurry amended treatment, the slurry-derived labile inorganic P, labile organic P, moderately labile P, stable P represented 40%, 29%, 2% and 29% of soil TP respectively. This showed no significant differences with values of 27%, 31%, 15%, and 27% respectively for the liquid fraction treatment (Chapter 7). So, in contrast to expectation, the liquid fraction did not cause greater labile inorganic P in soil than the solid fraction.

During incubation of the applied slurry-P in the soil, P solubility and availability is controlled by sorption/desorption, immobilisation/mineralisation, precipitation/dissolution (Bünemann, 2015). The soluble P content should decrease once it enters the soil, due to sorption of P to Fe and Al hydroxides and precipitation with Ca, and experiencing adsorption → precipitation onto mineral surface → crystallisation (Perassi & Borgnino, 2014). However, organic molecules from slurry may associate with Al and Fe, decreasing P sorption (Iyamuremye et al., 1996). Moreover, some organic P forms, e.g. phytate, from livestock slurry might preferentially sorb onto soil particles, and exchange for indigenous soil inorganic P (Celi et al., 1999; Marshall & Laboski, 2006; Karathanasis & Shumaker, 2009). Therefore, slurry would maintain soil solution P for a longer period than inorganic P fertiliser as demonstrated in other studies (Audette et al., 2016), although this was not tested in this thesis. According to the fitting of a sorption first order kinetics in Chapter 7, the IP-H_2O concentrations, representing soil solution P, in the solid and liquid cattle slurry treatments had half-lives of 65 and 69 days, respectively.

Mineralisation and immobilisation occur simultaneously in soil. Buehler et al. (2002) found inorganic ^{33}P was immobilised to the OP-NaOH and OP-HCl forms after 14 d of incubation in soil. Organic materials with >2 mg kg^{-1} of TP are believed to show net P mineralization (Floate, 1970; Roboredo et al.,2012). Addition of livestock slurry can accelerate organic P mineralisation in the soil, especially in long-term repeat application experiments (Whalen et al., 2001; Leytem et al., 2002; Pagliari & Laboski, 2014). Liquid manure tends to have greater microbial activity and a lower C/P ratio than solid manure

(Magid et al., 1996; Turner et al., 2003, Chapter 3). Cumulative P released in soil was negatively correlated with the C/P ratio (Mafongoya et al., 2000). Thus, after application, the liquid slurry fraction is expected to cause greater net mineralisation than the solid fraction.

After 126 days of incubation (Chapter 7), the labile inorganic P proportions of both untreated solid and liquid slurry treatments decreased significantly, and there was no difference in the reduction rates between these two treatments. However, the slurry-derived total inorganic P proportion showed a significant increase in the untreated liquid slurry treatment between day 1 and day 126, indicating rates of mineralization dominated those for immobilization, while there was no change in the untreated solid treatment (Chapter 7), implying greater P release from organic matter of the untreated liquid slurry treatment than the CS treatment, which is consistent with the hypothesis above. In Chapter 8, both the untreated solid and liquid slurry fractions increased soil pH, EC, and the Olsen-P content of the upper soil, and yielded slightly greater ryegrass P offtake, although there was no significant difference between the untreated solid and liquid treatments. Thus, the difference in P speciation between solid and liquid fractions was not large enough to make differences in speciation and transformation of added P in soil, when applied once on the same TP and water volume basis. Repeated long-term application may present differences. Further, in practice, solid and liquid fractions of slurry are applied to fields without maintaining the same water volume input as we did in the laboratory condition. The liquid fraction would add more water to soil, which may facilitate P desorption and movement through the soil.

9.2.3 Acidification

Acidification had little effect on the DM and TP distributions in the different particle size fractions (Chapter 3). However, the labile inorganic P proportion increased from 45% to 55% of TP of whole acidified cattle slurry (Chapter 3), mainly due to P desorption and the dissolution of dominant precipitates, i.e. struvite, Ca-P and other occluded P (Güngör et al., 2007; Güngör & Karthikeyan, 2008; Christensen et al., 2009). In contrast, the proportion of stable P decreased from 21% to 15% of TP of whole acidified slurry (Chapter 3). Following separation of the acidified slurry, the P form proportions in the solid fractions (> 2000, >500, >63 µm), from three sieve sizes, showed little change (Chapter 3). However, in the liquid fractions (<2000, <500, <63 µm), the labile inorganic P proportion of TP increased from 46%, 46%, and 45% to 58%, 56%, and 56%, respectively, and the moderately labile P proportion increased from 10%, 10%, and 10% to 11%, 12%, and 12%, respectively. While the stable P proportions in the liquid fractions decreased from 20%, 21%, and 21% to 14%, 15%, and 15%, respectively (Chapter 3).

