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USING BIO-MANIPULATION TO OPTIMISE NUTRIENT MANAGEMENT WITHIN INTENSIVE FARM SYSTEMS

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Abstract

Optimising the use of organic amendments, such as livestock slurry, on commercial farms represents one route through which the reliance of agricultural production on inorganic fertiliser use might be reduced. For economic, environmental and geopolitical reasons, decoupling future agricultural production from inorganic fertiliser use is desirable, particularly if increases in future demand for food at global scale are to be met sustainably. However, there remains substantial uncertainty surrounding the impacts of organic amendments on many of the key physico-chemical and microbial properties of agricultural soils. This uncertainty reduces the likelihood that land owners and land managers will adjust farming practices in order to deliver more widespread use of organic amendments to support production. In this context, the research reported in this thesis sought to understand how the management of livestock slurry within intensive grassland systems can be optimised to support production. The thesis had a particular focus on understanding how the soil microbial community mediates the input of livestock slurry, in terms of the influence of this community on the cycling and crop-availability of macronutrients within soil. The thesis first examined the impact of a biological slurry additive, SlurryBugs, on the nutrient content of livestock slurry during storage, finding positive effects of the additive particularly with respect to the total phosphorus (P), where an increase by 27% was observed compared to the control slurry treatment, and the total solids contents of slurry during storage. It was hypothesised that the SB additive may have altered the emission of phosphine (PH_3) from slurry during storage. Subsequently, the impacts of slurry application, both with and without the biological additive, on soil organic matter (SOM), as well as on the nitrogen (N) and P content of grassland soils were examined, in comparison to inorganic fertiliser and control treatments. Positive

effects following slurry application were observed, spanning SOM, Olsen P, mineral N and soil pH conditions.

Finally, the impacts of applying slurry alongside a range of carbon (C) substrates of different quality (glucose, glucose-6-phosphate (G6P), and cellulose) to a grassland soil were examined, in terms of the partitioning of C within soil as mediated by the microbial community and in terms of changes in the structure and biomass of the soil microbial community. The results revealed an increase in the soil microbial biomass, as well as a decrease in the cumulative respiration, following the application of both slurry types, alongside a carbohydrate, compared to the treatment with the carbohydrate alone, likely due to a microbial metabolic mechanism known as preferential substrate utilisation. In addition, a bacterial predominance within the soil microbial community was observed in all treatments, with increasing dominance of fungi toward the end of the 49-day incubations. This thesis also revealed that the quality of C substrates represented a major factor affecting both the extent of mineralisation and of incorporation of externally-derived C into microbial biomass. The application of ^{14}C-glucose or ^{14}C-G6P to soil resulted in a significantly greater incorporation of ^{14}C into microbial biomass by 68 or 57%, respectively, compared to 41% following the ^{14}C-cellulose application. Further, the addition of US slurry alongside ^{14}C-glucose generated a significantly greater extent of mineralisation by 30%, compared to the treatments with AS slurry or with only ^{14}C-glucose added with 19 and 21%, respectively. Taken together, the data reported within this thesis have potentially important implications for the way in which livestock slurry is managed as a nutrient resource on commercial farms, as well as for broader environmental concerns including the acidification of agricultural soils and the impact of agricultural soils on the global C cycle.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and is less than 100,000 words in length. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except due reference has been made.

Vito Abbruzzese

Signature

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List of acronyms and abbreviations

The list below shows the acronyms used in this thesis and their definitions.

ANOVA	Analysis of variance
AS	Amended slurry
CL soil	Clay loam soil
C_{mic}	Microbial biomass C
dwe	dry weight equivalent
EA-IRMS	Elemental analyser - isotope ratio mass spectrometry
F/B	fungal/bacterial
FYM	Farmyard manure
GC-FID	Gas chromatography - flame ionisation detector
GC-MS	Gas chromatography – mass spectrometry
GHGs	Greenhouse gases
G -ve bacteria	Gram-negative bacteria
G +ve bacteria	Gram-positive bacteria
ICP-OES	Inductively coupled plasma – optical emission spectrometry
MANOVA	Multivariate analysis of variance
MR	Maintenance respiration
n.a.	Not applicable
O soil	Organic soil
PE	Priming effect
PI	Priming index
PLFA	Phospholipid fatty acid
P_{mic}	Microbial biomass P
PSB	Phosphate solubilising bacteria
PSU	Preferential substrate utilisation
RCF	Relative centrifugal force
RPM	Revolution per minute

SB	SlurryBugs-SlurryBooster
SOM	Soil organic matter
SL soil	Sandy loam soil
SSR	Soil with substrate respiration
TK	Total potassium
TMg	Total magnesium
TN	Total nitrogen
TNa	Total sodium
TOC	Total organic carbon
TP	Total phosphorus
TS	Total solids
TSP	Triple superphosphate
US	Unamended slurry
VFA	Volatile fatty acid
VOC	Volatile organic compound
WHC	Water holding capacity

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1 Introduction and objectives of the thesis

During human history, food availability has frequently been limited by natural phenomena, including crop pests or drought, and human-induced events, such as conflicts or economic crises. Today, with the world population already exceeding seven billion and predicted to reach almost 10 billion by 2050, global food demand is increasing rapidly and some parts of the world are likely to see reduced food security in the future compared to the present day (Rengel and Zhang, 2011). Commercial food production relies heavily on inputs of externally-derived nutrients to agricultural soil in order to support crop yields (Bouwman et al., 2013). As a result, a significant increase in nutrient input to soil is likely to be required in the future, in order to increase global food production at a rate that meets the growing demand for food from the global population (Van Vuuren et al., 2010). However, following widespread degradation of water resources and of agricultural soils, as well as the rise in energy consumption on farms, in food processing, and in the production of inorganic fertilisers, future agricultural practices are required to make food production more efficient per unit area of land, in order to sustainably meet the predicted increase in global demand for food (Tilman et al., 2002). Deriving greater benefit from existing nutrient stocks within agricultural systems, for example those within soil or within livestock slurry, represents one means through which reliance on the input of externally-derived nutrients to support food production could be reduced in the future (Garnett, 2011).

However, although the need to increase the efficiency of future food production is clear, farm systems are highly likely to continue to rely on a range of external inputs, key among which is inorganic fertiliser. Historically, increased input of inorganic

fertilisers to agricultural soils has been a critical factor in the increases in agricultural yields that have been observed across the UK, Europe and North America, particularly since the 1940s (Stewart et al., 2005). Today, very few agricultural soils can sustain commercial yields without the regular application of externally-derived, plant-available nutrients (Dawson and Hilton, 2011), meaning that very substantial amounts of inorganic fertiliser are used globally on an annual basis to support food production. Between 1960 and 1995, there was a sevenfold increase in the global use of nitrogen (N) fertiliser, whilst the use of phosphorus (P) fertiliser increased 3.5-fold in the same period. Further, the use of both N and P fertilisers is predicted to increase threefold by 2050, unless there is a substantial increase in fertiliser use efficiency within agriculture (Tilman et al., 2001). However, further increases in fertiliser application are unlikely to result in the widespread, positive yield responses that have been seen historically, due to ever decreasing returns in terms of crop yield per unit additional fertiliser application that characterises many agricultural regions (Tilman et al., 2002).

However, a range of other nutrient resources beyond inorganic fertilisers exist within farm systems, including crop residues, food waste, compost, farmyard manure (FYM) and slurry. Optimising the use of these alternative nutrient resources should be a target for work that seeks to support future food production, in order to reduce reliance on externally-derived inorganic fertiliser resources and to better close the loop on farm nutrient cycles (Petersen et al., 2007). In particular, if these organic substrates are recycled for use on the same farm from which they are originally derived, high nutrient use efficiency can be delivered with respect to the original import of nutrient resources through the farm gate, with consequent reductions in the adverse environmental impacts associated with inorganic fertiliser use on farms (Petersen et al., 2007). Further, organic substrates potentially offer a number of

additional advantages over inorganic fertiliser use, due to the effects that organic substrates may have on the biological, chemical and physical properties of soils (Van-Camp et al., 2004). In turn, these effects may translate into enhanced primary production and carbon (C) sequestration in plant biomass and, ultimately, increased crop yields (Acharya et al., 1988, Fraser et al., 1988, Latif et al., 1992, Versini et al., 2013). However, substantial challenges and uncertainties continue to surround the use of organic substrates to support production within agriculture. For example, these substrates are normally added in large quantities to soil, due to their lower nutrient content per unit mass compared to inorganic fertilisers (Hati and Bandyoopadhay, 2011). Because slurry and FYM are predominantly applied to soils according to their N content, and because these materials possess a N:P ratio of between 2:1 to 6:1, P is often added to soil in excess of N following slurry/FYM application (Eck et al., 1995). Compounding the issue of imbalanced N:P in slurry/FYM, the ratio of N to P uptake by crops, including grass, is often even greater, ranging from 7:1 to 11:1, thereby further enriching soils in terms of P content (Heathwaite et al., 2000). In the broad context outlined above, this thesis focusses on determining how the use of organic substrates, in particular livestock slurry, can be optimised to support production in grassland systems, thereby reducing reliance on finite, geo-politically constrained and environmentally costly inorganic fertiliser resources.

1.1 The importance of native organic matter in the availability of plant nutrient within soil

The application of organic substrates, such as livestock slurry, to soil represents an important practice to increase available carbon (C) for soil microorganisms and,

thereby, to alter decomposition pathways of the soil organic matter (SOM) pool (Haynes and Naidu, 1998, Lal, 2004). The SOM pool represents the largest reservoir of C in terrestrial ecosystems, with a magnitude that is three times larger than the C retained in plant biomass and twice the pool present in the atmosphere as CO_2 (Amundson, 2001, Lal, 2004). SOM is also a key factor influencing many ecosystem functions within soil, due to its capacity to bind inorganic ions, such as metals, to reduce erosion, as well as to store nutrients in forms that may subsequently become available to plants through the activity of soil biota (Pascault et al., 2013). Further, SOM plays a crucial role in determining soil quality in relation to water retention, due to the large volume of mesopores and micropores within the soil aggregates associated with SOM (Nanzyo et al., 1993). Alongside allochthonous inputs of organic matter, for example associated with the application of FYM/slurry, autochthonous rhizodeposition, including root exudation (Jones et al., 2009, Nguyen, 2009) and root and mycorrhizal hyphal turnover (Gill and Jackson, 2000, Wallander, 2006), represents a further process through which labile C substrates may enter the SOM pool.

Among the wide range of organic compounds present in soil, the majority of SOM is composed of high molecular weight compounds, including chitin, protein and cellulose, as well as recalcitrant humic substances, all with relatively slow turnover rates (van Hees et al., 2005, Vinken et al., 2005). However, despite often being a relatively small proportion of the total SOM pool, the turnover of low molecular weight organic compounds, such as organic acids, sugars, amino sugars, amino acids and nucleotides, dominates the C released from soil as a result of respiration (van Hees et al., 2005, Rousk et al., 2011). The rate at which different components of the SOM pool are turned over, as mediated by soil biota, is critical for the availability of

plant nutrients within soil, including N and P, as well as for determining the impact of agricultural soils on the global C cycle (Altieri, 1999). Beyond the composition of the SOM pool itself, SOM turnover is also significantly influenced by the physical and chemical properties of soil, including pH, moisture content, temperature, salinity and aeration, as well as the physical accessibility of SOM to microorganisms and enzymes, due to the protection of SOM offered by the mineral matrix and soil minerals (Sollins et al., 1996, Jastrow and Miller, 1997, Baldock and Skjemstad, 2000, Gleixner et al., 2001). In particular, the chemical and physical nature of the soil mineral fraction, as well as the architecture of the soil matrix, represent important factors that promote the stabilisation of SOM (Baldock and Skjemstad, 2000).

To ensure that arable and grassland soils reach maximum productivity, it is not only critical that adequate levels of SOM are maintained, but also that adequate bio-availability of nutrients within soil is maintained (Baligar et al., 2001). The application of organic amendments to soil offers potential benefits because these substrates are important sources of both SOM and of key plant nutrients (Adegbidi et al., 2003, Cordell et al., 2011). However, the fate of OM and of nutrients supplied to soil through FYM or slurry is fundamentally governed by soil biota. Therefore, it is critical that the interactions between soil microbiota and organic substrates, such as FYM/slurry, are robustly understood. This understanding may subsequently be used to manipulate the interactions between soil biota and the input of organic substrates to soil, in order to enhance the availability of plant nutrients within soils and to reduce the reliance of agricultural production on external inputs of inorganic fertilisers.

1.2 Role of soil microorganisms in the cycling of soil organic matter and the availability of plant nutrient from soil stocks

The turnover of SOM is predominantly governed by the activity of soil decomposers, mainly soil microorganisms (bacteria and fungi), which simultaneously control: (i) the storage of SOM for sustaining soil structure and fertility (Le Guillou et al., 2012); (ii) the re-cycling of nutrients in agricultural soil (Pascault et al., 2013); and (iii) the emission of greenhouse gases (GHGs) from soil to the atmosphere (Schlesinger and Andrews, 2000). In this context, soil decomposers must hydrolyse high molecular weight compounds into molecules of lower molecular weight that are capable of being transferred into microbial cells (Glanville et al., 2012).

According to Hütsch et al. (2002), 64–86% of organic compounds that are derived from root exudates are rapidly transported to the intracellular environment and respired by soil microorganisms, whilst the residual of this root-borne C, alongside the OM synthesised by microbial fauna during decomposition, accumulates within the soil matrix. The quantity, form, and distribution of root exudates, as well as the products from subsequent decomposition of microbial fauna, are affected by a number of biotic and abiotic factors from soil and plant (Jones et al., 2004). Among the most important soil biotic factors, microbial community size, structure and activity represent key elements for the turnover of root exudates in soil, due to rapid mineralisation by soil micro-organisms of these root-born substances (Jones et al., 2004). For example, Ryan et al. (2001) reported a half-life of between 0.5 and 2 h for most amino and organic acids, as well as carbohydrates released as root exudates by plants. Whilst rapid mineralisation consumes much of the substrates exuded by roots into soil, some of the exuded C will become incorporated into microbial biomass and enter a pool with a slower turnover time, typically between 30 and 90 days (Ryan et al., 2001).

Because externally-derived organic substrates, such as FYM/slurry, are respired by soil microorganisms alongside native SOM and root exudates, it is important to understand the relative importance of the decomposition of SOM versus decomposition of a substrate that is added to soil with respect to total CO_2 efflux to the atmosphere (Kuzyakov, 2006). This enables an assessment to be made of the likely contribution of susbtrates, such as livestock slurry, to the long-term accumulation of SOM versus short-term respiration. To determine the fate of C applied through amendments to soil, a number of studies have been conducted that assess the partitioning of the C associated with the added substrate and that associated with native SOM, as governed by the microbial community within soil (Hill et al., 2008, Schneckenberger et al., 2008, Dungait et al., 2013, Verastegui et al., 2014). Among the different methods that have been used to probe the partitioning of C, incubation experiments are common (Kuzyakov, 2006). These experiments involve the application of isotopically-labelled substrates to soil, such as ^{13}C and ^{14}C, followed by subsequent determination of the labelled-C fractions in different pools (incorporated within microbial biomass; mineralised and released as CO_2 to the atmosphere; and retained within soil).

As understanding of the dynamics of SOM has developed, different pools of SOM have been defined that distinguish living from non-living components (Condron et al., 2010). In this context, microbial biomass has been defined as one of the major driving forces in the decomposition of SOM (Fan and Liang, 2015). This vision of SOM decomposition as driven by microbial activity is distinct from the traditional view of SOM decomposition as solely temperature- and moisture-driven, as underpins many models simulating C and N dynamics in soil (Molina and Smith, 1997, Smith et al., 1998c, Manzoni and Porporato, 2009). Further, in many soils the majority of total N,

sulphur (S) and P are linked with the microbial biomass, with interactions between SOM and the microbial community controlling the fluxes and bioavailability of these key nutrients (Bünemann and Condron, 2007, McNeill and Unkovich, 2007). In particular, the varying distribution of soil microorganism taxa has potentially important implications for SOM and nutrient dynamics in soil. For example, changes in the relative importance of bacteria versus fungi between near-surface soil horizons (often dominated by fungi) compared to the subsoil (often dominated by bacteria), may have important implications for SOM stored within microbial biomass. For example, fungi are often assumed to have a higher C storage capacity than bacteria (Hendrix et al., 1986), but also for the translocation of nutrients between surface horizons and the subsoil through fungal hyphae. (Oehl et al., 2005).

The application of organic amendments to soil, such as slurry or FYM, may lead to changes in soil microbial community biomass, size and activity. For instance, a number of studies have described modifications to the microbial structure, size and activity of soil microflora, as well as to extracellular enzyme activities, following the application of fresh slurry/FYM to different types of soil (Bol et al., 2003a, Bol et al., 2003b, Plaza et al., 2004, Aguilera et al., 2010, Kheyrodin et al., 2012, Balota et al., 2014). Equally, several studies have reported that the specific nature of changes in the microbial community structure and activity depend on the quality of organic substrates added to soil, such organic acids, carbohydrates and amino acids, as well as plant-derived inputs, including plant litter, crop residues and root exudates (Falchini et al., 2003, Mondini et al., 2006, de Graaff et al., 2010, Eilers et al., 2010, Garcia-Pausas and Paterson, 2011, Paterson and Sim, 2013). Furthermore, a positive relationship has been identified between SOM content and microbial biomass size, with greater SOM content normally being linked to larger microbial biomass, and with

the addition of organic substrates to soil resulting in the accumulation of microbial biomass (Gunapala and Scow, 1998, Nannipieri et al., 2003, Bastida et al., 2008, Plassart et al., 2008).

However, the impact of applying organic amendments on SOM and on soil nutrient cycles, as mediated by changes in the soil microbial community, remains to be fully elucidated. Therefore, this thesis seeks to advance understanding of the interactions between organic amendments, soil microbial communities, the turnover of SOM and nutrient bioavailability in grassland soils.

1.3 Thesis objectives

The aim of this thesis is to understand how production within intensive grassland systems can make better use of organic materials as nutrient resources, thereby reducing reliance on externally-derived inorganic fertilisers. More specifically, the thesis will focus on how the management of livestock slurry, alongside the application of slurry to grassland soil, might be optimised to increase the availability of key macronutrients to grass plants. The thesis has a specific focus on understanding how the soil microbial community responds, in terms of structure and function, to the input of organic amendments to soil and the consequences of changes in the microbial community for macronutrient cycles in grassland soil.

In order to achieve these aims, four objectives were developed. The thesis structure and the main objectives of each chapter are summarised below:

- **Chapter 2.** The objective of this chapter was to review and synthesise: i) current knowledge relating to the management of slurry during storage in grassland

production systems, specifically associated with the use of slurry additives; ii) the effects of the application of organic amendments, including slurry, on soil nutrient cycles and microbial community structure and function; and iii) the environmental and economic implications of slurry application within grassland systems;

- **Chapter 3.** The objective of this chapter was to determine whether the treatment of livestock slurry with a biological slurry additive significantly influenced the nutrient content of livestock slurry during storage. In addition, this chapter focussed on changes in the nutrient content of grassland soil following the application of organic amendments (livestock slurry with and without a slurry additive applied during storage) compared to inorganic fertiliser treatment;
- **Chapter 4.** The objective of this chapter was to determine the extent to which the addition of C sources of different complexity and degree of microbial availability to grassland soil, spanning glucose, glucose-6-phosphate and cellulose, alongside livestock slurry amended or not-amended with a biological additive, affected the activity of the soil microbial community and the resulting partitioning of C within soil;
- **Chapter 5.** The objective of this chapter was to determine the extent to which the addition of C sources of different complexity and degree of microbial availability to grassland soil affected the biomass and the structure of the soil microbial community;
- **Chapter 6.** The objective of this chapter was to synthesise the effects following the application of livestock slurry to grassland soil, based on the outcomes reported in Chapters 3 to 5. Further, the chapter also places the findings from the thesis in the broader context of key debates surrounding the

accumulation/reduction of SOM in agricultural soil; soil acidification; gaseous phosphine emission; enhancement of soil quality; and the relevance of the thesis findings to environmental and geopolitical concerns surrounding the depletion of phosphate rock reserves.

2 Application of slurry to grassland soil: potential implications for key crop nutrients and the soil microbial community

2.1 The role of slurry and slurry additives in agricultural production

Increased availability and application of inorganic fertilisers has arguably been the key factor underpinning the substantial increases in food production seen in many parts of the globe over the past century (Matson and Parton, 1997). In order to deliver such increases in food production within the UK, total inorganic fertiliser application has increased substantially, particularly since the 1940s (Johnston and Dawson, 2005). For example, there has been a sustained increase in the quantities of inorganic nitrogen (N) fertilisers applied, particularly as ammonium sulphate ($(NH_4)_2SO_4$) and sodium nitrate ($NaNO_3$), in UK agriculture, rising from approximately 2.5 10^9 to 1.7 10^{10} kg during the period 1913-1985 (Johnston and Dawson, 2005). Parallel increases in the quantities of phosphorus (P) fertiliser, as phosphorus pentoxide (P_2O_5), and potassium (K) fertiliser, as potassium oxide (K_2O), have also been observed in UK agriculture during the same period, with peak applications of 5.0 10^9 kg of nutrients applied during the years 1960-1990 (Johnston and Dawson, 2005).

However, organic amendments have always represented an additional, in many cases essential, source of carbon and nutrients within agriculture (Parr and Hornick, 1992). A range of organic amendments, including farmyard manure (FYM), different types of slurry (the mixture of manure, urine and rainwater/farmyard washings) including from livestock, pig or chicken sources, green manure, biochar, compost, sewage sludge, wastes from dairy, vegetable, fish meat, poultry processing industries, and anaerobic digestate, have been applied to agricultural soils (note that the term ‘slurry’ will be used throughout the remainder of this thesis to indicate livestock

slurry). A number of potential advantages have been reported in the literature that support the importance of making maximum use of organic amendments in agriculture, in addition to the opportunity to reduce reliance on inorganic fertilisers. For example, organic amendments are widely seen as important for the enhancement of soil organic matter (SOM), soil structure and water retention, for increasing cation exchange capacity and for contributing to soil fertility through the supply of essential crop nutrients including, but not limited to, N and P (Iyamuremye et al., 1996, Rochette and Gregorich, 1998, Albiach et al., 2000, Leifeld et al., 2002, Montemurro et al., 2008, Annabi et al., 2011, Patel et al., 2015, Molnár et al., 2016). Figure 2.1 synthesises a range of effects that are thought likely to be associated with the application of organic amendments to arable or grassland soils, as reported previously in the literature.

Among the available organic amendments, FYM and slurry represent some of the most commonly used in agriculture across the globe (Chandra, 2005). Due to the increase in fossil fuel prices and subsequent significant increases in the cost of inorganic fertilisers, as well as the environmental and broader economic costs of manufacturing and applying inorganic N and P fertilisers, there is increasing interest within agriculture in the role of FYM and slurry as sources of OM and nutrients, rather than predominantly as waste materials to be disposed of to agricultural land (Dordas et al., 2008). In particular, as agricultural systems have developed into more industrialised and commercial units (Bittman et al., 2014), the recycling of internal inputs, such as FYM and slurry, in order to close C, N and P loops within farm systems has become essential, in order to enhance nutrient use efficiency and reduce costs to farm businesses associated with inorganic fertiliser use (Petersen et al., 2007). Table 2.1 reports indicative physico-chemical properties for cattle, pig and chicken

slurry, emphasising the significant nutrient resource contained within such materials. Nutrient use within animal production systems that produce these organic materials continues to be optimised through improved design of feeding schedules and through manipulation of feed such that N and P use efficiency is increased. For example, the addition of exogenous enzymes, such as phytase or protease, to cattle, pig or poultry diets is utilised to increase the efficiency of P uptake (Selinger et al., 1996, Acamovic, 2001).

However, the processes before slurry application to soil, including slurry collection and storage, as well as the technique of slurry application to land and any manipulation of slurry immediately after application, strongly affect the efficiency with which nutrients and OM within slurry are recycled within farm systems through return to soil and uptake by crops. Further, environmental risks, for example associated with unacceptably high levels of N and P accumulating within soil that increase the probability of nutrient export to water or release to the atmosphere as GHGs including nitrous oxide (N_2O) or methane (CH_4), can arise as a consequence of sub-optimum practices in terms of slurry management (De la Torre et al., 2000, Amon et al., 2006). Beyond GHG emissions, emissions of NH_3 during slurry storage or following slurry application to land play a crucial role in the decline of biodiversity, being a major contributor to soil acidification and N deposition within ecosystems (Berg et al., 2006). Further, the emission of offensive odours during the storage of slurry in tanks and lagoons, is associated with a complex mixture of volatile organic compounds (VOCs), including volatile fatty acids (VFAs), alcohols, aldehydes, amides, amines, aromatic compounds, carbonyls, esters, ethers, halogenated hydrocarbons, hydrocarbons, ketones, nitriles, olefins, paraffins, phenols and indoles (Ni et al., 2012). According to Blanes-Vidal et al. (2009a), sulphur-containing

compounds, such as hydrogen sulphide, dimethylsulphide, dimethydisulphide and dimethyltrisulphide, which may be emitted from stirred slurry, can also be an important challenge for slurry management. Therefore, whilst organic amendments such as slurry represent a potentially significant nutrient resource within farm systems, understanding how this resource can be used to optimum effect whilst minimising any associated environmental risks, remains a significant challenge for agriculture.

Slurry additives, applied to slurry during storage, represent a potential way in which nutrient use efficiency within slurry-based systems might be significantly enhanced (Wheeler et al., 2011). A summary of available slurry additives is provided in Table 2.2. Several additives, both biological and chemical in nature, have been developed in an attempt primarily to reduce NH_3 volatilisation and odour emission during FYM and slurry storage (Schils and Kok, 2003). McCrory and Hobbs (2001) categorised both biological and chemical additives, according to their ways of action: digestive additives, acidifying additives, disinfectants, oxidising agents, adsorbents and masking agents. For instance, disinfectants inhibit the natural degradation of solids by acting on the microbially-mediated processes occurring in livestock slurry, whereas adsorbents use their high adsorptive capacities to increase total solids (TS) in manure storage (Wheeler et al., 2011). The manipulation of the balance between NH_3 and NH_4^+ by reducing the slurry pH through application of inorganic acids represents another way to control emissions (Stevens et al., 1989, Oenema and Velthof, 1993, Hendriks and Vrielink, 1997, Kroodsma and Ogink, 1997, Martinez et al., 1997, Beck and Burton, 1998, Pedersen, 2003, Pedersen et al., 2004).

Digestive or biological additives represent a mixture of microbial strains and/or enzymes that increase the decomposition of livestock waste. In addition, they seek to control NH_3 volatilisation and to reduce the release of odorous compounds during

slurry storage (McCrory and Hobbs, 2001). Biological additives are a potentially attractive alternative to chemical additives, due to the risks associated with secondary environmental pollution following the application of chemically-amended slurry to soil, alongside the high costs associated with producing chemical additives themselves (Zhu et al., 2006). Further, the extensive use of acidifying chemical additives, such as aluminium (Al) and iron (Fe) salts, can risk the degradation of soil quality, by increasing sulphate and chloride concentrations in soil and reducing soil pH following the application of slurry (Kox, 1981, Fangueiro et al., 2015).

In order to reduce NH_3 emissions, biological additives seek to promote the metabolism of N in combination with the decomposition of OM, and then to stabilise N as organic N compounds within biomass in slurry, rather than remaining as NH_3 to be volatilised (Wheeler et al., 2011). To control odour emission, a number of biological additives have been developed that seek to reduce the production, or to increase the degradation, of odorous volatile compounds (Nykänen et al., 2010). It has been observed that odorous compounds are produced under anaerobic conditions within slurry, mainly by *Clostridium* and *Eubacterium* spp. that favour high pH (Zhu, 2000). Therefore, lowering slurry pH and adjusting the conditions during slurry storage to favour lactic acid bacteria should avoid conditions under which anaerobic bacteria release odorous compounds (Nykänen et al., 2010). Further, the use of aeration to influence the bacterial community in slurry, mainly composed of *Bacillus* spp. that produces malodorous compounds such as VFAs, has led to the selection of specific *Bacillus* strains that drastically decrease the level of VFA production within slurry during storage (Hanajima et al., 2009).

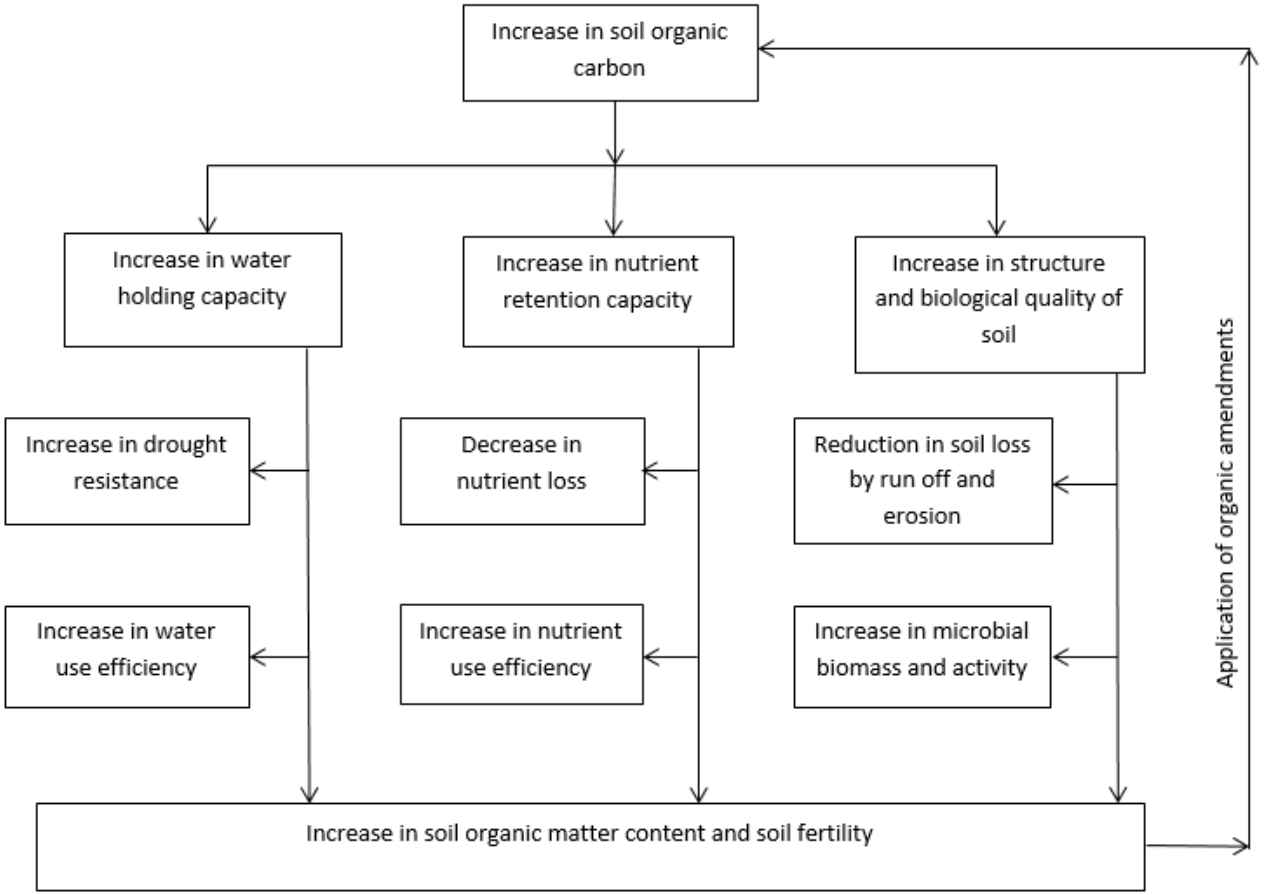

Figure 2.1. Effects of increasing soil organic matter content and soil fertility through the application of organic amendments to agricultural or grassland soils. Modified from Lal (2006).

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Table 2.1. Typical composition of cattle, pig and chicken slurry (adapted from Salazar et al. (2007), Blanes-Vidal et al. (2009b), Suresh et al. (2009a), DEFRA (2010), Suresh and Choi (2012), Ch'ng et al. (2013); Villamar et al. (2013), Ch'ng et al. (2014), Christel et al. (2014), Provenzano et al. (2014), Villamar et al. (2014), Cabassi et al. (2015), Dale et al. (2015), Kumari et al. (2015), Antezana et al. (2016), Cocolo et al. (2016), Omar et al. (2016)).

Parameters	Slurries		
	cattle	pig	chicken
Dry matter (%)	8.88 ± 2.90	9.10 ± 0.00	8.07 ± 0.51
pH	7.49 ± 0.30	7.00 ± 0.20	7.52 ± 0.09
Total N (%)	3.75 ± 0.96	7.50 ± 0.00	4.65 ± 0.10
NH_4-N (mg kg^{-1})	1510 ± 0.45	5500 ± 0.00	1288 ± 2.02
Soluble P (mg g^{-1})	n.a.	1900 ± 0.01	n.a.
Total P (mg kg^{-1})	640 ± 0.23	1490 ± 235	2960 ± 1.05
Total K (mg g^{-1})	2540 ±0.79	3500 ± 0.00	167 ± 0.64
Total Mg (mg g^{-1})	250 ±0.00	704 ± 632	257 ± 7.88
Total Na (mg kg^{-1})	1520 ±1.06	542 ± 241	5002 ± 1.50

Table 2.2. Summary of the available slurry additives in the worldwide market according to the type of additive, type of slurry and their purpose during slurry storage (adapted from McCrory and Hobbs (2001) and Wheeler et al. (2011)).

Type of additive	Slurry type	Main purpose	Commercial name	Composition	Author
Chemical	cattle feedlot manure	reduction of odorous compounds	AGCO	natural plant extract	Miner and Stroh (1976)
	cattle slurry	reduction in NH_3 emission	Kemira No. 2	superphosphate ($Ca(H_2PO_4)_2$) and gypsum $CaSO_4 \cdot 2H_2O$	Andersson (1994)
			Kemira No. 5	peat impregnated of calcium chloride ($CaCl_2$) and superphosphate	
			Kemira No. 15	H_2O_2, $CaCl_2$, and propionic acid	
			Penac G	silicon dioxide and "oxygen treated"	
			Stalosan	superphosphate and copper sulphate ($CuSO_4$)	
		reduction in NH_3 emission and GHGs, and in odorous compounds	AMD	abandoned mine iron-rich sediments	Wheeler et al. (2011)
			Anthium dioxcide	5% aqueous stabilised chlorine dioxine	
			Borax	sodium tetraborate decahydrate	
			Carvacrol + pinene	essential oils of *Origanum vulgare* (oregano) and *Pinus sylvestris* (pine)	
			CAS=Air solution R305 deamine	proprietary mixture of chemicals	
			CBP=Biostreme 222 Pond-X	proprietary chemicals/ micronutrient concentrate	
			CBS=Biostreme 101	proprietary chemicals/ micronutrient concentrate	

Table 2.2. Continued.

Type of additive	Slurry type	Main purpose	Commercial name	Composition	Author
Chemical	cattle slurry	reduction in NH_3 emission and GHGs, and in odorous compounds	CGE=Greaseater	proprietary mixture of chemicals in isopropyl alcohol	Wheeler et al. (2011)
			CPR=Predator	proprietary complex triazine mixture	
			CSE=Septi-sol	proprietary dipole dibase formulation	
			Eugenol	essential oil of *Syzygium aromaticum*	
			Glycerol	glycerin	
			Hydrogen peroxide	hydrogen peroxide	
			MUN=UNLOK	proprietary chemicals and surfactants for facultative bacteria	
			Ocimum basilicum	essential oil of *Ocimum basilicum* (basil)	
			Peppermint black mitcham	essential oil of *Mentha piperita* (Peppermint)	
			Zeolite	clinoptilolite, K-Ca-Na aluminosilicate	
		reduction in odorous compounds	Bio-Gest	no information available	Yu et al. (1991)
			Nature-Aid	no information available	
	pig slurry	reduction in odorous compounds, retain NH_3 and OM	Agri-Scents	Yucca plant extract	Patni (1992)
			Bio-Surge	nutrient combination	
			Hydrogen cyanamide	chemical (CH_2N_2)	
			Micro-Aid	saponin surfactant, urease inhibitor	

Table 2.2. Continued.

Type of additive	Slurry type	Main purpose	Commercial name	Composition	Author
Chemical	pig slurry	reduction in odorous compounds, retain NH_3 and OM	Natural Odor Catalyst Peat	solution of amino acids, vitamins, trace minerals and enzymes Sphagnum peat for Shippagan	Patni (1992)
		reduction in NH_3 emission and GHGs	AMGUARD	lactic acid	Hendriks and Vrielink (1997)
		increases oxygen level in liquid to support bacterial activities	CPPD	chemical oxidising agent	Zhu et al. (1997)
		reduction in odorous compounds	MPC	chemical emulsifier	
		enhances biological and chemical processes to reduce odour	Shac	natural coal product (enzyme)	
Biological	cattle feedlot manure	reduction in odorous compounds	Odor Control Plus	mixture of dried bacterial and enzyme	Miner and Stroh (1976)
	pig manure	reduction in odorous compounds	ADD CATADD	mixture of aerobic bacteria	Zhu (2000)
	cattle slurry	reduction in NH_3 emission	Add A	mixture of anaerobic bacteria	Andersson (1994)

Table 2.2. Continued.

Type of additive	Slurry type	Main purpose	Commercial name	Composition	Author
Biological	cattle slurry	reduction in NH_3 emission and GHGs, and in odorous compounds	MAC=Alken Clear-Flo 8000 MAE=Alken Enz-Odor 5 & Alken Enz-Odor 9 MAF=Alken Clear-Flo 7110 & Alken Enz-Odor 5 & 9	proprietary aerobic/facultative microbes with growth factors	Wheeler et al. (2011)
			MBR=Bio-Regen Animal Waste	proprietary aerobic/facultative microbes	
	pig slurry	reduction in odorous compounds, retain NH_3 and OM	Roebic	mixture of aerobic, anaerobic and facultative bacteria	Patni (1992)
		reduction in NH_3 emission and GHGs	6806405 Instra. Pro Specimen	mixture of bacteria	Liao and Bundy (1994)
		reduction in odorous compounds	Bio-Safe	Enzymes and microorganisms	Zhu et al. (1997)
		breaks down volatile organic compounds	X-Stink (LF1)	Aerobic bacteria	

2.2 Application of slurry to soil in farm systems

The application of FYM and slurry to soil represents a valid alternative to inorganic fertilisers in order to meet crop demand for available nutrients (Stockdale et al., 2001). Much effort has been invested in enhancing the nutrient recycling in farm systems through the application of slurry and manure so as to minimise reliance on external inputs, because of environmental, economic and geopolitical concerns over continued access to, and use of, inorganic fertilisers (Chambers et al., 2000, Stockdale et al., 2002). Although inorganic fertilisers may be necessary to sustain intensive, high-yield crop production, widespread concerns have been raised with respect to the environmental and economic sustainability associated with future production of inorganic fertilisers (Dawson and Hilton, 2011). Most inorganic N fertilisers involve N fixation through the Haber–Bosch process. This synthesis is an energy-intensive process, when compared to the production of other inorganic fertilisers, even though enormous progresses have been made in enhancing the energy-efficiency of the N-fixation process (Smil, 2001). For example, according to Dawson and Hilton (2011), the production of N fertilisers requires, globally, over 90% of the overall energy input needed for fertiliser production. Although fossil fuels can be replaced by many other energy sources for NH_3 synthesis, there are no imminent alternatives to the increasing reliance on the Haber process to meet demand for N fertilisers (Smil, 1999).

Differently from the synthesis of NH_3, inorganic P fertiliser production is reliant on P extracted from phosphate rock, which is a non-renewable and finite P resource on human timescales (Cordell et al., 2009), due to the extremely long time required for P to cycle between the lithosphere and the hydrosphere (Cordell and White, 2011). Further, because of the highly uneven distribution of phosphate rock reserves in the world, with five countries controlling approximately 88% of remaining reserves and

Morocco alone controlling 74% of these reserves, there is a significant power imbalance in terms of the control over the supply and the price of inorganic P fertilisers (Cordell and White, 2015). In addition, significant uncertainty continues to surround the size and depletion time for remaining phosphate rock reserves (Cordell and White, 2014). Therefore, the use of organic inputs, such as slurry, could be a valid route through which agricultural production systems are able to reduce reliance on finite and geo-politically constrained phosphate rock resources.

Slurry and FYM represent an important source of OM and nutrients for farm systems, in order to replace OM and nutrients removed in crops and thereby to maintain and enhance soil fertility and crop growth (Goulding et al., 2008). When slurry/FYM that is generated within a farm system is recycled within the boundaries of that farm system, this maximises the efficiency of nutrient use within the system and, in turn, reduces the cost of transporting these organic amendments elsewhere, for example to off-site anaerobic digesters, alongside the farm-business expense associated with inorganic fertiliser purchase and application (Oenema, 2006, Goulding et al., 2008). Several studies have already described increases in SOM and SOC following the application of slurry or FYM to soil (Haynes and Naidu, 1998, Kapkiyai et al., 1999, Morari et al., 2006, Rasool et al., 2008, Huang et al., 2010, Mellek et al., 2010, Peng et al., 2012). Greater concentrations of Ca, K, Mg and Mn have also been found in soil amended with different types of slurry and manure (Bulluck III et al., 2002, Adegbidi et al., 2003, Soumaré et al., 2003, Gil et al., 2008, Suresh et al., 2009b, da Veiga et al., 2012, Vanden Nest et al., 2014, Lima et al., 2015).

Similarly to the other soil parameters, the effects of the addition of slurry/FYM to soil have been observed in terms of total N and of mineral N (NH_4-N and NO_3-N) associated with the direct input of NH_4-N via slurry/FYM alongside the mineralisation

of organic N within these materials (Eghball, 2002, Mäder et al., 2002, Bittman et al., 2005, Fließbach et al., 2007, Birkhofer et al., 2008, Duan et al., 2011, Yang et al., 2015). However, as N inputs increase in farm systems, the risk of greater total N losses also increases (Oenema, 2006). Losses of N may occur via NH_3 emissions, denitrification, leaching and run-off (Kirchmann and Lundvall, 1993, Loro et al., 1997, Beckwith et al., 1998, Smith et al., 2001c, Sommer and Hutchings, 2001). Ammonia emissions are mostly associated with slurry storage and the period immediately following the application of slurry to land (Oenema, 2006). Reducing such nutrient losses, particularly from livestock production systems, has been a significant concern both at UK and at European levels. Regulations at the EU level, as well as policy and management practices at local and national level, have been adopted in order to minimise NH_3 emissions, as well as N losses to groundwater and to surface water bodies (Erisman et al., 2008, EU Council Decision, 2009). One potentially important route through which the export of N from farm systems may be realised is through more efficient utilisation of slurry/FYM in balance with inorganic N fertilisers (Hatch, 2004, Rotz, 2004). For example, the application of slurry to soil through injection may reduce NH_3 volatilisation compared to surface application of slurry by almost 50% (Kuipers et al., 1999). Further, the amendment of slurry with additives may have the potential to enhance nutrient use efficiency, for example by mitigating NH_3 emissions from slurry (McCrory and Hobbs, 2001).