Acidification did not alter the DM quantity of colloids, but the TP quantity of the colloidal fraction decreased from 62% of TP of whole untreated slurry to 37% of TP of whole acidified slurry. The P form composition of the untreated colloidal fraction was 46% of labile inorganic P, 20% of labile organic P, 12% of moderately labile P, and 22% of stable P, and changed to 33%, 22%, 19%, and 26%, respectively, in the acidified colloidal fraction, due to labile P being dissolved and transferred into the <0.45 µm fraction. At the same time, the TP content of the <0.45 µm fraction increased >8 times, and the increased P was mainly in the soluble fraction of <0.001 µm. The nanoparticle-associated P was interpreted by the significant difference between the <0.45 and <0.03 µm or between the <0.03 and <0.001 µm fractions. However, as a result of this substantial increase in soluble P and its relatively large variability, no significant differences were monitored in TP and IP concentrations between the <0.45 and <0.03 µm or between the <0.03 and <0.001 µm fractions. We could not conclude if the nanoparticle-associated P content had changed or not. But even if the nanoparticle associated P was changed by acidification, it was not large enough to result in greater potential P leaching from soil.

Therefore, it was expected that acidified slurry would result in a greater labile P content of the soil and greater plant P uptake than untreated slurry, but would not show differences in potential P leaching from soil compared to the untreated slurry. However, in Chapter 7, the differences in labile and stable P contents between untreated and acidified slurries were not big enough to be reflected in the slurry-derived labile and stable P proportions of soil on day 1, and the slurry-derived P change during the soil incubation showed little difference to untreated slurry treatments. However, Roboredo et al. (2012) showed that an acidified pig slurry treatment resulted in greater labile P than from an untreated slurry treatment, with double the P application rate we used. Thus, our low P application rate may be one of the reasons for the insignificant differences in labile P comparison between untreated and acidified slurry treatments in Chapter 7. In addition, the acidified treatments did not result in greater plant P offtake than untreated slurry (Chapter 8). But in Chapter 8, 12 weeks after slurry applied, the Olsen-P content of the 2-3 cm soil layer was significantly greater in the acidified liquid slurry treatment than the untreated liquid slurry treatment. The acidified solid and liquid slurry treatments resulted in significantly lower pH in the upper soil compared with the untreated solid and liquid slurry treatments.

Moreover, although no difference was seen between the EC values of untreated and acidified slurry before application, the low pH in acidified slurry dissolved and released salt ions in soil (Hjorth et al., 2015), resulting in significantly greater EC values of all depths of soil columns compared to the untreated slurry treatments. In soils with <10% clay, P sorption increased with decreasing pH (Gahoonia et al., 1992; Holmén & Casey, 1996; Waldrip-Dail et al., 2009; Gustafsson et al., 2012). But potential P leaching results from both Chapter 6 and Chapter 8 showed no significant differences between untreated and acidified slurry treatments.

9.2.4 Anaerobic Digestion

Anaerobic digestion reduced the cattle slurry DM content by 40%. Further, because the degradation of organic matter occurred unevenly across the different slurry particle size fractions, the DM distribution was also altered. In anaerobic digesters under the condition of stirring and high temperatures, microbial degradation of large particles is relatively fast compared to small particles (Hjorth et al., 2010). In our results, the DM reduction extent was greatest for larger particles >500 µm than smaller particles <500 µm. As a result, the proportions of >2000, 500-2000, 63-500, and <63 µm fractions of total DM of the anaerobically digested slurry changed from 40%, 14%, 12% and 35% to 24%, 9%, 17% and 50%, respectively. Similarly, Masse et al. (2005) found particles <10 µm proportion of the DM of whole pig slurry increased after anaerobic digestion. Less change was found in the TP distribution in these particle fractions, compared to DM. The proportions of >2000, 500-2000, 63-500 and <63 µm fractions in TP of whole anaerobically digested slurry were 19%, 5%, 10%, 66%, respectively.

The labile inorganic P and moderately labile P proportions of TP of whole cattle slurry increased to 53% and 12% respectively after anaerobic digestion, while labile organic P proportion decreased to 13%. The proportion of stable P did not change much. In the solid fractions from the three separating sieve sizes, the labile inorganic P proportions also increased to 47%, 48% and 49%, respectively. Moderately labile P proportions in the solid fractions increased to 15%, 15% and 14% respectively. The labile organic P decreased to 19%, 18%, 16% respectively. In the liquid fractions from the three separation sieve sizes, the labile inorganic P proportions increased to 55%, 53% and 53%, respectively. The moderately labile P increased to 12%, 12%, 12%. The labile organic P of solid fractions decreased to 11%, 13%, 13%.

The DM mass of the colloidal fraction decreased during anaerobic digestion by 18%, which was lower than the reduction in other larger particle size fractions. Consequently, its proportion of total DM of the whole slurry increased from 28% to 37%, with no change in the colloid proportion of the DM of the <63 µm fraction. The TP of the colloidal fraction still represented the same proportion of TP of the whole slurry after anaerobic digestion. The P form change of the colloidal fraction was mainly in the reduction of labile organic P and an increase of labile inorganic P content. The proportions of labile inorganic P, labile organic P, moderately labile P, and stable P of the colloidal fraction of digested slurry changed to 54%, 10%, 14%, and 22%, respectively. Colloid-facilitated P leaching following application of the digested slurry may result in greater inorganic P loss. In the nanoparticlulate fraction, anaerobic digestion resulted in only a small (insignificant) decrease in the inorganic P content of the 0.03-0.45 µm fraction. The organic P and TP contents of the <0.001 µm fraction were significantly reduced by anaerobic digestion. The inorganic P content of the <0.001 µm fraction was also reduced, but not significantly. The magnitude of these changes in the nanoparticulate and soluble fractions was small and would not be expected to influence P dynamics in the soil after application.

Therefore, it was expected that application of anaerobically digested slurry to soil would cause greater labile inorganic P and moderately labile P contents of the soil, greater plant P uptake and potential inorganic P leaching from soil than untreated slurry. However, in Chapter 7, on the day 1, there was no difference in the slurry-derived labile inorganic P proportion of the soil between untreated and anaerobically digested treatments. In contrast, the slurry-derived moderately labile P proportion of the anaerobically digested liquid treatment was a negative value and lower than the untreated liquid treatment, which may be because degraded organic products in digested slurry resulted in greater P desorption from Fe- and Al-P in soil. After 126 days of incubation, the anaerobically digested solid and liquid slurry treatments resulted in greater slurry-derived labile inorganic and organic P proportions than untreated solid and liquid slurry treatments. While the untreated liquid slurry treatment resulted in greater slurry-derived moderately labile P proportion than the anaerobically digested liquid slurry treatment. However, in Chapter 8, 12 weeks after slurry application, the soil Olsen-P content did not result in significant differences between the digested and the untreated slurry treatments. In addition, the measured differences in the available P content of anaerobically digested slurry compared to untreated slurry were not large enough to influence the cumulative ryegrass P offtake and cumulative P leaching over the 12-week experiment. Other researchers have also reported field experiments where they did not find significant effects of anaerobic digestion on P availability (Loria and Sawyer, 2005; Möller and Stinner, 2010; Bachmann et al., 2011). However, in Chapter 6, the two-day experiment did imply anaerobically digested liquid fraction (<63 μm) caused greater potential inorganic and organic P leaching facilitated by colloids. The different time intervals between slurry application and leachate collection may be the main reason leading to the different results from Chapter 6 and 8. In Chapter 8, there was one week between slurry application and water event, allowing sufficient time for slurry P bound with soil. Thus, the slurry application should be recommended with consideration of irrigation or local rainfall events to reduce P loss risk.

9.2.5 Discussion and implication

P recycling from manure

In China < 50% of manure P is being recycled to cropland (Bai et al., 2016), which is much smaller than the nearly 100% recycling rate in the UK and EU (Ott & Rechberger, 2012). Efforts should be made within the manure management chain, i.e. ‘feed-excretion-housing-storage-treatment-application’, to reduce P loss and improve P cycling efficiency (Bai et al., 2016).