With regard to P, a number of studies have shown that the application of manure, slurry or other organic amendments may enhance P availability to crops in different soil types (Iyamuremye et al., 1996, Guppy et al., 2005, Agbenin and Igbokwe, 2006, Jiang et al., 2006, Negassa et al., 2008, Gichangi and Mnkeni, 2009, Gichangi et al., 2010, Šimon and Czakó, 2014, Vilela Penha et al., 2015). Largely in contrast to N, the

application of P to agricultural land through slurry/FYM, alongside inorganic P fertilisers, over several decades in what were originally P-deficient soils, has largely exceeded P offtake in crops and led to significant accumulation of P in agricultural soils (Hooda et al., 2001). Therefore, due to this large P surplus, many agricultural soils do not require further inorganic P fertiliser applications, particularly given slurry/FYM inputs, or at most they require only maintenance applications (Condron, 2004). However, according to Zhang and Schroder (2014), within soils in which slurry/FYM application rates are primarily determined based on crop N requirements, significant surpluses of P are applied with respect to crop requirements. The resulting P accumulation in soil can significantly increase the potential for P export through runoff and leaching from these soils (Kleinman et al., 2002). As a result, a reduction in P surpluses in soil by matching crop requirements with P inputs is needed for effective soil quality management in intensively managed agroecosystems (Condron, 2004). However, whilst this target could easily be achieved in soils with inorganic P fertiliser applications, it will be difficult in lands receiving significant amounts of slurry/FYM applications where P application is likely to remain in excess of crop requirements (Hooda et al., 2001, Withers et al., 2001, Csathó and Radimszky, 2012).

2.3 Impact of slurry application on soil microbial composition and activity

Organic amendments, including slurry and FYM, represent a key source of energy and nutrients for soil microorganisms (Condron et al., 2010). Bacteria and fungi comprise 85% of the soil biomass, and their interactions with the soil faunal community in complex food-web systems regulate the turnover of OM and associated

nutrients in soil (Wardle, 2002, Coleman and Wall, 2007). Decomposition of organic C and nutrients in soil is mainly driven by the activities of bacteria and fungi, because the heterotrophic nature of most of these organisms means that they rely on SOM as a source of energy and of nutrients (Hopkins and Gregorich, 2005, Winding et al., 2005). Soil microorganisms are usually classified with respect to their ecological characteristics, corresponding to the classification of copiotrophic and oligotrophic groups used for animals and plants in relation to resource availability (Fierer et al., 2007). Microorganisms whose relative abundance in C-rich soils is high are classified as copiotrophs, whilst oligotrophs have been observed to grow and reproduce in extremely C-poor soils (Langer et al., 2004). Furthermore, whereas copiotrophs are also classified as r-strategist or zymogenous, oligotrophs correspond to K-strategists or autochthonous (Hopkins and Gregorich, 2005). Table 2.3 summarises several ecological, morphological and biochemical traits that are assocaited with r- and K-strategist organisms as they exist within the soil microbial community.

The application of organic amendments, such as slurry and FYM, to soil has been observed to generate a microbial succession during the decomposition process. Copiotrophs/r-strategists, largely corresponding to gram-negative (G –ve) bacteria, dominate the early stages of decomposition, due to their adaptation to the organic amendments added to soil (Fierer et al., 2003, Fontaine et al., 2003, Cleveland et al., 2007, Fierer et al., 2007, Kramer and Gleixner, 2008, Fanin et al., 2014). In contrast, as substrate quantity and/or quality declines over time, oligotrophs/K-strategists, mainly consisting of gram-positive (G +ve) bacteria and fungi, become increasingly dominant, because of their tollerance towards environmental stress, such as low resource concentration. Therefore, these organisms are able to derive sufficient energy and nutrients from the decomposition of the older and more recalcitrant SOM (Fierer

et al., 2003, Fontaine et al., 2003, Cleveland et al., 2007, Fierer et al., 2007, Kramer and Gleixner, 2008, Fanin et al., 2014). However, along with biosynthetic processes that lead to increasing microbial biomass, dissimilation processes can also occur following the addition of organic amendments to soil, with such processes being favoured when energy constraints exist or when energy demands are high (Geyer et al., 2016). Among these dissimilatory processes, maintainance respiration, represnting the basal energy requirement for purposes other than biomass production, and overflow respiration, the respiratory mechanism used by nutrient-limited microorganisms to mine SOM in search of N, P or other nutrients, are among the most important processes (Geyer et al., 2016).

According to Fierer et al. (2007), even though it is unlikely that a whole phylum would respond similarly to changes in C availability, for example following FYM/slurry application, and there is enormous physiological and phylogenetic diversity whithin each phylum, most of the microorganisms in the phyla studied in previous research presented common ecological traits in relation to C availability. Several studies have described bacteria belonging to *Acidobacteria* that were most abundant in C-poor soils, whereas α-, β-, γ-*Proteobacteria* and Bacteroidetes displayed a higher relative abundance in soils with high C availability, either as an intrinsic soil property or because of organic amendments (Marilley and Aragno, 1999, McCaig et al., 1999, Axelrood et al., 2002, Padmanabhan et al., 2003, Héry et al., 2005, Cleveland et al., 2007, Nemergut et al., 2010, Wang et al., 2016). In contrast, Fierer et al. (2007) did not find any significant change in the overall abundance of α-*Proteobacteria*, *Firmicutes* and *Actinobacteria* to changes in C availability in soil.

Table 2.3. Ecological, morphological and biochemical traits that are likely to correspond to r- and K-strategists. Modified from Fierer et al. (2007).

Traits	**r-strategists**	**K-strategists**
Corresponding microbial groups	Gram negative bacteria	Gram positive bacteria and fungi
Ecological groups	Copiotrophs	Oligotrophs
Growth rates	High growth rate when resources are non-limiting, e.g. after addition of organic amendment	Low growth rate, predominant with recalcitrant SOM and outcompeted by r-strategists in rich-nutrient soils
Growth yield	Low, inefficient biomass accumulation per unit substrate	High, efficient substrate conversion into cell biomass, efficient resource utilisation
Maintenance requirements	High, cells remain viable only when substrates are supplied at a sufficiently high rate	Low, rates of substrate can also be low to maintain viability
Substrate uptake systems	Low cell specific affinity for substrates, low competition with limited substrates [1]	High specific affinity, high capacity of simultaneous uptake of mixed substrates [1]
Receptivity to substrate applications	Short lag time before growth after application of organic amendments, constitutive production of enzymes	Long lag in growth rates on organic amendments, induced production of enzymes
Metabolic quotient (qCO_2, respiration rate per unit of biomass)	High[2]	Low[2]
Temporal variability in population size	High, pulsed substrate supply, fast rates of population turnover, short generation times	Low, fairly constant substrate availability, slow rates of population turnover, long generation time
Ease of cultivation	High, best isolated in nutrient-rich media, visible colonies with short-duration incubation	Low, visible colonies slow to appear, optimal growth with nutrient-poor media

Table 2.3. Continued.

Traits	r-strategists	K-strategists
Cell chemistry and morphology	Low C:N and C:P owing to protein content and high intracellular nucleic acid, spherical cells with low surface area: volume ratio [3]	Long or filamentous cells (hyphae in fungi) with high surface area: volume ratio [3], high intracellular storage capacity of nutrient reserves [4]
Tolerance to environmental stressors (e.g., pH, temperature)	High sensitivity to environmental stress, spore formation in suboptimal environment	Viability under stressful environmental conditions

[1] Button (1993); [2] Dilly (2005); [3] Matin (1979); [4] Hirsch et al. (1979).

2.4 Impact of slurry application on the priming effect and SOM turnover

It has also been demonstrated that the addition of organic amendments, such as slurry and FYM, to soil may result in changes in SOM decomposition due to microbial metabolism, as characterised by a phenomenon known as the priming effect (PE) (Löhnis, 1926, Jenkinson et al., 1985, Kuzyakov et al., 2000). The PE is a strong (from 4- to 11-fold larger than in an unamended soil) and relatively short (from a few days up to several months after the application) modification in the turnover rate of native OM caused by, often on relatively limited, addition of substrates to soil (Kuzyakov et al., 2000). The concept underlying PE is consistent with the notion of SOM as a highly recalcitrant substrate for microorganisms that necessitates greater investment of cellular resources, such as enzyme synthesis, to hydrolyse compared to the resource retrieved from metabolism of this substrate (Garcia-Pausas and Paterson, 2011). In contrast, the application of organic amendments, particularly in soils where there is nutrient deficiency, offers the energy resources needed to mineralise SOM and mobilise nutrients that would otherwise limiting microbial growth and activity (Fontaine et al., 2003, Paterson et al., 2009). According to Fontaine and Barot (2005) and Wutzler and Reichstein (2008), PE is conceived with the decomposition rate that is not only determined by the amount of substrate added and SOM, but also by the microbial biomass pool. Therefore, PE essentially depends on three factors: the quality of an added substrate, the microbial community composition and the availability of soil nutrients to the microbial community (Chowdhury et al., 2014).

Since it is not possible to determine SOM turnover rate directly, this parameter is quantified through changes in CO_2 efflux rates. However, the amount of C evolved from soil can be attributed to different microbial processes (Blagodatskaya and

Kuzyakov, 2008). Indeed, it is possible to distinguish real from apparent PE. In the former, CO_2 evolves directly from SOM decomposition, whereas under an apparent PE CO_2 is released in response to the activation of microbial metabolism and higher microbial biomass turnover with no 'real' effects on SOM decomposition (Blagodatskaya and Kuzyakov, 2008). Further, a real PE can either be positive, in which the addition of an organic amendment causes an acceleration in the mineralisation of SOM, or negative, whereby a reduction in the mineralisation of native soil C occurs after the addition of organic amendments (Kuzyakov et al., 2000).

A number of hypotheses have been proposed to explain potential changes in PE following the application of organic amendments. Depending on the amount of organic amendment added to soil, a succession of mechanisms may occur (Fontaine et al., 2003, Kuzyakov and Bol, 2006), as summarised in Figure 2.4. The first phase relies on the amount of an amendment applied to soil. In this phases, the application of more readily available organic amendments, such as slurry/FYM, compared to SOM can induce the growth of r-strategists that, in turn, can extend their activity by degrading SOM once any added substrates have been completely exhausted (Blagodatskaya and Kuzyakov, 2008). Based on this, two apparent PEs can be observed during the first days after the application of organic amendments, with a triggering effect, showing a small and brief increase in CO_2 efflux, that can be observed when the amount of added amendment is much lower than the microbial biomass C (C_{mic}), whereas a pool substitution after the triggering effect can be identified when the amount of added substrate is less but comparable with C_{mic} (Blagodatskaya and Kuzyakov, 2008). By contrast, when the quantity of an added substrate is higher than C_{mic}, a preferential substrate utilisation has been observed, with a temporary decrease in the decomposition of SOM (negative PE), due to

microbial utilisation of the organic amendments added to soil, followed by a later increase in the decomposition of SOM (Sparling et al., 1982, Billes et al., 1988, Cheng, 1999).

The activation of the most reactive components of the microbial community has been observed following the addition of organic amendments to soil and, given a sufficiently high addition of substrate, the active microflora can grow (Helal and Sauerbeck, 1984, Sallih and Bottner, 1988, Cheng and Coleman, 1990, De Nobili et al., 2001, Mondini et al., 2006). Changes in the relative proportions of soil microbial communities, such as increase in the fungal population, have also been observed following the addition of organic amendments to soil (Griffiths et al., 1999, Broeckling et al., 2008, Chigineva et al., 2009, de Graaff et al., 2010, Berthrong et al., 2013, Zhang et al., 2016). Once the most readily available substrates are exhausted, the activated microflora will use the more recalcitrant substrates (Kuzyakov and Bol, 2006) through the release of extracellular enzymes, resulting in a further SOM decomposition by co-metabolism, in particular when nutrients are in limiting amounts (Blagodatskaya and Kuzyakov, 2008). Further, PE has been associated with the acquisition of N or P through mineralisation of recalcitrant OM in the processes of microbial N and P mining (Moorhead and Sinsabaugh, 2006, Craine et al., 2007, Guenet et al., 2010, Hartley et al., 2010, Fontaine et al., 2011, Wang et al., 2014, Zhang et al., 2016). Finally, microbial biomass and activity will decrease and return to the initial condition, thus, the initial relationship between SOM and the microbial community is potentially re-established (Stenström et al., 2001) within few days or a few weeks of the addition of an organic substrate (Kuzyakov and Bol, 2006).

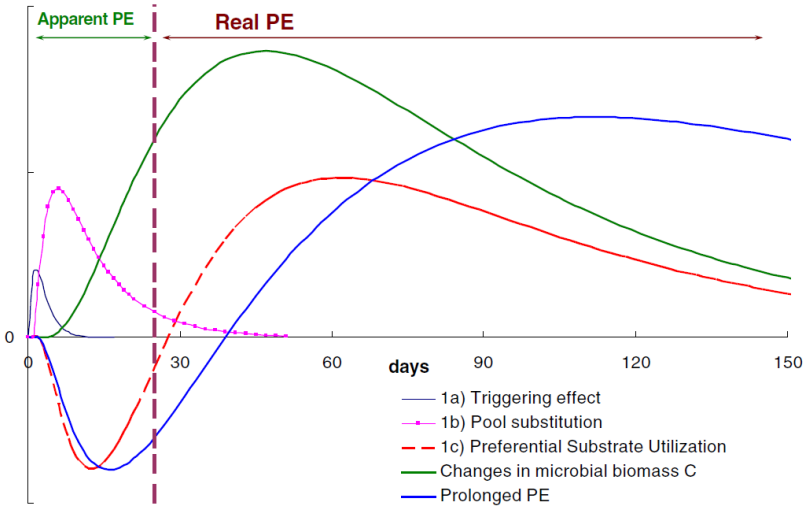

Figure 2.2. Sequence of mechanisms during priming effect (Blagodatskaya and Kuzyakov, 2008).

3 The effects of amended slurry application on soil nutrient availability

3.1 Introduction

The application of farmyard manure (FYM) and slurry to soil is a common practice to replenish nutrient offtake in agricultural products, alongside the loss of bioavailable nutrients to more recalcitrant pools in soil or to surface water and groundwater. Farmers and other land managers increasingly recognise the beneficial effects of nutrients supplied via FYM and slurry, even though some concern continues to surround the reliability of nutrient supply to crops following the application of FYM/slurry as compared to inorganic fertiliser (Smith et al., 2000; 2001a; 2001b). However, in some locations, such China, parts of the Netherlands or the southeast USA, FYM and slurry are treated largely as a waste product from intensive livestock production, with the application to land of large quantities of these organic materials adversely affecting environmental quality and agricultural productivity (Westerman and Bicudo, 2005, MacDonald et al., 2011, Szögi et al., 2015). Optimising application of these organic materials to agricultural land is of increasing importance, not least due to the volatile and, often, unaffordable price of inorganic fertilisers, particularly for farmers in developing countries. This, coupled with increasing awareness of the importance of environmental sustainability for agriculture and the desire to close nutrient loops within agricultural systems, has led to growing interest in maximising the utility of cheaper, locally-sourced inputs of nutrient resources to support agricultural production (Opala et al., 2012).

The application of FYM and slurry has the potential to contribute both to farm-scale and to soil nutrient requirements, due to the considerable nutrient content of

these organic materials. Notably, the use of livestock slurry and FYM has been shown to positively influence plant growth and crop yields (Rahman et al., 2008). This is mainly due to the supply of key macronutrients to plants via slurry application, including nitrogen (N), phosphorus (P) and potassium (K) (Culley et al., 1981, Beauchamp, 1986, Sutton et al., 1986, Matsi et al., 2003, Grignani et al., 2007, Lithourgidis et al., 2007). A number of long-term studies, predominantly focussed on arable soils, have reported increases in soil pH, organic matter (OM) content as well as N mineralisation following the addition of FYM (Mäder et al., 2002, Bittman et al., 2005, Fließbach et al., 2007, Birkhofer et al., 2008, Citak and Sonmez, 2011, Azeez and Van Averbeke, 2012). Similarly, manure and other organic amendments have been shown to enhance P availability in different soil types (Iyamuremye et al., 1996, Guppy et al., 2005, Agbenin and Igbokwe, 2006, Jiang et al., 2006, Gichangi and Mnkeni, 2009, Šimon and Czakó, 2014).

Slurry is also an important source of micronutrients, such as iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn), which at low soil concentrations are necessary to support plant growth and crop yields (Berenguer et al., 2008, Moral et al., 2008, Nikoli and Matsi, 2011). Greater concentrations of calcium (Ca), K, Magnesium (Mg) and Mn have been reported within soils treated with different types of organic inputs, including cattle and composted poultry manure (Bulluck III et al., 2002, Adegbidi et al., 2003, Soumaré et al., 2003, Gil et al., 2008, Vanden Nest et al., 2014, Lima et al., 2015). Other research has reported changes to the chemical properties of soil, including increase in the concentration of carbon (C) and sulphur (S), alongside increases in the C/N ratio, after the addition of pig slurry to soil, primarily because dissolved organic matter within the slurry was incorporated into the soil C cycle (Giusquiani et al., 1998, Plaza et al., 2002). However, although a number of studies

have been conducted to determine the impacts on soil of applying FYM versus inorganic fertiliser, less is known about the application of slurry versus inorganic fertiliser on the availability of nutrients within soil, particularly within grassland systems.

Further, before application to soil, slurry is usually stored in tanks or lagoons. Under certain circumstances, slurry may be stored for a significant period of time, for example up to 5-6 months in the context of farms within a Nitrate Vulnerable Zone as designated in the UK under the European Nitrates Directive (OJEC, 1991). During storage, slurry may be subjected to modifications of its chemical and physical properties, such as phase separation and crust formation (Smith et al., 2007, Hjorth et al., 2010). Odour issues from slurry tanks and lagoons can also be generated during slurry storage (McCrory and Hobbs, 2001). Previous work has demonstrated that ammonia (NH_3) volatilisation from slurry may be of concern during storage, not least because volatilisation reduces the N content of slurry prior to application to land (Sommer et al., 1993). The emission of NH_3 is also a particular concern during and immediately after slurry application to soil, with more than half of the N applied potentially lost due to NH_3 volatilisation (Sommer et al., 2003). FYM and slurry often possess a nutrient stoichiometry that differs considerably from crop requirements, particularly in terms of N to P ratio (Edmeades, 2003). In particular, due to an excess of P relative to N in many slurries, long-term application of substantial quantities of slurry can lead to significant net accumulation of P within soil (Sharpley et al., 1994, Schröder, 2005). Therefore, care must be taken when applying FYM and slurry to soil in order to avoid imbalanced application of certain elements (Vitale et al., 2011).

Slurry additives that are used during slurry storage represent one way in which nutrient use efficiency within slurry-based systems might be enhanced. Different types

of additives, both biological and chemical, have been developed and utilised globally (see Section 2.1). These additives may help to reduce NH_3 volatilisation, odour emission, and handling problems caused by crust formation and phase separation during slurry storage (McCrory and Hobbs, 2001). Among the different types of slurry additives that are available, biological additives represent a mixture of microbial strains and enzymes that are designed to both control NH_3 volatilisation and to reduce the release of odorous compounds during slurry storage (McCrory and Hobbs, 2001).

To reduce NH_3 emissions, biological additives seek to stimulate immobilisation of N as NH_4^+ by microorganisms, through the mineralisation of decomposable organic molecules with low N content or recalcitrant organic matter with high C:N ratios, thereby decreasing NH_3 concentration in livestock slurry. Another mechanism utilised by biological additives is to increase the uptake of NH_4 into microbial biomass and the immobilisation of N as organic N compounds. In terms of controlling odour emission, it is hypothesised that biological additives alter the microbial community within slurry in such a way as to reduce the production, or to increase the degradation, of odorous volatile compounds (Wheeler et al., 2011). The amendment of slurry with additives has also been undertaken to mitigate gaseous emissions from slurry, owing to increased pressure from regulatory agencies to decrease greenhouse gas (GHG) emissions, including nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4), alongside NH_3 (Wheeler et al., 2011). In particular, GHG emission targets from sectors, such as agriculture, have been established by the EU Effort Sharing Decision for all Member States for the period 2013-2020 (EU Council Decision, 2009). In addition, a number of European Commission policy instruments, as well as several national policies, have influenced emissions of GHGs and NH_3 from the agricultural sector, (Erisman et al., 2008).

Although the manufacturers of slurry additives assert that positive effects are delivered by their products to farm businesses and to the environment, and it is clear that farmers invest in slurry additives, the effectiveness of these additives, both in terms of slurry nutrient dynamics and in terms of nutrient availability in soil following slurry application, remains uncertain (van Vliet et al., 2006). According to McCrory and Hobbs (2001), further research is needed to fully understand the effects of biological slurry additives, both in terms of reducing NH_3 emissions and controlling offensive odours, due to inconsistent results observed in existing research to date. The need for further research in this area is illustrated by DeLaune et al. (2004) who found that the application of a microbial mixture to poultry litter did not reduce NH_3 losses compared to the application of chemical additives. In contrast, more recent studies (Amon et al., 2005, Amon et al., 2006, Sasaki et al., 2006, Lee et al., 2007, Van der Stelt et al., 2007, Wang et al., 2009, Kuroda et al., 2015) have demonstrated more encouraging results with regard to reduction of NH_3 emissions when biological additives were applied during the storage of cattle and pig slurry. However, it remains the case that relatively few studies have sought to understand the effects of the addition of biological additives, hereafter termed 'additives', on the nutrient content of livestock slurry. Further, much more research is required to establish whether the use of additives during slurry storage significantly influences nutrient availability within soil following slurry application to land.

In this context, the first objective of the research reported in this chapter was to determine whether treatment of livestock slurry with the commercial additive SlurryBugs™ (SB) significantly influenced the nutrient content of livestock slurry during storage, focusing on key chemical parameters that were hypothesised to be likely to vary during slurry storage. The second objective of the research reported here

was to determine the effects on soil nutrient properties within a grassland system of applying slurry, with or without the SB additive, in comparison to inorganic fertiliser. The specific hypotheses that were evaluated in this chapter are:

i. the application of the additive SB to slurry results in significantly higher total nutrient contents, compared to a control slurry that does not receive the SB additive, during slurry storage;
ii. the addition of slurry, both with and without the SB additive, to grassland soil results in significantly higher concentrations of bioavailable nutrients within soil, compared to treatment of soil with inorganic fertiliser;
iii. soils treated with SB-amended slurry contain a significantly higher concentration of bioavailable nutrients compared to soils treated with a control slurry.

3.2 Materials and methods

This study was divided in two distinct trials and related analyses. The first trial concerned the inoculation of slurry with the biological additive, SB. The second trial involved the application of slurry, both amended and non-amended with SB, versus the addition of inorganic fertiliser to soil.

3.2.1 Slurry and slurry additive

Livestock slurry for use in these trials was provided by Myerscough College farm, Bilsborrow, Preston, Lancashire. The slurry was produced by Holstein cows fed with a straw-based ration combined with grass silage, whole crop maize silage and feed supplements (Myerscough College, 2014). The slurry was not treated with any slurry additive prior to the trial reported here.

The slurry additive used in this research was a mixture of SlurryBugs^{TM} and SlurryBooster^{TM} (hereafter abbreviated to SB), two products developed and commercialised by EnviroSystems UK Ltd. SB is a microbial and enzyme preparation intended to enhance the nutrient content of slurry during storage. The specific microbial community in the product is hypothesised to retain N through NH_3 sequestration within slurry, fixing N in organic compounds which are subsequently made available to crops by slow release in the soil following slurry application to land (EnviroSystems, personal communication). SlurryBooster^{TM} is a specific micro-nutrient complement, which has been specifically designed to increase microbial activity within slurry during storage. The combination of SB and SlurryBooster is also hypothesised to reduce odour and GHG emissions from slurry during storage (EnviroSystems, personal communication).

3.2.2 Soils and soil sampling

Three soil types were included in this research, described as clay loam (CL), organic (O) and sandy loam (SL) based on qualitative textural analysis. All soils were sampled from fields on Mr James Rogerson's farm, Singleton, Lancashire, UK. Where fields contained more than one predominant soil type, soils were sampled only from the CL, O or SL sub-areas of each field. Samples of O and SL soils were collected on 11th July 2013, whilst CL soil samples were collected on 18th July 2013. All fields from which soil samples were collected for this research only received slurry that had not been amended with SB, and soil sampling occurred immediately before the second silage cut of the 2013 season. Within each field on each sampling date, soil sampling followed a 'W' sampling design involving 51 individual cores (17 core positions with three replicates at each position). All soil cores were taken to a depth of 7.5 cm using a gouge auger. Cores within an individual field were combined into a single bulk soil sample of some 10 kg, homogenised and stored at 4 °C in the dark prior to the experiments.

3.2.3 Experimental design of the slurry trial

The slurry trial was undertaken at Myerscough College Farm for 9 weeks, from August to October 2013. Two treatments were used, an unamended livestock slurry (control) and a slurry treated with SB. Six identical 60 L cylindrical plastic open top drums (three replicates per treatment), each containing 45 L of slurry at the start of the experiment, were placed in the field at ambient temperatures. The drums were left without lids during the entire 9 week trial to allow rainwater to enter and to allow evaporation and gaseous exchange between the slurry and the atmosphere, in order to replicate as far as possible the natural conditions occurring in slurry tanks during

storage. Three times per week, 33 μL of SlurryBooster and 33 mg of SlurryBugs were added to 50 mL of de-ionised water and poured into the 45 L of SB-treated slurry. The mixture of SB was applied across the surface of slurry in each drum. The same quantity (50 mL) of de-ionised water was also added to each drum containing control slurry at the same time. An additional 1 L of fresh slurry was added to each drum three times per week in an attempt to replicate periodic additions of fresh slurry to slurry storage tanks. After addition of materials to the drums, the SB-de-ionised water mixture or the de-ionised water alone were briefly stirred to mix into the slurry. Slurry samples for analysis of approximately 200 mL were collected using a plastic beaker at 0, 2.5, 5, 8 and 9 weeks of storage from the bottom of the slurry profile. All slurry samples were transferred to plastic bottles in the field, capped with screwcaps and refrigerated in a cold room before being sent for analysis. In addition, 30 L of control slurry and 30 L of SB-treated slurry were collected at the end of the trial after 9 weeks of storage and placed in two drums for the soil trial (see Section 3.2.4 below). Slurry samples were sent to an independent laboratory (NRM Ltd) and each sample was analysed for pH, total solids (TS), total P (TP), total N (TN), NH_4-N, total K (TK), total Mg (TMg) and total Na (TNa).

3.2.4 Experimental design of the soil trial

Four treatments: soil treated with control slurry; soil treated with SB-amended slurry; soil treated with inorganic fertiliser and soil alone (control), were established. All treatments were incubated in triplicate within a laboratory for 85 days, from November 2013 to February 2014. Bulk samples of the three soil types described in Section 3.2.2 were thoroughly mixed to ensure homogenisation and the treatments were then applied to 9750 g of each soil type. The slurry application rate (v/w) was

calculated on the basis of the typical slurry-to-soil application ratio used by contractors at Mr James Rogerson's farm (3.37 L m^{-2}), assuming a bulk density of 1.28, 1.30 and 1.44 g cm^{-3} for CL, O and SL soil respectively. The inorganic fertiliser applied to soils was a mixture of urea (CH_4N_2O) (46-00-00), triple superphosphate (TSP, monocalcium phosphate, $Ca(H_2PO_4)_2$) (00-46-00) and potassium chloride (KCl, muriate of potash) (00-00-60). The N application rate to soils replicated the typical application rates used on the farm from which soil samples were collected (8.7 g urea m^{-2} for CL and O soils; 7.6 g urea m^2 for SL soil). The application rates for inorganic P and K fertilisers were calculated based on the recommendations given in RB209 (Defra, 2010). Target indices for soil P for CL, O, and SL soils were 1, 2 and 4, whilst for soil K the target indices were 1, 3 and 3, respectively. Based on these indices, and the number of silage cuts taken from each field, the application rate of TSP to CL, O, and SL soil was 2.17, 0 and 2.17 g m^{-2}, whilst the application rate of KCl to CL, O, and SL soil was 11.67, 3.33, and 3.33 g m^{-2} respectively. The application rates for slurry and for inorganic fertiliser in the trial are summarised in Table 3.1.

Table 3.1. Slurry and NPK fertiliser application rates for the three soil types.

Soil type	**Clay loam**	**Organic**	**Sandy loam**
Slurry application rates (v/w)	0.035 L kg^{-1}	0.035 L kg^{-1}	0.031 L kg^{-1}
Inorganic fertiliser[1] application rate (w/w)	0.11 g kg^{-1}	0.11 g kg^{-1}	0.10 g kg^{-1}

[1] urea, KCl and TSP

Plastic containers of 150 mL volume were used for the soil incubation experiment. The treatments were divided to give 36 containers (four treatments * three soil types *

three replicates) for each day of analysis (days 0, 1, 5, 15, 25 and 85). The amount of soil required per container for each day of analysis was 125 g. This corresponded to 1,625 g in total per soil type, and to 4,875 g considering the 5 dates for analysis. Another 125 g was also required at day 0 for the preliminary analysis directly from the bulk soil sample collected as detailed in Section 3.2.1.2. After treatments were applied, the samples were left at ambient temperature, with the lids placed on top of each container but not sealed in order to allow for gas exchange, and wrapped with aluminium foil to avoid light exposure. The average temperature measured with a Level TROLL 500 Data Logger (In-Situ, USA) during the 85 days of the soil trail was 14.00 ± 0.04 ºC, with 22.59 and 8.09 ºC as the maximum and minimum temperature recorded respectively (Figure 3.1). Before the soil trail was conducted, soil moisture content at saturation and at field capacity was determined for the three soil types. Soil moisture content was maintained at 70% of saturated soil moisture content throughout the 85 day incubation, based on periodic weighing of each container and manual addition of deionised water as required in order to maintain the desired soil moisture conditions.

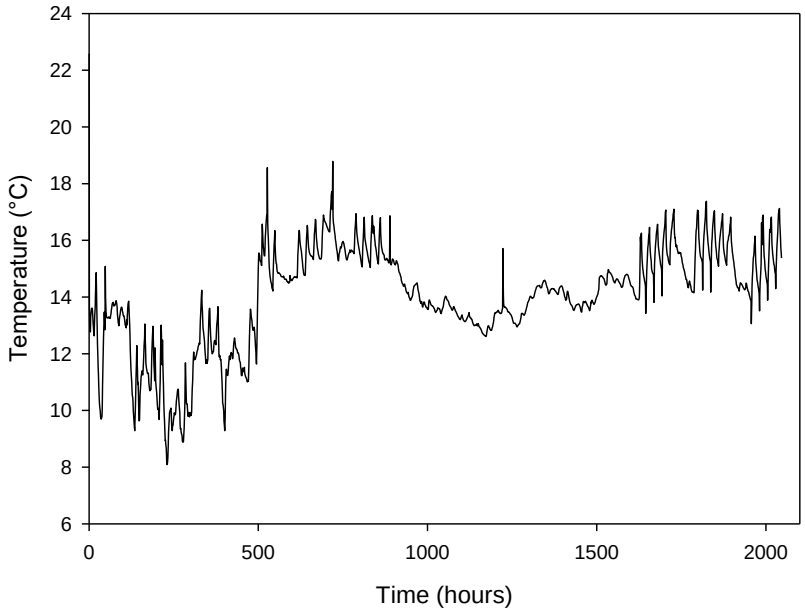

Figure 3.1. Ambient temperature measured during the 85-day soil incubation.

3.2.5 Soil moisture content

Soil moisture content was determined gravimetrically. For each sample, 10 g of moist soil was added into a pre-weighed foil tray. Samples were subsequently placed in an oven at 105 °C. After 24 hours, samples were removed and allowed to cool in a desiccator prior to being weighed. Samples were then place back in the oven and re-weigh to check for constant weight. The moisture content was then determined using Equation 3.1 (Gardner and Klute, 1986):

$$\% \text{ moisture content} = \frac{(\text{wet weight} - \text{dry weight})}{\text{dry weight}} \text{ x } 100. \qquad (3.1)$$

3.2.6 Soil pH

Soil pH was determined by adding 25 mL of de-ionised water to 10 g of air-dried soil (<2 mm) within a 50 mL polypropylene centrifuge tube. The suspension was

stirred periodically and allowed to stand for 1 hour. Soil pH was measured by mixing the soil and solution when a measurement was taken with a pH meter (PHM 220) that had been calibrated before each set of readings using pH buffers 7.0 and 4.0 (Radojevic and Bashkin, 1999).

3.2.7 Soil organic matter content

The organic matter (OM) content was measured through oxidation using the loss-on-ignition method. For each sample, a crucible was weighed and 10 g of air-dried soil was added to each crucible. Crucibles containing soil samples were then added to an oven for 24 h at 105 °C, placed in a desiccator, and then weighed. The crucibles containing oven-dry soil samples were then put into a furnace for at least 18 h at 450 °C. Following removal from the furnace, the crucibles were placed in a desiccator and then weighed again. Organic matter content of the samples was determined using Equation 3.2:

$$\text{OM } \% = \frac{\text{soil weight after oven drying} - \text{soil weight after the furnace}}{\text{soil weight after oven drying}} \text{ x } 100. \qquad (3.2)$$

3.2.8 Mineral-N in soil

Soil mineral-N, the combination of NH_4-N and nitrate-N (NO_3-N), was extracted from fresh soil using a 2 M potassium chloride solution (MAFF, 1986). An aliquot of 15 g of moist fresh soil was weighed and sieved through a 5.6 mm mesh sieve. 10 g of the sieved soil was then transferred into a 50 ml polypropylene centrifuge tube and 30 ml of 2M KCl was added. The tubes were capped and then shaken on a roller shaker for 2 hours, followed by filtration through a Whatman No 40 filter paper into a new

centrifuge tube. Prior to analysis, all extracts were diluted 1:2 with Milli-Q water in order to reduce the KCl molarity to 1M, the required sample matrix for analysis, and a further 1:5 with 1M KCl to bring the sample concentration within the calibration range.

The concentration of NH_4-N and NO_3-N in the KCl extracts was determined in a continuous flow stream using a Bran + Luebbe Auto analyser 3. The concentration of NH_4-N was calculated after reaction with dichloroisocyanuric acid and salicylate and with nitroprusside used as a catalyst to form a blue compound and measured at 660 nm (ISO11732, 2005). The concentration of NO_3-N was determined after the reduction of nitrate to nitrite by hydrazine sulphate in alkaline solution with a copper catalyst. The nitrite then reacted with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride to produce a pink compound measured at 550 nm (ISO13395, 1996). Phosphoric acid was added at the final stage in order to reduce the pH. As a result, precipitation of magnesium and calcium hydroxide were avoided. Five calibration standards were used for the spectrophotometric analysis. They were prepared from a 1000 ppm mixed stock solution using ammonium sulphate, potassium nitrate and Milli-Q water, and then diluted using 1M KCl to produce a matrix-matched calibration range of 0-5 ppm for both NH_4-N and NO_3-N. The NH_4-N and NO_3-N content in soil, expressed as mg kg^{-1} soil, were calculated using Equations 3.3 and 3.4, respectively:

$$\mathrm{NH_4 - N\ (mg/kg\ dry\ soil)} = \frac{1000 * \text{mg } NH_4 \text{ released in 30 mL of extractant}}{\text{equivalent dry soil mass extracted (g)}}. \qquad (3.3.)$$

$$\mathrm{NO_3 - N\ (mg/kg\ dry\ soil)} = \frac{1000 * \text{mg } NO_3 \text{ released in 30 mL of extractant}}{\text{equivalent dry soil mass extracted (g)}}. \qquad (3.4)$$

3.2.9 Olsen P in soil

In order to determine Olsen P, 2 L of Olsen's reagent was prepared dissolving 84 g of sodium hydrogen carbonate in de-ionised water. 20 mL of 1M sodium hydroxide was then added to the solution to adjust the pH to 8.5. Subsequently, 20 mL of Olsen's reagent was added to 1 g of air-dried and sieved soil (<2 mm) within a centrifuge tube. The tubes were capped and shaken for 30 minutes on a roller shaker and then centrifuged at 3000 RPM for 5 minutes. The extracts were filtered through a Whatman No 2 filter paper into a second centrifuge tube. Soil samples were then treated with 1.5M sulphuric acid, ammonium molybdate working reagent (0.15% w/v), and ascorbic acid as the reducing agent to determine P concentration by colourimetry. The five PO_4-P calibration standards used for the spectrophotometric analysis were matrix-matched with the Olsen's reagent and they were prepared by dilution of a stock standard with the Olsen's reagent to give a range 0-4 mg P L^{-1}. 2.5 mL of the filtered soil extracts, blanks and calibration standards were pipetted into a third centrifuge tube. 0.5 mL of 1.5M sulphuric acid was slowly added to the tubes and gently swirled to release carbon dioxide. 10 mL of 0.15% w/v ammonium molybdate reagent and 2.5 mL of ascorbic acid solution were then added to the tubes. The tubes were mixed and allowed to stand for 30 minutes. Finally, the absorbance was measured at 880 nm using a Jen Way 6300 Spectrophotometer, Spectronic Analytical Instruments, zeroing the instrument with de-ionised water. Olsen P (expressed as mg kg^{-1} soil) was calculated using Equation 3.5:

$$\text{Olsen P (mg/kg soil)} = \frac{(\text{mgP/L in sample} - \text{blank}) * \text{volume of extract 0.02 L}}{\text{weight of soil taken (kg)}}. \qquad (3.5)$$

3.2.10 Extractable cations in soil

The extractable cations Ca^{2+}, Mg^{2+}, Mn^{2+}, Na^{+} and K^{+} in soil were determined through inductively coupled plasma-optical emission spectrometry (ICP-OES). Samples (5 g) of air-dried soil (<2 mm) were weighed into a 50 mL polypropylene centrifuge tube. Subsequently, 25 mL of ammonium nitrate solution (160 g ammonium nitrate in 1 L of Milli-Q water) was added and the suspension was shaken on a roller shaker for 30 minutes and then centrifuged at 3000 RPM for 5 minutes. The suspension was then filtered through a Whatman No 2 filter paper, Whatman, UK, into a second centrifuge tube (MAFF, 1986). Four calibration standards were used with a range between 5 to 20 ppm for Ca, 1 to 5 ppm for Mg and K, and 0.25 to 1 ppm for Mn and Na, using 0.1 M HNO_3 as standard matrix. The extracts were then acidified to the same 0.1 M HNO_3 matrix. Before analysis, instrument stability and sensitivity was also evaluated by running a zinc (Zn) test using a 2 ppm Zn solution in 2% nitric acid. Finally, in order to detect possible drifts of the analyser during each sample run, a check standard at 1 ppm was run every 12 samples for all cations.

3.2.11 Total phosphorus in soil

Soil samples were digested using a modified Kjedahl method according to Rowland and Grimshaw (1985) prior to the quantification of total P. A sulphuric-peroxide digestion mixture was prepared by mixing 350 mL of hydrogen peroxide, 0.42 g of selenium, 14 g of lithium sulphate and 420 mL of sulphuric acid in 2 L flask within a fume cupboard. The mixture was then stirred and allowed to cool on ice. Samples (0.30 g) of soils that have been previously ground using a ball-mill were weighed and transferred into digestion tubes. Subsequently, 4.4 mL of the digestion mixture was added to the tubes and a glass “tear-drop” stopper was placed on each

tube. Each digestion was conducted using two reference soils at known concentrations of total P (600 and 1200-1300 mg kg^{-1} soil). The samples and the digestion mixtures were heated within a fume cupboard in a block digestor, BD28s/BD50s Block Digestion System (SEAL Analytical Ltd, UK). The two-stage digest first ramped heat up to 250 °C, then the temperature was kept constant for 15 min before being ramped to 400 °C for two hours. The digests were then allowed to cool, diluted to 50 mL by adding Milli-Q water and left overnight to settle. The supernatants were poured into a set of plastic containers, and then diluted adding 8 mL of hydrogen peroxide to 2 mL of supernatant within a different set of plastic containers.

Total P was measured using an optimised version of the standard US EPA 365.1 methodology (O'Dell, 1993). This methodology is based on a reaction between ammonium molybdate and antimony potassium tartrate with dilute P solutions in an acidic matrix in order to form an antimony-phosphomolybdate complex. This complex is in turn reduced by ascorbic acid to a blue-coloured complex, with the colour intensity proportional to the P concentration in solution. This complex was measured spectrophotometrically at 880 nm using a Seal Analytical AQ2+ discrete analyser. The five calibration standards (range 0 to 0.2 mg L^{-1}) for the spectrophotometric analysis were obtained using the stock anion standard 1000 g L^{-1} PO_4-P (Ion Chromatography SPEX CertiPrep, Thermo Fisher Scientific, USA). The reference standards WR1 and WR2 were obtained using 15 mg L^{-1} P and 7.5mg L^{-1} P, respectively (Custom Ion Standard – Ion Chromatography SPEX CertiPrep, Thermo Fisher Scientific, USA). In order to detect possible drifts of the analyser during each sample run, reference standards WR1 and WR2 were added every 12 samples. Matrix matched calibration and external reference standards to the digest solution.

3.2.12 Total organic carbon and total nitrogen in soil

Total Organic Carbon (TOC) and Total N contents were quantified by elemental analysis. For each sample, 1 g of milled soil was weighed into a glass beaker. 4 mL of 10% hydrochloric acid was then added to the beaker in order to remove inorganic carbonate and left overnight. The samples were then tested with pH paper to ensure that the acid had not been fully neutralised by carbonate. The acidified soils were then transferred into 50 mL polypropylene centrifuge tubes, rinsed three times with 25 mL of de-ionised water and centrifuged at 3500 rpm for 10 min between each rinse. Samples were left overnight to dry at 40 °C, subsequently homogenised and then left to dry at 40 °C until analysis. Subsamples of the acidified soils were weighed (approximately between 4-5 mg for O soil and 17-20 mg for CL and SL soils) and placed into tin capsules. The sample capsules were lowered into the combustion reactor of the vario EL III elemental analyser (Elementar Analysensysteme GmbH, Germany), in which the samples underwent combustion at high temperatures in an oxygen atmosphere to be transformed into NO_x, CO_2 and H_2O and then analysed by gas chromatography – mass spectrometry (GC-MS).

3.2.13 Statistical analysis

The dependent variables both in the slurry and in the soil trials were checked for normal distributions using the Shapiro-Wilk test. Because a normal distribution was observed in all data, the effects of treatment (control and SB-amended slurry) and of time on the nutrient content of slurry were assessed using parametric tests. Equally, the effects of treatment (control, inorganic fertiliser, slurry and slurry amended with SB), of soil type and of time on soil properties were assessed using parametric tests. One-way repeated measures analysis of variance (ANOVA) and two-way repeated

measures ANOVA were performed for the slurry and the soil trial respectively. Mauchly's Test of Sphericity was conducted to check for homogeneity of variances for each parameter. When the assumption of sphericity (ε) was violated, Greenhouse-Geisser correction was applied for $\varepsilon < 0.75$, whilst Huynh-Feldt correction was applied for $\varepsilon > 0.75$ (Girden, 1992). Further, when both the Greenhouse-Geisser and the Huynh-Feldt corrections were unable to account for violation of the assumption of sphericity, a multivariate analysis of variance was performed. However, all data from ANOVA and MANOVA were compared and, because it was ascertained that both analyses provided the same outcome, the data from ANOVA were reported although assuming $p < 0.001$ was required to indicate a significant effect. Pairwise comparisons were conducted using Bonferroni post-hoc tests for those factors for which significant differences were observed. Analyses were conducted using IBM SPSS Statistics 22, assuming a significant effect at $p < 0.05$.

3.3 Results

3.3.1 Effects of the SlurryBugs additive on the nutrient content of dairy livestock slurry during storage

The decomposition of organic matter in slurry was observed by tracking slurry TS content over the 9-week storage period, as displayed in Figure 3.2.a. Overall, no significant difference in the TS content was observed between the two slurry treatments. However, TS content varied significantly through time within the two treatments ($F(4, 1) = 363.044$, $p = 0.039$, $\eta^2 = 0.999$). In particular, TS content decreased substantially in both treatments comparing week 0 and 2.5 and week 0 and 9. Over the entire 9-week storage period, a decrease in TS content of 21% compared to week 0 for the control treatment, and approximately 12% for the SB-amended slurry, was observed. Although no significant difference was observed between the two treatments, from week 2.5 onwards the TS content in SB-amended slurry did maintain higher values than within the control slurry. Overall, no significant difference was observed in pH between the SB-amended and the control slurry through the incubation (Figure 3.2.b). No significant decrease was also observed in slurry pH across the 9-week storage period in both treatments. Finally, there was no significant interaction effect between slurry treatment and time in the incubation.

The effects of SB on slurry TN content are reported in Figure 3.2.c. No significant differences were identified across the storage period between the SB-amended and the control slurry. Further, no significant variation in slurry TN content was also observed through time, although an average 32% decrease in the TN content for control slurry and 29% for the SB-amended slurry was observed, when comparing the start to the end of the 9-week storage period. However, a significant treatment*time effect was

observed ($F(1, 4)$, = 8.727, p = 0.042, η^2 = 0.686), with higher TN content in SB-amended slurry compared to control slurry towards the end of the experiment. In particular, a significant difference was observed at week 8, when SB-amended slurry contained 0.36 ± 0.02 kg N m^{-3} slurry, whilst the control slurry contained 0.28 ± 0.02 kg N m^{-3} slurry. Despite the fact that no significant difference was observed in TN content between the two slurry treatments, NH_4-N concentration did vary significantly between the control and the SB-amended slurry ($F(1, 4)$ = 30.143, p = 0.005, η^2 = 0.883), as shown in Figure 3.3.a. A significant decrease in the NH_4-N concentration within both slurries was also observed through time in the order week 0 > week 2.5, week 5 > week 8, week 9 ($F(4, 1)$ = 910358.095, p = 0.001, η^2 = 1.000). Interestingly, at the end of the slurry storage approximately 70 and 66% of the initial TN content was measured as NH_4-N in the control and in the SB-amended slurry, respectively. However, no significant interaction effects between treatment and time factors in terms of NH_4-N was observed.

As illustrated in Figure 3.3.b, a significantly higher TP content was found within the SB-amended slurry compared to the control slurry during the storage experiment ($F(1, 4)$ = 16.124, p = 0.016, η^2 = 0.801). Significant variation in the TP content of slurry through time was also observed across both treatments ($F(4, 1)$ = 27523927.6, p < 0.000, η^2 = 1.000), with a general decrease in TP content from week 5 onwards. Further, there was a significant treatment*time effect for TP ($F(1, 4)$ = 17.549, p = 0.014, η^2 = 0.814), illustrated by the approximately 27% higher TP content in SB-amended slurry compared to the control slurry at week 8. As a result, comparing the start to the end of the 9 week storage period, TP content had decreased by an average of 19% in the control slurry treatment compared to an average of only 6% in the SB-amended slurry. No significant treatment or time effects were found for TK, TMg or

TNa (Figures 3.3.c, 3.4.a, 3.4.b, respectively), despite an average 25% decrease in slurry TK content comparing the start to the end of the 9-week storage period across both slurry treatments.

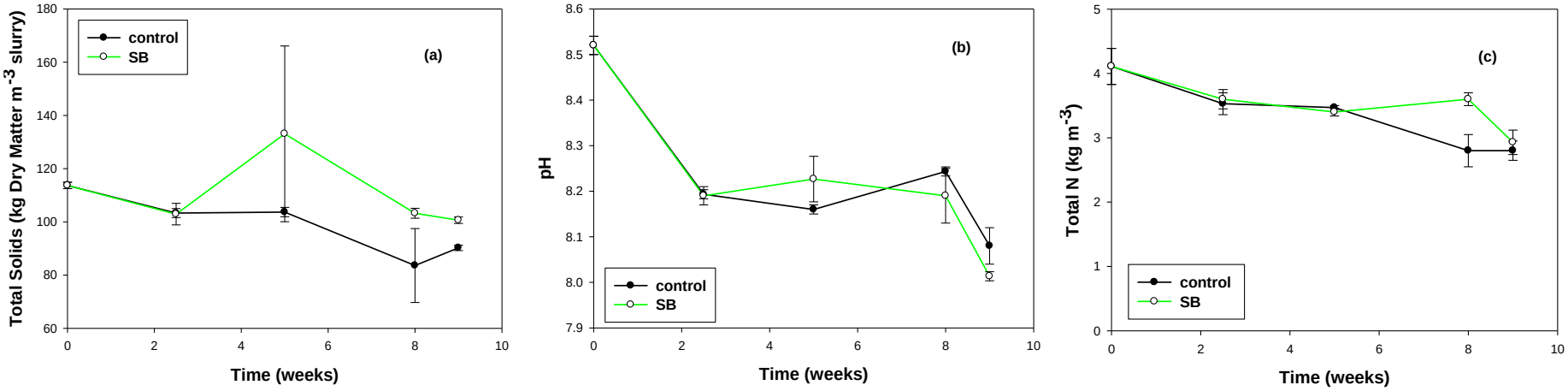

Figure 3.2. Total solids in control and SlurryBugs-SlurryBooster (SB)-amended slurry over time (a); pH in control and SB-treated slurry over time (b); Total N in control and SB-treated slurry over time (c). Average values of measured data are presented as symbols: black line and filled circles indicate control slurry, green line and empty circles indicate SB-amended slurry, error bars indicate standard error of the mean (n = 3).

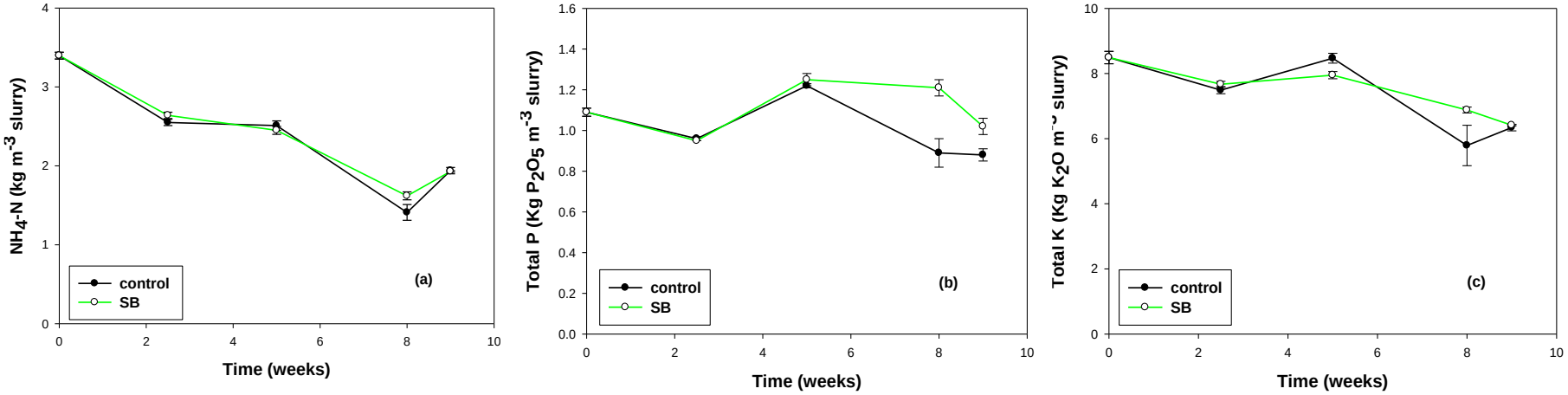

Figure 3.3. NH_4-N in control and SlurryBugs-SlurryBooster (SB)-treated slurry over time (a); Total P in control and SB-treated slurry over time (b); Total K in control and SB-treated slurry over time (c). Average values of measured data are presented as symbols: black line and filled circles indicate control slurry, green line and empty circles indicate SB-amended slurry, error bars indicate standard error of the mean (n = 3).

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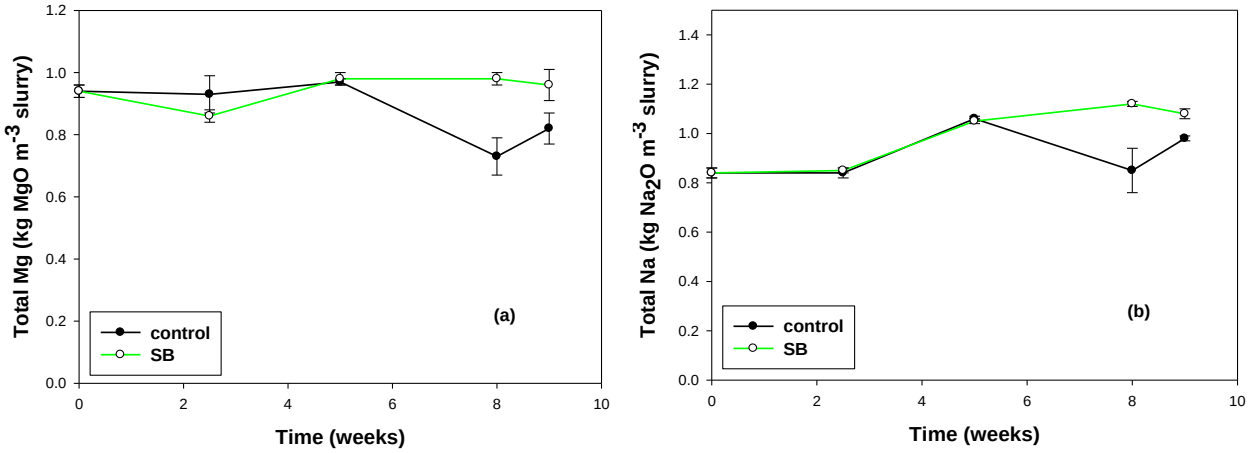

Figure 3.4. Total Mg in control and SlurryBug-SlurryBooster (SB)-treated slurry over time (a); Total Na in control and SB-treated slurry over time (b). Average values of measured data are presented as symbols: black line and filled circles indicate control slurry, green line and empty circles indicate SB-amended slurry, error bars indicate standard error of the mean (n = 3).

3.3.2 Changes in soil nutrient concentrations following application of slurry and inorganic fertiliser

3.3.2.1 Soil pH

Significant differences in soil pH (Figures 3.5.a-c) were observed between the three soil types used in the incubation ($F(2, 24) = 15536.934$, $p < 0.000$, $\eta^2 = 0.999$), with average pH decreasing in the order CL > O > SL. However, significant differences in soil pH were also observed between treatments ($F(3, 24) = 601.651$, $p < 0.000$, $\eta^2 = 0.987$). In particular, a significant decrease in pH was observed in the order SB-amended slurry> control slurry > control > inorganic fertiliser. Further, significant differences in soil pH were observed through time across all treatments ($F(5, 20) = 1777.254$, $p < 0.000$, $\eta^2 = 0.998$), with pH measured at day 85 lower than that measured on all other days. A significant time*treatment effect on soil pH was also observed ($F(15, 55) = 30.736$, $p < 0.000$, $\eta^2 = 0.871$). From day 1 onwards, the treatment of soil with inorganic fertiliser resulted in significantly lower pH than following treatment with either slurry or within the control treatment. The application of both slurries to soil caused a significantly higher pH compared to the control treatment, but only at days 15 and 85. The only significant difference in soil pH between the two slurry treatments occurred at day 85, with higher pH following the addition of the SB-amended slurry to soil.

3.3.2.2. Soil organic matter and total organic carbon

Soil type significantly influenced both SOM (Figures 3.6 a-c) ($F(2, 24) = 7480.175$, $p < 0.000$, $\eta^2 = 0.998$) and TOC content ($F(2, 24) = 9656.150$, $p < 0.000$, $\eta^2 = 0.999$) (data not reported), with higher SOM and TOC contents in O soil, followed

by SL and then CL soils. No significant effect of treatment was observed for either SOM or TOC content. Significant changes were observed in SOM ($F(5, 20) = 43.280$, $p < 0.000$ $\eta^2 = 0.915$) and TOC contents ($F(5, 20) = 11.382$, $p < 0.000$ $\eta^2 = 0.740$) with time. A significant increase in SOM content was found for all days of analysis compared to day 0. In contrast, TOC contents at days 0 and 5 were found to be significantly higher than at days 25 and 85. Finally, there were no significant interaction effects between treatment and time on either SOM or TOC content.

3.3.2.3 Nitrogen

In this research, N was determined as mineral-N (NH_4-N and NO_3-N) and as total N (TN). No significant differences in NH_4-N content (Figures 3.7a-c) were observed across the three soil types used in the incubation. Significant differences in NH_4-N concentration were identified between treatments ($F(3, 24) = 114.741$, $p < 0.000$, $\eta^2 = 0.935$). As expected, the application of both control and SB-amended slurries and also of inorganic fertiliser caused a significantly higher NH_4-N concentration compared to the control treatment. However, a significantly higher NH_4-N concentration was also observed under both slurry treatments compared to the inorganic fertiliser treatment. Significant differences in NH_4-N concentration were also found through time ($F(5, 20) = 273.706$, $p < 0.000$, $\eta^2 = 0.986$), with a significant increase in NH_4-N concentration observed from day 0 to day 1, followed by a significant decrease until day 15, and with a final significant increase from day 25 until the end of the incubation period. Further, there was a significant interaction effect between time and treatment on NH_4-N concentration ($F(15, 55) = 12.694$, $p < 0.000$, $\eta^2 = 0.745$).

In contrast to NH_4-N, NO_3-N concentration (Figures 3.8.a-c) differed significantly between individual soil types ($F(2, 24) = 863.185$, $p < 0.000$, $\eta^2 = 0.986$), with a decrease in NO_3-N concentration in the order O > SL > CL. The concentration of NO_3-N was also found to differ significantly between treatments ($F(3, 24) = 344.746$, $p < 0.000$, $\eta^2 = 0.977$), with the application of both slurries and of inorganic fertiliser to soil causing a significantly higher NO_3-N concentration compared to the control treatment. However, no significant difference in the NO_3-N concentration was observed comparing the inorganic fertiliser to the two slurry treatments. The concentration of NO_3-N increased significantly through time ($F(5, 20) = 1809.682$, $p < 0.000$, $\eta^2 = 0.998$). However, a significant time*treatment interaction effect on NO_3-N concentration was also observed ($F(15, 55) = 32.315$, $p < 0.000$, $\eta^2 = 0.877$), resulting in a significantly higher NO_3-N concentration towards the end of the incubation following the application of both control and SB-amended slurry, compared to either inorganic fertiliser or to the control soil.

Similarly to NO_3-N, TN content (Figures 3.9.a-c) differed significantly between individual soil types ($F(2, 24) = 6218.151$, $p < 0.000$, $\eta2 = 0.998$) and decreased in the order O > SL > CL. A significant change in TN content was found through time across treatments and soil types ($F(5, 20) = 8.357$, $p < 0.000$, $\eta2 = 0.676$), with higher TN content at days 0 and 5 than at days 25 and 85 of the incubation. However, no significant differences in TN content between treatments was observed, nor was there a significant time*treatment interaction effect.

3.3.2.4 Phosphorus

Olsen P (Figures 3.10.a-c) differed significantly between individual soil types ($F(2, 24) = 784.428$, $p < 0.000$, $\eta^2 = 0.985$) in the order SL > CL > O. Significant variations in Olsen P were also found between treatments ($F(3, 24) = 22.439$, $p < 0.000$, $\eta^2 = 0.737$), with Olsen P under both slurry treatments significantly exceeding that of the control treatment. Whilst no significant change in Olsen P was observed comparing the control slurry treatment versus the inorganic fertiliser treatment, the application of SB-amended slurry resulted in a significantly higher Olsen P concentration compared to inorganic fertiliser treatment. Significant decrease in Olsen P concentration were also observed through time ($F(5, 20) = 513.085$, $p < 0.000$, $\eta^2 = 0.992$), in the order day 85 > day 15, day 1, day 5, day 25 > day 0. There was also a significant time*treatment interaction effect on Olsen P concentration ($F(15, 55) = 6.513$, $p < 0.000$, $\eta^2 = 0.607$). The application of both slurries caused significantly higher Olsen P at days 1, 25 and 85 of the incubation compared to the control treatment. A significantly higher Olsen P concentration following the addition of both slurries to soil compared to the addition of inorganic fertiliser was only found at day 25, whilst the application of inorganic fertiliser produced a significantly higher Olsen P compared to the control treatment only at day 0 and 85 of the incubation. Finally, a significantly higher Olsen P occurred only at day 5 under SB-amended compared to control slurry application.

As for Olsen P, TP content (Figures 3.11.a-c) varied significantly between soil types ($F(2, 24) = 2770.256$, $p < 0.000$, $\eta^2 = 0.996$) in the order O > SL > CL. However, no significant differences in TP content were observed between treatments. Significant variations in TP content were observed through time ($F(5, 20) = 27.261$, $p < 0.000$, $\eta^2 = 0.872$), with a significant increase in TP content from day 1 until the end

of the incubation. Further, a significant time*treatment interaction effect was observed for TP ($F(15, 55) = 7.555$, $p < 0.000$, $\eta^2 = 0.640$), with a higher TP content in both slurry-treated soils compared to the control treatment during days 25 and 85 of the incubation, whereas the control treatment contained a higher TP content than slurry-treated soils at day 15. The concentration of TP was also found to be significantly higher under both slurry treatments than under inorganic fertiliser treatment at days 1 and 5.

3.3.2.5 Other elements

Focussing here on the effects of treatment rather than soil type or time alone, significantly higher concentrations of available K (data not reported) were observed across all soil types following the application of slurry, compared to either the inorganic fertiliser or the control treatments ($F(3, 24) = 4888.255$, $p < 0.000$, $\eta^2 = 0.998$). Further, higher concentrations of available K were found under SB-amended than unamended slurry treatment. A significant treatment*time interaction effect was also observed for available K ($F(15, 55) = 75.248$, $p < 0.000$, $\eta^2 = 0.940$), with the application of both slurries resulting in significantly higher available K concentrations than either inorganic fertiliser or control treatments from day 1 until the end of incubation. The addition of SB-amended slurry to soil resulted in significantly higher available K concentration compared to the addition of unamended slurry, but only at days 5 and 25.

Available Ca, Mg, Mn and Na concentrations (data not reported) varied significantly under different treatments ($F(3\ 24) = 17.668$, $p < 0.000$, $\eta^2 = 0.688$), ($F(3\ 24) = 205.982$, $p < 0.000$, $\eta^2 = 0.963$), ($F(3\ 24) = 558.768$, $p < 0.000$, $\eta^2 = 0.986$), and

($F(3\ 24) = 926.239$, $p < 0.000$, $\eta^2 = 0.991$), as the statistical results for available Ca, Mg, Mn and Na, respectively. The application of both slurries resulted in higher concentrations of available Mg, Mn and Na compared to the control soil treatment, and significantly higher available Mg and Na concentrations compared to the inorganic fertiliser treatment. In contrast, higher available Ca and Mn concentrations were observed after inorganic fertiliser treatment compared to either slurry treatment. Compared to the control slurry treatment, the application of SB-amended slurry to soil increased significantly only the concentrations of available Ca and Mg. A summary of the composition of the slurry additive SB, of the composition of the two slurry types, as well as of the composition of the three soil types with the four treatments is listed in Table 3.2.

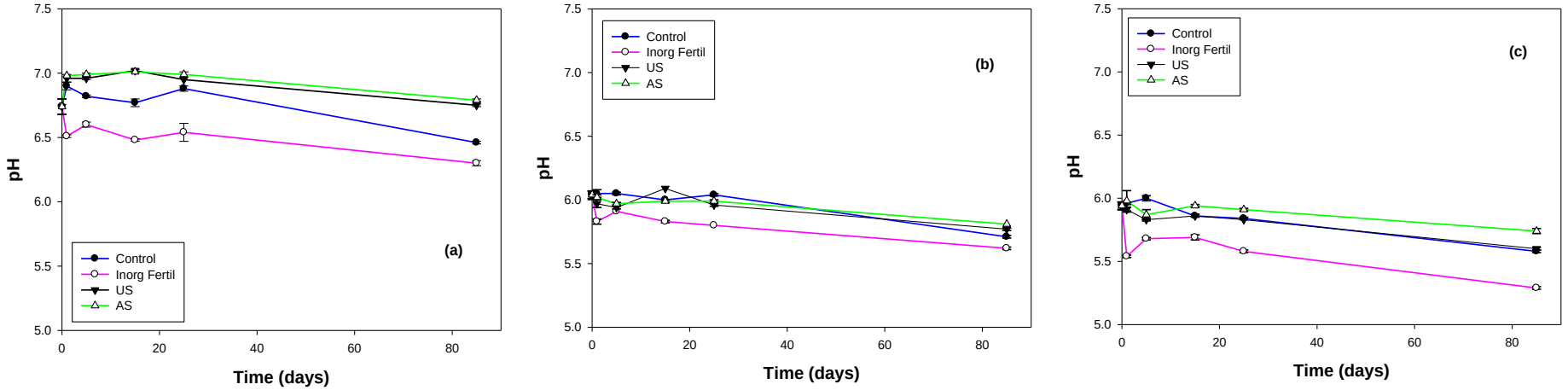

Figure 3.5. pH in clay loam (a), organic (b), and sandy loam soil (c) over time. The four treatments are: control soil (control), with blue line and filled circles, soil with inorganic fertiliser (Inorg. Fertil), with pink line and empty circles, soil with unamended slurry (US), with black line and filled triangles and soil with SlurryBugs-SlurryBooster (AS)-amended slurry, with green line with empty triangles. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

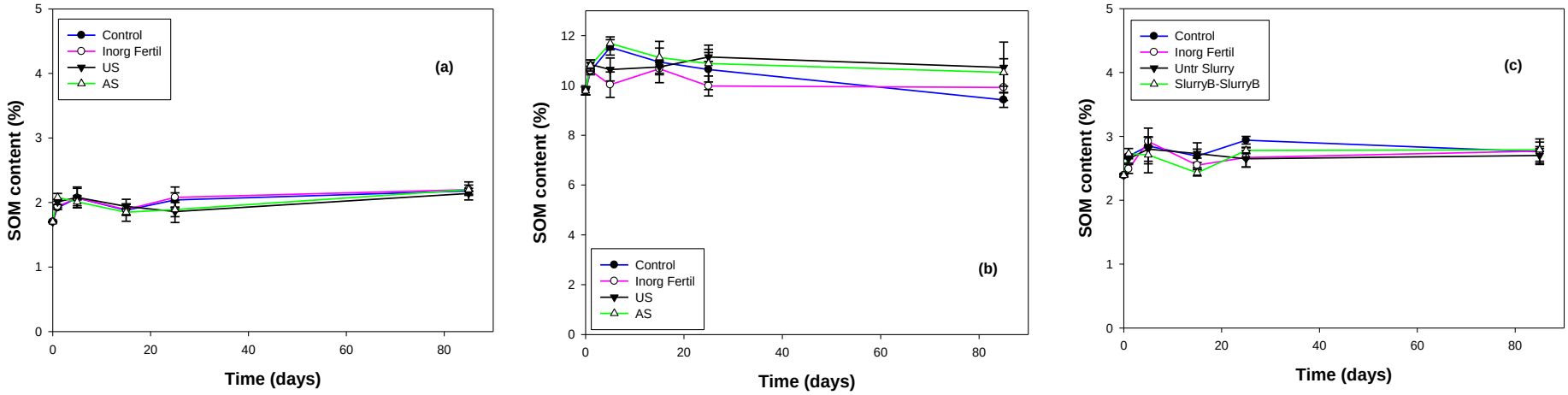

Figure 3.6. SOM in clay loam (a), organic (b), and sandy loam soil (c) over time. The four treatments are: control soil (control), with blue line and filled circles, soil with inorganic fertiliser (Inorg. Fertil), with pink line and empty circles, soil with unamended slurry (US), with black line and filled triangles and soil with SlurryBugs-SlurryBooster (AS)-amended slurry, with green line with empty triangles. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3). Note that y-axis scales differ between individual plots.

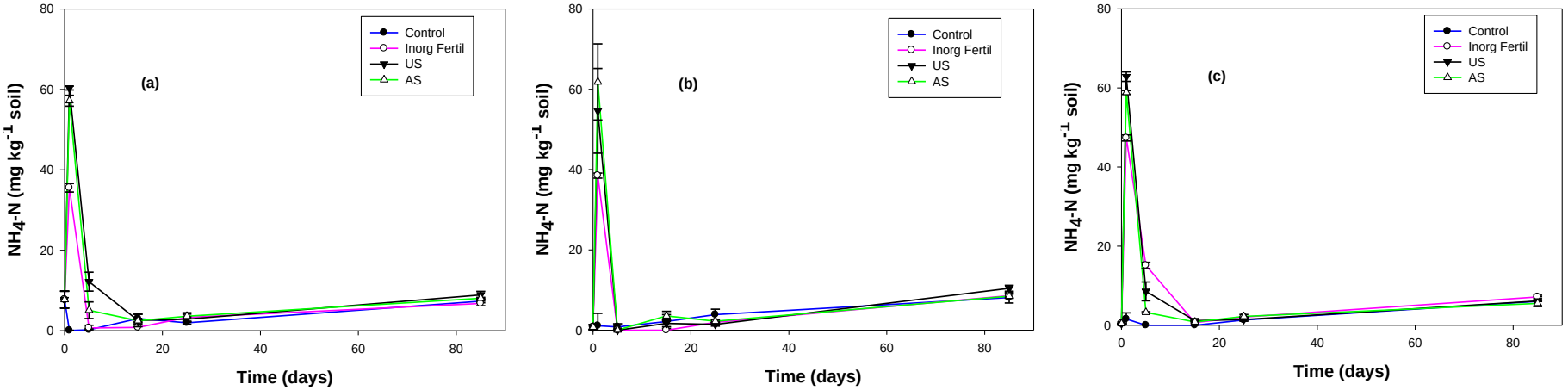

Figure 3.7. NH_4-N in clay loam (a), organic (b), and sandy loam soil (c) over time. The four treatments are: control soil (control), with blue line and filled circles, soil with inorganic fertiliser (Inorg. Fertil), with pink line and empty circles, soil with unamended slurry (US), with black line and filled triangles and soil with SlurryBugs-SlurryBooster (AS)-amended slurry, with green line with empty triangles. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

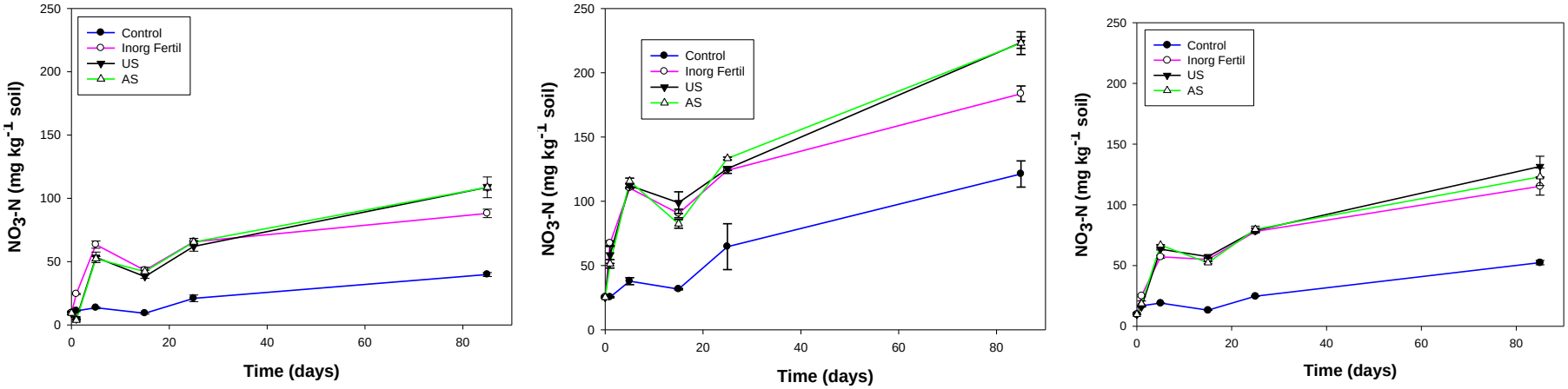

Figure 3.8. NO_3-N in clay loam (a), organic (b), and sandy loam soil (c) over time. The four treatments are: control soil (control), with blue line and filled circles, soil with inorganic fertiliser (Inorg. Fertil), with pink line and empty circles, soil with unamended slurry (US), with black line and filled triangles and soil with SlurryBugs-SlurryBooster (AS)-amended slurry, with green line with empty triangles. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

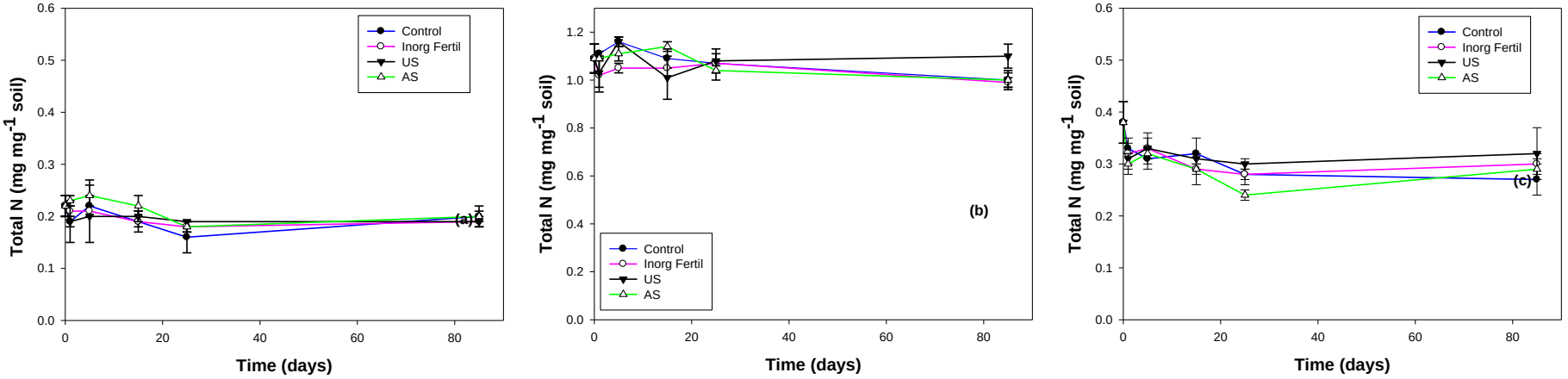

Figure 3.9. Total N in clay loam (a), organic (b), and sandy loam soil (c) over time. The four treatments are: control soil (control), with blue line and filled circles, soil with inorganic fertiliser (Inorg. Fertil), with pink line and empty circles, soil with unamended slurry (US), with black line and filled triangles and soil with SlurryBugs-SlurryBooster (AS)-amended slurry, with green line with empty triangles. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3). Note that y-axis scales differ between individual plots.

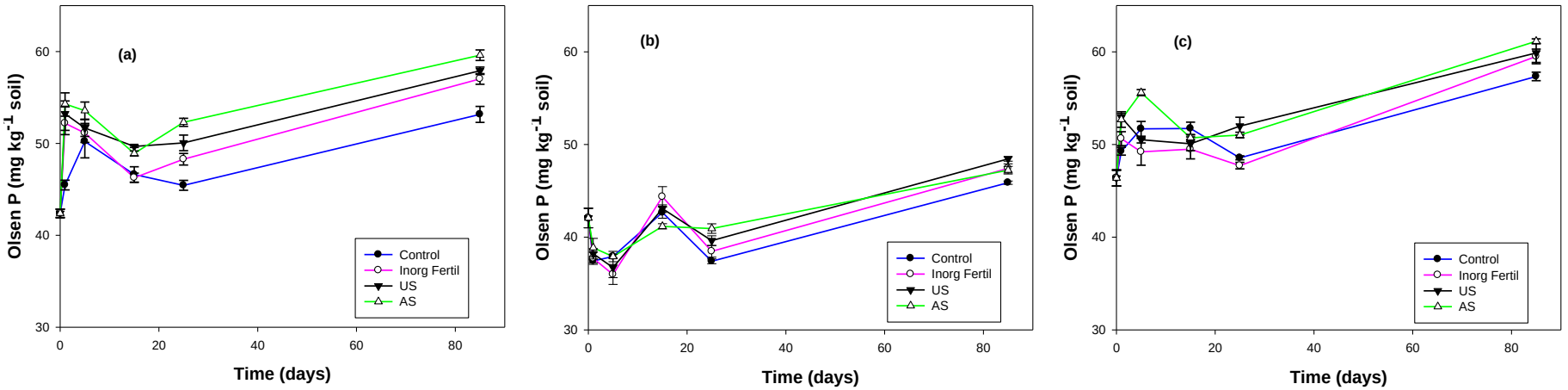

Figure 3.10. Olsen P in clay loam (a), organic (b), and sandy loam soil (c) over time. The four treatments are: control soil (control), with blue line and filled circles, soil with inorganic fertiliser (Inorg. Fertil), with pink line and empty circles, soil with unamended slurry (US), with black line and filled triangles and soil with SlurryBugs-SlurryBooster (AS)-amended slurry, with green line with empty triangles. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

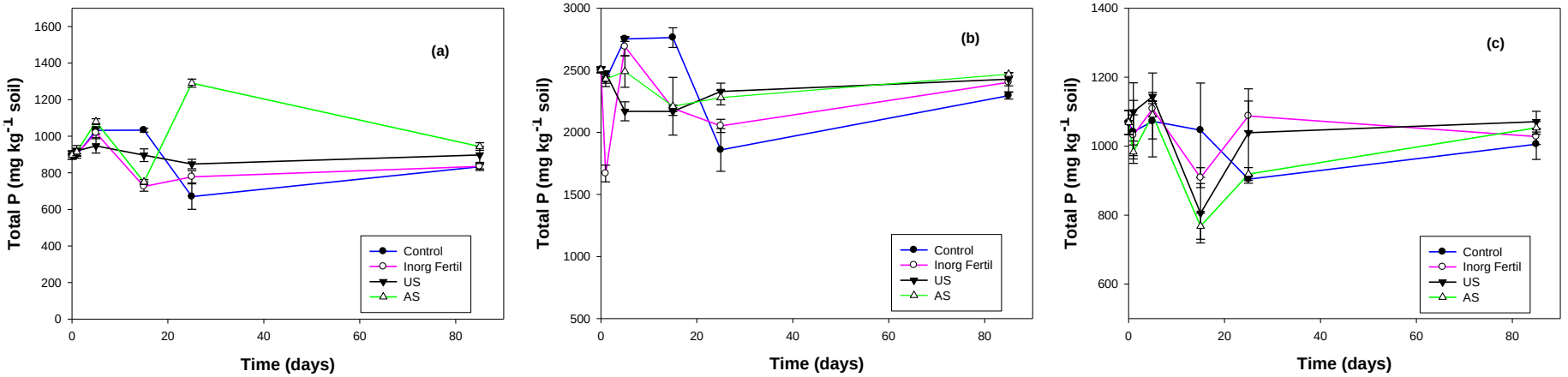

Figure 3.11. Total P in clay loam (a), organic (b), and sandy loam soil (c) over time. The four treatments are: control soil (control), with blue line and filled circles, soil with inorganic fertiliser (Inorg. Fertil), with pink line and empty circles, soil with unamended slurry (US), with black line and filled triangles and soil with SlurryBugs-SlurryBooster (AS)-amended slurry, with green line with empty triangles. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3). Note that y-axis scales differ between individual plots.

Table 3.2. Summary of the composition of the slurry additive SlurryBugs-SlurryBooster (SB), of the composition of control and SB-amended slurry, and of the composition of *clay loam (CL), organic (O), and sandy loam (SL) soils with the four treatments*, control soil (Control), soil with inorganic fertiliser (Inorg Fertil), with unamended slurry (US), and with SB-amended slurry (AS), at the end of the 9-week slurry and 85-day soil trials, respectively.

Slurry additive	
SlurryBugs	Freeze dried *Bacillus* spp. and enzyme combination.
SlurryBooster	Mixture of plant extracts, polymer, proteins, vitamins and minerals (EnviroSystems, personal communication).

Slurry			
	units	**Control**	**SB-amended slurry**
pH		8.08 ± 0.04	8.01 ± 0.01
Total Solids	%	90.23 ± 1.03	100.63 ± 1.23
TN	Kg m^{-3} slurry	2.80 ± 0.15	2.93 ± 0.19
NH_4-N	Kg m^{-3} slurry	1.94 ± 0.04	1.93 ± 0.01
TP	Kg P_2O_5 m^{-3} slurry	0.88 ± 0.03	1.02 ± 0.04
TK	Kg K_2O m^{-3} slurry	6.34 ± 0.10	6.41 ± 0.02
TMg	Kg MgO m^{-3} slurry	0.82 ± 0.05	0.96 ± 0.05
TNa	Kg Na_2O m^{-3} slurry	0.98 ± 0.01	1.08 ± 0.02

Table 3.2. Continued.

Soil						
	pH					
	CL soil	**O soil**	**SL soil**			
Control	6.46 ± 0.01	5.71 ± 0.01	5.58 ± 0.01			
Inorg Fertil	6.30 ± 0.02	5.62 ± 0.01	5.29 ± 0.01			
US	6.75 ± 0.01	5.77 ± 0.01	5.60 ± 0.01			
AS	6.79 ± 0.00	5.81 ± 0.00	5.74 ± 0.02			
	SOM (mg kg^{-1} soil)			**TOC** (mg mg^{-1} soil)		
	CL soil	**O soil**	**SL soil**	**CL soil**	**O soil**	**SL soil**
Control	2.18 ± 0.14	9.42 ± 0.30	2.76 ± 0.15	2.18 ± 0.22	13.70 ± 0.52	2.63 ± 0.30
Inorg Fertil	2.20 ±0.07	9.92 ± 0.52	2.77 ± 0.19	2.05 ± 0.02	13.48 ± 0.26	2.95 ± 0.17
US	2.14 ± 0.03	10.72 ± 1.02	2.70 ± 0.14	2.11 ± 0.09	15.01 ± 0.78	3.26 ± 0.56
AS	2.20 ± 0.02	10.52 ± 0.55	2.79 ± 0.02	2.14 ± 0.10	13.60 ± 0.46	2.98 ± 0.09
	NH_4-N (mg kg^{-1} soil)			**NO_3-N** (mg kg^{-1} soil)		
	CL soil	**O soil**	**SL soil**	**CL soil**	**O soil**	**SL soil**
Control	7.33 ± 0.38	8.11 ± 1.34	6.03 ± 1.18	39.83 ± 1.52	121.23 ± 10.19	52.42 ± 1.78
Inorg Fertil	6.77 ± 0.58	8.59 ± 0.61	7.14 ± 0.43	88.13 ± 3.33	183.67 ± 6.06	115.29 ± 7.28
US	8.89 ± 0.87	10.43 ± 0.73	6.13 ± 0.14	108.73 ± 2.30	223.47 ± 4.51	131.53 ± 8.50
AS	8.05 ± 0.22	8.45 ± 0.52	5.52 ± 0.80	108.70 ± 8.21	223.06 ± 8.84	123.09 ± 7.18
	TN (mg kg^{-1} soil)			**Olsen P** (mg kg^{-1} soil)		
	CL soil	**O soil**	**SL soil**	**CL soil**	**O soil**	**SL soil**
Control	0.20 ± 0.02	1.00 ± 0.04	0.27 ± 0.03	53.16 ± 0.87	45.88 ± 0.18	57.34 ± 0.46
Inorg Fertil	0.19 ± 0.00	0.99 ± 0.02	0.30 ± 0.02	57.02 ± 0.58	47.42 ± 0.49	59.50 ± 0.79
US	0.19 ± 0.01	1.10 ± 0.05	0.32 ± 0.05	57.91 ± 0.40	48.45 ± 0.25	59.88 ± 1.03
AS	0.20 ± 0.01	1.00 ± 0.03	0.29 ± 0.02	59.60 ± 0.57	47.23 ± 0.41	61.14 ± 0.26

Table 3.2. Continued.

Soil						
	TP (mg kg^{-1} soil)			**K** (mg kg^{-1} soil)		
	CL soil	O soil	SL soil	CL soil	O soil	SL soil
Control	833.28 ± 12.18	2295.44 ± 26.97	1005.68 ± 44.37	46.46 ± 0.95	481.67 ± 4.44	136.91 ± 1.51
Inorg Fertil	835.03 ± 22.00	2402.75 ± 76.19	1027.85 ± 23.17	80.48 ± 2.66	528.75 ± 2.43	166.02 ± 2.27
US	897.46 ± 46.46	2428.45 ± 54.24	1071.02 ± 30.01	183.90 ± 1.81	767.67 ± 7.06	272.42 ± 3.90
AS	944.26 ± 20.62	2468.27 ± 13.49	1053.01 ± 18.33	188.42 ± 1.83	769.08 ± 4.47	285.58 ± 10.14
	Ca (mg kg^{-1} soil)			**Mg** (mg kg^{-1} soil)		
	CL soil	O soil	SL soil	CL soil	O soil	SL soil
Control	1752.75 ± 13.75	3766.67 ± 36.01	1596.38 ± 9.63	74.40 ± 0.13	311.42 ± 3.67	134.64 ± 1.16
Inorg Fertil	1815.00 ± 34.18	3714.17 ± 24.85	1514.00 ± 16.25	80.04 ± 1.93	305.33 ± 1.47	127.03 ± 2.23
US	1666.08 ± 13.92	3600.83 ± 5.46	1465.92 ± 15.31	90.38 ± 0.80	332.58 ± 0.65	144.05 ± 1.14
AS	1678.17 ± 9.20	3648.33 ± 25.67	1495.58 ± 19.70	90.44 ± 0.96	342.92 ± 2.33	147.43 ± 1.75
	Mn (mg kg^{-1} soil)			**Na** (mg kg^{-1} soil)		
	CL soil	O soil	SL soil	CL soil	O soil	SL soil
Control	1.03 ± 0.17	2.69 ± 0.01	5.29 ± 0.16	14.03 ± 0.29	70.86 ± 0.61	14.78 ± 0.38
Inorg Fertil	1.37 ± 0.08	3.22 ± 0.04	9.52 ± 0.21	14.96 ± 0.57	70.32 ± 0.13	14.24 ± 0.56
US	0.70 ± 0.04	2.71 ± 0.07	5.48 ± 0.09	40.98 ± 0.46	110.35 ± 0.60	39.41 ± 0.53
AS	0.53 ± 0.02	2.53 ± 0.07	5.24 ± 0.19	44.28 ± 0.88	112.43 ± 0.93	43.14 ± 1.51

3.4 Discussion

3.4.1 Effects of the SlurryBugs additive on the nutrient content of dairy livestock slurry during storage

The addition of SB to slurry resulted in a significantly higher TP content compared to the control slurry treatment, by up to a maximum of 27% after 8 weeks of storage (Figure 3.3.b). These data indicate that SB had a positive effect on the TP concentration in slurry during storage, suggesting potentially beneficial effects of biological slurry additives, such as SB, on the availability of P within farms that run slurry systems. However, increased TP concentration is not always found in slurry following the use of additives. For example, Chapuis-Lardy et al. (2003) found no significant differences in the TP content of cattle slurry treated with a chemical additive at the end of a 3-week storage period, compared to a control slurry treatment. Further, whilst slurry TP content can vary as a result of multiple factors, including animal diet, alongside the techniques used during slurry collection, treatment and storage (Barnett, 1994, Eck et al., 1995), the mechanism responsible for differences in slurry TP concentration between the two treatments in the experiment reported in this chapter is not immediately obvious.