60%-90% of nutrients in feed are typically excreted via dung and urine and vary for different animal species and managements (Suttle, 2010). For example, ruminant livestock such as cattle typically have a lower P content in their manure because of their more efficient digestion system than non-ruminant animals such as pigs (Pagliari & Laboski, 2012). Poultry manure contains greater Ca-P than swine and cattle manure (He et al., 2006; Pagliari & Laboski, 2012). In addition, mineral supplements are sometimes added to improve livestock performance and health (Liu et al., 2017). Added elements may affect P use efficiency in livestock feed and thereafter influence excreted P speciation and solubility in manure. It has been reported that the increase in dietary Ca reduces phytate adsorption efficiency for animals (Suttle, 2010). Balanced element supplements and phytase additives are important to improve feed P adsorption efficiency and reduce nutrient excretion.

Our results on P speciation and particle size composition of cattle slurry are applicable to the slurry from only one farm, and cattle slurry particle size composition may vary due to the different practices and management such feeding, cleaning water volume and bedding materials on different farms. Some farms may also apply mechanical separation to generate solid and liquid manure fractions. But we have generated useful information on P form composition in terms of P solubility and availability for each range of particle sizes, which could be used to estimate P solubility of certain cattle slurry samples by its water content and approximate particle size composition if cows were on a similar diet. Similarly, the performance of mechanical separation and P in separated fractions could also be estimated as discussed in section 9.2.2.

Manure/slurry is typically stored for several months before being recycled to cropland and grassland. This is especially the case if a farm is in a Nitrate Vulnerable Zone, where farmers with high available N content manures are prohibited in spreading manure to land during the winter months when the risk of nitrate leaching is greatest. In such circumstances, there is a requirement to store these high available N content manures for 6-9 months (Oenema, 2004). During storage, slurry composition may change vertically in the store, due to the various densities of different slurry particles. Light fibrous material such as straw may float on the surface and form a crust, whist heavier material may sink within the depth of slurry (Burton & Turner, 2003). Based on our observation, the smaller particle size fractions (above 0.45 µm) had a greater density than the larger particle size fractions in the cattle slurry. Old natural crusts are a porous matrix in contact with the air, allowing some diffusion of oxygen into the crust (Nielsen et al., 2010). Thus, aerobic and anaerobic microorganisms are distributed differently throughout the slurry depth, so biological processes are also different throughout the slurry store. In the deeper depths under anaerobic condition, small particles may degrade to a greater extent than larger particles (Hjorth et al., 2010). Therefore, the P proportion represented by small particulate or colloidal fractions may decrease during storage. On the other hand, pH may also increase during storage, resulting in precipitation of Ca-P and struvite, replenishing colloidal P or particulate P.

Anaerobic digestion maximises the slurry conditions to generate biogas. It is a green energy technology producing biogas for heat and power generation, and benefit in reducing odour and pathogens in livestock slurry (Smet et al., 1999; Sahlström, 2003; Clemens et al., 2006). Under the warm and stirred condition in the digester, large particles degrade faster than small particles (Hjorth et al., 2010). Organic P is mineralised, with the formation of struvite and other P precipitates (see section 4.4.2). Anaerobically digested slurry with reduced dry matter is also be mechanically separated into solid and liquid fractions.

Separated liquid slurry cannot form crusts due to its low fibre content, but sedimentation of heavier, more dense particles may still occur. Based on our results, a large proportion of the slurry P is distributed in this liquid fraction. Its high-water content (and volume) means storage and transportation costs are high, whilst its high P content means that application rates are limited. Therefore, strategies to recover P from the liquid fraction of separated slurry prior to spreading may be attractive in some situations where the local landbank is limited by high soil P status. In addition, the efficiency of recycling manure P to cropland is only about 30%-62% in China (Bai et al., 2016). The main loss pathway is direct discharge to watercourses. Without appropriate and effective regulation, the benefit in recycling manure as a substitute for inorganic fertilisers is not attractive to Chinese farmers, especially as the costs of constructing large storage tanks or paying for the transportation of manure to distant nutrient-demanding fields are prohibitively high.

Perhaps the upcycling of these ‘wastes’ into high-value chemical products would be more profitable. Chemical treatment processes include addition of cations for formation of Ca-P, Fe-P or struvite ($MgNH_4PO_4.6H_2O$). Struvite recovers both P and NH_4^+ from slurry, and it has been demonstrated to be a good low-release fertiliser (Talboys et al., 2016). Acidification increases the solubility of P and both the NH_4^+ and P content in dissolved fraction in cattle slurry is high (3.4.1). Thus, acidification could be a pre-treatment before separation, followed by an adjustment of the pH and addition of Mg for an optimal formation of struvite.