The slurry that was used to create the control and SB-amended treatments in this storage experiment was derived from a common source, i.e. from the same animals that had been fed the same diet, and was collected from the livestock shed using the same method (periodic, automatic scraping of the sheds). Therefore, differences in the TP content of slurry at source cannot explain the differences in TP concentration between control and SB-amended slurries that emerged during storage.

Perhaps the only viable explanation for the observed differences in slurry TP concentration during storage is variation in gaseous emissions of P between the control and SB-amended slurries. Phosphine (PH_3) is a gaseous and toxic form of P, with PH_3 release having been reported from animal slurry during storage (Glindemann and Bergmann, 1995, Eismann et al., 1997a). According to Eismann et al. (1997a) and Jenkins et al. (2000), PH_3 is generated by different groups of anaerobic fermentative bacteria and, among the genera observed, *Salmonella* is one of the dominant groups of microorganisms detected in pig and livestock manure (Miller and Varel, 2011).

A possible reduction in PH_3 emission, due to the dominance of specific microbial groups in slurry following application of the SB additive, could explain the maintenance of higher TP concentrations in the SB-treated slurry compared to the control slurry. In particular, the addition of SB that already contains a number of *Bacillus* spp. (EnviroSystems, personal communication), alongside those *Bacillus* spp. already in slurry (Peu et al., 2006, Swain and Ray, 2009, Kim et al., 2012), could inhibit or limit the activity of PH_3-generating bacteria. Further, the addition of slurry to soil is considered to be another cause of PH_3 emissions to the atmosphere (Eismann et al., 1997b, Cao et al., 2000). If changes in the microbial community within slurry during storage are also transferred to changes in soil microbial communities following slurry application, the use of slurry additives such as SB may ultimately influence PH_3 emissions from soils to the atmosphere. However, further research to determine emissions of PH_3 from slurry during storage, and specifically whether these emissions are influenced by the use of slurry additives, is required in order to test this hypothesis.

However, no significant difference in the TN content was also found between the control slurry and the SB-amended slurry in the experiment reported in this chapter

(Figure 3.2.c). This is consistent with Van der Stelt et al. (2007) who demonstrated that the addition of two microbial additives did not significantly change the TN content in livestock slurry, compared to a control slurry, after a 232-day storage period. Similarly, Provolo et al. (2016) observed no significant differences in the TN content between a control pig slurry and slurry treated with a biological additive during a 155-day storage period. The experimental results reported in this chapter are also consistent with those reported by Regueiro et al. (2016), in which no significant increase was observed in the TN content after treating dairy and pig slurries with five different chemical additives (acetic acid, citric acid, lactic acid, sulfuric acid, and alum) compared to control slurry. The gradual decrease in the TN content of slurry observed over the 9-week storage period in both treatments reported in this chapter reflected the decrease in NH_4-N concentration during slurry storage, due to the high proportion (70%) of TN present as NH_4-N in slurry.

During slurry storage, NH_4-N may be generated through two distinct processes, microbial hydrolysis of urea and mineralisation of faecal protein N (Béline et al., 1998). The former process consists of a rapid and complete transformation of urea N to ammonium, potentially within 1 day (Béline et al., 1998). However, due to the complexity of the faecal material, the mineralisation of N in proteins is a slower pathway with respect to NH_4-N production, thus requiring longer slurry storage periods to produce an equivalent mass of NH_4-N (Muck and Steenhuis, 1982, Béline et al., 1998). Despite the potential for production of NH_4-N during slurry storage, the decrease in slurry NH_4-N (and TN) concentration reported in this chapter suggests that there was a net loss of ammonium from slurry during storage. Whilst NH_4-N may be taken up and immobilised as organic N compounds within microbial biomass, thus leading to a reduction in NH_4-N concentration in slurry, this would not result in the

reduction in TN content in slurry that was reported in the current chapter. Instead, the volatilisation of NH_3 from slurry was presumably responsible for the reduction in both NH_4-N and TN concentrations, with volatilisation occurring at a sufficiently high rate to account for any NH_4-N produced during slurry storage.

No significant differences were identified in NH_4-N concentration between the control slurry and the SB-amended slurry over time, suggesting that the SB additive did not significantly influence the volatilisation of NH_3 during storage. According to McCrory and Hobbs (2001), several additives, including biological additives, that are suggested to reduce NH_3 volatilisation from cattle and pig slurry, by promoting NH_4-N uptake and storage in organic compounds during slurry storage, may not be particularly effective. One of the reasons for the low efficacy of biological additives could be their proposed mechanism, as NH_4-N removal from solution through microbial immobilisation of N can only be a temporary solution. On death, the decomposition of microbial necromass allows stored N to be degraded by other microorganisms and, ultimately, for NH_3 volatilisation to occur (McCrory and Hobbs, 2001). Further, a lack of sufficient stimulation of the microbial community in the additive, compared to the community in the control slurry, may also be responsible for the non-significant differences in NH_4-N concentration between the two slurry treatments reported in the current chapter. According to Grubbs (1979), biological additives are effective only when the microbial communities that they introduce to slurry become dominant with respect to the indigenous microbial communities in slurry.

A range of environmental factors in slurry, including pH, temperature, salinity, dissolved oxygen concentration or nutrient availability, as well as toxins or potential pathogens, have the potential to limit the ability of the microorganisms within a slurry

additive to dominate the indigenous microbial community in slurry. For example, Ottosen et al. (2009) observed a significant change in microbial community composition and activity in acidified pig slurry, due to the strong pH sensitivity of the microbial community. However, the microbial communities present within the control and the SB-amended slurries were not analysed directly as part of the research reported in this chapter. Therefore, it is not possible to determine whether the SB-additive successfully led to changes in the composition or activity of the microbial community within slurry. Despite this, it is clear that the SB additive did not result in significant changes in the NH_4-N or TN content of livestock slurry in this research. Clearly, further work is required to more broadly assess the impact of slurry additives on the N content of slurry, alongside the microbial and/or physicochemical mechanisms that are responsible for any impacts.

The research reported here demonstrates that the application of SB to slurry did not cause any significant difference in the TS content compared to a control treatment in which slurry did not receive the additive. This finding is consistent with work previously reported by Smith et al. (1980), Warburton et al. (1980), Patni (1992) and Zhu et al. (1997), as reviewed by McCrory and Hobbs (2001), as well as by van Vliet et al. (2006), and van der Stelt et al. (2007). These previous studies also suggested that no significant effects on the TS content of pig or dairy cattle slurry was associated with a number of biological additives, over a range of slurry storage periods. However, in contrast, Provolo et al. (2016) showed a significant decrease (by an average of 18% over a 6-month storage period) in the TS content of a pig slurry treated with a biological additive compared to a control slurry. These authors explained differences in slurry TS content on the basis of variation in OM degradation rates as stimulated by microorganisms in the additive. However, similar patterns were

not observed in the research reported in this chapter. Indeed, for the majority of the 9-week storage period, a higher TS content was observed in the SB-treated slurry, although this difference only became significant towards the end of the storage period. It is possible that differences in physicochemical properties between the two slurry treatments, such as pH or salinity, influenced microbial activity within the slurry during storage and, thereby, the TS content. For example, according to Kumari et al. (2015), pH is the main physicochemical variable regulating microbial community activity in pig slurry. However, no significant differences in pH were observed between the control and SB-amended slurry in the research reported here. Alternatively, a higher TS content in SB-amended slurry compared to the control slurry may reflect greater microbial biomass accumulation following application of the SB additive to slurry. However, microbial biomass was not directly determined in the experiments reported here. Further research would be required in order to understand the mechanism responsible for differences in slurry TS content as governed by the use of additives such as SB.

No significant differences in pH were observed between the SB and control slurry treatments during storage. In contrast, van Vliet et al. (2006) reported a significant increase in pH in slurry treated with two different biological additives compared to a control slurry over a 6-week storage period. However, the data reported in the current chapter suggest that the same acidification process occurred in both slurry treatments during storage, likely associated with the formation of acetic and further organic acids as a result of the microbial decomposition of organic matter in slurry (Matulaitis et al., 2013). This acidification process was not enhanced, but neither was it significantly reduced, through application of the SB additive during the 9-week storage experiment reported here.

3.4.2 Changes in soil nutrient concentrations following application of slurry and inorganic fertiliser

The application of both control and SB-amended slurry to grassland soil resulted in significantly higher soil pH compared to soils that received inorganic fertiliser treatment or compared to the control soil treatment. This finding is consistent with results reported by van Eekeren et al. (2009) where, at the end of a 5-year incubation experiment, the addition of either FYM manure or cattle slurry to soil resulted in significantly less acidic soil pH in a temperate grassland soil than under inorganic N fertiliser or control soil treatments. In contrast, Matsi et al. (2015) observed no significant difference in the pH of a Mediterranean arable soil between treatments that included liquid dairy cattle manure, NP inorganic fertiliser and control soil, after an 11-year incubation experiment. The decrease in soil pH through time for soils receiving organic amendments, including the slurry treatments reported in this chapter, may reflect the microbial decomposition of organic N compounds in the organic amendment to NH_4-N, followed by nitrification, or the release of organic and inorganic acids upon oxidation of the organic amendment within soil (Tunney, 1981, Helyar and Porter, 1989, Chang et al., 1991, Eghball, 1999).

This pH effect was observed across all soil types, but was particularly pronounced within the clay loam where a maximum pH difference of >0.5 pH units was observed between the SB-amended slurry and inorganic fertiliser treatments. The addition of slurry to soil did not result in any increase in soil pH compared to the start of the incubations. However, it is likely that the hydrolysis of urea fertiliser to NH_4-N through soil urease activity, followed by nitrification of ammonium to NO_3-N may have contributed to acidification within soils that received inorganic fertiliser (Omar and Ismail, 1999, Zhang et al., 2008). There is widespread concern regarding the

potential for inorganic fertiliser application to lead to soil acidification, with important consequences for crop production. These consequences for agricultural production stem from lower availability of a number of key nutrients, including P, Ca and Mg, under acidic soil conditions, alongside concern that some other elements, such as Mn and Al, may reach toxic levels under sufficiently low soil pH (Velthof et al., 2011). In addition, leaching of cations under low soil pH conditions may negatively affect water quality (Velthof et al., 2011). Low soil pH may also limit the microbial processes involved in the soil N cycle, such as biological N fixation and organic N mineralisation (Raubuch and Beese, 2005). Therefore, the potential to reduce soil acidification through slurry application to land may represent a valuable route through which to not only reduce reliance on inorganic fertilisers as nutrient resources, but also to mitigate adverse impacts that follow the reductions in soil pH through inorganic fertiliser application (see also Ezekiel, 2010).

With regard to N, a significant initial increase in NH_4-N concentration was observed across both slurry treatments compared to the inorganic fertiliser and control treatments. This result contradicts previous studies where the amount of available N, mainly in the form of NH_4-N, which was observed in slurry-treated soils was significantly lower compared to inorganic fertiliser treatments (Beauchamp, 1983, Jokela, 1992). The increase observed in the current chapter can be attributed to the input of NH_4-N already present in the slurry. In fact, in both slurry treatments at the end of the slurry storage period, approximately 70% of the TN content was present as NH_4-N. However, a significant initial increase in NH_4-N concentration was also observed in the fertiliser treatment compared to the control treatment. The results in this chapter suggest rapid hydrolysis of the inorganic urea fertiliser occurred within

soil. Despite this, a greater initial increase in NH_4-N concentration in the slurry treatments was observed compared to the fertiliser treatment.

The application of both slurry types and the inorganic fertiliser to soil resulted in a significant initial increase in NO_3-N concentration compared to the control treatment, although no difference were observed between the two slurry types. This initial increase in NO_3-N concentration that was observed across both slurry treatments and the fertiliser treatment is consistent with nitrification of NH_4-N. However, longer-term increases in NO_3-N concentration in both slurry treatments compared to the fertiliser treatment suggests mineralisation of organic N, nitrification and continued supply of NO_3-N occurred in soil. Similarly, Bechini and Marino (2009) observed similar N dynamics, in terms of both NH_4-N and NO_3-N concentrations, across an 181-day soil incubation with five different liquid livestock manures. In particular, these authors reported: i) a significant increase in NH_4-N concentration in the first four-to-seven days compared to a control treatment; and ii) a significant increase in the NO_3-N concentration throughout the incubation compared to the control treatment, with a rapid increase during the first four-to-seven days followed by a slower increase thereafter.

The results from this chapter suggest the potential for accumulation of organic N in soils following the application of organic amendments, alongside longer-term supply of bioavailable NO_3-N because of the nitrification process acting on these organic N pools, in contrast to fertiliser treatments. According to Bechini and Marino (2009), NH_4-N in slurry is only partially available to crops during the first hours or days after addition to soil, due to the possibility that it is either incorporated within microbial biomass, volatilised, nitrified, or trapped within soil through sorption. Notably, volatile fatty acids (VFAs), which are formed during decomposition of organic

compounds in slurry storage, are hypothesised to cause N immobilisation immediately after slurry application to soil (Sørensen, 1998). However, a slow remineralisation of immobilised NH_4-N has also been observed in soil treated with slurry (Bechini and Marino, 2009). Therefore, this N remineralisation from the NH_4-N pool that is originally supplied to soil through slurry, or from the organic N within SOM or within slurry itself, may contribute to the increase in NO_3-N in both slurry treatments during the later stages of the incubation reported in this chapter. Further, this N remineralisation may also explain the significant increase in NH_4-N concentration that was observed from day 5 until the end of the incubation both in the inorganic fertiliser and in the two slurry treatments.

Finally, no significant change was observed in TN content across the soil treatments. However, in contrast to this short-term study where a single input of slurry or inorganic fertiliser was added to soils, repeated applications of organic materials over a longer-term incubation may generate a different scenario. Specifically, repeated applications may result in the build-up of organic N and therefore increases in TN compared to fertiliser treatment. In particular, according to Diacono and Montemurro (2010), repeated long-term additions of organic-rich material, such as slurry and FYM, not only has the effect of enhancing the size of the organic N pool in soil, but also to produce extremely large variations in additional soil properties that modify N dynamics and may result in further net accumulation of N. For example, a significantly greater microbial biomass was observed in soil treated with slurry compared to inorganic fertiliser at every year across a 6-year incubation, thereby leading to the potential for significantly higher organic N accumulation in these slurry-treated soils (Bittman et al., 2005).

Application of the SB-amended slurry to soil also resulted in significantly higher available P (Olsen P) concentrations in CL and SL soil types, compared to either inorganic fertiliser or control treatments. These results are consistent with those reported previously from manured soils that showed greater available P concentrations compared to treatment with monopotassium phosphate fertiliser (Laboski and Lamb, 2003). Two reasons are suggested to explain the greater available P concentration in soil following the application of SB-amended slurry compared to inorganic fertiliser or control treatments. Firstly, because most of the P in manure/slurry is in inorganic form (Turner and Leytem, 2004), this suggests that available P within slurry is mainly in the form of inorganic P which will contribute directly to increases in Olsen P within soil. This hypothesis is in good agreement with the results reported by Sharpley et al. (2004), where, in contrast to soils that were not treated with manure in which inorganic P comprised 26 to 57% of TP, in manured soils 49 to 80% of TP was present in the form of inorganic P. Secondly, Laboski and Lamb (2003) observed that the microbial community in slurry may contribute to increase P availability when slurry is applied to soil through release of organic acids, compared to treatment of soil with inorganic fertiliser.

Therefore, it could be hypothesised that, due to the fact that significantly higher available P was only observed in the SB-treated soil, both the indigenous microbial population in slurry and the community in the SB additive contributed to the increase in Olsen P concentration in this treatment, compared to either treatment with control slurry or with inorganic fertiliser. In particular, *Bacillus*, one of the microbial genera that is present in the SB additive (EnviroSystems, personal communication), has already been studied as phosphate solubilising bacteria (PSB) in slurry (Swain and Ray, 2009). Further, some bacterial inoculants with *Bacillus* gen. or mixed with other

bacteria and/or fungi have been considered as valid alternatives to phosphate fertilisers and have commonly been used to increase P uptake and crop yield in soil (Rodríguez and Fraga, 1999, Velineni and Brahmaprakash, 2011, Mohammadi and Sohrabi, 2012). The mechanism proposed to explain such increase in P uptake by the plants is due to release of a range of organic acids, such as formic, citric, acetic, propionic, malic, succinic, fumaric, glycolic and gluconic acids, that efficiantly solubilised insoluble P within soil (Velineni and Brahmaprakash, 2011).

A significant increase in SOM content in O soil was observed as a result of SB-amended slurry application compared to the inorganic fertiliser treatment, likely due to the addition of OM from the slurry. This greater SOM concentration following the application of slurry compared to inorganic fertiliser was not observed within the other two soil types. This may be due to more rapid SOM decomposition after slurry application in CL and SL soil types, compared to the O soil type. As suggested by Bell et al. (2003), differences in the composition of the soil microflora can play an important role in determining the rate of mineralisation of SOM after slurry application. Indeed, the activity and composition of both bacterial and fungal species in the microbial community across CL and SL soil types may have contributed to the rapid degradation of the organic compounds entering soil following slurry application, thereby resulting in no significant differences in SOM concentration in these soil types.

However, importantly, this short-term study with only one application of the individual treatments, generated a higher SOM content in O soil following slurry application. Therefore, it is conceivable that higher SOM content may also be expected across a longer-term study following repeated additions of organic materials to the other two soil types. Further studies are required to establish the SOM dynamics

across different soil types following the application of organic amendments, such as slurry and FYM. The loss of SOM from agricultural soils owing to land use change and intensification of agricultural production represents a serious concern for soil quality and food production (Carter, 2002, Bhattacharya et al., 2016). In the context of an increasing desire from economic and environmental perspectives to reduce reliance on inorganic fertiliser to support production, it is likely that agricultural practices will increasingly have to take into account and exploit the application of organic-rich materials, such as slurry and FYM, to soil in order to replenish SOM and thereby maintain soil quality and crop yields (Edmeades, 2003, Paterson et al., 2011).

4 The effects of organic amendments on microbial activity within soil

4.1 Introduction

The application of fresh substrates, such as cattle slurry, crop residue or root exudates, to soil can strongly affect carbon (C), nitrogen (N) and phosphorus (P) cycling and therefore soil fertility (Kumar and Goh, 1999, Ludovici and Kress, 2006, Wang et al., 2015). When these readily available substrates are added to soil, decomposition of the added C substrate and mineralisation of more recalcitrant C-compounds in the native soil organic matter (SOM) are typical processes that follow, as governed by soil microbial activity (Condron et al., 2010). Changes in microbial activity following substrate addition are also responsible for a priming effect (PE), in which modifications of both the fractions and the rate of SOM decomposition are observed, following the addition of organic or mineral substrates to soil (Blagodatskaya and Kuzyakov, 2008). In particular, it has been demonstrated that the addition of fresh C substrates can activate microbial groups in soil that were otherwise inactive or dormant, with a broad range of both extracellular and intracellular enzymes synthesised for SOM decomposition (Blagodatskaya and Kuzyakov, 2008). Evidence suggests that SOM decomposition initially involves extracellular enzymes, such as hydrolases and oxidoreductases, followed by intracellular enzymes (Marxsen and Witzei, 1991).

According to Fontaine and Barot (2005), an increase in the extracellular activity of enzymes responsible for the degradation of recalcitrant compounds, such as lignin and cellulose, is believed to be the cause of a PE, due to the involvement of these enzymes in the decomposition of SOM. Further, it has been observed that Fe^{2+} can stimulate phenol oxidase activity in soil, thus leading to an increase in the degradation of other

organic compounds in soil, such as phenols, and to contribute to the PE (Van Bodegom et al., 2005, Emsens et al., 2016). However, the addition of organic amendments not only results in acceleration of SOM mineralisation (a positive PE), but may also cause a reduction in the rate of decomposition of native soil C (a negative PE) (Kuzyakov et al., 2000). For example, with a level of added substrate C of two to five times microbial biomass C (C_{mic}), soil microorganisms are thought to switch from degradation of recalcitrant SOM to the more available added substrate, in turn resulting in a lower rate of SOM decomposition and a negative PE (Blagodatskaya and Kuzyakov, 2008).

However, owing to the microbial metabolic demand for C and/or energy, assimilation of both SOM and added substrate into microbial biomass may also occur, alongside dissimilation processes including maintenance respiration (MR) (Kim and Gadd, 2008). Whilst assimilation occurs under conditions favourable to microbial biomass synthesis, such as increased substrate availability, and accounts for the growth of a microbial population, dissimilation processes take place when energy demands are high or energy limitation exists (Geyer et al., 2016). In particular, MR represents the basal energy requirement for purposes other than biomass production (Wang and Post, 2012). In intensively managed agricultural grasslands in temperate latitudes, microorganisms can be C-limited (Jones and Donnelly, 2004) or co-limited by C in combination with N and/or P (Jones et al., 2004, Demoling et al., 2007). Such limitations by C may mean that a rapid response is observed following the addition of readily decomposible sources of C to soil, associated with stimulation of microbial growth and activity, in turn leading to a PE (Blagodatskaya and Kuzyakov, 2008). Therefore, in order to determine the fate of the C applied through amendments to the soil, such as livestock slurry, it is critical to measure the partitioning of the C

associated with the added substrate and associated with SOM into both catabolic and anabolic pathways, as driven by the microbial community in soil (Hill et al., 2008).

Measurements of this partitioning have typically relied on incubation experiments through the application of isotopically labelled substrates, such as ^{13}C and ^{14}C, to soil, followed by subsequent determination of the fractions of ^{13}C or ^{14}C activity in three pools: mineralised; incorporated within microbial biomass; and retained within soil. A number of studies have examined the effects of the quality of organic inputs applied to soil on decomposition of the added substrate and SOM respiration (Schutter and Dick, 2001, Dilly, 2004, Orwin et al., 2006, Hernández and Hobbie, 2010, Jagadamma et al., 2014, Elmajdoub and Marschner, 2015). Other studies have examined the impacts of substrates of different lability on the microbial turnover of the substrates and of SOM (Carreiro et al., 2000, Rovira and Vallejo, 2002, 2007, Wild et al., 2014). However, substantial uncertainty remains surrounding the impacts of adding organic compounds of different composition on microbial activities, such as respiration and assimilation, in C- and nutrient-limited soils.

In addition, little is known about the influence of the P moiety on the mineralisation/assimilation of phosphorylated substrates added to grassland soils in which soil microbial populations may be co-limited by C and nutrients. Among the organic compounds added to soil, phosphosugars and nucleotides, represent a considerable input of organic P to soil (Turner et al., 2005). Therefore, determining how the P moiety within added substrates is partitioned once within the soil environment is important in order to understand the impacts of substrate addition on soil fertility, alongside the risk of P export from agricultural soils. Spohn and Kuzyakov (2013) found that microbial mineralisation of organic P compounds within soil was driven by the microbial need for C, following the addition of glucose-6-

phosphate (G6P) in temperate forest soils, resulting in the incorporation of only a small proportion of P within microbial biomass. Therefore, if P moieties are not required by microbial populations then P availability in soils is likely to increase as a result of the addition of compounds such as G6P. Whilst this might initially be positive in terms of plant P availability and crop production, it may ultimately increase the risk of P export from agricultural soils if the accumulation of P moieties continues (Heckrath et al., 1995).

However, no studies have been conducted to measure the extent of C respired and incorporated into microbial biomass following the application of organic amendments of different quality, such as glucose, G6P, and cellulose, alongside slurry, to grassland soil. Further, no information is available regarding the impact of biological additives, used during slurry storage, on the soil microbial community and how any impact on this community influences the fate of fresh substrate and SOM. For example, whilst van Vliet et al. (2006) examined the effects of applying a biological additive to slurry on microbial diversity within the slurry, alongside how slurry additives impacted grass production, no analyses were conducted on the soil microbial community or on the fate of C within substrate and SOM. Therefore, the objective of this chapter is to determine how the quality of organic substrates, such as carbohydrates and slurry, added to grassland soil significantly affects the partitioning of elements within the added substrate and the native SOM, including C and P, as influenced by microbial activity in the soil. In this chapter, it is hypothesised that:

i. the balance between mineralisation, assimilation or retention in soil will be significantly influenced by the quality of a substrate added to soil, comparing simple carbohydrates, such as glucose and G6P, to more complex carbohydrates, such as cellulose;

ii. a significantly higher concentration of available P within soil will be observed following the application of a phosphorylated C substrate, compared to application of the non-phosphorylated counterpart, due to the microbial requirement for C, but not for P;

iii. the addition of livestock slurry, either amended or not amended with the biological additive SB, alongside carbohydrates to soil, will result in a significantly lower mineralisation and assimilation rate of added substrate and SOM compared to the addition of carbohydrates alone, due to the presence of more recalcitrant C compounds in slurry;

iv. the addition of slurry that has received a biological additive during storage will result in significantly higher C cycling in soil, as driven by changes in the microbial community within slurry and within soil associated with the biological additive, compared to an unamended slurry.

4.2 Materials and methods

The experimental work reported in this chapter was divided in two distinct laboratory incubations and related analyses of soil that received different treatments. The first incubation concerned the inoculation of soil with non-radiolabelled carbohydrates (glucose, G6P and cellulose), alongside livestock slurry, that had either been amended or not amended with SB, in order to determine the microbial respiratory activity. The second incubation concerned the inoculation of soil with ^{14}C-labelled carbohydrates (^{14}C-glucose, ^{14}C-G6P, and ^{14}C-cellulose), alongside the same unlabelled substrates utilised in the first incubation, in order to measure the extent of ^{14}C-mineralisation, ^{14}C incorporation into microbial biomass and the residual ^{14}C activity in soil.

4.2.1 Soil and soil sampling

Clay loam soil was selected for use in the current chapter. Due to possible C-limitation in intensively managed agricultural grasslands in temperate latitudes (Jones and Donnelly, 2004), a clay loam soil was selected according to the low total organic C (TOC) content measured during the previous experiment, described in Section 3.4.2. Further, a clay loam soil was been selected because it is representative of many grassland soils in the UK. Bulked soil samples were collected from a grassland field in Myerscough College Farm, Bilsborrow, Preston, Lancashire, that was not previously treated with inorganic fertiliser or slurry amended with SB. The sampling followed a 'W' sampling design, with 51 individual cores (17 sites along the 'W', with 3 replicates at each site). All soil cores were taken to a depth of 7.5 cm using a gouge auger. Cores were combined into a single bulk sample of some 10 kg, passed through

a 2.0 mm sieve in order to remove roots and other vascular material, as well as to homogenise the sample, and then stored in plastic bags at 4 °C prior to the experiments.

4.2.2 Experimental design for the soil incubations

Twelve treatments were established for the soil incubations for total respiration and for the ^{14}C-labelled study, as summarised in Table 4.1. All treatments were incubated in triplicate within a laboratory for 18 days, with both incubations occurring between May and June 2015. The incubation for the microbial respiratory activity (Section 4.2.4) required 252 g dry weight equivalent $_{(dwe)}$ of soil (7 g_{dwe} of soil per treatment * 3 replicates * 12 treatments), whereas the incubation for the ^{14}C mineralisation (Section 4.2.5) required 504 g_{dwe} of soil (14$_{dwe}$ g of soil per treatment * 3 replicates * 12 treatments). An initial amount of 800 g_{dwe} of soil was divided in four fractions, of which one was used for the control soil and the soils with US or AS slurries, whilst the other three fractions were utilised for the treatments with the three carbohydrates with or without the two slurries. The treatments were designed in order that soil was amended with 0.3 mg C g^{-1} $_{dwe}$ soil, to remain comparable with the rate of glucose or cellulose amendments reported in previous studies (Shen and Bartha, 1996, Blagodatskaya et al., 2014, Jagadamma et al., 2014).

Table 4.1. Summary of the twelve treatments for the incubations for the total respiration and the ^{14}C mineralisation. Each treatment is incubated in triplicates.

Treatments
Soil alone (control)
Soil + unamended slurry (US)
Soil + slurry amended with SB (AS)
Soil + glucose
Soil + glucose + US
Soil + glucose + AS
Soil + G6P
Soil + G6P + US
Soil + G6P + AS
Soil + cellulose
Soil + cellulose + US
Soil + cellulose + AS

A glucose and a G6P solution of 6.8 * 10^{-5} and 6.7 * 10^{-5} M, respectively, were made using 0.14 g of glucose and 0.2 g of G6P in 57 mL of Milli-Q water. Each solution was added to a different soil fraction of 200 g_{dwe} and then thoroughly mixed to ensure homogenisation. Another soil fraction was treated with 0.14 g of cellulose that was added directly as powder and then thoroughly mixed alongside 57 mL of Milli-Q water. An aliquot of 65 g_{dwe} of soil from each of the four fractions was treated with 45.5 µL of US slurry from the slurry inoculation (see Section 4.2.3), wherease another soil aliquot of the same amount was treated with the same volume of AS slurry. Finally, a third aliquot of soil was left either untreated (control soil) or treated with one of the three carbohydrates alone in order to have the 12 treatments for both incubations. From each aliquot, 21 g_{dwe} of soil was used for each of the three replicates in the incubation for cumulative respiration (see Section 4.2.4), whereas 42 g_{dwe} of soil was used for the three replicates in the incubation involving ^{14}C mineralisation (see Section 4.2.5). Each fraction of 42 g_{dwe} of soil for the ^{14}C mineralisation incubation that had previously been treated with carbohydrates was

subseqently spiked with the corresponding ^{14}C-labelled carbohydrates, before being separated into replicates and placed into respirometers. For these experiments, D-Glucose was obtained from BDH Laboratory Supplies, UK. D-Glucose-6-phosphate disodium salt hydrate and α-Cellulose were obtained from Sigma-Aldrich Co. Ltd., UK. [^{14}C(U)]-D-Glucose (>98% pure), [^{14}C(U)]-D-Glucose-6-phosphate (>97% pure) and [^{14}C(U)]-Cellulose (*Nicotiana tobacum*) were obtained from BIOTREND Chemikalien GmbH, Germany.

4.2.3 Slurry and slurry additive

Following the slurry trial described in Section 3.2.1, livestock slurry provided by Myerscough College farm, Bilsborrow, Preston, Lancashire, was used for the slurry inoculation prior to the soil incubation experiments described in this chapter. The slurry was produced by Holsteins cows fed with conserved forage (grass silage). Further, the slurry was not treated with the slurry additive SB prior to the trial. The biological additive used for this study was SlurryBugs (SB), a product developed and commercialised by EnviroSystems UK Ltd. A more advanced version of SB was used in the research reported in this chapter compared to that described in Section 3.2.3. In the current chapter, inoculation of slurry used SB alone, due to the incorporation of the organic booster (SlurryBooster, see Section 3.2.1) into the additive itself. The slurry inoculation was carried out at Myerscough College Farm for 8 weeks, from March to May 2015. Details of the slurry inoculation used in the research reported in this chapter matched those described in Section 3.2.1. Slurry samples were collected at weeks 0 and 8 of the incubation from the bottom of the slurry using a plastic container and then mixed thoroughly. All slurry samples were collected in plastic bottles, refrigerated in a cold room before being sent for external analysis at NRM

laboratories for pH, total solids, total P, total N, NH_4-N, total K, total Mg and total Na. In addition, 5 L of US slurry and 5 L of AS slurry were collected at the end of the 8-week storage trial and kept at 4 ºC prior to application to soil as part of the treatments described above.

4.2.4 Determination of the microbial respiratory activity following amendment of soil with unlabelled glucose, glucose-6-phosphate and cellulose, alongside amended and unamended slurry

The microbial basal and substrate-induced respiration were determined by measuring the cumulative CO_2 (mg C g^{-1} $_{dwe}$ soil) released in the headspace of the experimental containers following the application of organic amendments to soil. The soil moisture content was checked and kept at approximately 70 % of water holding capacity. 252 g_{dwe} of soil were used for the mineralisation assay (7 g_{dwe} of soil per pot * 3 replicates * 12 treatments). Each soil sample of 7 g_{dwe} was placed in a 250 mL Kilner jar with metal screw band and vacuum seal. A rubber septum in the centre of the seal allowed headspace gas released during the respiration to be sampled via a gas-tight syringe. The bottles were incubated in a Panasonic MIR-154-PE Cooled Incubator in the dark at 20 ºC for 18 days. 9 mL of headspace gas was collected at day 0, 1, 2, 4, 8, 11 and 18 of the incubation and injected into 3 mL Labco Exetainer flat bottomed soda glass vials. Vacuum to 10^{-3} mbar was applied to the exetainers by a vacuum pump RZ 2.5 VACUUBRAND GMBH + CO KG, Germany. Three evacuated exetainers were filled with each of three standard gases from 200 bar BOC gas cylinders at 514.6, 1038 and 4198 CO_2 ppm. All exetainers without standard gases were then filled with 9 mL of headspace gas from the Kilner jars. Subsequently, all the exetainers were analysed by a PerkinElmer AutoSystem XL Gas Chromatograph

plus HTA Headspace Autosampler to quantify the amount of CO_2 released as a result of microbial respiration activity. Dilutions of the headspace gas injected in the exetainers were required from day 3 of the incubation, using oxygen-free nitrogen from a 200 bar BOC gas cylinder.

4.2.5 Determination of the mineralisation rate of ^{14}C-Glucose, ^{14}C-Glucose-6-phosphate and ^{14}C-Cellulose (*Nicotiana tobacum*) to $^{14}CO_2$

A mineralisation assay was performed for 18 days using the respirometric method of Reid et al. (2001) in order to determine the mineralisation rate of [^{14}C(U)]-D-Glucose, [^{14}C(U)]-D-Glucose-6-phosphate and [^{14}C(U)]-Cellulose (*Nicotiana tobacum*) to ^{14}C-CO_2. By doing so, the catabolic potential of the soil microbial community for the added substrates was established, by quantifying the ^{14}C-CO_2 released over the course of the incubation (West and Sparling, 1986, Reid et al., 2001). The utilisation of ^{14}C-labelled compounds allows the fate of substrates applied to soil to be tracked successfully (Chotte et al., 1998).

A soil amount of 504 g_{dwe} (14 g_{dwe} of soil * 3 replicates * 12 treatments) was used for the radio-labelled mineralisation assay. The soil fractions that had already been treated with the three carbohydrates were spiked with their ^{14}C-labelled analogues. The specific ^{14}C activity measured for the labelled ^{14}C-glucose, ^{14}C-G6P and ^{14}C-cellulose was 108.42, 95.67 and 199.83 Bq g^{-1}_{dwe} soil, respectively, using 2 mL of ethanol:H_2O for ^{14}C-glucose and ^{14}C-G6P, and 2 mL of NaOH for ^{14}C-cellulose, as the carrier solvents. After spiking, the carriers were allowed to volatilise over two hours before soils were added to the respirometric flasks.

The respirometric flask consisted of a 250 mL Schott bottle with a Teflon-lined screw threaded lid. The lid was drilled in the centre and a stainless steel studding was inserted to attach a crocodile clip. The clip held a CO_2 trap, composed of a 7 mL glass scintillation vial containing 1 M NaOH (1 mL). All soil samples of 14 g_{dwe} were placed in the flasks and the lids were then closed tighly. Any $^{14}CO_2$ released from the microbial catabolism was then trapped into the vial located in the middle of the respirometric flask and above the soil. The respirometers were then placed on an orbital shaker (Janke and Kunkel, IKA-Labortechnik KS250, Germany) and shaken at 100 RPM at a temperature of 20 ± 2 ºC. Sampling was performed at 0 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, 7 d, 8 d, 9 d, 10 d, 15 d and 17 d. At any timepoint of analysis, the lid of the flask was unscrewed and the vial was removed and replaced with a new one. The vial that had been removed was wiped with tissue soaked in acetone in order to remove any ^{14}C activity. 5 mL of liquid scintillation cocktail was then added to the vial, followed by resting the vial overnight in a dark cupboard. The liquid scintillation solution was then counted in order to quantify the ^{14}C activity, using a Canberra Packard Tri-Carb 2250A liquid scintillation counter.

4.2.6 Determination of ^{14}C-Glucose, ^{14}C-Glucose-6-phosphate and ^{14}C-Cellulose (*Nicotiana tobacum*)-associated activity in soil

The soil treatments from the 250 mL Schott bottles described above in Section 4.2.5 were analysed for residual ^{14}C-activity in soil through combustion of approximately 1 g of dry soil plus 200 µL combust-aid for 3 minutes to approximately 125ºC, using a Packard 307 Sample Oxidiser. The samples were taken a few days after the end of the mineralisation assay, with the bottles stored at 4 ºC until analysis. The evolved $^{14}CO_2$ as a result of combustion was trapped using 10 mL of PermaFluor-

E and 10 mL of Carbo-sorb with a trapping efficiency > 95%. The samples were kept in the dark for 16 hours to minimise the effects of chemi-luminescence and, then, analysed using a Canberra Packard Tri-Carb 2250A liquid scintillation counter to determine the residual ^{14}C activity in soil.

4.2.7 Determination of ^{14}C-Glucose, ^{14}C-Glucose-6-phosphate and ^{14}C-Cellulose (*Nicotiana tobacum*)-uptake into microbial biomass

The uptake of ^{14}C into microbial biomass was determined using the fumigation and K_2SO_4 extraction method (Vance et al., 1987). 4 gd_{we} of soil from each soil treatment in the 250 mL Schott bottles described above in Section 4.2.5 was weighed and placed in 10 mL beakers for fumigation extraction, whilst a further 4 g_{dwe} was weighed and placed in plastic tubes for non-fumigation extraction. The samples were taken a few days after the end of the mineralisation assay, with the bottles stored at 4 ºC until analysis. The extraction for the non-fumigated soil samples was carried out using 20 mL of 1 M K_2SO_4. The samples were shaken on an orbital shaker at 100 RPM for 30 minutes at room temperature. The supernatant was filtered and 5 mL of supernatant was then added into a 20 mL vial with 15 mL of GS1 Gold Star liquid scintillation cocktail. The samples were kept in the dark overnight before counting for the ^{14}C activity incorporated in microbial biomass using Canberra Packard Tri-Carb 2250A liquid scintillation counter. The samples for fumigation were placed in a desiccator with wet paper towels, along with a beaker containing 75 mL ethanol-free chloroform ($CHCl_3$) and a few anti-bumping granules. $CHCl_3$ was allowed to boil vigorously in the desiccator for a few minutes, then samples were left for 24 hours. The pressure from the desiccator was then released slowly once time elapsed and residual $CHCl_3$ from soil was removed by evacuating the desiccator five to six times. The soil samples

were extracted and ^{14}C activity determined as described above for the non-fumigated samples. ^{14}C activity associated with microbial biomass was then calculated as follow:

^{14}C-activity within microbial biomass= ^{14}C-activity in fumigated soil – ^{14}C-activity in non-fumigated soil. (4.1)

4.2.8 Soil chemical analyses

Soil chemical analyses were carried out, as detailed in Sections 3.2.9 and 3.2.12, to determine the concentrations of TOC, TN, Olsen P, and the C/N ratio prior to the microbial respiration incubation in the control soil ($S_{in.}$) and at the end of the incubation for each soil treatment, as described in Table 4.2.

4.2.9 Calculation of the priming index

The priming index (PI) accounts for any increase or decrease in mineralisation of organic matter in soil per unit of substrate added per unit of time against the control soil that does not receive any substrate addition. The PI was defined by Shen and Bartha (1996, 1997), with later modifications for negative priming effects (PE) by Kuzyakov et al. (2000), so that a negative PI describes the immobilisation or the reduced decomposition of SOM compared to a control treatment:

$$PI(t) = \frac{netCO_2}{^{14}C\text{-}CO_2} - 1 \qquad (4.2)$$

where ^{14}C-CO_2 is the labelled CO_2 respired from the soil expressed as a percentage of the total ^{14}C initially spiked to the soil, whereas netCO_2 represents the cumulative CO_2

respired from the soil as a percentage of the total C initially added to the soil in a treatment that has received ^{12}C and ^{14}C, defined as:

$$netCO_2 = \frac{(SSR-MR)}{Cs*100\%} \qquad (4.3)$$

where SSR (soil with substrate respiration) is the cumulative CO_2 released from the soil with the substrate added, MR (maintenance respiration) is the basal cumulative CO_2 released from the soil with no substrate added, whilst C_s is the amount of C in the substrate calculated at any timepoint, with all these values expressed as mg C g^{-1} $_{dwe}$ soil.

4.2.10 Statistical analysis

The t-test was performed to evaluate whether significant differences between the beginning and the end of the incubations existed across each chemical parameter. The effects of applying different carbohydrates and slurry (amended or non-amended with SB) on total respiration, on partitioning of C across microbial biomass, soil and evolved as CO_2, as well as on soil nutrient concentrations, were evaluated by a two-way analysis of variance (ANOVA), with carbohydrate and slurry as the two independent factors, using IBM SPSS Statistics 22. A value of $p < 0.05$ was considered the threshold value for significance. Pairwise comparisons were conducted using Bonferroni correction at $p < 0.05$ for those factors for which significant effects were determined.

4.3 Results

4.3.1 Soil chemical analyses

The concentration of available P, TOC, TN and the C/N ratio were measured in the control soil treatment before and at the end of the incubation. Further, the same chemical parameters were measured at the end of the 18-day incubation across all soil treatments (Table 4.2). No significant change was observed in the Olsen P concentration in the control soil between the beginning and end of the incubation. The application of carbohydrates to soil resulted in significant differences from each other in the pairwise comparison in Olsen P concentration between treatments, in the order G6P > cellulose > glucose > control ($F(3, 21) = 568.269$, $p < 0.000$, $\eta^2 = 0.988$). Significant differences in Olsen P concentration were also observed following the addition of slurry across all AS and US treatments, with higher concentrations in AS slurry and control treatments than the treatment with US slurry ($F(2, 21) = 27.325$, $p < 0.000$, $\eta^2 = 0.722$). Significant impacts on Olsen P concentration following the application of slurry alongside cellulose or G6P were also found, with the Olsen P concentration in the cellulose and cellulose + AS treatments being higher than in the cellulose + US treatment ($F(2, 21) = 30.400$, $p < 0.000$, $\eta^2 = 0.743$). With respect to the G6P treatments, Olsen P concentration was found to differ significantly in the order G6P > G6P + AS > G6P + US ($F(2, 21) = 68.975$, $p < 0.000$, $\eta^2 = 0.868$).

No significant changes were observed either in the TOC or TN concentrations in the control soil treatment between the beginning and the end of the incubation. In addition, neither the different carbohydrate treatments nor the different slurry treatments led to significant differences in TOC or TN after the 18-day incubation. A significant increase in the C/N ratio was observed between the beginning and the end

of the incubation period in the control soil treatment ($F(1, 5) = 12.308$, $p = 0.025$, $\eta^2 = 0.602$). At the end of the 18-day incubation, the application of either carbohydrate or slurry treatments did not result in any significant change in the C/N ratio between treatments. Finally, interactions between carbohydrate and slurry treatments did not cause any significant effect on the C/N ratio at the end of the incubation.

4.3.2 Cumulative CO_2 production from grassland soils following slurry and carbohydrate application

Figure 4.1 reports the cumulative efflux of CO_2 from the control soil treatment, corresponding to the maintenance (basal) respiration, versus the treatments with three carbohydrates of differing quality (glucose, G6P and cellulose). In addition, for each individiaul carbohydrate, this figure reports CO_2 efflux from treatments that received either an unamended slurry, or a slurry that had been amended with the SB additive, alongside the respective carbohydrate. To account for the additional C supplied within the slurry itself, the values of the cumulative CO_2 from the treatments that received slurry in addition to carbohydrates were divided by two, in order to normalise all data to the concentration of C supplied by the addition of carbohydrate to soil alone (0.3 mg C $g^{-1}_{\ dwe}$ soil).

A significant reduction in the microbial respiratory activity was observed following the addition of each carbohydrate to soil compared to the control treatment ($F(3, 24) = 3093.433$, $p < 0.000$, $\eta^2 = 0.997$). Further, the response of microbial respiratory activity to the application of carbohydrate depended on the quality of the C substrate added to soil, with CO_2 effluxes varying in the order glucose > G6P > cellulose, with 73, 56 and 50 % of the cumulative CO_2 measured for the control treatment,

respectively, at the end of the soil incubation. The addition of slurry alongside each carbohydrate treatment seemed to further reduce microbial respiratory activity, producing a significantly lower cumulative CO_2 efflux over the 18-day incubation compared to the treatments with carbohydrates alone and to the control treatment ($F(3, 24) = 462.612$, $p < 0.000$, $\eta^2 = 0.973$). Significant differences between AS and US treatments were only observed following the addition of glucose or G6P to soil, with the application of US slurry resulting in lower microbial respiration compared to AS slurry. In contrast, no significant difference was observed between CO_2 efflux following cellulose+US and cellulose+AS treatments.

Table 4.2. Concentrations of Olsen-P, TOC, TN and the C/N ratio in the different treatments prior to and at the end of the substrate-induced respiration experiment (S_{in}= control soil before the incubation, S= control soil at the end of the incubation, US= unamended slurry, AS= slurry amended with the biological additive SlurryBugs, Glu= glucose, G6P= glucose-6-phosphate, Cel= cellulose). All numbers are expressed as means of triplicates ± standard error of the mean.

Treatments	**Olsen-P** (mg kg^{-1} soil)	**TOC** (%)	**TN** (%)	**C/N ratio**
$S_{in.}$	73.41 ± 0.72	3.29 ± 0.11	0.40 ± 0.01	8.22 ± 0.06
S	76.95 ± 0.52	3.52 ± 0.15	0.40 ± 0.01	8.85 ± 0.17
S+US	81.36 ± 1.88	3.74 ± 0.08	0.43 ± 0.01	8.78 ± 0.12
S+AS	81.44 ± 1.01	3.83 ± 0.32	0.41 ± 0.02	9.21 ± 0.35
S+Glu	84.58 ± 1.46	3.40 ± 0.16	0.38 ± 0.02	8.92 ± 0.08
S+Glu+US	87.47 ± 0.75	3.40 ± 0.12	0.40 ± 0.01	8.46 ± 0.06
S+Glu+AS	86.90 ± 0.58	3.54 ± 0.02	0.40 ± 0.01	8.95 ± 0.12
S+G6P	121.51 ± 1.16	3.28 ± 0.09	0.39 ± 0.01	8.45 ± 0.08
S+G6P+US	102.16 ± 1.92	3.42 ± 0.25	0.39 ± 0.01	8.71 ± 0.40
S+G6P+AS	110.05 ± 0.90	3.49 ± 0.22	0.42 ± 0.03	8.27 ± 0.04
S+Cel	84.88 ± 0.16	3.61 ± 0.11	0.41 ± 0.01	8.88 ± 0.10
S+Cel+US	76.47 ± 0.38	3.82 ± 0.07	0.43 ± 0.01	8.83 ± 0.16
S+Cel+AS	87.45 ± 0.50	3.26 ± 0.18	0.39 ± 0.02	8.26 ± 0.10

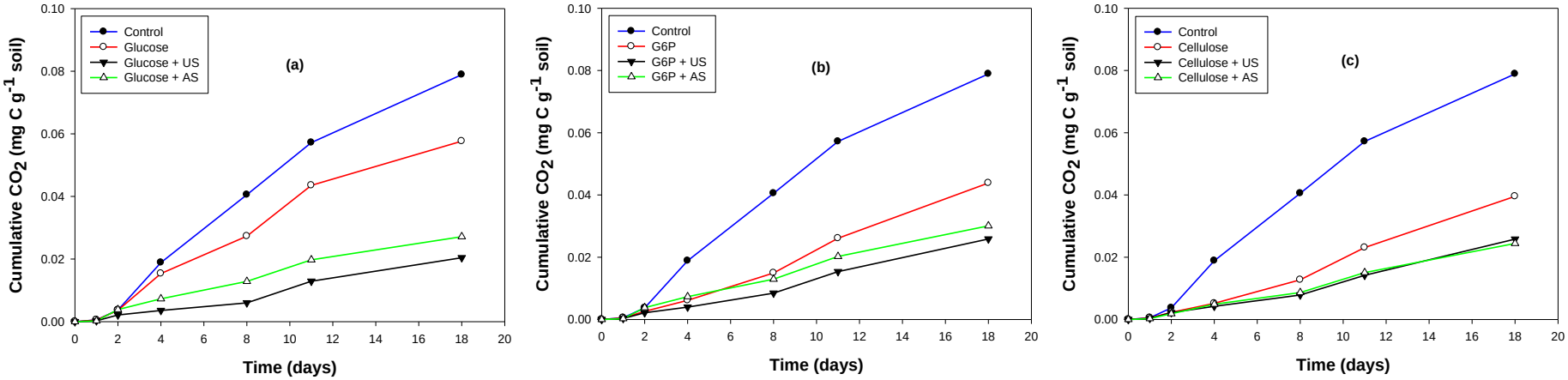

Figure 4.1. Cumulative CO_2 efflux from control soil and from soil treated with glucose and slurry amended (AS) and unamended (US) with the biological additive SlurryBugs (a); from control soil and from soil treated with glucose-6-phosphate (G6P) and AS and US slurry (b); from control soil and from soil treated with cellulose and AS and US slurry (c) during 18 days of incubation. Average values of measured data are presented as symbols (n = 3).

4.3.3 Maximum mineralisation rate and C-partitioning associated with soil microbial activity following carbohydrate and slurry application to grassland soil

Using ^{14}C-labelled carbohydrates, the partitioning of C applied to soils over the 18-day incubation period was ascribed to three pools: $^{14}CO_2$ evolved via mineralisation; ^{14}C in biomass uptake; and residual ^{14}C remaining in soil (Table 4.3). The highest rate of ^{14}C mineralisation was measured during the first hour of the incubation for every treatment. The addition of either simple ^{14}C-carbohydrates (^{14}C-glucose or ^{14}C-G6P) caused a significantly higher initial mineralisation rate compared to the application of the more complex ^{14}C-cellulose ($F(2, 18) = 192.124$, $p < 0.000$, $\eta^2 = 0.955$) (Table 4.3). A significant increase in the maximum mineralisation rate was also observed following the application of US slurry compared to the treatment with AS slurry, or that with only ^{14}C-glucose added ($F(2, 18) = 49.209$, $p < 0.000$, $\eta^2 = 0.845$). Significant interaction effects between ^{14}C-carbohydrate and slurry were observed for the maximum mineralisation rate ($F(4, 25) = 79.872$, $p < 0.000$, $\eta^2 = 0.947$). In particular, whilst no significant differences were observed in the ^{14}C-cellulose treatments with or without slurry application, the addition of AS slurry to the soil treated with ^{14}C-G6P resulted in a significantly higher initial mineralisation rate compared to the treatment with ^{14}C-G6P alone. Finally, significant differences in the initial mineralisation rate were found in ^{14}C-glucose treatments following the addition of both slurries in the order ^{14}C-glucose + US > ^{14}C-glucose > ^{14}C-glucose + AS.

The extent of mineralisation differed significantly across the three ^{14}C-carbohydrates added to soil, in the order ^{14}C-glucose > ^{14}C-G6P > ^{14}C-cellulose ($F(2, 18) = 58.727$, $p < 0.000$, $\eta^2 = 0.867$). A significantly greater extent of mineralisation was also observed following the application of US slurry compared to the treatments

with AS slurry or those with only a ^{14}C-carbohydrate added (F(2, 18) = 27.333, p < 0.000, η^2 = 0.752). Further, significant interaction effects between ^{14}C-carbohydrate and slurry were observed for the extent of mineralisation (F(4, 18) = 27.333, p < 0.000, η^2 = 0.905). Notably, whilst no significant variation was found for the extent of mineralisation following the application of ^{14}C-cellulose and slurry treatments, the addition of AS slurry to the ^{14}C-G6P-treated soil caused a significantly greater extent of mineralisation compared to the treatments with ^{14}C-G6P alone or with ^{14}C-G6P and US slurry. In contrast, the addition of either slurry to the ^{14}C-glucose-treated soil led to significant differences in the extent of mineralisation in the order ^{14}C-glucose + US > ^{14}C-glucose > ^{14}C-glucose + AS.

The application of either ^{14}C-glucose or ^{14}C-G6P to soil resulted in a significantly greater incorporation of ^{14}C into microbial biomass compared to ^{14}C-cellulose (F(2, 18) = 60.865, p < 0.000, η^2 = 0.871). A significant different biomass uptake was also observed following the application of either slurry treatments in the order ^{14}C-carbohydrate > ^{14}C-carbohydrate + AS > ^{14}C-carbohydrate + US (F(2, 18) = 227.530, p < 0.000, η^2 = 0.962). Significant interaction effects between ^{14}C-carbohydrate and slurry were observed for the incorporation of ^{14}C into microbial biomass across treatments (F(4, 18) = 11.468, p < 0.000, η^2 = 0.718). In particular, significantly higher biomass uptake was observed in the treatments without slurry compared to treatment with either slurries for each ^{14}C-carbohydrate. Significant differences were also observed in residual ^{14}C activity in soil between the different carbohydrate treatments, in the order cellulose > G6P > glucose (F(2, 18) = 119.513, p < 0.000, η^2 = 0.930). The addition of slurry, whether amended or non-amended with SB, alongside a carbohydrate treatment, also resulted in significantly greater residual ^{14}C activities in soil compared to the treatments involving the carbohydrate alone (F(2, 18) = 222.887,

$p < 0.000$, $\eta^2 = 0.961$). Further, significant interaction effects were observed between carbohydrate and slurry for the residual ^{14}C activities in soil ($F(4, 18) = 222.887$, $p < 0.001$, $\eta^2 = 0.628$). In particular, the addition of either slurry to each ^{14}C-carbohydrate treatment always resulted in significantly higher residual ^{14}C activity in soil compared to the application of the three ^{14}C-carbohydrates alone.

4.3.4 Effect of carbohydrate and slurry application to grassland soil on the priming index

The microbial activity toward both different substrate types added to soil and SOM was characterised through calculation of the PI (Figure 4.2). Significant differences in PI were observed between the three carbohydrate treatments, with decreasing PI in the order glucose > G6P > cellulose ($F(2, 18) = 58.531$, $p < 0.000$, $\eta^2 = 0.867$). The application of slurry to soil also revealed a significant effect on the PI, with lower values of PI observed following the application of either slurry types compared to treatments that only included a carbohydrate ($F(2, 18) = 51.262$, $p < 0.000$, $\eta^2 = 0.851$). Significant decreases in PI were observed through time across the treatments ($F(4, 15) = 2598.200$, $p < 0.000$, $\eta^2 = 0.999$). Furthermore, significant interaction effects between carbohydrate and slurry were observed for the PI ($F(4, 18) = 30.305$, $p < 0.000$, $\eta^2 = 0.871$). In particular, a significantly lower PI was observed following the application of either slurry types to soil alongside cellulose, compared to treatment with cellulose alone. With respect to G6P, the application of US slurry resulted in a significantly lower PI compared to the treatment with AS slurry or G6P alone. Finally, a significant decrease was found in PI following the addition of either slurry types alongside glucose, in the order glucose > glucose + US slurry > glucose + AS slurry.

Table 4.3. Mineralisation rate, extent of mineralisation, percentage of ^{14}C activity incorporated in microbial biomass, residual ^{14}C activity in soil and respiratory quotient (mineralisation extent divided by biomass uptake) at the end of the incubation time for each treatment.

Treatment	**Maximum mineralisation rate** (% h^{-1})	**^{14}C mineralisation extent** (%)	**^{14}C biomass uptake (%)** (Fixed k_{EC}[a]; k_{EC}= 0.35)	**Residual ^{14}C activity in soil** (%)	**Respiratory Quotient**
Soil + Glu	5.08 ± 0.53	21.10 ± 0.94	68.00 ± 0.70	10.40 ± 4.33	0.31
Soil + Glu + US	11.41 ± 0.48	29.98 ± 0.84	22.76 ± 0.09	47.24 ± 0.63	1.32
Soil + Glu + AS	2.49 ± 0.21	18.48 ± 0.20	40.99 ± 1.00	40.53 ± 0.67	0.45
Soil + G6P	5.79 ± 0.32	18.28 ± 0.39	57.09 ± 0.38	22.66 ± 1.11	0.32
Soil + G6P + US	6.97 ± 0.42	20.35 ± 0.72	25.46 ± 1.52	54.07 ± 1.44	0.80
Soil + G6P + AS	7.51 ± 0.16	22.84 ± 0.47	34.50 ± 0.16	40.98 ± 0.84	0.66
Soil + Cel	2.30 ± 0.21	17.93 ± 0.54	41.11 ± 0.83	40.91 ± 0.96	0.44
Soil + Cel + US	1.30 ± 0.01	17.41 ± 0.47	20.81 ± 0.96	61.78 ± 1.25	0.84
Soil + Cel + AS	2.50 ± 0.22	18.13 ± 0.52	19.46 ± 1.82	62.26 ± 1.87	0.93

[a] k_{ec} = (^{14}C-flush)/(initial ^{14}C-activity added - ^{14}C respired - ^{14}C-activity in unfumigated soil) (Boucard et al., 2008).

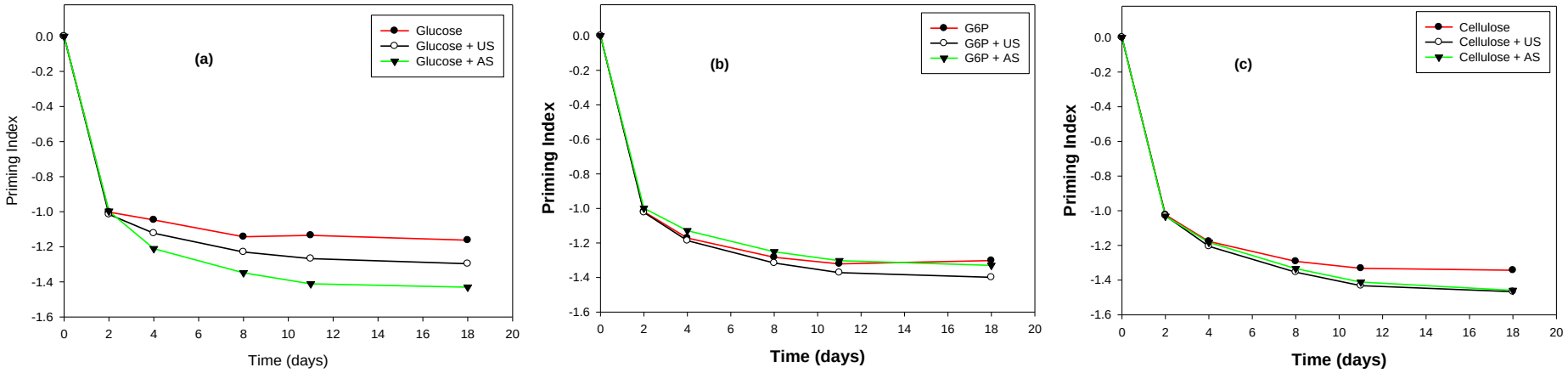

Figure 4.2. Priming index (PI) for soils treated with glucose, glucose alongside either unamended (US) slurry or slurry amended with the biological additive SlurryBugs (AS) (a); for soils treated with glucose-6-phosphate (G6P), G6P alongside either US or AS slurry (b); for soils treated with cellulose, cellulose alongside either US or AS slurry (c) during an 18-days incubation. Average values of measured data are presented as symbols (n = 3).

4.4 Discussion

4.4.1 Total respiration following the addition of organic compounds to soil

The aim of this chapter was to determine how the quality of substrates, such as carbohydrates and slurry, added to grassland soil affects the partitioning of C within the added substrates and the native SOM, based on microbial processes in soil. The data reported in this chapter are among the first of their kind because no previous studies have investigated the fate of C from labile versus recalcitrant carbohydrates applied to a temperate grassland soil. In addition, the research reported in this chapter examined the impact of applying these carbohydrates alongside livestock slurry that had either been amended or not amended with a biological additive during slurry storage. The impacts on soil C cycling following the application of slurry that has received a biological additive during storage have also remained poorly constrained in previous research to date.

Alongside glucose, the phosphosugar G6P was selected to represent a labile carbohydrate in this experiment, because of the central role played by G6P in two microbial pathways of carbohydrate metabolism – glycolysis and the pentose phosphate pathway. G6P is the product of the initial reaction of phosphorylation by ATP from glucose in the glycolysis catalysed by the enzyme hexokinase and is also the initial reactant in the pentose pathway (Cohen, 2011). Furthermore, both glucose and G6P take part in the sequence of reactions for the synthesis of cellulose (Moat et al., 2003) that is a typical product of several bacterial species (Shoda and Sugano, 2005). Cellulose was selected as one of the most recalcitrant carbohydrates in soil, primarily input in the form of litter, with several microbial species in soil having developed specific strategies to utilise this substrate (Lynd et al., 2002). This chapter,

with the focus on C cycling following the input of substrates to soil, may also be relevant given the growing interest in organic amendments being applied to soil, including manure, compost, and anaerobic digestate. Under these practices, understanding the impacts on the soil C cycle, as mediated by the soil microbial community, is important.

An initial 3.3 and 0.4 % of TOC and TN concentration, respectively, were found in the soil used in the experiments reported above (Table 4.2). In consequence, higher total respiration was observed in the control treatment (when neither carbohydrate nor slurry was supplied to soil), compared to all other treatments (Figure 4.1). The CO_2 respired in the control treatment represents the MR corresponding to the energy demand for all non-growth, maintenance-related activities of soil microorganisms (Geyer et al., 2016). A significantly lower total respiration was also found as the complexity of the carbohydrate added to soil increased, in the order glucose > G6P > cellulose. These findings are in contrast with previous studies in which cumulative respiration generated by glucose application to soils was lower than following the application of more complex substrates, such as cellulose, starch, lignin, and chitin, both in arable and forest soils (Schutter and Dick, 2001, Dilly, 2004, Orwin et al., 2006, Hernández and Hobbie, 2010, Jagadamma et al., 2014, Elmajdoub and Marschner, 2015). This pattern in which lower cumulative respiration is induced by the addition of progressively more complex carbohydrates to grassland soil is consistent with the first hypothesis detailed in this chapter.

This finding can be attributed to the higher lability of glucose compared to cellulose, and the fact that glucose can be rapidly mineralised by most soil microorganisms in contrast to cellulose (Dungait et al., 2009). Within increasing complexity of the carbohydrate applied to soil, the hydrolysis pathway becomes more

complex and, therefore, the range of extracellular enzymes required in order to catalyse the hydrolysis of the carbohydrate to CO_2 increases. Indeed, whilst glucose is directly taken up by microorganisms, G6P requires the enzyme glucose-6-phosphatase for its hydrolysis (Cohen, 2011). Cellulose degradation is an even more complex process, due to the longer and more recalcitrant structure of this carbohydrate. It requires the combined activity of three types of enzymes, an *endo*-β-1,4-glucanase degrading the polysaccharide into smaller oligosaccharides, then an *exo*-β-1,4-glucanase, removing disaccharide units from both the ends of the oligosaccharide chains, and, ultimately, β-glucosidase, that hydrolyses the disaccharides to glucose (Moat et al., 2003). Therefore, the chemical composition of a carbohydrate added to soil is generally the main driver of the extent to which a substrate will be respired and released from soil through CO_2 efflux. Interestingly, more complex hydrolysis pathways ultimately result in less rapid respiration and lower total CO_2 efflux from soil following the addition of carbohydrate. Therefore, the application of complex substrates to soil can be an effective practice within intensive agricultural production systems due to a slower release of C into soil and it can represent a solution for long-term SOM accumulation.

Substrate quality also affected P availability in soil during the incubations. In particular, a higher concentration of Olsen P was observed following the application of G6P to soil compared to the application of glucose to soil. In fact, it is unlikely that, due to the incubation period of 18 days, the data from Olsen P simply reflect extraction of the G6P that was added to the soil. Instead, these observations are consistent with the second hypothesis detailed in this chapter and suggest an increase in bioavailable P as a result of microbial dephosphorylation of G6P. The data from the incorporation of ^{14}C into microbial biomass, indicating that 57% of the initial ^{14}C-G6P

spiked to soil was incorporated into microbial biomass (see Table 4.3), also support this hypothesis.

These findings extend those reported by Spohn and Kuzyakov (2013) from temperate forest soils and indicate that microorganisms in some temperate grassland soils may use the organic moiety of G6P as a C source to increase microbial biomass. These data suggest that the microbial demand for C can drive a parallel release of P into the bioavailable soil pool, following the application of a phosphorylated C compound to soil. This increased bioavailable P in grassland soil may well deliver initially beneficial results through increased plant-available P. However, long-term increases in bioavailable soil P concentrations also increase the risk of P transfer from soils to solution and ultimately to surface and ground water (McDowell et al., 2001). These findings also seem to challenge the conceptual model proposed by McGill and Cole (1981), in which microbial C demand drives only N and S mineralisation, but not P mineralisation. Indeed, according to McGill and Cole (1981), similar mechanisms appear to control the release of inorganic forms of N and S from organic compounds during C oxidation by soil microorganisms in search of energy (biological mineralisation). However, further research is required to confirm the mechanistic basis to a possible coupling between C and P mineralisation in grassland soils that is suggested by the data reported in this chapter.

The data reported in this chapter also suggest that the addition of slurry, alongside a carbohydrate, resulted in lower cumulative respiration compared to the treatment with the carbohydrate alone. This finding suggests suppression of the extent to which an added carbohydrate is subject to microbial respiration, caused by competition between the microorganisms introduced into soil through the application of slurry and the indigenous soil microbial community. In general, soil is assumed to be a hostile

environment for faecal microorganisms, with faecal microbial populations normally decreasing rapidly after the addition of materials such as slurry to soil (Unc and Goss, 2004). However, the survival rate for some slurry microorganisms in soil can be extremely variable and crucial for the net effect of slurry application on soil microbial activity (Unc and Goss, 2004). In particular, the mixture of slurry with soil can increase the potential for some slurry microorganisms to survive, including *Escherichia coli*, *Streptococcus faecalis* and *Enterococcus* spp., due to adsorbance of these microorganisms onto soil particles (Patni et al., 1985). According to Chenu et al. (2002), some possible mechanisms, including release of organic compounds, such as enzymes and other polymers, as well as physicochemical interactions, are the strategy adopted by slurry microorganisms to increase their survival rate.

The subsequent release of suppressing factors from slurry microorganisms in response to the competition from native soil microorganisms is potentially a mechanism through which to reduce the microbial respiration of the carbohydrate by native soil microorganisms and to allow accumulation of substrate-C into the cells of the slurry microorganisms. An extraordinary array of secondary metabolites, including antimicrobial polyketides, peptides, antibiotics, toxins, as well as volatile organic compounds (VOCs), have been identified as factors produced by some microbial species to inhibit the growth of other microbial species in soil (Hibbing et al., 2010, Schulz-Bohm et al., 2015). Finally, the addition of slurry that had been treated with the SB additive to soil resulted in significantly higher cumulative CO_2 efflux compared to unamended slurry for both the glucose and G6P treatments. These findings suggest that the SB additive potentially reduced the suppressive effects of slurry microorganisms acting on native soil microorganisms, thus allowing soil microorganisms to respire more CO_2 from the two simple carbohydrates compared to

the treatments with US slurry. However, further research into the role of microbial suppression following slurry application to soils would be required to test these possible mechanistic explanations for differences in cumulative CO_2 efflux.

4.4.2 Carbon partitioning following the addition of labelled carbohydrates to grassland soil

A mineralisation assay was used in this chapter to track the fate of the three ^{14}C-labelled carbohydrates following their addition to soil. Because none of the carbohydrates used in this research was charged, it was assumed that they were not sorbed onto soil particles. Therefore, the fraction of each carbohydrate spiked to soil that was not evolved as CO_2 due to microbial respiration, was assumed to either be assimilated into microbial biomass or to remain in the soil as C-substrates incorporated within the humified SOM pool (Hoyle et al., 2008). A high mineralisation rate, in particular for the G6P treatments, was observed one hour after the substrate-spiking. The final mineralisation extent for the two simple carbohydrates was 21 and 18% of the spiked ^{14}C for glucose and G6P, respectively, levels that are consistent with a number of previous studies. For example, van Veen et al. (1985) and Bremer and van Kessel (1990) measured mineralisation of glucose-C of 37% compared to the initial labelled input after 101 and after 7 days of incubation, respectively. Saggar et al. (1999) observed a mineralisation of 25-44% of ^{14}C from glucose after 35 days, Schneckenberger et al. (2008) found a mineralisation of 26-44% within 22 days, whilst Gunina et al. (2014) reported a decomposition of 25% within 10 days. The results from the current chapter also showed a high mineralisation rate for the cellulose treatments, with > 17% of the spiked ^{14}C. These results are

compatible with those measured by Crawford et al. (1977), where a mineralisation of 19-45% of ^{14}C from cellulose was observed after a soil incubation of 700 hours.

However, as a proportion of the C spike added to soil, considerably more ^{14}C was incorporated into microbial biomass than was evolved as ^{14}C-CO_2. The assimilation of a considerable fraction of labelled glucose and G6P into microbial biomass, as observed in the experiment reported in this chapter, is consistent with the model of short-term glucose utilisation proposed by Nguyen and Guckert (2001). In this model, once a carbohydrate is taken up from soil solution and temporarily allocated to an intermediate pool within a cell, it is then partitioned between respiration and incorporation into biomass as structural C. In turn, this stored glucose fraction can be transferred toward anabolic pathways to produce polymeric carbohydrates, such as cellulose, or directed to the synthesis of intracellular, dissolved compounds, depending on the cellular C demand (Gunina et al., 2014). Due to high degree of similarity between glucose and G6P, this model is also assumed to operate for G6P, after dephosphorylation. The lower assimilation of cellulose into microbial biomass compared to either glucose or G6P is expected, due to the three-stage, enzyme-mediated hydrolysis pathway required to degrade the polysaccharide prior to microbial uptake (Lynd et al., 2002, Moat et al., 2003).

An apparent suppression of biomass uptake of added substrate was observed following the addition of both types of slurry to the three ^{14}C-carbohydrate treatments (Table 4.3). However, these findings are not consistent with the concept of slurry application stimulating the soil microbial community and activity that has been reported in some research previously (Kandeler and Eder, 1993, Paul and Beauchamp, 1996, Saviozzi et al., 1997, Lalande et al., 2000, Peacock et al., 2001, Murugan et al., 2014). The high heterogeneity of slurry, due to different food and animal species, can

account for differences in microbial communities that, in turn, explain distinct soil microbial responses between the results from the current chapter and the literature following slurry application. As discussed in Section 4.4.1, competition in soil between the indigenous soil microbial community and the microbial community derived from slurry, involving the release of different suppressing factors from slurry microorganisms, is also thought likely to account for suppression of the activity of soil microorganisms, both in terms of respiration and uptake of the added carbohydrate. Further, differences in the organic C composition of slurry may be related to stimulation or suppression of microbial activity in soils that receive slurry applications. For example, according to Paul and Beauchamp (1989), microbial-mediated processes, such as denitrification, in manured soil can be positively related to the total water-soluble organic C and to the volatile fatty acid (VFA) concentration in manure. Slurry that is particularly rich in certain organic compounds may induce increases in microbial activity in soil.

Therefore, it is reasonable to hypothesise that the suppression of microbial respiration and assimilation of labile C reported in this chapter may be associated with the specific chemical characteristics of the slurry used in the experiments, for example due to particularly low VFA concentrations. However, further research is required to establish the impact of variations in the composition of organic compounds in slurry, such as carbohydrates and lignocellulosic materials (lignin, hemicellulose and cellulose) that represent the largest fraction of the organic compounds in slurry (Møller et al., 2004, Christensen et al., 2009), on microbially-mediated processes such as respiration or biomass uptake within soil. Regardless of the mechanism responsible, as a result of the slurry-based inhibition of respiration and assimilation of labile C substrates, greater accumulation of the added C in the soil pool was observed

compared to treatments in which no slurry was applied (Table 4.3). Consequently, a longer period of time following treatment of soil with slurry, either with or without the SB-additive, would appear to be necessary for either mineralisation or assimilation of the ^{14}C-substrates to achieve levels comparable to those observed in treatments without slurry addition. However, this study evidences that slurry alongside carbohydrate application appears to stabilise C within soil pools, at least over the timescales involved in this experiment, thus stimulating the accumulation of soil C if this effect persists.

The effect of adding carbohydrates and slurry to soil was also assessed in this chapter through calculation of the RQ, defined as the ratio of respired-to-incorporated ^{14}C-carbon. With the apparent outlier associated with the treatment that included glucose and US slurry, all the RQ values suggested that biomass accumulation (RQ < 1) was stimulated by the treatments applied to soil. These values contrast with those reported by Dilly (2001; 2003; 2004), where values of RQ > 1, suggesting microbial respiration, were observed after the application to arable, grassland and forest soils of glucose and more recalcitrant compounds, such as cellulose and humic acid, at a comparable rate to those used in this chapter during a short-term incubation experiment. In contrast to what observed by Dilly, glucose addition to the soil resulted in stimulation of respiration, as well as induction of microbial growth (Stenström et al., 1998).

The microbial activity towards both the different substrates added to soil and SOM was determined through the priming index (PI). The PI accounts for any increase or decrease in mineralisation of SOM per unit of substrate added per unit of time. This represents a robust method for a quantitative assessment of the priming effect (PE), due to the measurement both of net CO_2 and ^{14}C-CO_2 evolution (Kuzyakov et al.,

2000). The PI for each treatment examined in the research reported in this chapter was always < 0 over the course of the incubation, corresponding to immobilisation of C from the added substrate or a decrease in SOM decomposition compared to the control soil treatment that received no added substrate. Notably, more negative PIs were associated with treatments in which SOM mineralisation was lower, as revealed in the total respiration data to be in the order cellulose<G6P<glucose.

Further, matching the apparent suppression effect due to the slurry application that was described for total respiration, the PI suggested that lower SOM decomposition was associated with each slurry-amended treatment compared to the corresponding treatment that only included the addition of a carbohydrate. Therefore, both the addition of carbohydrates and of slurry resulted in a negative PI, although due to different causes. The negative PI following the addition of carbohydrates to soil depended on the cumulative CO_2 evolved from the respiration of both SOM and the more labile C substrates added to soil, as well as from the ^{14}C-CO_2 respired as a fraction of the labelled carbohydrate spiked to soil. In particular, the addition of carbohydrates resulted in a microbial metabolic switch, involving decreased respiration of SOM as respiration switched to the more labile carbohydrates, alongside incorporation of a substantial fraction of the labile carbohydrate into microbial biomass. This metabolic switch is known as ‘preferential substrate utilisation’ (PSU) (Sparling et al., 1982, Billes et al., 1988, Cheng, 1999). In contrast, the negative PI that was observed following slurry addition to the carbohydrate treatments is attributed to the difference in the cumulative respiration that, in turn, is due to differences in the SOM respiration. In contrast, Table 3 suggests that PI was not due to differences in the respiration of the added 14C-labelled carbohydrate, comparing the carbohydrate alone to the carbohydrate + slurry treatment.

In summary, the highest total respiration of SOM was measured in the control soil treatment, due to the MR performed by the microbial community in soil to maximise the catabolic harvesting of energy in a soil with low C and N. The pulsed input of substrates to soil generated a sudden increase in labile substrate availability, with the amount of substrate C added corresponding to 31% of the C_{mic} at the start of the incubation. As a result, a rapid decomposition rate of the added C substrate was measured, especially in the treatments with glucose and G6P. The total respiration was inversely proportional to the complexity of the unlabelled carbohydrates applied to soil. The decrease in the total respiration across the increasingly complex carbohydrate treatments could be attributed to the PSU from the respiration of SOM to the utilisation of more labile added substrates. The incorporation into the microbial biomass of a considerable fraction of all substrates that were spiked to soil, as observed at the end of the ^{14}C incubation, could also reflect this metabolic switch. Therefore, PSU can cause a decrease in the SOM decomposition, and accounts both for the lower cumulative CO_2 measured for each carbohydrate treatment compared to the control soil, and for the negative PI that was observed in the experiments reported above. However, this metabolic switch normally occurs when the added substrate C is greater than the C_{mic} existing within the soil (Kuzyakov, 2002, Cheng and Kuzyakov, 2005). In contrast, in the experiments reported in this chapter, the amount of substrate C added to soil was lower than C_{mic}.

Possible explanations for the apparent activation of PSU despite the relatively low amount of substrate C added to soil, in proportion to soil C_{mic}, may be related to the amount of available N in soil (Blagodatskaya and Kuzyakov, 2008). Decreases in PE have also been observed in a number of studies when organic C-substrates that are applied to soil contain available N (Liljeroth et al., 1994, Cardon, 1996, van Ginkel et

al., 1997, Martín-Olmedo et al., 2002, Blagodatskaya et al., 2007). Due to an initially low TN content of the soil used in the current chapter, soil microorganisms may be activated to decompose the N-rich livestock slurry to acquire N, rather than to continue the decomposition of SOM, thereby leading to a reduction in the PE. However, additional environmental processes that have not been investigated in this chapter, including interactions between the added substrates and the humic fraction in soil that can inhibit respiration by promoting the formation of stable aggregates and organo-mineral associations (Geyer et al., 2016), may also be responsible for the negative values of PI that were observed. Further investigation is required to verify if PSU continues to be evident with relatively low substrate C added to soil as a proportion of C_{mic} across different temperate grassland soils, with and without slurry application.

Finally, with the decreased availability, as the added materials are increasingly respired or taken up through time, alongside the growth of microbial biomass, a return to the initial state of SOM decomposition and a positive PE may be expected (Stenström et al., 2001, Kuzyakov and Bol, 2006). However, due to the short length of the incubation reported in this chapter, a positive PE was not observed. Presumably, the addition of substrate to soil was not sufficient to increase the microbial biomass in 18 days sufficiently to enhance SOM decomposition and, therefore, to cause a positive PE. Further research is required to investigate whether longer-term incubation experiments with the same amount of substrate C added to soil ultimately stimulate greater SOM decomposition compared to an 18-day incubation experiment and, therefore, promote a positive PE.

5 The impacts of organic amendment on microbial biomass and community structure in grassland soils

5.1 Introduction

Microorganisms play a pivotal role in the soil environment. They are one of the main regulators of soil organic matter (SOM) decomposition, nutrient cycling, and bioremediation of contaminated soils (Larkin, 2003, Aislabie and Deslippe, 2013, Teng et al., 2015). Changes in the soil microbial community are therefore relevant indicators of changes in soil quality, soil biological activity and the likely productivity of terrestrial agro-ecosystems (Brussaard et al., 2004, Birkhofer et al., 2008). Several factors have been suggested to drive change in soil microbial communities, including those associated with environmental conditions within soils and with broader land-use (Zhou et al., 2002, Lauber et al., 2008, Van Horn et al., 2013). For example, changes in soil pH, salinity, water content, as well as variation in land-use across forest, arable and livestock production, are among the factors known to shape the structure of soil microbial communities (Lauber et al., 2008, Van Horn et al., 2013).

Further, the addition of organic amendments, including crop residues and farmyard manure (FYM), to soil has been shown to influence soil microbial communities. Indeed, such addition has predominantly been associated with increases in soil microbial biomass and changes in microbial community structure, particularly the relative abundance of bacteria and fungi within a community (Frostegård et al., 1997, Ritz et al., 1997, Dinesh et al., 2000, Peacock et al., 2001, Lupwayi et al., 2005, Toyota and Kuninaga, 2006, Calbrix et al., 2007, Enwall et al., 2007, Kallenbach and Grandy, 2011, Kätterer et al., 2014, Blaud et al., 2015). In turn, the modifications of microbial biomass and community composition that result from the addition of

organic amendements to soil can result in shifts in nutritional competition between r- and K-strategists. Specifically, r-strategists, mainly corresponding to gram-negative (G –ve) bacteria, dominate the early stages of decomposition following the addition of a substrate to soil. In particular, increases in the proportions of some groups of G –ve bacteria, such as Bacteroidetes, α-, β-, and γ-*Proteobacteria*, have been found in soil in response to the application of substrates that contain labile C (Cleveland et al., 2007, Fierer et al., 2007, Nemergut et al., 2010). In contrast, K-strategists, consisting of gram-positive (G +ve) bacteria and fungi, tend to prevail during the later stages of decomposition, due to adaptations that provide a competitve advantage when nutritional resources are associated with more recalcitrant SOM (Fierer et al., 2003, Fontaine et al., 2003, Cleveland et al., 2007, Fierer et al., 2007, Kramer and Gleixner, 2008, Fanin et al., 2014).

A number of studies have previously been conducted into the critical role played by soil microorganisms in the acquisition and transfer of nutrients in soil (Parton et al., 1988, Rodríguez and Fraga, 1999, Bengtsson et al., 2003, Dannenmann et al., 2009, Hinsinger et al., 2011). In particular, soil microorganisms are involved in a range of processes affecting the availability of soil P to plants, including solubilisation and mineralisation, or the immobilisation of readily available sources of P (Richardson, 2001, Richardson and Simpson, 2011, Yevdokimov et al., 2016, Zeng et al., 2016). In addition, the soil microbial biomass contains a significant proportion of the immobilised P in soil, potentially accounting for 1-10% of the total soil P pool that is potentially available to plants (Hedley and Stewart, 1982, Brookes et al., 1984). As reviewed by Richardson (1994), this microbial biomass P (P_{mic}) is a dynamic component of the soil P cycle, varying in response to farming practices, soil fertility status and seasonal variation in environmental factors. However, further research is

required to understand how the addition of substrates containing C, and in particular those substrates that also contain a P moiety, influence P_{mic} within different soils.

However, compared to the impacts due to the application of FYM and other organic amendments, relatively little attention has been given to the effects on microbial biomass and community structure of applying slurry to soil. Acea and Carballas (1988a) found that 180 days of cattle slurry treatment to soil enhanced a number of populations of microorganisms involved in the soil N-cycle, such as proteolytic, ammonifying and nitrifying bacteria, as well as denitrifying and anaerobic free-N fixing bacteria. However, Acea and Carballas (1988b) observed different responses for microorganisms associated with the soil C-cycle, such as aerobic and anaerobic cellulolitic bacteria, and with the soil S-cycle, such as sulphate reducing, elemental S oxidising and anaerobic organic S mineralising bacteria. Specifically, across a soil incubation of comparable time to that described above following the application of cattle slurry, an initial growth in these groups of microorganisms was observed, followed by a rapid decline, resulting in no significant changes in the populations examined by the end of the incubation, presumably due to the rapid exhaustion of labile substrate.

A different response following the addition of slurry to soil was observed by Opperman et al. (1989), where a significant and rapid increase in presumptive coliforms was recorded, compared to a soil that did not receive slurry, followed by a decrease at similar rates for both treatments. Further, these authors observed no change in the soil fungal population over a 135-day incubation following the application of slurry to soil. More recently, an initial increase in microbial biomass C (C_{mic}) was found in an agricultural soil during a 120-day incubation following the application of pig slurry (Plaza et al., 2007). Similarly, Pezzolla et al. (2013) showed

that the addition of different forms of labile organic matter, including pig slurry, to arable soils resulted in a general increase in C_{mic} and in G -ve bacteria across a 45-day incubation, whereas a decline in G +ve bacteria was observed.

A number of studies have also been conducted into the impacts of applying different carbohydrates to soil on microbial biomass and community structure. Increases in soil microbial biomass content both in a beech forest soil and in an arable soil were observed following glucose and cellulose additions (Dilly, 2004). Similarly, after the application of cellulose to both temperate arable and grassland soils, Blagodatskaya et al. (2014) observed an increase in G -ve bacteria and fungi during the intensive phase of cellulose decomposition, whilst the growth of G +ve bacteria and fungi was observed during the slow phase of cellulose degradation. Further, Mondini et al. (2006) found that the response of microbial biomass to the application of substrates, including carbohydrates, depended on the complexity and degree of degradability associated with the carbohydrate added to soil.

However, despite such research, relatively little remains known about the structural changes in both the bacterial and fungal community that follow the addition of slurry and carbohydrates of different molecular complexity, particularly within temperate grassland soils. Therefore, the potential to interpret changes in soil nutrient cycles and nutrient bioavailability that follow the addition of carbohydrates and slurry to soil, such as those reported in Chapters 3 and 4, through changes in the soil microbial community, remains limited. Further research is needed to better understand the short-term variations in microbial biomass and in the composition of the microbial community, including across bacteria, consisting of G +ve and G –ve bacteria, and fungi, in grassland soils following the application of such amendments. In addition, little evidence is available to constrain the effects on microbial biomass and

community structure exerted by the P moiety within compounds, such as glucose-6-phosphate (G6P), compared to non-phosphorylated counterparts. Therefore, the objective of this chapter is to determine the extent to which the addition to soil of C and P sources of different complexity and degree of microbial availability, as well as livestock slurry amended or not amended with the biological additive SB, significantly affected the biomass and the structure of the microbial community in a temperate grassland soil. The specific hypotheses that are tested within this chapter are:

i. the application to soil of C substrates of increasing lability will result in significantly greater increases in C_{mic}, alongside a shift in the soil microbial community structure towards a significantly greater prevalence of G –ve bacteria over G +ve bacteria and fungi;
ii. the addition of phosphorylated compounds to soil will not result in any significant difference in P_{mic}, compared to the addition of the non-phosphorylated counterpart, due to the microbial requirement for C but not for P in a typical grassland soil;
iii. the application of slurry to soil, either amended or not amended with the biological additive SB, alongside a carbohydrate, will result in a significantly greater increase in both C_{mic} and in G +ve bacteria and fungi, compared to the addition of a carbohydrate alone, due to the addition of more recalcitrant C compounds to soil contained within slurry.