The separated slurry solid fraction can be further processed by composting to reduce its volume, odour and pathogen content, and stabilise the organic matter (Chowdhury et al., 2013). Materials such as straw break down in the manure, and the bulk density of this solid fraction increases during composting (Chowdhury et al., 2013). The water extractable P content has been reported to decrease after composting, but the composted solid fraction did not result in any P deficiency after applied to soil (Jorgensen et al., 2010).

P loss from agricultural fields

Besides the challenge of the low recycling rate of manure in China, the low P use efficiency and P losses from agriculture land following manure applications is also a serious issue both in China and the UK in terms of water quality. After manure or fertiliser applications, high nutrient concentrations can be found in runoff water (Smith et al., 2007; Allen & Mallarino, 2008). Vadas et al. (2015) used a modelling approach to estimate P runoff from pasture, and showed that ca. 30% was from dung, 10% from fertilizer, 15% from soil dissolved P, and 45% from soil erosion.

Organic and inorganic P are transported by water flow, either in solution, associated with particles, or incorporated in microorganisms (Schoumans et al., 2014). The concentration of soluble inorganic P in runoff water depends highly on the amount of adsorbed P. The amount of soluble inorganic P in surface runoff shows a good relationship with the soil P status of the plough layer (Allen et al., 2006). Manure/slurry addition instantly increases the P content of the upper soil layer, especially with labile inorganic P (see section 7.4.1; Chardon et al., 2007). Moreover, organic P in runoff may dominate inorganic P because of its lower sorption affinity to soil minerals. In addition, coarse fractions of cattle slurry are more likely to be retained on the soil surface if surface applied, and are at greater risk of being lost via runoff due to its low density. Thus, shallow injection of slurry would be a better practice, avoiding coarse fraction runoff (assuming injection slots are across the slope) and benefiting root uptake.

Acidified slurry and anaerobically digested slurry have different pHs, which may influence P sorption in soil and thereafter affect P loss with water flow. In calcareous soil, Ca-P sorption dominates and the addition of the high pH of digested slurry would increase P sorption, while acidified slurry would reduce P sorption and increase P mineral dissolution. Acidification of slurry may result in increased P availability to crops (i.e. it may have a greater fertiliser value), but it also represents a greater potential risk of P loss. In contrast, in acid soil, Fe-P and Al-P prevails in P sorption. The addition of digested slurry would reduce P sorption, while acidified slurry would increase P sorption in acid soil. The digested slurry could also be a slow-release fertiliser in this case.

Dissolved inorganic P leaching seems to be strongly influenced by the phosphate saturation of the soil (Schoumans and Groenendijk, 2000), and many studies have analysed the critical threshold soil P test values in different soils. As shown in section 7.4.2, P solubility in soil decreases with time after slurry application, so avoiding rainfall events immediately after application may help to reduce P leaching losses. However, colloidal P could be important in transporting P through and over soil due to the relatively high mobility of colloids. We showed that *ca.* 3% of inorganic P and 2% of organic P from liquid slurry could be transported with colloids to soil depths below 15 cm (see section 6.4.2), which may contribute to leached P. Perhaps recovery of P from liquid slurry, especially colloidal P, or chemical aggregation of colloidal P in slurry could benefit in reducing colloidal P loss.

9.3 Conclusions

In response to the original objective and hypothesis of the thesis that small particle size fractions of cattle slurry dominate the total P content, and have great mobility in soil, our results positively showed that the majority of P in cattle slurry, untreated and treated, was labile P (>65%), and the TP distribution was dominated by the smaller particle size fraction (<63 µm, >65%). The colloid-associated P accounted for a large proportion of the TP in cattle slurry and has the potential to increase P leaching from soil. In agreement with the hypothesis that slurry acidification and anaerobic digestion modify the slurry P speciation, we found acidification increased the labile inorganic P content and decreased the stable P content of cattle slurry, whilst anaerobic digestion increased the labile inorganic P and moderately labile P contents, and decreased the labile organic P content. However, in contrast to the hypothesis that slurry acidification and anaerobic digestion affect P availability and mobility in soil, the changes in slurry P characteristics resulting from acidification and anaerobic digestion did not necessarily influence plant P uptake and potential P leaching after only one slurry application. More factors, such as water/rain event, should be considered when recommending slurry application and predicting crop production and P loss risk.