5.2 Materials and methods

5.2.1 Experimental design for the soil incubations

Bulk samples of the clay loam (CL) soil described in Section 4.2.1 were thoroughly mixed to ensure homogenisation. Twelve treatments were used for the experiments reported in the current chapter, following the same experimental design described in Chapter 4 (see Table 4.1). Carbohydrates and slurry were derived from the same sources, and were applied to soil at the same application rates (v/w), as described for the incubations in Chapter 4 (see Section 4.2.2). Plastic containers of 150 mL volume were used for the experiment. An initial 120 g (fresh weight) of soil was placed in each individual container at the beginning of the incubation experiment. All treatments were incubated in triplicate within a laboratory for 49 days, from June to August 2015. Containers were kept in the dark within a temperature-controlled incubator at 20 ºC. On each day of analysis (days 10, 20 and 49), 1.5 g (fresh weight) of soil was sub-sampled from each container for phospholipid fatty acid (PLFA) analysis, 10 g (fresh weight) for C_{mic} determination, and 15 g (fresh weight) for P_{mic} determination. All soil samples for analysis were immediately analysed or stored for a short time in a refrigerated cold room before analysis.

5.2.2 Microbial biomass carbon

To measure the amount of C bound in soil microbial biomass, the fumigation-extraction method (Vance et al., 1987) was used. The fumigation procedure lyses microbial cells and releases C for extraction with K_2SO_4. 5 g of fresh soil was weighed for each sample into a beaker. All beakers were placed into a desiccator, alongside a beaker containing amylene-stabilised $CHCl_3$ and a few boiling chips. The

desiccator was evacuated using a water vacuum pump until $CHCl_3$ boiled. $CHCl_3$ was allowed to boil for one minute, then the water tap was closed, the water pipe was removed and the pump was turned off. The samples were left in the desiccator at 25 ºC for 18 – 24 hours. The pressure was then released slowly and residual $CHCl_3$ from the soil was removed by evacuating the desiccator five or six times.

To extract C from soil microbial biomass, a 0.5 M K_2SO_4 solution was prepared, and pH adjusted to 6.8 – 7.0 using a NaOH solution from NaOH pellets. 5 g of each sample of non-fumigated soil was weighed into a plastic bottle. Subsequently, 25 mL of the K_2SO_4 solution was added to each of the non-fumigated soil samples and to the soil samples that had been previously fumigated with $CHCl_3$, mixed thoroughly and placed on an orbital shaker for 30 minutes. The extract solutions were filtered through Whatman No 1 filter papers with the filtrate collected in Sterilin sample bottles. Filtrates were analysed to determine the C_{mic} using a Total Organic Carbon Analyzer. C_{mic}, expressed as mg g^{-1} dry soil, was calculated using Equation 5.1:

$$C_{mic} = \left[\left(\frac{Total\ C}{g\ dry\ fumigated\ soil}\right)\right] - \left[\left(\frac{Total\ C}{g\ dry\ non-fumigated\ soil}\right)\right] \qquad (5.1)$$

5.2.3 Microbial biomass phosphorus

To measure the amount of phosphate bound in soil microbial biomass, a fumigation-extraction method similar to that performed for C_{mic} was conducted, following the method of Brookes et al. (1982). The fumigation procedure lyses the cells and releases P for subsequent extraction with $NaHCO_3$. A third set of samples with an added P spike to account for P sorption to the soil during extraction was included. The fumigation of soil followed the procedure described in Section 5.2.2 for C_{mic}. To extract P from soil microbial biomass, a 0.5 M $NaHCO_3$ solution was

prepared, with the pH adjusted to 8.5 using a NaOH solution from NaOH pellets or a concentrated NaOH solution. Two 5 g (fresh weight) samples of non-fumigated soil for each sample were weighed into separate plastic bottles. 100 mL of the $NaHCO_3$ solution was added to each of the non-fumigated soil samples, in addition to a further bottle that contained the fumigated soil sample. For one non-fumigated sample, 1 mL of a phosphate spike solution (125 µg P mL^{-1}) was added. For the other non-fumigated sample, the equivalent volume of Milli-Q water to that added to the P-spiked sample was added. All bottles were mixed thoroughly and placed on an orbital shaker for 30 minutes, then allowed to settle before filtering. Soil solutions were filtered through Whatman No 42 filter papers and the filtrates were collected in Sterilin bottles. The filtrates were analysed for P using an XY-2 Sampler + AA3 Auto-Analyser. The extractable P, expressed as mg P g^{-1} soil, was calculated using Equation 5.2, whilst P_{mic}, expressed as mg P g^{-1} soil, was calculated using Equation 5.3:

$$Extractable\ P = \frac{(mg\ P\ per\ L\ in\ sample - blank) * extractant\ volume\ (L)}{weight\ of\ dry\ soil\ (g)} \quad (5.2)$$

$$P_{mic} = \frac{25 * (fumigated\ extractable\ P - non-fumigated\ extractable\ P)}{0.1 * (P\ spike\ extractable\ P - non-fumigated\ extractable\ P)} \quad (5.3)$$

where, 25 in Equation 5.3 refers to the P concentration in a blank sample that had received the P spike, whilst the 0.1 in Equation 5.3 refers to volume of the extractant in litres.

5.2.4 Phospholipids fatty acids analysis

To measure PLFAs in soil, a three-stage analysis was conducted. Stage one involved lipid extraction, weighing 1.5 g (fresh weight) of soil for each sample and

placing into 50 mL Pyrex glass tubes that had previously rinsed with $CHCl_3$. A blank tube was also included. In order to extract lipids, 1.5 mL citrate buffer (0.15M) at pH 4 was added to all tubes, alongside 1.9 mL $CHCl_3$, 3.8 mL methanol (MeOH) and 2.0 mL of extractant ($CHCl_3$:MeOH:citrate buffer, 1:2:0.8 v/v/v). Tubes were left for 2 hours, followed by centrifugation at 650 relative centrifugal force (RCF) for 10 minutes. Tubes were then left overnight to ensure phase separation. Subsequently, 3 mL of the lower phase were transferred by pipetting to a clean small glass test tube that had previously been rinsed with $CHCl_3$. The tubes were then placed in a heating block under a stream of compressed air entering the tubes to evaporate the liquid and with the heating block turned off. When all liquid had evaporated, the tubes were capped, labelled, bagged and frozen, or immediately used for stage two of the extraction process.

During the second stage (lipid fractionation), lipids were separated into different classes with increasing polarity: neutral lipids, such as hydrocarbons, free fatty acids and sterols, glycolipids and polar lipids, such as phospholipids. Columns (Isolute SI 500 mg 6 mL SPE columns) were activated using 2.5 mL of $CHCl_3$. The dry lipid material remaining from stage one of the protocol was dissolved by adding 0.5 mL of $CHCl_3$, then transferred carefully to each column using glass Pasteur pipettes. All tubes were then rinsed in sequence with $CHCl_3$ to elute the neutral lipids, acetone to elute the glycolipids, then MeOH to elute the phospholipids. The solvent from the tubes was evaporated under compressed air as described above, but with the heating block turned on to 40ºC, leaving the dried phospholipid fraction in the tubes. The tubes were then capped, labelled and either frozen or immediately used for stage three of the PLFA protocol.

In stage three (mild alkaline methanolysis), two internal standards, C13 (Methyl tridecanoate) and C19 (Methyl nonadecanoate), were prepared by adding 22.67 mg of C13 standard and 23.08 mg of C19 standard to 100 mL of hexane. 30 µL of both standards were added to each tube, then the samples were dissolved in 1 mL of a MeOH:toluene (1:1, v/v) solution and in 1 mL of KOH 0.2 M solution that had been previously prepared, and then incubated in a water bath at 37 ºC for 15 minutes. Subsequently, 2 mL of a hexane:$CHCl_3$ (4:1, v/v) solution, 0.3 mL of a 1 M acetic acid solution, and 2.0 mL of Milli-Q water were added to the samples. The samples were then vortexed for 1 minute and centrifuged at 650 RCF for 5 minutes. The upper organic phase was transferred using glass Pasteur pipettes to a clean set of test tubes that had been previously rinsed with hexane. The lower layer in the previous set of tubes was washed with a 2 mL portion of hexane:$CHCl_3$ (4:1, v/v), vortexed and then centrifuged, as above. The upper phase was then transferred to the small test tubes.

The liquid in the tubes was evaporated under a stream of compressed air with the heating block turned off. The pellets in the tubes were re-suspended in 150 µL of hexane, mixed for 20 seconds, then transferred using glass Pasteur pipettes to 150 µL Polyspring Thermo Fisher Scientific glass inserts, placed in Chromacol glass GC vials. The liquid in the insert was evaporated under a stream of compressed air, as above. The pellet resuspension and liquid evaporation in the insert were then repeated five times in order to ensure complete sample transfer to GC inserts. Once completed, the last evaporation the glass GC vials was stored at -20°C or re-suspended in 25 µL of hexane for immediate analysis by gas chromatography with flame ionisation detector (GC-FID). The GC-FID analysis was conducted using a 6890N GC analyser, in conjunction with an HP 7683 Series injector. All samples were run dissolved in 25

µL of hexane. The internal standards C13 and C19 were also run dissolved in 25 µL of hexane, alongside three blanks as hexane.

5.2.5 Statistical analysis

The normality of distributions was checked for C_{mic} and P_{mic}, as well as for each microbial community PLFA both graphically, using normal Q-Q plots, and statistically, through the Shapiro-Wilk test and assuming significant effects where $p < 0.05$. The independent T-test for two samples was conducted to check for statistically significant differences between the means at day 0 and day 10 for each parameter. A two-way, repeated measures analysis of variance (ANOVA) was then conducted to check for statistically significance differences between the means at days 10, 20 and 49. It was assumed that soil sample removed from different sample containers at these times could effectively be treated as repeated sub-samples from a single container related to a specific treatment, thereby supporting repeated measures analysis.

Prior to conducting the ANOVA analysis, when the assumption of normality was violated, the dataset was transformed in order to ensure all data were normally distributed. In particular, a root squared-transformation was used for the datasets for P_{mic} and bacterial PLFA, a log-transformation was used for total PLFA and G +ve PLFA, an exponential-transformation was used for G –ve PLFA, G +ve / G –ve ratio and F/B ratio, whilst the dataset for fungal PLFA was raised to the third power before the ANOVA analysis. Further, significance effects were assumed at $p < 0.01$ for all datasets in the ANOVA analyses in order to avoid Type 1 errors. Mauchly's Test of Sphericity was also conducted to check for homogeneity of variances for each parameter. When the assumption of sphericity (ε) was violated and the Mauchly's Test

of Sphericity was statistically significant, Greenhouse-Geisser correction was applied for $\varepsilon < 0.75$, whilst Huynh-Feldt correction was applied for $\varepsilon > 0.75$ (Girden, 1992). Where these corrections did not address the issue of a significant Mauchly's test statistic, a two-way multivariate analysis of variance (MANOVA) was conducted. Pairwise comparisons were conducted using Bonferroni post-hoc tests using $p < 0.01$ to determine significance. All statistical analyses were conducted using IBM SPSS Statistics version 22, IBM, US.

5.3 Results

5.3.1 Microbial biomass carbon

The application of carbohydrate had a significant effect on C_{mic} ($F(3, 24) = 7.383$, $p = 0.001$, $\eta^2 = 0.480$; see Figures 5.1 - 5.2), with significantly higher C_{mic} under the control treatment compared to the addition of glucose. In contrast, no significant differences in C_{mic} were observed comparing control and glucose treatments with G6P and cellulose treatments. The application of slurry also significantly affected C_{mic} ($F(2, 24) = 34.894$, $p < 0.000$, $\eta^2 = 0.744$), with significantly higher C_{mic} following the addition of both slurry types compared to the control treatment that received no slurry. Significant changes in C_{mic} were also observed through time, regardless of carbohydrate or slurry application ($F(3, 22) = 581.086$, $p < 0.000$, $\eta^2 = 0.988$), with C_{mic} increasing over the 49 day-incubation following the order day 0 < day 20, day 10 (no significant difference between day 20 and day 10) < day 49. A significant interaction between carbohydrate and slurry was also observed in terms of C_{mic} ($F(6, 24) = 8.170$, $p < 0.000$, $\eta^2 = 0.671$). The application of both slurry types to the glucose treatment resulted in significantly higher C_{mic} compared to the treatment with glucose alone. Similarly, the addition of either type of slurry to the cellulose treatment resulted in significantly higher C_{mic} compared to the treatment with cellulose alone.

Significant interaction effects were also observed between carbohydrate treatment and time for C_{mic} ($F(9, 53) = 38.834$, $p < 0.000$, $\eta^2 = 0.805$). At day 10, the application of glucose to soil resulted in significantly higher C_{mic} compared to the application of cellulose, whereas no significant difference was observed between the other two carbohydrate treatments and between the other two carbohydrate and control treatments. At day 20, C_{mic} decreased significantly in the order G6P > cellulose >

control and glucose, with no significant difference between control and glucose treatments. At day 49, C_{mic} decreased significantly in the order control > cellulose, glucose > G6P. Significant interactions were also observed between slurry treatments and time with respect to C_{mic} ($F(6, 44) = 35.367$, $p < 0.000$, $\eta^2 = 0.828$). At day 10, the addition of AS slurry to soil resulted in a significantly higher C_{mic} compared to either the control treatment with no slurry application or the US slurry treatment. In contrast, by day 20 C_{mic} varied significantly in the order US > AS > control. At day 49, the application of AS slurry to soil generated a significantly higher C_{mic} compared to the addition of US slurry, whereas no significant difference was observed between control and each of the slurry treatments.

5.3.2 Microbial biomass phosphorus

No significant effect was observed on P_{mic} (Figures 5.3 - 5.4) associated with either carbohydrate or slurry application alone. In contrast, time was found to be a significant factor with respect to P_{mic}, regardless of carbohydrate or slurry application ($F(3, 23) = 58.683$, $p < 0.000$, $\eta^2 = 0.836$). Indeed, at day 49 a significantly lower P_{mic} was observed compared to the other three days of analysis. In contrast, significant interaction effects were observed between carbohydrate and time factors in terms of P_{mic} ($F(6, 46) = 28.568$, $p < 0.000$, $\eta^2 = 0.788$). At day 49, a significantly higher P_{mic} was observed under control treatment compared to cellulose treatment. Further, significant interaction effects were observed between slurry and time factors in terms of P_{mic} ($F(4, 46) = 11.136$, $p < 0.000$, $\eta^2 = 0.492$). At day 49, a significantly lower P_{mic} was observed following US slurry treatment compared to either the control treatment without slurry or the treatment receiving AS-slurry.

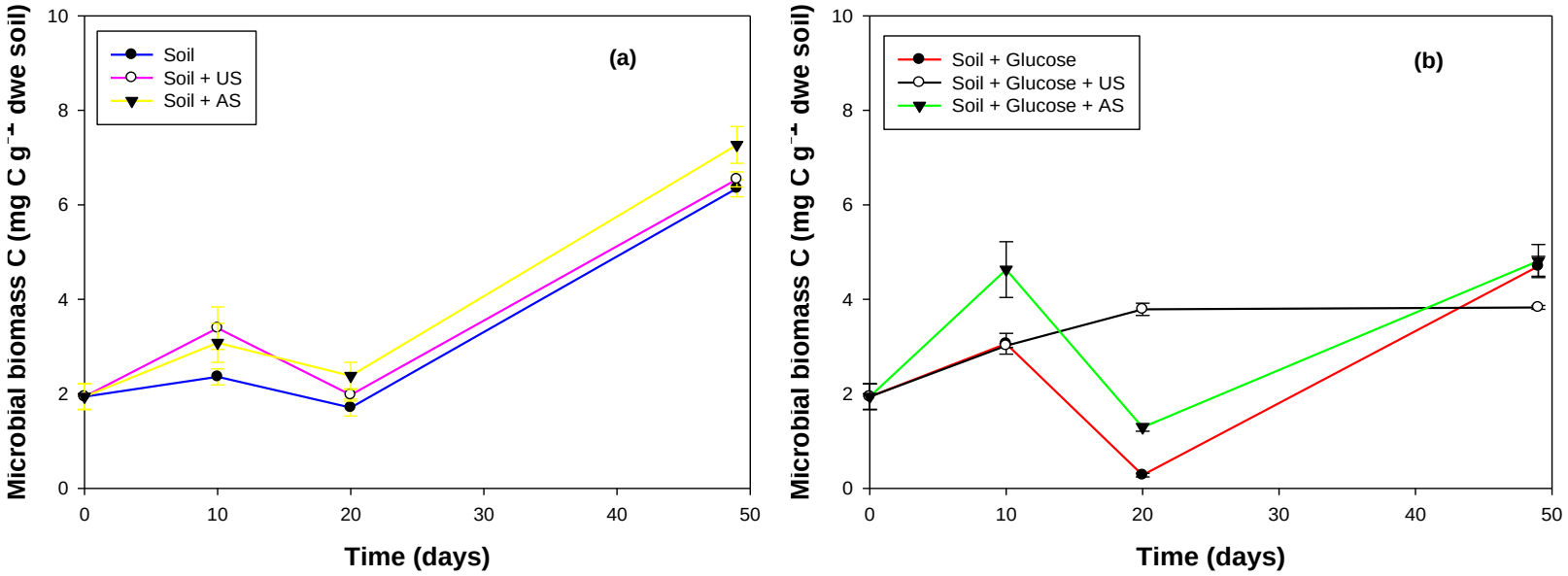

Figure 5.1. Microbial biomass C (mg C g^{-1} dry weight equivalent soil) using (a) control soil (soil), soil with unamended slurry (US), or slurry amended with the biological additive SlurryBugs (AS), (b) soil amended with glucose, soil with glucose and US or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

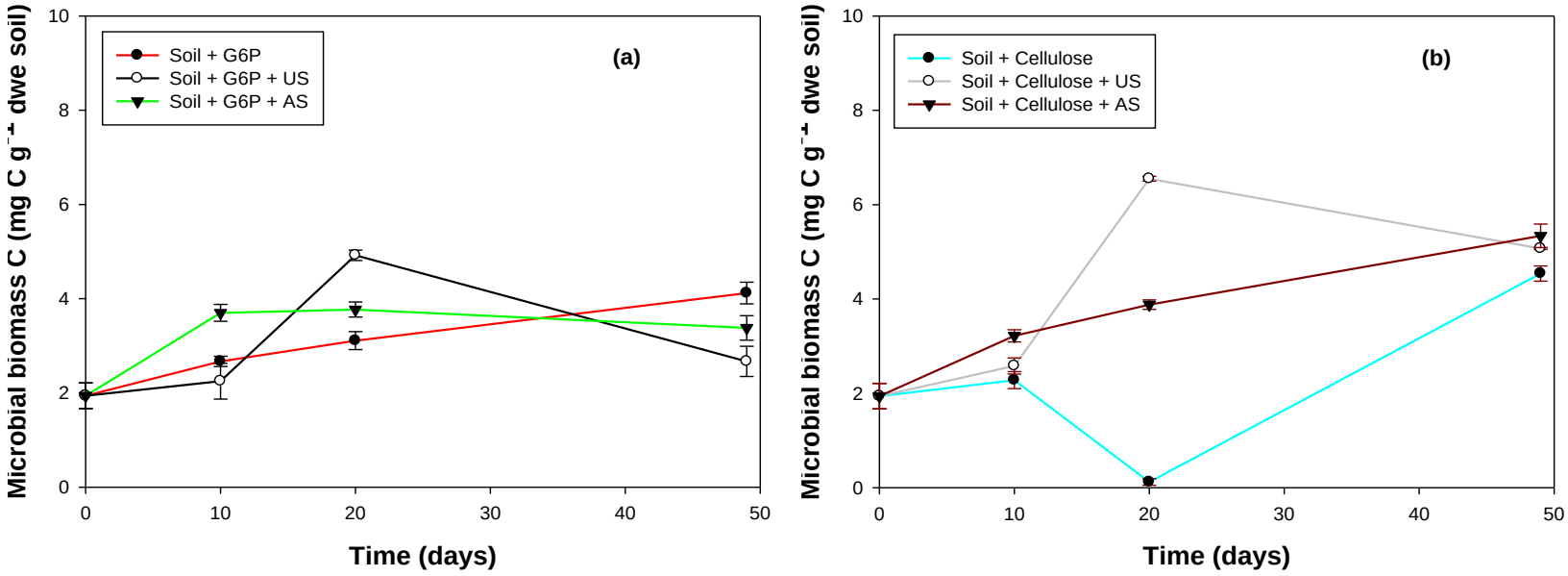

Figure 5.2. Microbial biomass C (mg C g^{-1} dry weight equivalent soil) using (a) soil with glucose-6-phosphate (G6P), soil with G6P and unamended slurry (US), or slurry amended with the biological additive SlurryBugs (AS), (b) soil amended with cellulose, soil with cellulose and US, or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

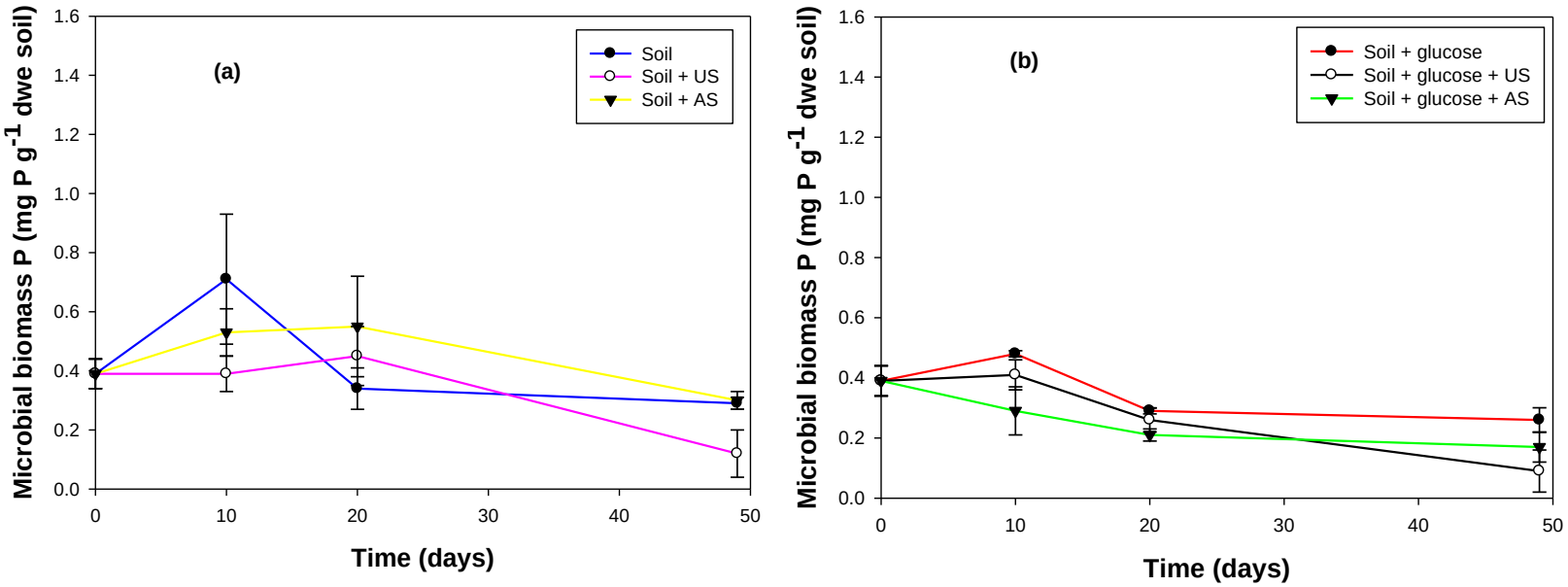

Figure 5.3. Microbial biomass P (mg P g^{-1} dry weight equivalent soil) using (a) control soil (soil), soil with unamended slurry (US), or soil with slurry amended with the biological additive SlurryBugs (AS), (b) soil amended with glucose, soil with glucose and US or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

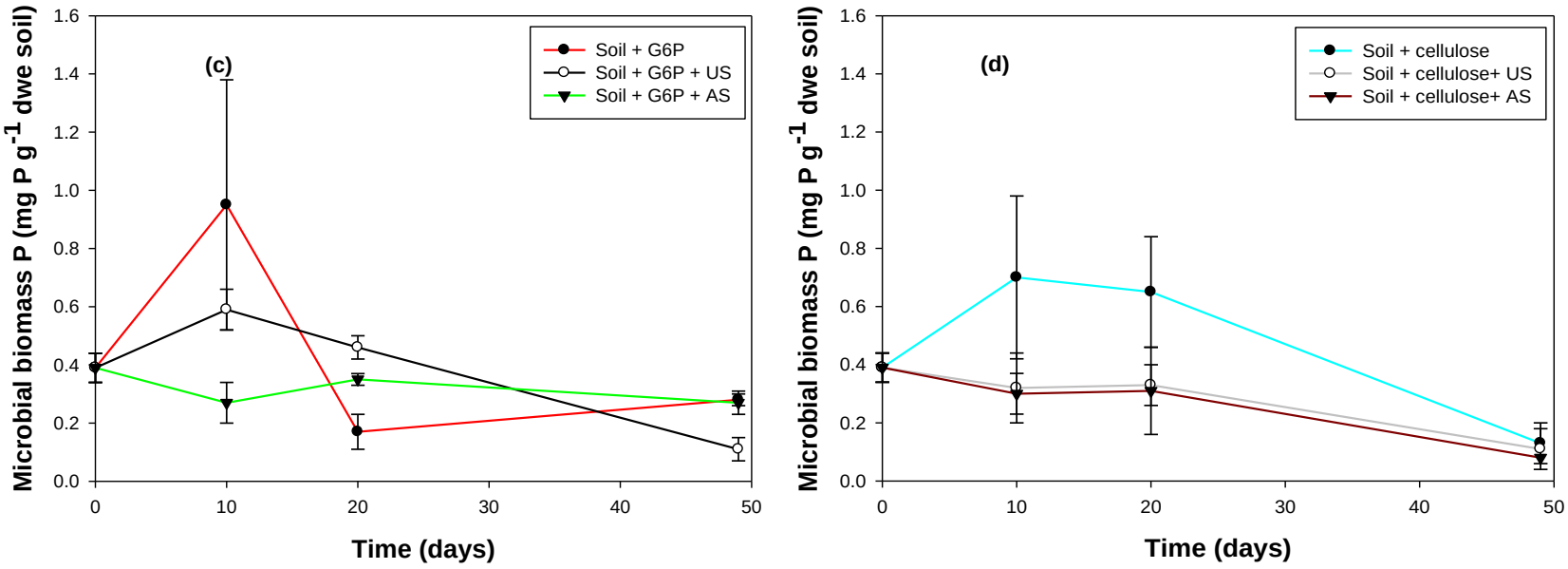

Figure 5.4. Microbial biomass P (mg P g^{-1} dry weight equivalent soil) using (a) soil with glucose-6-phosphate (G6P), soil with G6P and unamended slurry (US), or soil with G6P and amended slurry with the biological additive SlurryBugs (AS), (b) soil with cellulose, soil with cellulose and US, or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

5.3.3 Phospholipid fatty acids

5.3.3.1 Total phospholipid fatty acid profiles

No significant effect was observed on total PLFA (Figures 5.5 - 5.6) following either carbohydrate or slurry addition alone. In contrast, time was found to be a significant factor for total PLFA, regardless of carbohydrate or slurry application ($F(3, 22) = 224.457$, $p < 0.000$, $\eta^2 = 0.968$). Indeed, total PLFA changed significantly over the 49 day-incubation, in the order day 10, day 20 (no significant difference between days 10 and 20) > day 0 > day 49. No significant interaction effects between carbohydrate and time were observed for total PLFA. In contrast, a significant interaction effect was observed between slurry and time factors in terms of total PLFA ($F(6, 44) = 3.163$, $p = 0.011$, $\eta^2 = 0.301$). By day 49, a significantly higher total PLFA was observed under the control treatment without slurry compared to US slurry treatment.

5.3.3.2 Bacterial phospholipid fatty acid profiles

The application of carbohydrates significantly affected bacterial PLFA ($F(3, 24) = 8.127$, $p = 0.001$, $\eta^2 = 0.504$) (Figures 5.7 – 5.8). The application of cellulose resulted in significantly lower bacterial PLFA compared to either control or G6P treatments, whilst no significant difference between glucose treatment and treatments with the other two carbohydrates and the control were observed. The application of slurry also generated significant changes in bacterial PLFA ($F(2, 24) = 7.087$, $p = 0.004$, $\eta^2 = 0.371$), with bacterial PLFA being significantly lower following the addition of AS slurry compared to the control treatment that received no slurry, whereas no significant differences were observed following the addition of US slurry. Time

significantly influenced bacterial PLFA, regardless of carbohydrate or slurry application ($F(3, 22) = 175.202$, $p < 0.000$, $\eta^2 = 0.960$). Bacterial PLFA changed significantly across the 49 day-incubation, with a significant decrease at day 49 compared to all other days of analysis. Significant interaction effects between carbohydrate and slurry factors for bacterial PLFA were also observed ($F(6, 24) = 4.324$, $p = 0.004$, $\eta^2 = 0.519$). The addition of both slurry types to the G6P treatment resulted in significantly lower bacterial PLFA compared to the treatment with G6P alone.

A significant interaction effect between carbohydrate and time was also observed for bacterial PLFA ($F(9, 53) = 5.493$, $p < 0.000$, $\eta^2 = 0.411$). At day 20, a significantly lower bacterial PLFA was observed under glucose and cellulose treatments compared to the control treatment, whilst no significant difference was observed under G6P treatment compared to the control. By day 49, the application of cellulose resulted in a significantly lower bacterial PLFA compared to either control or G6P treatments, whereas no significant difference was observed in bacterial PLFA following glucose treatment compared to G6P treatment. Furthermore, a significant interaction effect between slurry and time factors was observed for bacterial PLFA ($F(6, 44) = 8.21$, $p < 0.000$, $\eta^2 = 0.528$). At day 20, a significantly higher bacterial PLFA was observed under the AS slurry treatment compared to the control treatments without slurry, whilst no significant difference was observed under US slurry addition. In contrast, by day 49, the application of both slurry types resulted in significantly lower bacterial PLFA compared to the control treatment.

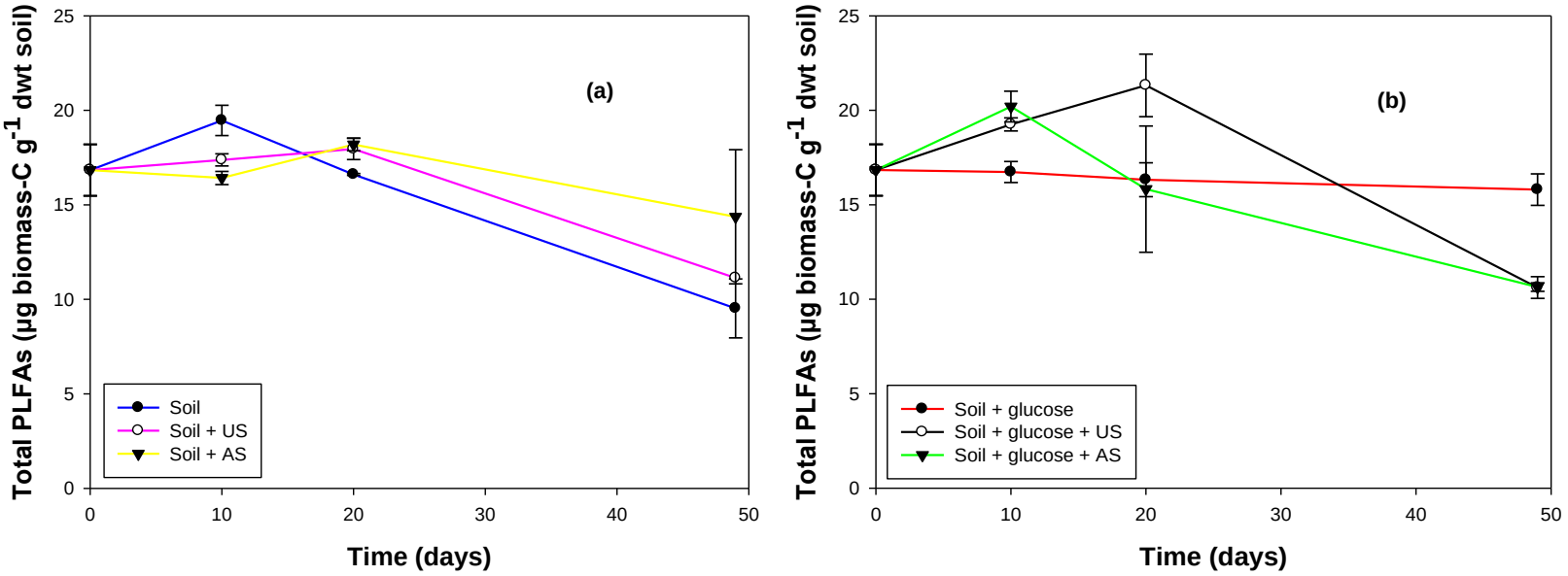

Figure 5.5. Total phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) control soil (soil), soil with unamended slurry (US), or soil with slurry amended with the biological additive SlurryBugs (AS), (b) soil with glucose, soil with glucose and US or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

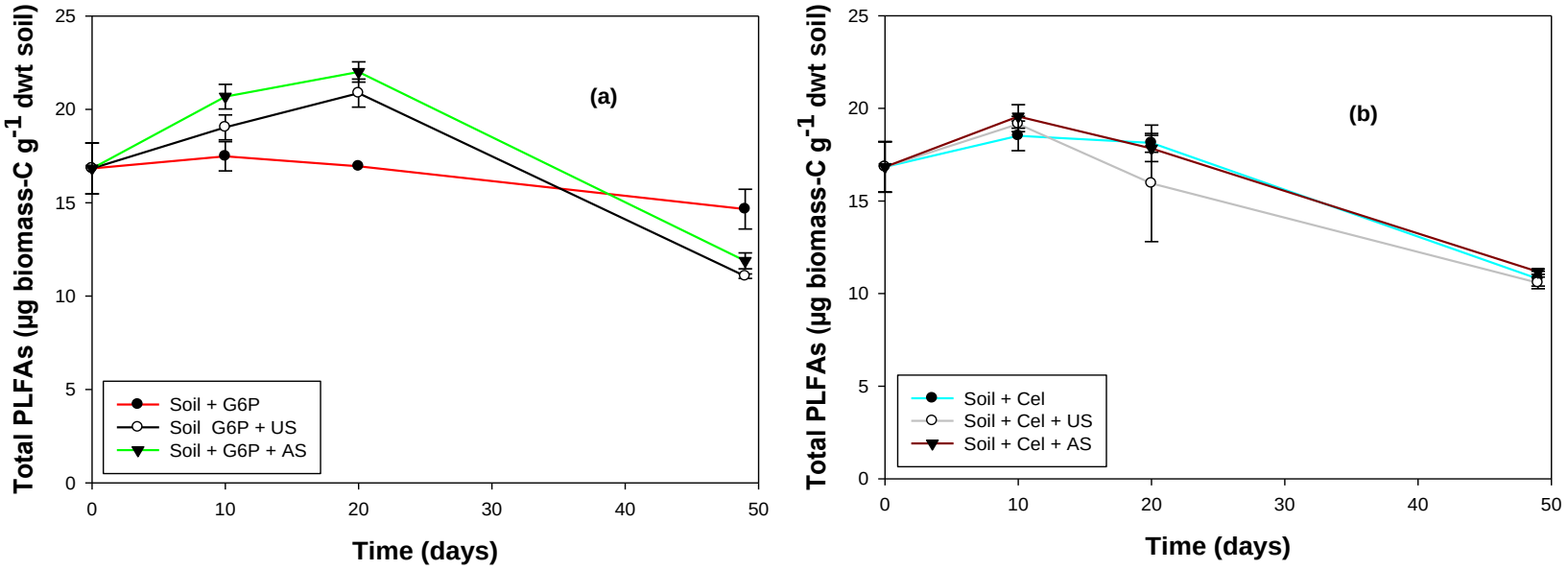

Figure 5.6. Total phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) soil with glucose-6-phosphate (G6P), soil with G6P and unamended slurry (US), or soil with G6P and slurry amended with the biological additive SlurryBugs (AS), (b) soil with cellulose, soil with cellulose and US, or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

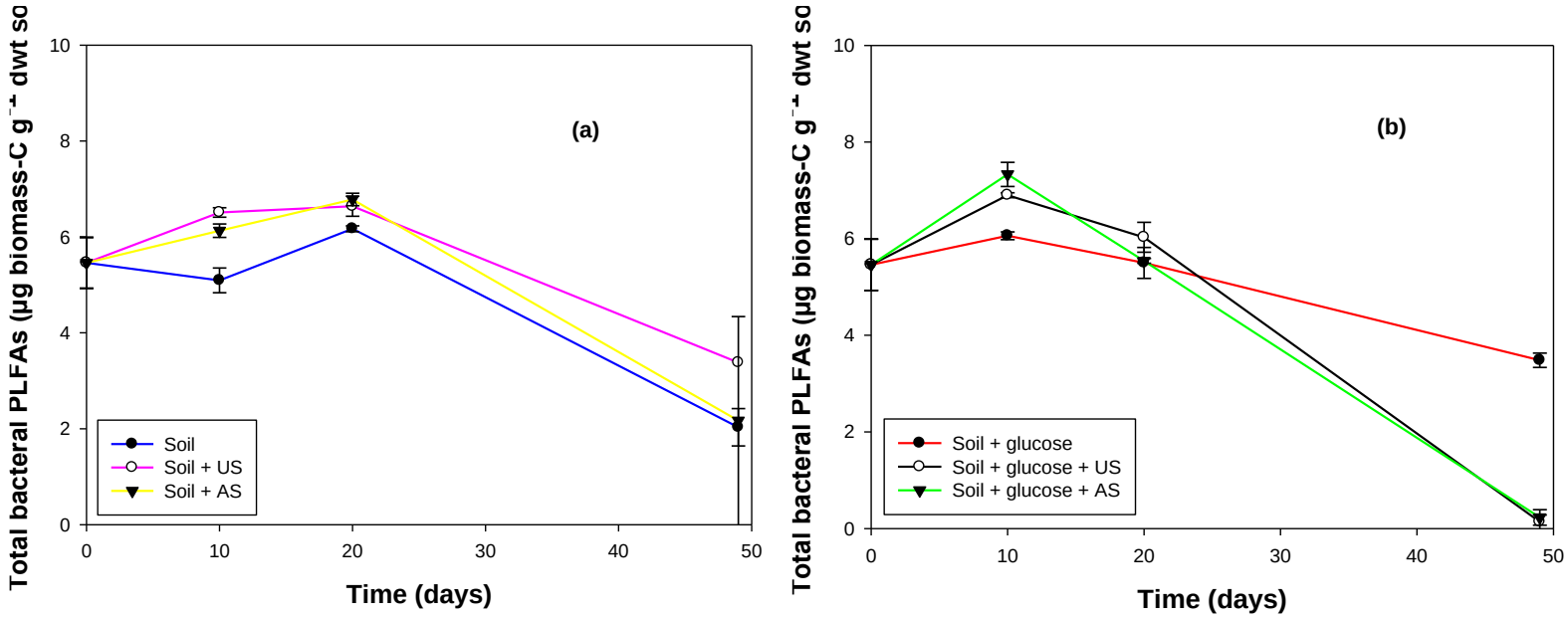

Figure 5.7. Total bacterial phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) control soil (soil), soil with unamended slurry (US), or soil with slurry amended with the biological additive SlurryBugs (AS), (b) soil with glucose, soil with glucose and US or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

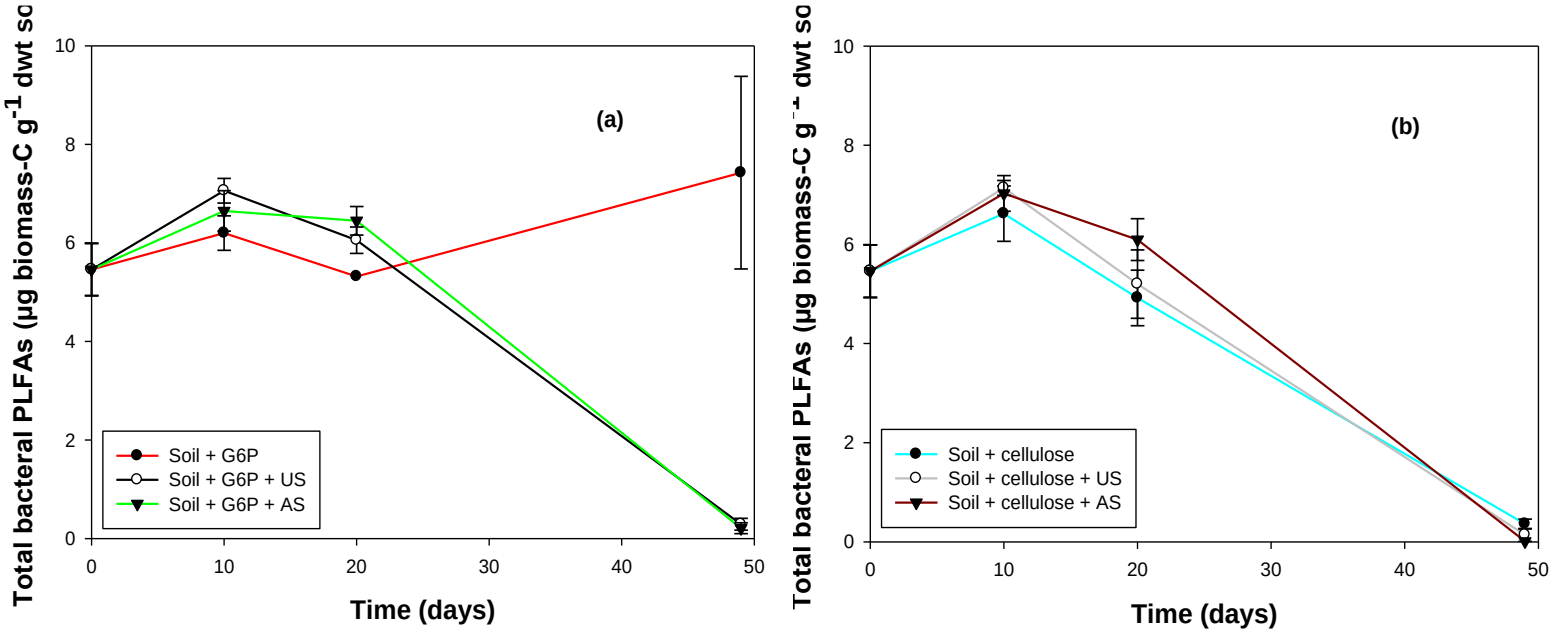

Figure 5.8. Total bacterial phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) soil with glucose-6-phosphate (G6P), soil with G6P and unamended slurry (US), or soil with G6P and slurry amended with the biological additive SlurryBugs (AS), (b) soil with cellulose, soil with cellulose and US, or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

5.3.3.3 Gram-positive phospholipid fatty acid profiles

No significant effect was observed on G +ve PLFA (Figures 5.9 - 5.10) following either carbohydrate or slurry application. In contrast, time was observed to significantly affect G +ve PLFA ($F(3, 22) = 115.575$, $p \leq 0.000$, $\eta^2 = 0.940$), with G +ve PLFA at days 10 and 20 exceeding that at days 0 and 49. Further, G +ve PLFA was not significantly affected by the interaction between carbohydrate and slurry treatment. In contrast, a significant interaction effect was observed between carbohydrate treatment and time in terms of G +ve PLFA ($F(9, 53) = 11.048$, $p \leq 0.000$, $\eta^2 = 0.573$). At day 10, the application of cellulose generated a significantly higher G +ve PLFA compared to the control treatment with no carbohydrate added. In contrast, no significant differences were observed between glucose or G6P and cellulose or control treatments. At day 49, a significant change in G +ve PLFA was observed across the carbohydrate treatments in the order control > G6P, glucose (no significant difference between G6P and glucose) > cellulose. Finally, the interaction between slurry and time resulted in significant effects on G +ve PLFA ($F(6, 44) = 10.462$, $p \leq 0.000$, $\eta^2 = 0.588$). By day 49, the addition of both slurry types caused significantly lower G +ve PLFAs compared to the control treatment with no slurry applied.

5.3.3.4 Gram-negative phospholipid fatty acid profiles

No significant effect was observed on G –ve PLFA (Figures 5.11 - 5.12) following carbohydrate or slurry addition alone. In contrast, time was observed to be a significant factor, regardless of carbohydrate or slurry application, in terms of G -ve PLFA ($F(3, 22) = 306.198$, $p \leq 0.000$, $\eta^2 = 0.977$), with G -ve PLFA at day 49 being significantly lower than at all other times in the incubation. A significant interaction

effect was observed between carbohydrate and time for G -ve PLFA ($F(9, 53) = 7.163$, $p \leq 0.000$, $\eta^2 = 0.473$). At day 20, a significantly higher G –ve PLFA was observed under the control treatment compared to each of the three carbohydrate treatments. By day 49, G –ve PLFA was significantly higher in G6P and control treatments than within glucose and cellulose treatments. Further, a significant interaction effect was observed between slurry and time for G -ve PLFA ($F(6, 44) = 7.120$, $p \leq 0.000$, $\eta^2 = 0.493$). At day 10, the addition of both slurry types resulted in significantly higher G –ve PLFAs compared to the control treatment without slurry, whereas no significant difference was observed between AS and US slurry treatments. Similarly, at day 20 the application of AS slurry resulted in a significantly higher G –ve PLFA compared to the treatment without slurry. In contrast, by day 49 significantly lower G –ve PLFA was observed following the application of both slurry types compared to the control treatment.

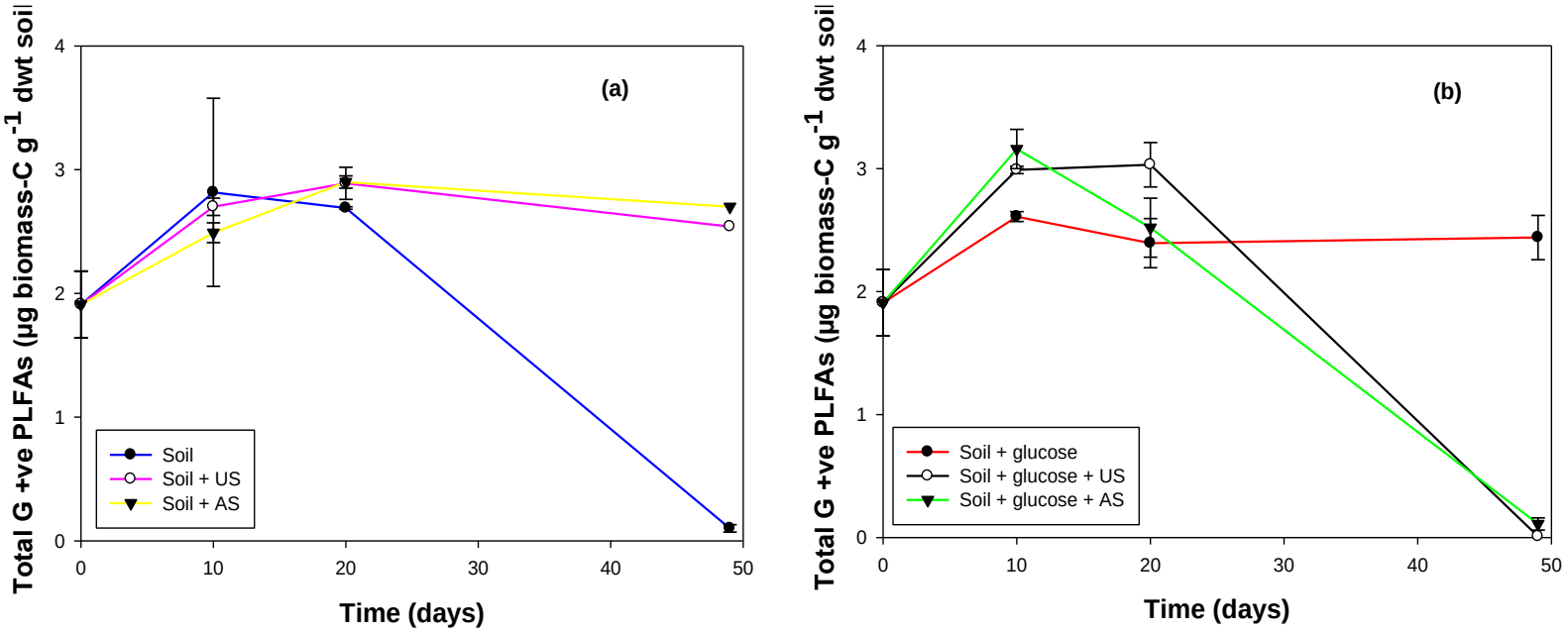

Figure 5.9. Total gram-positive (G +ve) phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) control soil (soil), soil with unamended slurry (US), or soil with slurry amended with the biological additive SlurryBugs (AS), (b) soil with glucose, soil with glucose and US or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

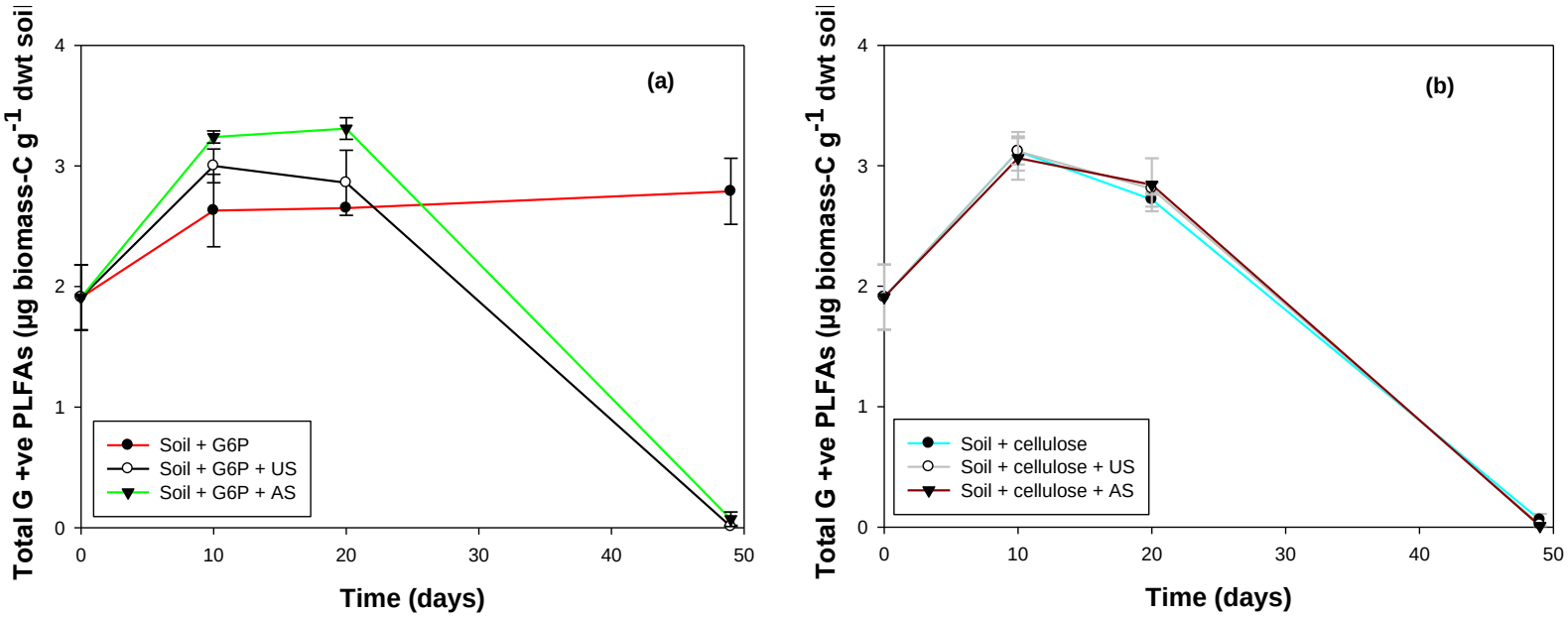

Figure 5.10. Total gram-positive (G +ve) phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) soil with glucose-6-phosphate (G6P), soil with G6P and unamended slurry (US), or soil with G6P and slurry amended with the biological additive SlurryBugs (AS), (b) soil with cellulose, soil with cellulose and US, or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

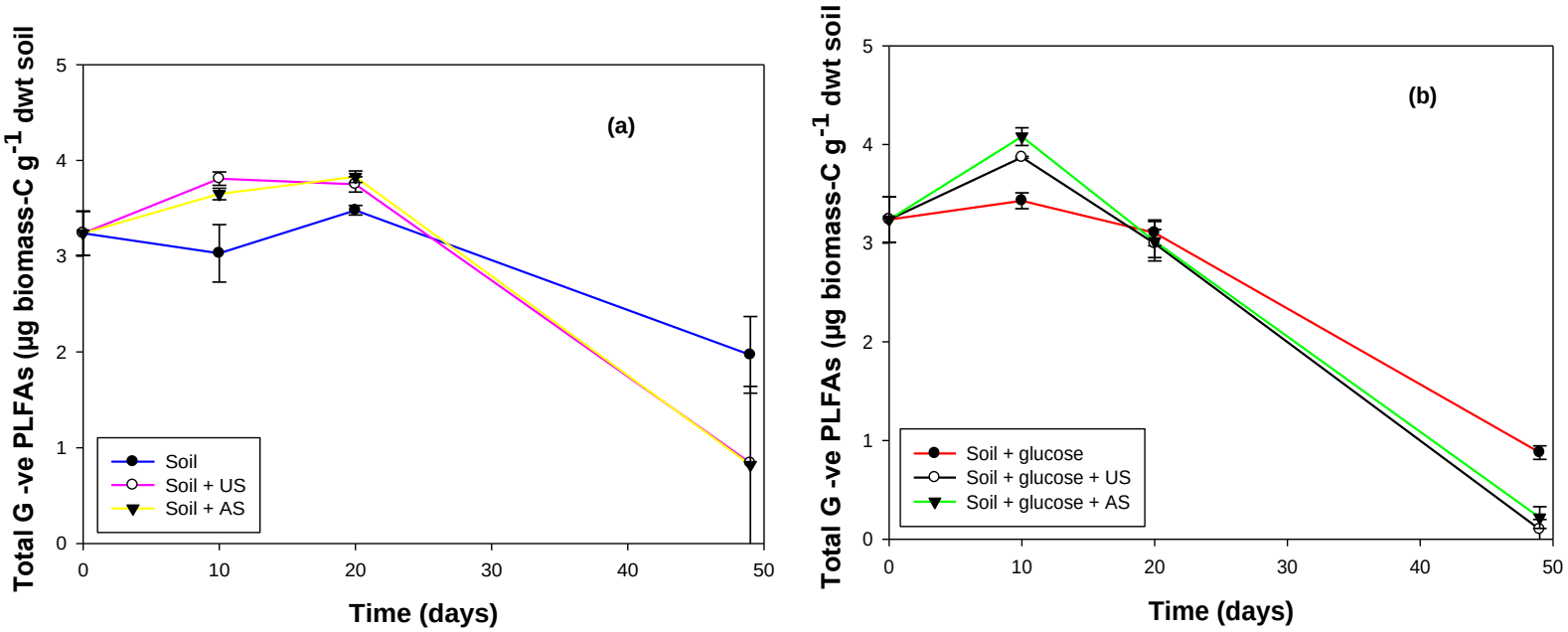

Figure 5.11. Total gram-negative (G –ve) phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) control soil (soil), soil with unamended slurry (US), or soil with slurry amended with the biological additive SlurryBugs (AS), (b) soil with glucose, soil with glucose and US or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

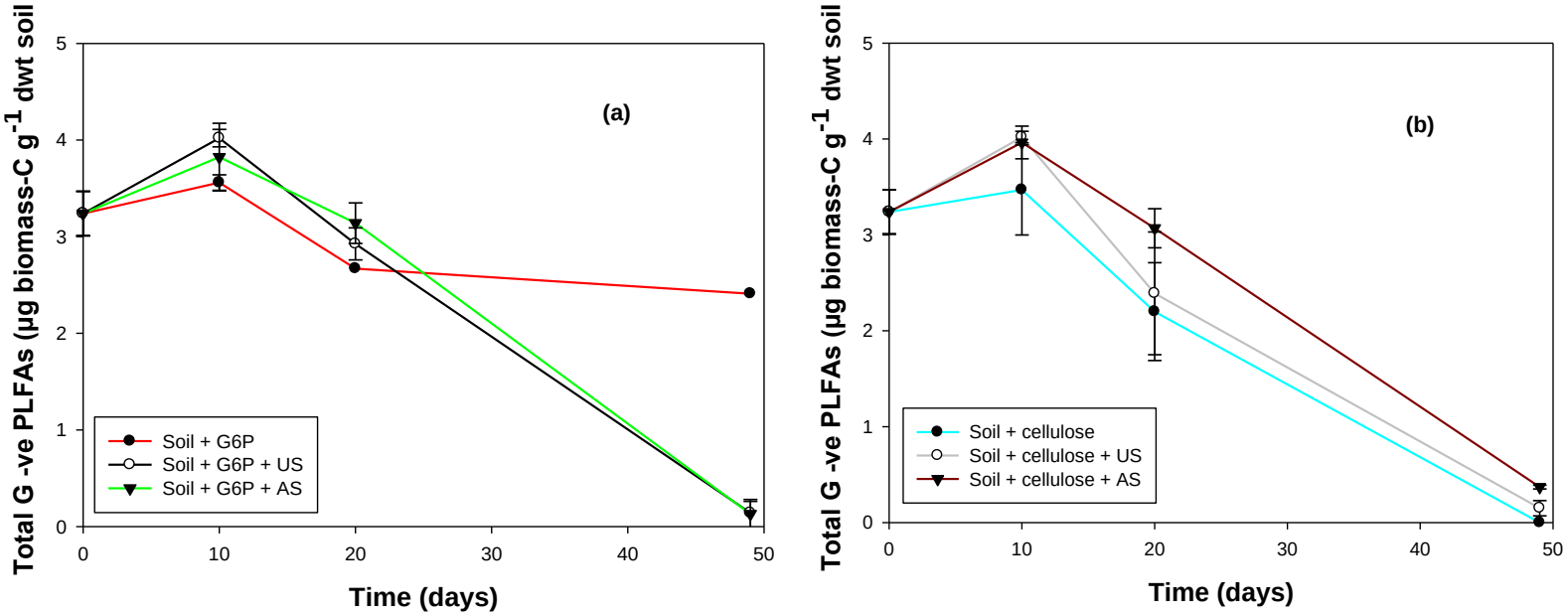

Figure 5.12. Total gram-negative (G –ve) phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) soil with glucose-6-phosphate (G6P), soil with G6P and unamended slurry (US), or soil with G6P and slurry amended with the biological additive SlurryBugs (AS), (b) soil with cellulose, soil with cellulose and US, or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

5.3.3.5 Gram-positive / Gram-negative phospholipid fatty acid ratio profiles

The addition of carbohydrate resulted in significant changes in the G +ve / G -ve ratio ($F(3, 24) = 62.366$, $p \leq 0.000$, $\eta^2 = 0.886$), with significantly higher ratios in the control and glucose treatments compared to the cellulose and G6P treatments. The application of slurry also resulted in significant effects on the G +ve / G –ve ratio ($F(2, 24) = 60.775$, $p \leq 0.000$, $\eta^2 = 0.835$), with the addition of AS slurry resulting in a significantly lower ratio compared either the control treatment or the treatment with US slurry. Time significantly influenced the G +ve / G -ve ratio ($F(2, 24) = 98.479$, $p \leq 0.000$, $\eta^2 = 0.931$), with a significant increase in the ratio throughout the 49 day incubation.

A significant interaction effect between carbohydrate and slurry factors was observed for the G +ve / G -ve ratio ($F(6, 24) = 134.391$, $p \leq 0.000$, $\eta^2 = 0.971$). The application of both US and AS slurry to the glucose treatment resulted in significantly lower G +ve / G -ve compared to the treatment with glucose alone. Further, the addition of US slurry to soil produced a significantly higher ratio compared to the treatments with either AS slurry or without slurry (control). A significant interaction effect was also observed between carbohydrate and time for G +ve / G –ve ratio ($F(9, 53) = 14.062$, $p \leq 0.000$, $\eta^2 = 0.626$). At day 20, the addition of cellulose resulted in a significantly higher ratio compared to either glucose or control treatments. By day 49, the application of carbohydrate resulted in a significant higher G +ve / G –ve ratio in control and glucose treatments compared to treatments with G6P or cellulose. Finally, a significant interaction effect between slurry and time was observed for G +ve / G –ve ratio ($F(6, 44) = 12.778$, $p \leq 0.000$, $\eta^2 = 0.635$). By day 49, the addition of AS slurry to soil caused a significantly lower ratio compared to the treatments either with US slurry or without slurry.

5.3.3.6 Fungal phospholipid fatty acid profiles

No significant effect of the carbohydrate treatment was observed for fungal PLFA (Figures 5.13 - 5.14). However, the application of slurry significantly affected fungal PLFA ($F(2, 24) = 5.801$, $p < 0.009$, $\eta^2 = 0.326$), with the addition of AS slurry resulting in a significantly higher fungal PLFA compared to the control treatment without slurry, whilst no significant difference was observed following the application of US slurry compared to either AS slurry or control treatment. Fungal PLFA was also significantly affected by time, regardless of carbohydrate or slurry application ($F(3, 22) = 105.769$, $p < 0.000$, $\eta^2 = 0.935$), with higher fungal PLFA at days 10, 20 and 49 compared to day 0.

No significant interaction effects between carbohydrate and slurry or carbohydrate and time were observed for fungal PLFA. In contrast, significant interactions effects between slurry and time factors were observed for fungal PLFA ($F(6, 44) = 4.233$, $p = 0.002$, $\eta^2 = 0.366$). At day 10, a significantly higher fungal PLFA was observed following AS slurry treatment compared to the control treatment. At day 20, the addition of US slurry resulted in a significantly higher fungal PLFA compared to the control treatment without slurry applied, whereas no significant differences were observed between US slurry and control or AS slurry treatments. No significant differences were observed at day 49 between each slurry or control treatment.

5.3.3.7 Fungal / bacterial phospholipid fatty acid ratio profiles

No significant differences in the fungal / bacterial (F/B) ratio were observed across carbohydrate or slurry treatments alone, or through time across the 49 day incubation. Further, no significant interaction effects on the F/B ratio were observed.

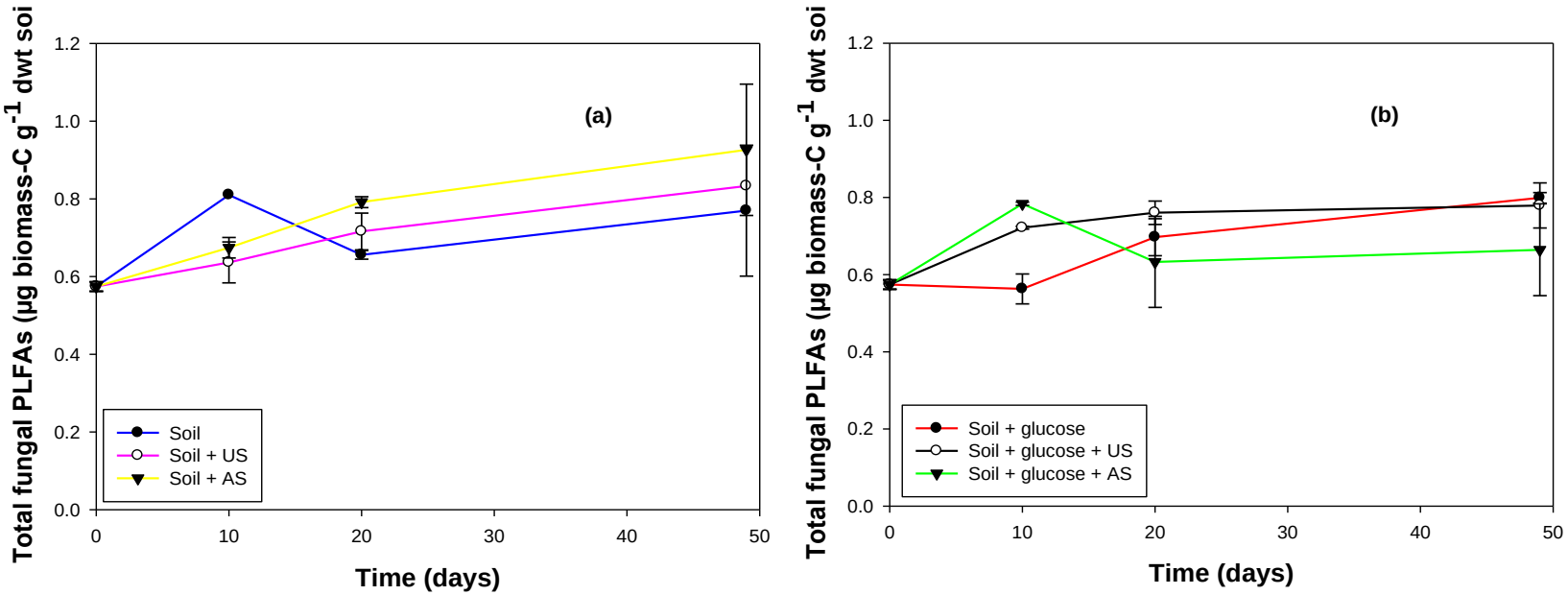

Figure 5.13. Total fungal phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) control soil (soil), soil with unamended slurry (US), or soil with slurry amended with the biological additive SlurryBugs (AS), (b) soil with glucose, soil with glucose and US or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

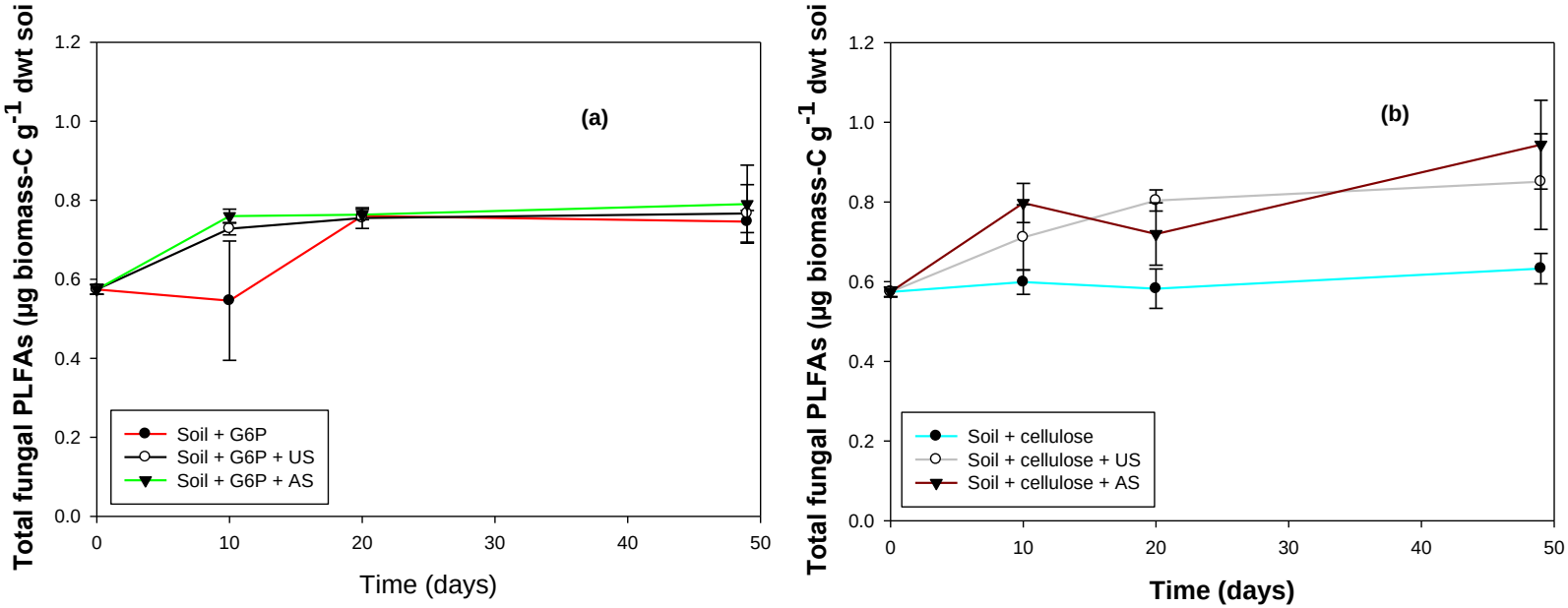

Figure 5.14. Total fungal phospholipid fatty acids (PLFAs) (µg biomass-C g^{-1} dry weight equivalent soil) using (a) soil with glucose-6-phosphate (G6P), soil with G6P and unamended slurry (US), or soil with G6P and slurry amended with the biological additive SlurryBugs (AS), (b) soil with cellulose, soil with cellulose and US, or AS slurry. Average values of measured data are presented as symbols, error bars indicate standard error of the mean (n = 3).

5.4 Discussion

5.4.1 Effects of carbohydrate and slurry application on soil microbial biomass

Changes in C_{mic} were not uniform for all carbohydrate or slurry treatments across every sampling day during the incubation (Figures 5.1 – 5.2). However, the addition of either US or AS slurry to soil resulted in significant increases in C_{mic}, compared to the control treatment with no slurry addition. Further, for glucose and cellulose, the addition of either AS or US alongside the carbohydrate resulted in significantly higher C_{mic} than the corresponding carbohydrate-only treatment. These findings are generally consistent with previous short-term incubation experiments, where an increase in C_{mic} appeared to be an important response to the application of cattle or pig slurry to different arable soils (Kandeler and Eder, 1993, Paul and Beauchamp, 1996, Saviozzi et al., 1997, Lalande et al., 2000, Murugan et al., 2014). The experimental results in the current chapter are also consistent with the results of a 59-year long-term experiment, where the addition of cattle slurry or FYM generated higher C_{mic} compared to inorganic fertiliser or control treatments (Šimon and Czakó, 2014). However, Plaza et al. (2007), in a 120-day incubation with a pig slurry-treated soil, found an increase in C_{mic} over the first 14 days compared to a control soil, followed by a decrease compared to the control soil during the subsequent 45 days, with no significant differences in C_{mic} between the slurry-treated and the control soils across the remainder of the experiment.

As proposed by Sakamoto and Oba (1994), the observation reported in the current chapter of a significant increase in C_{mic} in treatments with AS or US slurry, compared to a number of the carbohydrate-only or to the control treatments where no slurry was applied, may be attributed to the addition of a readily biodegradable organic C fraction

to soil via slurry application. Subsequently, this likely stimulated r-strategists with adaptations that give competitive advantage following the addition of labile C substrates to soil. A number of studies have demonstrated that, due to a range of readily available forms of C in slurry including organic and amino acids, carbohydrates, fatty acids and peptides, the addition of slurry to soil can induce microbial biosynthesis, involving increase in the soil microbial biomass through direct incorporation of the added substrates (Fraser et al., 1988, Reganold, 1988, Paul and Beauchamp, 1989, Sørensen, 1998, Chantigny et al., 2002, Bol et al., 2003a). In Chapter 4 (see Sections 4.4.1 and 4.4.2), competition in soil between the indigenous microbial community and the microbial community derived from slurry was proposed to account for suppression of the activity of soil microorganisms, both in terms of respiration and uptake of an added carbohydrate. However, in the current chapter, an increase in the soil microbial biomass was observed following the application of slurry to soil, including when slurry was applied alongside a number of carbohydrates.

The potential explanation for these differences is not immediately obvious. It is likely that the addition of labile compounds in slurry to soil resulted in the increase in C_{mic} observed in this chapter and in previous studies, because of the direct uptake and synthesis of labile C from the slurry into biomass. Therefore, because of the labile C provided by slurry, it is likely that the microbial community effectively 'switches' from the carbohydrate to the labile C in slurry, in terms of uptake and synthesis in new biomass. This explains the less incorporation of ^{14}C-labelled carbohydrates observed in Chapter 4 (see Section 4.4.2) when slurry was also applied, because the microbial community had switched focus onto the labile C in slurry, and the higher C_{mic} in the current chapter in slurry-amended treatments compared to control or to carbohydrate-only treatments. Further experimental work is required to test the hypothesis of the

microbial switch from the carbohydrate to the labile C in slurry following addition of slurry.

According to Bittman et al. (2005), one cannot exclude the possibility that the increases in C_{mic} in slurry-treated soils reflect the direct addition of faecal bacteria from slurry to the soil microbial community. Indeed, several studies have described various enterococci, including *Escherichia coli* and *Salmonella typhimurium*, surviving in soil for at least 19 weeks after the application of cattle slurry (Chandler and Craven, 1978, 1980, Perucci, 1992, Lau and Ingham, 2001, Hodgson et al., 2016).

In general, by the end of the 49-day incubation, a significantly lower C_{mic} was observed in each carbohydrate treatment compared to the control soil treatment. The higher C_{mic} in the cellulose treatment at the end of the incubation than under more labile carbohydrate treatments differs from findings reported by Dilly (2004), where the application of glucose both to a beech forest soil and to an arable soil resulted in higher levels of C_{mic}, compared to C_{mic} following cellulose treatment. Despite this, a significant increase in C_{mic} was still observed by Dilly (2004) in the arable soil following cellulose application until the end of the 20-day incubation. Similarly, Schutter and Dick (2001) found significantly higher C_{mic} following the addition of glucose to soil compared to either cellulose or control treatments during an 80-day incubation with an arable soil. However, an increase in C_{mic} was still observed by these authors for soil treated with cellulose, from day 35 until the end of the incubation.

A number of mechanisms may explain the higher C_{mic} reported in the current chapter following the addition of cellulose to soil, compared to the labile substrates glucose and G6P. Firstly, recalcitrant substrate-degraders, the so-called K-strategists,

may have been preferentially stimulated in the soils used in the experiments reported here, compared to r-strategists that are adapted to degrade more readily available carbohydrates (Fontaine et al., 2003). According to Mondini et al. (2006), it is conceivable that stimulation of different microbial populations in soil can account for variations in microbial responses to the application of carbohydrates of differing complexity and degree of availability. The results reported in this chapter suggest that a greater stimulation of K-strategists in cellulose-treated soil occurred, compared to the stimulation of r-strategists in glucose or G6P treatments. In particular, both fungi and G +ve bacteria that belong to the ecological group of K-strategists are expected to be involved in the degradation of cellulose and, despite the fact that cellulose is decompose faster by cellulolytic filamentous fungi, by far the greatest proportion of cellulose degradation in soil is undertaken by bacteria (Lynd et al., 2002). The low concentration of C_{mic} observed at day 20 for the cellulose treatment without slurry addition may be attributed to the long and recalcitrant structure of cellulose, meaning that three types of enzymes are sequentially involved in its degradation from polysaccharide chains to glucose monomers (Moat et al., 2003) (for further details, see Section 4.5.1).

Secondly, according to Fontaine et al. (2004), extracellular enzymes produced for cellulose degradation can contribute to SOM decomposition. Therefore, this non-targeted hydrolysis of SOM components, coupled with the uptake of the more labile C compounds released from SOM degradation into microbial cells, may be another mechanism that accounts for the higher level of C_{mic} observed under the cellulose treatment, compared to the other carbohydrates treatments with no slurry added. Finally, as proposed by Schneckenberger et al. (2008), a possible explanation for the significantly higher C_{mic} observed in the control treatment compared to the treatments

with carbohydrates and no slurry applied is that glucose, either derived from cellulose degradation or from glucose and G6P application, were actually taken up into microbial cells, but glucose was incorporated into microbial biomass as non-chloroform labile compounds within the cellular structure of the microbial biomass.

In contrast to C_{mic}, a steady decrease in P_{mic} was observed through time, with no significant differences between control and carbohydrate treatments (Figures 5.3 – 5.4). The decrease in P_{mic} regardless of the carbohydrate applied to soil contrasts with results reported by Jonasson et al. (1996), where a significant increase in P_{mic} was observed after the addition of a labile C source (sucrose) to a grassland soil, whilst the application of a recalcitrant substrate (sawdust) did not cause any change in P_{mic}. According to Richardson and Simpson (2011), there is a direct coupling between C mineralisation following the addition of organic amendments to soil and P released into soil from microbial biomass, with a turnover time between 42 to 160 days depending on the type of organic substrate added to soil. In fact, significant amounts of P have been described to be released from soil microorganisms after the decomposition phase of fresh C-substrates added to soil, such as carbohydrates or slurry, due to microbial death and predation (Oehl et al., 2004).

The decrease in P_{mic} observed in the current chapter may be related to the re-mineralisation and release of microbial P into soil associated with the decomposition of microbial necromass, or with microbial grazing through biological or biochemical processes (Oehl et al., 2004). Therefore, it is expected such decrease in P_{mic} occurring at the same time as a general increase in C_{mic} that was observed in the experiment in the current chapter, being C_{mic} and P_{mic} the two moieties of a microbial system. Further, the lack of any significant difference in P_{mic} between the individual carbohydrate treatments, and in particular between glucose and the phosphorylated

counterpart G6P, confirms the hypothesis outlined at the start of this chapter, that the addition of G6P would not generate significant difference in P_{mic} compared to other carbohydrate treatments.

5.4.2 Effects of carbohydrate and slurry application on PLFAs

The effects of carbohydrate and slurry application to soil were also studied in terms of changes in the structure of the soil microbial community, using PLFA analysis. The data showed that, in contrast to C_{mic}, the total PLFA profiles within carbohydrate- and slurry-treated soils, as well as within control soils, initially evidenced a significant increase until day 20, followed by a significant decrease until the end of the 49-day incubation. The behaviour of total PLFA reflects the response of all components of the soil microbial community to the application of organic amendments to soil. The two-stage process observed in total PLFA reported in the current chapter is consistent with research reported by Hammesfahr et al. (2011) in which, during a 32-day incubation experiment with an agricultural soil, treatment by liquid pig manure resulted in an initial increase in total PLFA during the first 8 days, followed by a decrease in total PLFA until the end of the incubation. In both the current chapter and the study of Hammesfahr et al. (2011), the microbial growth as represented by increases in total PLFA during the early stages of an incubation was not sustained over the longer term, due to depletion of the labile organic substrates as the incubation progresses.

However, despite the general decrease in total PLFA observed across the 49-day incubation in the current chapter, the application of different carbohydrates to soil did not appear to cause any significant difference in total PLFA at any sampling time. These findings are consistent with those reported by Orwin et al. (2006), where the

addition of cellulose to a grassland soil did not produce significantly higher total PLFA compared to glucose treatment. Furthermore, the application of slurry to soil in the current chapter did not produce any significant change in the total PLFA compared to a control treatment with no slurry added.

The apparent two-stage process observed in total PLFA across all treatments (an initial increase, followed by a subsequent decrease until the end of the incubation), is mainly due to a parallel response found in the total bacterial PLFA, due to the F/B ratio showing a bacterial dominance in all treatments. Lower values were observed in total bacterial PLFA at day 49 than at day 10 and 20 across all treatments (see Figures 5.7 – 5.8). However, a steady increase through time during the incubation was observed in fungal PLFA across all treatments (Figures 5.13 – 5.14). Nevertheless, due to the predominance of bacterial PLFA in the total PLFA, the general increase observed in fungal PLFA across all treatments could not offset the overall decrease observed in total bacterial PLFA through time. Soils in which the fungal community predominates within the microbial community are normally expected to produce more C_{mic} for each unit of substrate C utilised, compared to bacterially-dominated microbial communities (Keiblinger et al., 2010, Strickland and Rousk, 2010).

However, the incorporation of a considerable amount of labile C into microbial cells following the application of carbohydrates and slurry to soil was reported (see section 4.4.2) within a soil that has been shown, on the basis of PLFA analysis in the current chapter, to be bacterially-dominated. This finding corroborates research reported by Six et al. (2006), where a number of studies were reviewed with little or no support for the hypothesis that a greater proportion of C is stored within microbial biomass in fungal-dominated soils compared to bacterially-dominated soils. Thiet et al. (2006) also found no difference in C storage with a predominance of fungi over

bacteria in the microbial community of two arable soils following treatment with glucose. Further studies are therefore required to understand how the relative proportions of fungi and bacteria can change in temperate grassland soils, under treatments with different quantities of carbohydrates as well as with amended and unamended slurry.

Existing evidence suggests that changes in the relative abundance of fungi or bacteria in soil microbial communities can significantly influence N and P availability, as a consequence of the specific physiology and differential interactions that these two microbial groups have with OM and nutrients in soil (Six et al., 2006). For example, a predominance of fungi over bacteria in temperate grassland soils is likely to be related to low levels of mineral N availability in soil and a lower N leaching potential, compared to soils with lower F/B ratios (Bardgett and McAlister, 1999, de Vries et al., 2006). In addition, according to Bardgett (1996), the F/B ratio can be lowered in fertilised soils because fungi are adversely affected by high amounts of mineral N. Similarly to N acquisition, a predominance of fungi over bacteria has been observed in forest soils, resulting in systems with greater P acquisition efficiency from organic P compounds, or systems in which P incorporation into fungal biomass is enhanced, compared to soils which exhibit bacterial predominance (Allison et al., 2007). Therefore, it is conceivable that grassland soils in which increases in fungal biomass occur, as in the research reported in this chapter on the basis of PLFA analysis, may ultimately be characterised by greater efficiency of P acquisition from more recalcitrant soil P pools.

The initial increase, followed by the decrease until the end of the incubation, observed in terms of total bacterial PLFA can be attributed to similar changes observed both in G +ve (Figures 5.9 – 5.10) and G -ve bacterial PLFAs (Figures 5.11

– 5.12). Further, G +ve/G –ve ratios showed an overall dominance of G –ve bacteria through time across all treatments. The increase observed during the first 10 days both in G +ve and G –ve bacteria is in partial agreement with the supposed two-stage decomposition process associated with the soil bacterial community. This involves G –ve bacteria prevailing during the early stages of decomposition, followed by G +ve bacteria and, subsequently, fungi at later stages (Moore-Kucera and Dick, 2008). This pattern is driven by the fact that G -ve bacteria that are predominantly r-strategists and have rapid growth rates, are adapted to metabolise readily available substrates such as glucose and G6P, as well as the most available fractions of C in slurry (Cleveland et al., 2007, Fierer et al., 2007, Nemergut et al., 2010). The results reported in the current chapter suggest that, after day 10 of the incubation, labile substrates were exhausted and G -ve bacterial PLFA decreased substantially, due to the inability of G-ve bacteria to gain competitive advantage from more recalcitrant substrates, including within SOM. Significantly higher G –ve bacterial PLFA was also observed following slurry treatments compared to the control treatment without slurry during the first 20 days of the incubation.

These data indicate significantly enhanced growth for these r-strategists in treatments with slurry application that presumably supplied more readily available substrates to soil, compared to the control treatments, until day 20 when the available substrate became exhausted. However, according to de Boer et al. (2005), several soil bacterial species present functionally equivalent cellulolytic systems to those in fungi, including G -ve *Cytophaga* gen. (Lynd et al., 2002). Therefore, the data reported for G -ve bacterial PLFA following the addition of cellulose suggest a possible bacterial-fungal competition for cellulose in soil, representing a possible explanation for the two-stage pattern observed in G -ve PLFA under all carbohydrate treatments,

regardless of the complexity of the added substrate. However, due to the limited level of taxonomic resolution achievable with PLFA analysis, further techniques would be required for complete analysis of the microbial community in soil (Nannipieri et al., 2003). Indeed, a number of limitations have been observed with PLFA analysis, including the limited number of fatty acids characteristic for specific microbial groups, potentially resulting in sub-optimal identification of differences in microbial community structure, a limited number of signature fatty acids for fungi that may lead to underestimation of fungal biomass, and a lack of information about species composition (Jandl et al., 2005, Marschner, 2007). Therefore, the combination of molecular approaches utilising PCR-based methods and metagenomic analyses, such as high-throughput sequencing, represent cost-effective options dealing with large datasets that could provide a higher resolution at species- or strain-level of soil microbial communities, compared to a traditional approach such as PLFA (Lemos et al., 2011, Zhang et al., 2011, Thies, 2014).

Differently from G -ve, G +ve bacteria and fungi are physiologically classified as K-strategists, due to their adaptation to utilise the almost inexhaustible and more recalcitrant SOM (Fontaine et al., 2003, Romaní et al., 2006). Thus, they are continuously active, yet grow slowly and, particularly for fungi, dominate the latter stages of substrate decomposition (Fontaine et al., 2003, Fierer et al., 2007, Kramer and Gleixner, 2008). The results reported in the current chapter are partially consistent with this theory. A significantly higher G +ve bacterial PLFA was observed at day 10 following cellulose treatment compared to the control treatment, consistent with the competitive advantage towards more recalcitrant substrates such as cellulose that is possessed by G +ve bacteria. However, in contrast to fungal PLFA, no increase was observed in the G +ve bacterial PLFA after the first 20 days, when the exhaustion of

more labile substrates might have been expected to stimulate this group of K-strategists through the release of cytoplasmic components after lysis undertaken by G -ve bacteria (Pezzolla et al., 2015). It may be hypothesised that a substrate competition between G +ve bacteria and fungi resulted in a decrease in this component of the bacterial community and a concomitant increase in the fungal community. According to Lynd et al. (2002), usually by far fungi have a greater ability compared to bacteria to access cellulose fibres through hyphae that bring cellulolytic enzymes into close contact with the cellulose polymers. Therefore, the increase in the fungal fraction of the microbial community in soil associated with a number of the treatments reported in the current chapter, for example through AS application, may increase the ability of the soil microbial community to access components of the SOM pool that would otherwise not be accessible. However, further analyses are needed to determine whether shifts in the G +ve:G –ve bacterial community, and in the balance between fungi and bacteria, can be induced in temperate grassland soils over the longer-term following the addition to soil of substrates of different complexity and degrees of microbial availability.