9.4 Further work and recommendations

In this thesis, cattle slurry was only collected from one farm, so representativeness may have been limited. A larger sampling size would be necessary to account for differences in farm management regimes, and different livestock types, e.g. pigs, should be explored. To simplify the work, particle size fractions could be reduced to only >63 and <63 µm.

In addition, the anaerobic digestion process in this thesis was conducted under constrained laboratory conditions. In the further work, samples could be collected directly from biogas industries from the influent and effluent.

P speciation of particle size fractions of cattle slurry was analysed by sequential fractionation in this thesis. Whilst it is a useful method to evaluate P solubility and availability, specific P compounds cannot be differentiated. In addition, the analytical accuracy could be affected by humic acid precipitation, acid hydrolysis of organic P, presence of pyro- and poly-phosphates, and complexation and occlusion of inorganic P and incomplete extraction (Condron & Newman, 2011). Further analysis work could use another technology such ^{31}P NMR and XANES to specify the bound of Fe-, Al-, Ca-, OM-P.

In the analysis of colloids of cattle slurry, the upper size range was operationally defined at 63 μm, which is larger than the commonly used definition size of 1 μm or 10 μm (Buddemeier & Hunt, 1988; McGechan & Lewis, 2002). This broader definition of colloids greatly contributed to the additional P leaching in Chapter 6, but the true colloidal faction may only have accounted for a part of the DM and C we defined in Chapter 5. Further detailed separations should be carried out within the 0.45-63 μm slurry fraction. With this additional information, the greater P leaching from the anaerobic digested slurry treatment in Chapter 6 could be better explained. A better understanding of the specific forms of the P associated with colloids and nanoparticles in differently processed slurry is needed to elucidate how acidification and anaerobic digestion affects P cycling in slurry and subsequently in soil. The Fe, Al, Mg, Si content should be determined by ICP for evaluation of P species. Field-flow fractionation coupled with ICP has been developed for river sample analysis. This technology has the potential to be used in the analysis of slurry colloids.

The P application rate used in Chapter 6 was small, and the nanoparticles contained a very small quantity of P. In contrast to other researchers, this experiment did not conclude the important contribution of nanoparticles to P leaching. This may have been due to the low P loading rate. Further research could use increased P application rates, or use of isotope tracking, e.g. specific ^{33}P forms to elucidate the nanoparticulate P movement in soil.

In Chapter 7, the transformation of P forms did not show changing patterns over times as clear as other researchers (Roboredo et al., 2012). Again, perhaps the P application rate was not high enough. But because the water content of the liquid slurry fraction was so high, to avoid exceeding the soil’s water holding capacity, the low rate was the most practical one. Perhaps freeze-drying of the liquid slurry fraction could have been used to reduce its water content. But this could have modified the shapes and sizes of the slurry particles.

After application of slurry P to soil, rapid changes in inorganic P solubility occur due to chemical, biological and physical processes, e.g. sorption/desorption and microbial immobilisation/mineralisation. Isotopes can help elucidate these processes. For example, the ^{33}P dilution method has been used to analyse microbial organic P mineralisation (Spohn et al., 2013), and could be used to explore other P cycling processes following the application of slurry-derived P to soil.

In Chapter 8, to maintain a simulated grassland condition, ryegrass was sown in pots and grown for 6 weeks before slurry application. When the slurry was applied to the grass sward, the root system was mature and located all depths of the pot. Therefore, the slurry was spread on the surface rather than incorporated into the soil. In addition, the application rate was not very high. As a result, the P uptake from the soil prevailed rather than from the slurry, and expected differences in P uptake between slurry treatments were difficult to measure. Long-term field trials with repeated application of differently processed slurries are needed to study whether and how untreated and treated slurry influence P speciation and mobility in soil. Different soil types, P saturation conditions, P levels, P application rates and water/rain events should also be included as factors. This thesis only studied P loss via leaching. Runoff, is another important loss pathway, and could also be studied with respect of the P distribution in particle sizes fractions of livestock slurry.

Since the colloidal fraction contained the majority of P in cattle slurry, further slurry separation development could focus on recovering P from these colloids. This may require multiple technologies, e.g. centrifugation, membrane filtration, coagulation and flocculation. Recovery of colloid-associated P from livestock slurries could help in reducing the risk of P transfers to watercourses via leaching and runoff, and hence reduce the potential for eutrophication.

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