6 Synthesis of thesis outcomes and discussion of the broader environmental context for livestock slurry application to grassland soil

6.1 Achievements of the thesis

This thesis aimed to examine whether treatment of livestock slurry with the commercial slurry additive SlurryBugs can enhance the total and the available nutrient content of livestock slurry during slurry storage. In addition, this thesis aimed to investigate whether the availability of key crop nutrients in temperate grassland soil can be enhanced through optimising the management of livestock slurry, as part of attempts to close nutrient loops within intensive agricultural production systems. A specific focus within the thesis was placed on how the soil microbial community responds to the input of slurry as one form of organic amendment to grassland soil, both in terms of the structure and the function of this community. The soil microbial community is a fulcrum, mediating the interface between the input of allochthonous material to agricultural soil and the availability of essential nutrients to crops growing within soil. In order to achieve this aim, four objectives were addressed through the thesis. Below, the major contributions of the thesis to each of these objectives are synthesised.

Chapter 3 examined the impact of a commercial slurry additive, SlurryBugs (SB), on the nutrient content of livestock slurry during storage, alongside the physico-chemical and nutrient properties of soil following slurry application to agricultural land. The first objective of the chapter was to determine whether treatment of livestock slurry with the SB additive influenced the nutrient content of slurry during storage. Treatment with SB resulted in a significantly higher concentration of total

phosphorus (TP) in slurry after nine weeks of storage, by 27% compared to a control slurry treatment that did not receive the SB additive. This is the first time that significant, positive effects on slurry TP content have been reported following treatment of slurry with a biological additive. Whilst some other research has examined the impacts of chemical additives applied to livestock slurry (e.g. Chapuis-Lardy et al., 2003), such work has generally found no significant effects on the TP content of slurry at the end of short-term storage periods. The mechanism responsible for the observed difference in slurry TP content was not identified within this thesis. However, it was hypothesised that the SB additive may have altered the emission of phosphine (PH_3) from slurry during storage, because a gaseous pathway is the only feasible route through which differences in slurry TP content could have been generated during the storage experiment. Whilst the extent of data describing PH_3 emissions from sources such as livestock slurry remains limited, those data that have been reported indicate that these emissions may be substantial (e.g. Glindemann et al., 1996). No significant differences were observed in slurry pH or in the total content of other nutrient elements following treatment with SB compared to control slurry. This suggests that the application of the microbial community in the biological additive to the indigenous microbial community in slurry did not stimulate sufficient changes in microbial processes to alter the physico-chemical properties and nutrient content of livestock slurry during storage.

The second objective within Chapter 3 was to determine the impacts of applying SB-amended slurry, control slurry and inorganic fertiliser on the physico-chemical and nutrient properties of grassland soils. Application of both the control and SB-amended slurry to soil resulted in significantly higher soil pH after an 85-day incubation than in soil treated with inorganic fertiliser or left as an unamended control

treatment. This pH effect was observed across clay loam, sandy loam and organic-rich soil types, but was particularly pronounced within the clay loam soil where a maximum pH difference of > 0.5 pH units was observed between the slurry and inorganic fertiliser treatments. Whereas no change in soil pH through time in the incubation followed the addition of slurry to soil, it is likely that the decrease in soil pH under the inorganic fertiliser treatment resulted from hydrolysis of urea fertiliser to NH_4-N through soil urease activity, followed by nitrification of ammonium to NO_3-N (Omar and Ismail, 1999, Zhang et al., 2008).

The treatment applied to soil in Chapter 3 also had significant effects on fractions of the P, N and C pools within grassland soils. With respect to P, significantly higher concentrations of Olsen P, representative of immediately plant-available P, were observed in clay loam and sandy loam soils at the end of the 85-day incubation following the addition of SB-treated slurry, compared to the addition of either control slurry or inorganic fertiliser. These differences in Olsen P concentration are likely due to changes in microbial turnover of P within the soils following the different treatments. Specifically, the microbial community in soils receiving SB-treated slurry, with *Bacillus* as the presumptive genus, is believed to be associated with microbially-driven increases in the availability of soil P, compared to conditions within soils receiving control slurry or inorganic fertiliser treatments.

In terms of mineral N within soil, slurry treatment (both SB- and control-slurry) resulted in higher concentrations of NH_4-N in soils during early stages of the incubation compared to the inorganic fertiliser treatment, likely as a result of the direct input of NH_4-N from slurry into the soil pool. During later stages of the soil incubation, there were no differences in NH_4-N concentration between different soil treatments, suggesting that additional NH_4-N within soil following the application of

slurry was either converted to other forms of N or assimilated within microbial biomass. However, the addition of slurry (both SB- and control-slurry) to soil resulted in significantly higher concentrations of NO_3-N in soils during later stages of the incubation, compared to either inorganic fertiliser or control treatments. It is hypothesised that this represents either a legacy of nitrification from the greater NH_4-N pools supplied to soil via slurry, or that mineralisation of organic N within soil organic matter and within slurry itself liberated greater NO_3-N in soils that received SB- or control-slurry, compared to other treatments. In contrast, the application of slurry, both amended and unamended with SB, did not generate any significant change in TN content across the soil treatments. Finally, a significant increase in SOM content in the organic-rich soil type was observed after applying SB-amended slurry, compared to the inorganic fertiliser treatment. However, no parallel increase in SOM content after SB-slurry application was observed in either clay loam or sandy loam soil types. It was hypothesised that a different composition within the soil microflora, and specifically a composition that supported a higher SOM decomposition rate, in these soil types compared to that in the organic-rich soil, was responsible for this observation.

The objective of Chapter 4 was to establish how the quality of organic substrates applied to grassland soil, specifically glucose, G6P, cellulose, and livestock slurry, influenced the fate of these externally-derived sources of C, as mediated by microbial activity within soil. Firstly, it was observed that the quality of C substrates was a major factor that influenced both the extent of mineralisation and of incorporation of externally-derived C into microbial biomass. Further, as the complexity of the C substrates applied to soil increased in the order glucose > G6P > cellulose, total respiration (of the added substrates in combination with SOM) decreased. Such a

decrease was also evident from the priming index calculated in Chapter 4, which indicated that the degradation of SOM decreased significantly (defined by a significantly more negative priming index) as increasingly complex C substrates were added to the soil. Preferential substrate utilisation, involving a microbial metabolic switch from decomposition of the added C substrate to SOM decomposition as the complexity of the added substrate increased, was proposed as the mechanism to explain the observed reduction in total respiration as more complex substrates were added to soil.

Chapter 4 also examined the partitioning of C and changes in the concentration of Olsen P in soils that received glucose and G6P, testing whether microbial demand for C in these grassland soils may drive increases in P availability in soil due to cleaving and release of the P moiety in added C substrates. Significantly higher Olsen P concentrations in soil were observed following the application of G6P compared to glucose, suggesting that microbial demand for C drove P release in the grassland soil, with only a fraction of the P made available following addition of a C substrate to soil subsequently being incorporated within microbial biomass. These observations suggest that the addition of substrates containing C and P moieties to grassland soils may result in an increase in the P status of soils, as a result of greater microbial demand for C compared to P. Further, if not utilised by the soil microbial community or by crops, the accumulation of available P within soil may ultimately increase the risk of P export to water from agricultural soils and the potential for adverse environmental impacts in receiving waters (Sharpley et al., 2001).

Chapter 4 also examined the impact on the partitioning of C within grassland soil of applying slurry, either amended with the SB-additive or non-amended, alongside C substrates. Compared to treatments in which only the relevant C substrate was applied

to soil, the addition of slurry alongside a C substrate resulted in lower total respiration and lower uptake of added C into microbial biomass, whilst a greater proportion of the added C substrate remained within the soil pool and was not involved in microbial activity. Therefore, following slurry addition, there is potential for stabilisation of C within SOM, rather than accumulation of C within microbial biomass or stimulation of microbial respiration. These data suggest that either a chemical (e.g. the presence of recalcitrant C compounds in the two slurries), or a microbiological (e.g. competition between soil and slurry microorganisms) mechanism may have reduced microbial respiration and the incorporation into microbial biomass of C substrates that were added to soil alongside slurry. Finally, the addition of slurry that has received an additive, such as SB, has the potential to alter the partitioning of added C to grassland soil, in comparison to slurry that has not been treated with an additive. Specifically, the effect of the slurry amended with SB was to stimulate microbial cycling of the added C substrate through respiration, but not through incorporation into biomass, although respiration was not stimulated to the same extent as observed in the C substrate-only treatments.

Chapter 5 sought to understand the effects of applying C substrates of varying complexity, in combination with livestock slurry, on the biomass and structure of the microbial community in a temperate grassland soil. In contrast to the original research hypothesis, a greater increase in microbial biomass C in soil was observed following the application of cellulose compared to either glucose or G6P. This response was attributed to biomass accumulation following cellulose addition, whereas the addition of glucose or G6P stimulated respiration, rather than accumulation of microbial biomass. Further, a two-stage behaviour was observed with respect to the total microbial community due to the response of soil microorganisms to the added C

substrates, with an initial increase in total PLFA until day 20 followed by a subsequent decrease until the end of the incubation at day 49. The addition of C substrates also altered the structure of the microbial community in soil. Specifically, G -ve (r-strategist) bacteria were initially stimulated by the addition of C substrates during the early stages of the incubations. However, in later stages of the incubation, G -ve bacteria declined, likely as a result of exhaustion of labile C that was added to soil, whilst G +ve bacteria and fungi began to increase. Apparent changes in the balance of G +ve bacteria and fungi were also observed, with increasing dominance of fungi toward the end of the incubation.

In contrast to microbial biomass C, microbial biomass P appeared to decrease steadily throughout the incubations reported in Chapter 5, with no significant differences observed through time between control treatments and soils that had received carbohydrate treatments. This decrease in microbial biomass P was attributed to release and re-mineralisation of synthesised organic P in soil microbial biomass, due to microbial death and predation. The data reported in this chapter indicate that the addition of G6P did not cause any significant increase in microbial biomass P compared to the addition of other C substrates, confirming the hypothesis from Chapter 4 that the soil microbial community was not constrained by the availability of P within the grassland soil used in these experiments.

Finally, the addition of slurry to soil in combination with a carbohydrate resulted in a significant increase in microbial biomass C compared to the corresponding treatment with carbohydrate alone. The addition of the readily biodegradable organic C fraction of slurry, in combination with the microbial community that existed within slurry itself, likely resulted in the observed increases in C_{mic}. The application of slurry to soil in combination with a carbohydrate also altered the soil microbial community under a

number of treatments, compared to the addition of a carbohydrate alone. For example, the application of both slurry types alongside glucose or G6P to soil caused significant decreases in G +ve bacterial PLFAs compared to the corresponding treatments with carbohydrates alone. Furthermore, the addition of SB-treated slurry alongside cellulose resulted in a significant increase in fungal PLFA compared to the treatment with cellulose alone. These data confirm partially the hypothesis that an increase both in G +ve bacteria and fungi was expected following slurry application, due to the stimulation of both microbial groups that are adapted to the recalcitrant C compounds that are present in SOM and that are added to soil following slurry application.

The findings reported within this thesis suggest that the effectiveness of biological additives applied to slurry to enhance nutrient content during storage remains uncertain. The addition of SB to slurry resulted in a significantly higher concentration of TP compared to the control slurry during a 9-week storage period. However, further analyses across a wider range of slurries are required to fully evaluate the effectiveness of additives such as SB on the total and bioavailable content of nutrients within slurry during storage. However, application of slurry, both amended with SB and unamended, to soil represents an important way in which to potentially enhance the availability of nutrients within soil, thereby reducing reliance on inorganic fertilisers whilst maintaining and increasing soil quality and crop yields. Significant positive effects on soil pH and on fractions of the C, N and P pool within grassland soil were generated following the addition of both slurry types, compared to inorganic fertiliser and control treatments. In particular, the application of SB-amended slurry to soil resulted in positive effects on SOM content and Olsen P concentrations, compared to the other treatments, including unamended slurry. Such findings illustrate the

potential value of applying SB-amended slurry to soil, compared to either control slurry or inorganic fertiliser treatments.

With respect to the soil microbial community composition and activity, the results from this research show that the addition of slurry, alongside the application of carbohydrates, to soil has the potential to stabilise C within SOM and to increase C_{mic}. The data suggest that either a competition between soil and slurry microorganisms, or the presence of organic C fractions of slurry, such as the readily biodegradable and/or the recalcitrant organic C fraction of slurry, resulted in lower cumulative respiration and greater C_{mic} after the application of slurry and carbohydrate to soil, compared to the addition of a carbohydrate alone. Furthermore, the addition of SB-amended slurry stimulated microbial cycling of the added C substrate through respiration and an apparent suppression of biomass uptake of the added substrate.

The bacterial dominance in the soil microbial community that was observed in all treatments following the application of slurry and carbohydrates, with G –ve prevailing during the initial stages of decomposition due to the utilisation of more readily available substrates until they became exhausted, was hypothesised to be the reason for the increase in C_{mic}. An increasing dominance of fungi over G +ve bacteria towards more recalcitrant substrates was observed toward the end of the incubation through AS application, thus suggesting low levels of mineral N availability in soil and a lower N leaching potential, compared to soils with bacterial dominance. This study also revealed that the quality of C substrates represented a major factor affecting both the extent of mineralisation and of incorporation of externally-derived C into microbial biomass, with a greater increase in C_{mic} following cellulose application than either glucose or G6P. In addition, as the complexity of the applied C substrates increased, total respiration and the priming effect were observed to decrease, with

preferential substrate utilisation as the microbial mechanism proposed to explain the observed reduction in total respiration, due to the microbial metabolic switch from the respiration of SOM to the more labile carbohydrates added to soil, alongside incorporation of a substantial fraction of the labile carbohydrate into microbial biomass.

Finally, an increase in the P status of soil as a result of greater microbial demand for C compared to P, as well as a decrease in P_{mic}, were observed over the course of the incubations, following the addition of substrates containing C and P moieties to grassland soils. These findings suggest that soil microbial community were not constrained by the availability of P within the grassland soil using in the incubations reported here. However, this increase in the P status of soil due to the microbial demand for C over P could ultimately increase the risk of diffuse P export from soil to water and the potential for adverse environmental impacts in receiving waters. Figure 6.1 displays a conceptual model of the major effects that SB-slurry additive cause on slurry nutrient content, and the major effects that both SB-amended and control slurry, carbohydrates, and NPK inorganic fertiliser cause on soil nutrient content, as well as on soil microbial composition and activity.

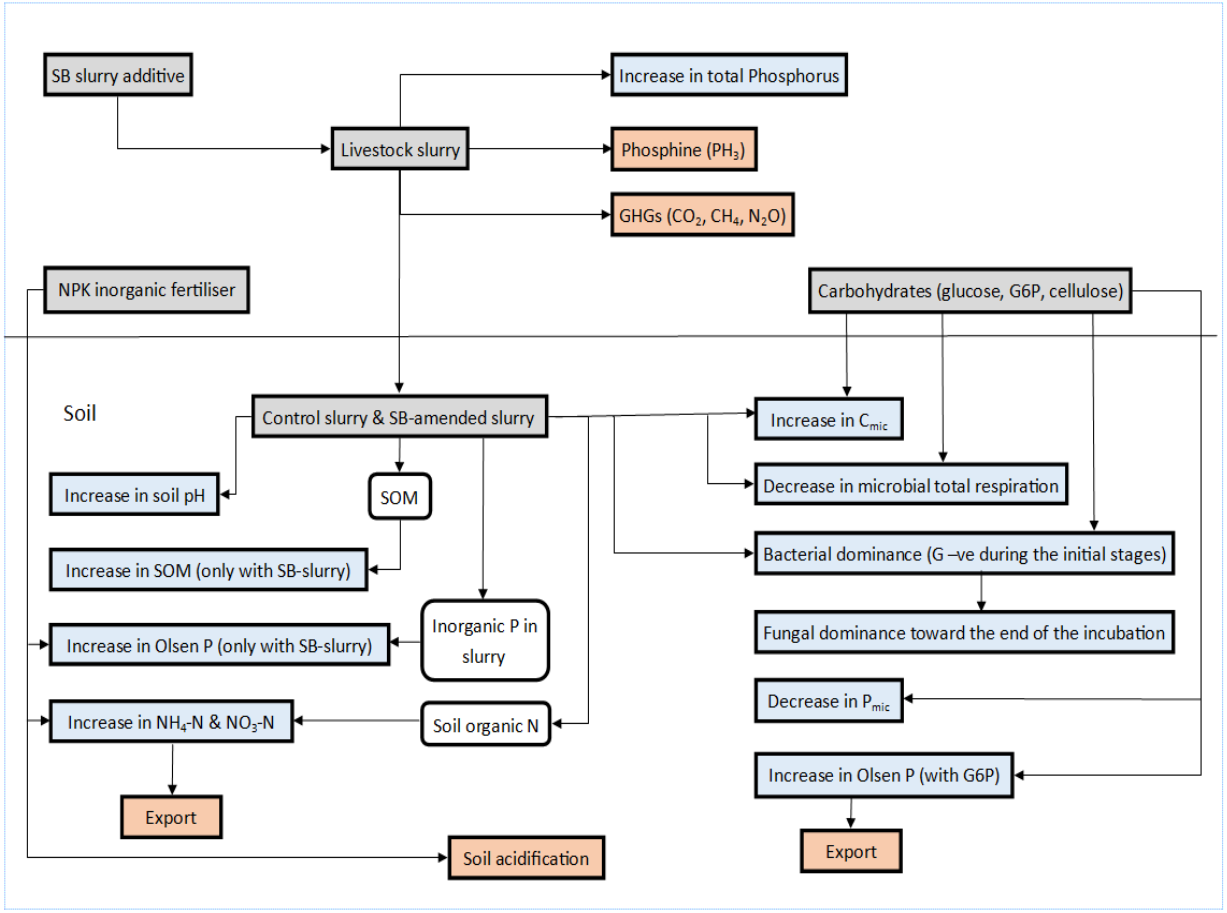

Figure 6.1. Conceptual model of the major effects that SlurryBugs-SlurryBooster (SB)-slurry additive cause on slurry nutrient content, and the major effects that both SB-amended and control slurry, carbohydrates (glucose, glucose-6-phosphate (G6P), and cellulose), and NPK inorganic fertiliser cause on soil nutrient content, as well as on soil microbial composition and activity. Rounded rectangles indicate pools, grey boxes indicate inputs, orange boxes indicate outputs, light blue boxes indicate processes.

6.2 Recommendations for future research

Although the application of the commercial slurry additive SB to slurry resulted in significantly higher concentration of TP compared to the control slurry, no analysis was performed on likely changes in the microbial communities present within the slurry following the treatment of slurry with SB, in order to account for the lack of significant difference in other nutrient elements. The mechanism responsible for the difference in TP content for SB-amended versus control slurry was not identified. However, it was hypothesised that the SB additive resulted in significantly reduced emissions of PH_3 from slurry during storage. Further experimental work is required to understand whether changes in the magnitude of PH_3 emissions are generated following the use of additives, such as SB, during longer-term storage (more than the 9-week storage undertaken in Chapter 3), alongside the specific mechanism responsible for any change in PH_3 emissions from slurry following the application of an additive. Similarly, further research is needed to determine whether changes in the microbial community within slurry during storage are also transferred to changes in soil microbial communities following slurry application, in order to use slurry additives such as SB to ultimately influence PH_3 emissions from soils to the atmosphere.

No significant changes in the NH_4-N or TN content of livestock slurry were observed between control and SB-amended slurries. However, additional work is required using different slurry types and different additives to more broadly assess the impact of slurry additives on the N content of slurry, alongside the microbial and/or physicochemical mechanisms that are responsible for any impact. A higher TS content was observed in SB-amended slurry compared to the control slurry, potentially reflecting greater microbial biomass accumulation following application of the SB

additive to slurry. In order to test this hypothesis, microbial biomass within slurry should be determined, alongside further experimental work to understand the mechanism responsible for higher TS content in slurry as governed by the use of additives such as SB.

With respect to changes in soil nutrient concentrations following application of slurry and inorganic fertiliser, in this thesis a significant increase in SOM content was observed only in O soil as a result of SB-amended slurry application, compared to the inorganic fertiliser treatment. In addition, this short-term study was designed with only one application of an individual treatment. Therefore, longer-term studies (more than 85 days) with repetead additions of organic amendments, such as slurry or FYM, would be useful to establish the SOM dynamics across different soil types in response to the application of organic substrates. In Chapter 3, the potential for accumulation of organic N in soils was also observed following the application of organic amendments, alongside a slow remineralisation of the immobilised NH_4-N pool, in turn contributing to an increase in soil NO_3-N in both slurry treatments during the later stages of the incubation. Thus, longer-term studies are needed with different types of organic amendments, such as slurry or FYM, in order to advise farmers on the importance of the application of these amendments to soil for accumulation of organic N and longer term changes in available N from these soil pools.

With regard to the quality of C substrates affecting the function of the soil microbial community, suppression of soil microbial respiration and assimilation of the added carbohydrate was observed in Chapter 4. It was hypothesised that this suppression is linked with the specific chemical characteristics of the slurry used in the experiments, for example due to particularly low VFA concentrations, or the release of suppressing factors from slurry microorganisms in response to the

competition with native soil microorganisms. However, further research is needed to establish whether variations in the composition of organic compounds in slurry, such as carbohydrates and lignocellulosic materials, or microbial suppression following slurry application to soils is the potential mechanism through which to reduce the microbial respiration of the carbohydrate by native soil microorganisms and to allow accumulation of substrate-C into the cells of the slurry microorganisms.

In Chapter 4 it was observed that, in contrast to previous studies in which PSU normally occurs when the amount of substrate C added to soil is greater than the C_{mic} existing within the soil, the microbial metabolic switch occurred when the added substrate C to soil was lower than C_{mic}. Therefore, further investigation is required to verify whether PSU continues to be evident with relatively low substrate C added to soil as a proportion of C_{mic} across different temperate grassland soils, with and without slurry application. Further research is also needed to test whether longer-term incubation experiments with the same amount of substrate C added to soil ultimately stimulate greater SOM decomposition compared to an 18-day incubation experiment and, therefore, promote a positive PE. Furthermore, a microbial demand for C was suggested to drive a parallel release of P into the bioavailable soil pool, following the application of a phosphorylated C compound to soil. However, further experimental work is needed to confirm the mechanistic basis to a possible coupling between C and P mineralisation in grassland soils.

In Chapter 5 it was hypothesised that the increase in C_{mic} that was observed following the addition of slurry to soil, including when slurry was applied to soil alongside a carbohydrate, was due to the microbial community that switched from the carbohydrates to the labile C in slurry. However, further research is required to test the hypothesis of this switch from the carbohydrate to the labile C in slurry following

slurry application. In addition, although a dominance of bacteria over fungi, and of G –ve over G +ve bacteria, was observed in all treatments, further analyses are needed to determine whether shifts in the balance between fungi and bacteria, and in the G +ve:G –ve bacterial community, can be induced in temperate grassland soils over longer-term incubation experiments (>49 days) under treatments with different quantities of carbohydrates, as well as with amended and unamended slurry. Finally, a limited taxonomic resolution has been observed with PLFA analysis. Therefore, molecular approaches using PCR-based methods and metagenomics analysis are expected to achieve higher resolutions at species- or strain-level for a complete analysis of the microbial community in grassland soil before and after slurry treatments.

6.3 Accumulation of soil organic matter following the application of slurry and other organic amendments to grassland soil

Compared with the application of inorganic fertilisers, the addition of slurry to grassland soil in Chapter 3 resulted in a significant increase in SOM and TOC concentrations during the 85-day incubation. In temporal terms, this type of input to soil is considered to be a pulse or occasional input, differing considerably from continuous or permanent inputs that are typically associated with inputs of leaf and shoot residues, dead roots, as well as some rhizodeposits (Kuzyakov, 2010). Indeed, whilst in the latter cases the organic substrates are often less immediately labile and, therefore, tend to be utilised by the soil microbial community over longer periods of time, pulse inputs can be associated with spatial hotspots of soil microbial activity over the timescale of a few days, in which the turnover rates of these substrates are

much higher than outside of the hotspot area, due to the ready availability of the added substrates themselves (Kuzyakov, 2010). Further, the extracellular enzymes produced to decompose continuous inputs of organic substrates to soil are likely to be more efficient at degrading SOM compared to the intracellular enzymes that hydrolyse easily available substrates associated with pulsed inputs (Fontaine et al., 2003).

A previous study has demonstrated that application of inorganic fertilisers to agricultural soil, in the absence of any organic amendment, can result in significant degradation of soil quality, in particular due to the loss of SOM (Fan et al., 2005). Alongside inorganic fertiliser application, additional agricultural practices can have detrimental effects on soil quality, including through promoting rapid mineralisation of SOM that decreases soil C stocks and increases CO_2 emissions to the atmosphere (Quinton et al., 2010, Bhattacharya et al., 2016). Specifically, tillage represents one of the main causes of SOM depletion, due to the imbalance between the mass of organic C from soil plus photosynthetically-fixed C that is removed from soil through harvesting of crops, compared to the mass of C returned to soil through the input of organic matter (Janzen, 2006). Additional land use practices, including overgrazing and excessive harvesting, are also considered important agricultural activities that severely degrade terrestrial ecosystems through depletion of SOM (Evrendilek et al., 2004). In the absence of regular inputs of organic matter to agricultural soils, the soil microbial community will continue to degrade SOM, resulting in depletion of SOM in grassland soils over time. This was emphasised through the significantly higher cumulative CO_2 released by soil microorganisms in the control treatment with no substrate added, compared to all other treatments, as reported in Chapter 4 of this thesis.

A number of studies have established that, compared to inorganic fertiliser addition, significant accumulation of fixed C as SOM may follow the application to soil of a range of organic amendments, including slurry/FYM, compost, sewage sludge, crop residue, anaerobic digestate, biochar and food waste (Gregorich et al., 2001, Benjamin et al., 2010, Roig et al., 2012, Kätterer et al., 2014, del Mar Montiel-Rozas et al., 2016, Parmar et al., 2016). In particular, work has sought to enhance the density of organic C in soil, improve its depth distribution and stabilise organic C within micro-aggregates in order to protect C from microbial degradation, or to decrease SOM turnover time through increasing the proportion of recalcitrant C in soil (Lal, 2004). In addition, it has been argued that the proportion of C from organic amendments that is retained in soil over longer timescales (decades or more), is dependent on the properties of the organic substrates themselves (Gerzabek et al., 1997, Peltre et al., 2012).

Bronick and Lal (2005) suggested that agricultural practices, such as the application of organic amendments to soil, that reduce decomposition rates both of SOM and added substrates, also help to enhance SOM storage in soil. This was observed in Chapter 4, with the addition of slurry alongside a carbohydrate tending to reduce respiration and incorporation into biomass of added C, resulting in accumulation of added C within more recalcitrant soil pools. Interestingly, after a 174-day incubation with different organic amendments, including chicken manure, wheat, peat and sawdust, Clark et al. (2007) observed two distinct phases of decomposition of organic residues added to soil. Specifically, these authors suggested a model of initial rapid decomposition, followed by subsequent protection of the residual C. Slower respiratory activity and, therefore, reduced loss of C from soil was also associated with application of less mature residues. This was likely due to physical protection

mechanisms, such as the formation of bacterial extra-cellular polysaccharides during bacterial dominance of the early stage of decomposition, because of the significantly higher concentration of readily degradable material within younger, more labile residues, compared to more mature residues, that promotes bacterial growth (Hu et al., 1999, Eiland et al., 2001). Consequently, these polysaccharides allowed the formation of soil aggregates (Chapman and Lynch, 1985, Amellal et al., 1999, Alami et al., 2000) and the accumulation of more stable SOM, due to reduced accessibility for larger soil microorganisms, such as fungi or nematodes, to these residues compared to more mature amendments to soil (Clark et al., 2007).

Composting is a traditional practice to stabilise and sanitise mixtures of organic substrates through biodegradation processes carried out by microbial communities (Insam and De Bertoldi, 2007). A significant increase in SOM content has been observed under repeated applications of farm compost compared to inorganic N fertiliser (D'Hose et al., 2014). Further, the increase in SOM and related soil quality properties, including water holding capacity and cation exchange capacity, were hypothesised to be the main factors responsible for an observed increase in crop production (D'Hose et al., 2014). Sauerbeck (1982) observed that when different organic amendments were applied to soil, accumulation of SOM increased in the order green manure < straw < fresh FYM < composted FYM. Similarly, Johnston (1975) found that the increase in SOC per ton of organic amendments applied to soil was significantly greater for composted compared to fresh inputs. Due to the increased hydrolysis of the organic substrates during composting, when composted materials are applied to soil they are relatively more resistant to further breakdown compared to fresh substrates, resulting in greater increases in SOM (Haynes and Naidu, 1998). However, the findings reported by Sauerbeck (1982) and Johnston (1975) are

apparently in contradiction with those of Clark et al. (2007), where the addition of less mature (i.e. not composted) materials to soil resulted in greater accumulation of SOM compared to the addition of more mature materials. The results within Chapter 4 of this thesis (see Sections 4.4.1–4.4.2) are in agreement with those reported by Sauerbeck (1982) and Johnston (1975), suggesting that, as the recalcitrance of an organic amendment increases, both the extent of mineralisation and of incorporation into microbial biomass of externally-derived C decreased, thus resulting in a SOM pool that was more stable and resistant to microbial degradation.

The application of crop residue to soil represents another common agricultural practice that has the potential to enhance SOM content. Within intensive farming systems, increases in crop yield may significantly raise SOM content, due to higher quantities of crop residues that are returned to soil compared to scenarios in which inorganic fertiliser application occurs without the return of any residue to land (Mandal et al., 2007). Mandal et al. (2007) also found that the quality of crop residues returned to soil can have significant impacts on the amount of C sequestered in soil. In fact, rice and wheat residues were observed to have a low N content, thus representing more effective ways of increasing SOM compared to residues, such as jute or berseem, that contain a higher N content and are, therefore, more likely to be decomposed by soil microorganisms. Further, Triberti et al. (2008) reported that after a 29-year soil incubation, the application of FYM to soil promoted more rapid SOM accumulation compared to cattle slurry or to crop residues, due to the greater proportion of less readily degradable SOM in FYM compared to in the other two substrates. However, this suggestion is somewhat in contrast to the data reported in Chapter 4, where reduced uptake of added C into microbial biomass was observed

following the application of slurry alongside a C substrate, compared to treatments with C substrates alone.

More recently, biochar (biomass-derived black C) has been studied as a further organic amendment that may be applied to soil and that may lead to the accumulation of SOM, because of the relatively inert forms of C present within biochar that represent a low risk of CO_2 emissions (Atkinson et al., 2010). Further, due to its porous nature and high affinity for SOM (Kasozi et al., 2010), biochar may sequester non-biochar OM in soil, protecting it from both microbial and abiotic degradation (Zimmerman et al., 2011). However, some studies have reported both rapid and slow decomposition of biochar (Shindo, 1991, Bird et al., 1999). Despite the uncertainty regarding the potential for turnover of biochar within soil, when this material has been applied to soil, black C has been observed to be one of the oldest and most stable forms of C in soil, due to aggregation and physical protection of black C particles (Pessenda et al., 2001). Therefore, biochar represents one of the lowest-risk strategies for long-term SOM accumulation, compared to the other options that have been previously described, including fresh and composted FYM, in which the risk of release of CO_2 from the materials added to soil may be greater (Lehmann, 2007).

In Chapter 3 it was observed that the addition of slurry to soil resulted in a significant increase in SOM in the organic-rich soil type. Organic C taken up by soil microbial biomass following the addition of organic substrates to soil is partitioned among microbial biomass production, respiration and metabolite excretion (Six et al., 2006). The proportion of substrate-C incorporated as microbial biomass versus respired as CO_2 depends on the efficiency with which organic amendments are incorporated into bacterial and fungal biomass. In turn, this relies on substrate quality (C/N ratio) and the capacity of soil to protect microbial biomass (Six et al., 2006).

6.4 Potential reductions in soil organic matter following the application of slurry and other organic amendments to soil

Although the application of organic amendments may result in SOM accumulation, as discussed in Section 6.2, the application of fresh substrate to soil can also trigger microbial activity that results in net SOM reduction, a phenomenon known as a positive priming effect (PE) (Löhnis, 1926, Jenkinson et al., 1985, Kuzyakov et al., 2000). However, a negative PE may also be measured under certain circumstances, representing a temporary decrease in the rate of decomposition of SOM within a soil that receives a substrate, compared to a control soil with no substrate addition, because microbial utilisation of an added substrate C may be two to five times higher than C_{mic} (Blagodatskaya and Kuzyakov, 2008). Further, the quantity of a substrate added to soil relative to C_{mic} can switch the direction of the PE (Blagodatskaya and Kuzyakov, 2008). In Section 4.5.2, a negative PE was determined in all treatments following the application of carbohydrates and slurry to grassland soil. Therefore, it appears possible that the input of substrates, including slurry, to soil can effectively 'protect' SOM from degradation, due to a negative PE. According to Kuzyakov and Bol (2006), this preferential substrate utilisation expressed by soil microorganisms towards the added substrate can last for a few days to a few weeks, before the microbial community returns to the initial pathways of SOM decomposition.

A number of studies have investigated the effects on PE of applying organic amendments of different quality to soil, including slurry, compost, biochar, sewage sludge, crop residues, anaerobic digestate and food waste (Bernal et al., 1998, Johnson et al., 2006, Fangueiro et al., 2007, Cross and Sohi, 2011, Luo et al., 2011,

Zimmerman et al., 2011). In a 56-day incubation with dairy slurry that had been previously passed through sieves before application to a UK grassland soil, producing six different size fractions (> 2000, 425 – 2000, 250 – 425, 150 – 250, 45 – 150 and < 45μm), Fangueiro et al. (2007) observed both positive and negative PE at different stages of the incubation, depending on the slurry fraction added to soil. In particular, the authors observed that a positive PE occurred later in the incubation in the treatment involving coarser slurry fractions, compared to the treatment with the finest size slurry fractions. Further, an earlier negative PE was observed in the treatment with the finest size slurry fraction (Fangueiro et al., 2007). In contrast to the study of Fangueiro et al. (2007), where both positive and negative PE was observed, Bol et al. (2003b) only observed a positive PE during a nine-day incubation with cattle slurry that was added to a UK grassland soil. Although the study of Bol et al. (2003b) did not analyse different slurry fractions, it may be assumed that differences in the sampling times of both slurry and soil (summer versus winter) can account for different observation in terms of PE for these two studies. These different sampling times may have affected the slurry and/or soil quality, resulting in a decrease in the readily mineralisable C that, in turn, produced only a positive PE in the study of Bol et al. (2003b).

The chain of mechanisms involved in the PE, as suggested by Kuzyakov and Bol (2006), can provide the explanation for both positive and negative PEs at different times during an incubation. In fact, the earlier negative PE observed in the finest slurry fractions during the first days of incubation by Fangueiro et al. (2007) may correspond to the preferential substrate utilisation, as reported in Section 4.5.2. This involves soil microorganisms switching from the decomposition of more recalcitrant SOM to the more labile compounds within slurry and specifically to those within the

finest fractions of slurry. In contrast, the delayed positive PE observed in the coarser particle fractions may be attributed to an increase in microbial activity towards the more recalcitrant coarser slurry fraction, after exhaustion of the more labile, smaller sized slurry fractions. Finally, by the end of the incubation, the initial state of the soil was re-established in all treatments as a result of the decline in microbial biomass and activity (Fangueiro et al., 2007). Consequently, due to both an increase and a decrease in SOM losses induced by applying slurry of different size classes, these findings have important implications for better optimisation of future slurry application to soil (Fangueiro et al., 2007). The application of slurry, in particular of the finest fraction (< 45µm), due to a possible decrease in C:N ratio of the remaining residue after the initial labile decomposable C losses from slurry, results in a negative PE that, in turn, drives SOM accumulation. Similarly to the results of Fangueiro et al. (2007), a negative PE was observed in Chapter 4, presumably because soil microorganisms preferentially degraded the carbohydrates added to the soil rather than the more recalcitrant SOM. However, such SOM accumulation as a result of the negative PE is not expected to persist, because the preferential degradation of an added substrate by soil microorganisms is only a temporary effect until that substrate is consumed.

As described in Section 6.2, the application of biochar to soil is a potentially important route for the sequestration of C. However, Zimmerman et al. (2011) observed seemingly contradictory results with both positive and negative PE following the addition of different types of biochar to different soils during a 548-day incubation. Specifically, positive PE was found during the first 90 days of incubation in soils receiving biochar produced from grasses at low temperatures, whereas negative PE occurred in soils treated with biochars from hard woods at high temperatures during later stages (day 250-500) of a soil incubation (Zimmerman et al.,

2011). The mechanism proposed by Fontaine et al. (2003), involving the growth of r-strategist soil microorganisms that are adapted to respond quickly to fresh substrates applied to soil, is consistent with the rapid increase in biochar decomposition and, thus with the positive PE reported by Zimmerman et al. (2011).

Although PE concerns the effect of an amendment on SOM degradation, rather than whether the substrate added is itself labile or recalcitrant, the type and quantity of biochar applied to soil caused the activation of part of the microbial community that resulted in a significantly higher SOM decomposition, compared to the treatment without biochar. Further, a negative PE was hypothesised to be largely the result of SOM sorption to biochar, either by encapsulation within the porous structure of biochar with no biotic response or by sorptive protection onto external biochar surfaces (Kaiser and Guggenberger, 2000). Therefore, the sorption of SOM to biochar protected the SOM from microbial degradation, reducing the rate of SOM mineralisation compared to a control soil and resulting in a negative PE. As a result, since the negative PE occurred during the later stage of the incubation, this is the direction of PE that might be expected to endure into the future, thus making biochar one of the best organic amendments in agriculture to deliver long-term increases in total soil organic C (Luo et al., 2011). Differently from biochar, slurry application is not expected to produce long-term SOM accumulation, due to the temporary microbial utilisation of the added substrate. Therefore, in contrast to biochar, in order to ensure long-term maintenance of SOM content, the frequency at which slurry is applied to soil will be critical in order to promote a negative PE for prolonged periods of time.

6.5 Effects of slurry versus inorganic fertiliser application to soil on nutrient management within grassland systems

The continued use of inorganic fertilisers as the dominant source of nutrients within production systems has generated a number of environmental and economic challenges that scientists, farmers and stakeholders have to face nowadays. In fact, the production of inorganic N fertilisers through N fixation (the Haber-Bosch process) represents the most energy-intensive input to modern agriculture, with over 90% of the worldwide energy required for fertiliser production and approximately 1.1% of energy use globally associated with synthesising inorganic N fertiliser (Dawson and Hilton, 2011). In contrast to inorganic N fertilisers, the production of inorganic P fertilisers is reliant on P extracted from phosphate rock deposits that are finite and geopolitically constrained in their global distribution (Cordell et al., 2009). In fact, although the mass of P present globally is never reduced, as phosphate rock mining continues, high-grade P reserves will become increasingly exhausted. In turn, this will increase reliance on remaining phosphate rock reserves with lower P concentration and higher contaminant concentration that are also physically harder to access, meaning the generation of more waste materials and increased extraction costs (Cordell and White, 2014). In addition, phosphate rock reserves are distributed unevenly across the globe, exposing the majority of countries which rely on inorganic phosphate fertiliser supplies, but which lack their own phosphate rock deposits, to geopolitical risks surrounding future access to inorganic P fertiliser. For example, 90% of the rock P reserves in the world are controlled by only six countries: Morocco, China, Algeria, Syria, South Africa and Jordan (Cordell and White, 2015). In turn, Morocco alone, because most of the reserves are situated in Western Sahara that is

currently occupied by Morocco, controls 74% of the worldwide P reserves (Cordell and White, 2015).

A number of advantages have already been described in applying slurry and FYM, as well as other organic amendments, including compost, sewage sludge, food waste, crop residues, anaerobic digestate and biochar, to soil compared to inorganic fertilisers (Steinbeiss et al., 2009, Alburquerque et al., 2012, Usman et al., 2012). As reported in Chapter 3 (see Section 3.4.2), the addition of slurry to grassland soil resulted in significantly higher NH_4-N and Olsen P concentrations compared to inorganic fertiliser through the 85-day incubation. A single application of slurry/FYM to the soil at the beginning of plant growth cycles may be sufficient to sustain the growth rate and have significantly longer effects compared to the application of inorganic fertiliser (Adegbidi et al., 2003). An example of single slurry application producing this type of effect is described in Chapter 3 (Section 3.4.2), particularly for mineral N in slurry-treated soils, compared to soils receiving inorganic fertiliser. With respect to N, prolonged positive effects on N availability in soils have been associated with slower N release following slurry/FYM compared to inorganic fertiliser application, primarily as a result of mineralisation of organic N compounds added to soil via the organic amendment (Adegbidi et al., 2003).

According to Flavel and Murphy (2006), the addition to soil of different types of organic amendments, such as poultry manure, green waste-based compost, straw-based compost, and vermicompost, resulted in significantly higher N concentrations compared to inorganic N fertilisers that, in turn, warranted a reduction in inorganic N fertiliser application. Therefore, there is an increasing tendency in organic farms to acquire the bulk of their N through organic inputs, instead of relying on manufactured N fertilisers (Badgley et al., 2007). However, research has also suggested that, in

common with inorganic fertiliser application, the application of organic amendments to agricultural soil should be conscious of the potential for over-application of N, resulting in adverse environmental impacts. For example, Flavel and Murphy (2006) suggested that a reduction in the application rate of poultry manure to soil, due to the high amount of N released from this organic input compared to inorganic fertiliser should be considered, in order to minimise possible leaching losses of N in areas where groundwater quality is of concern. The other common agricultural practice of growing temporary leguminous cover crops (green manure) has also been demonstrated to be extremely useful not only to minimise the reliance on inorganic fertilisers, but also to increase SOM concentration and to provide an energy source for soil biota that enables humus production through SOM decomposition (Smith et al., 2015).

The increase in energy costs and GHG emissions associated with future mining of lower-grade phosphate rock reserves, alongside geopolitical concerns linked with the distribution of and access to phosphate rock reserves, represent compelling incentives for optimising the use of organic sources of P on farms, including slurry/FYM, crop residues, food waste and compost, not least because these sources of P may be more cost-effective per unit of available P compared to mining, processing and shipping phosphate rock (Cordell et al., 2009). Indeed, differently from inorganic P fertilisers, organic amendments may be considered renewable sources of P (not considering the feed supplements for cattle that are themselves derived from phosphate rock reserves), and represent a viable route through which agricultural production systems may become less reliant on finite and geo-politically constrained inorganic P fertilisers (Cordell and White, 2011, Dawson and Hilton, 2011). In particular, in contrast to parts of Europe and North America where recent fertiliser demand within agriculture has

stabilised following decades of over-applications of inorganic P fertilisers compared to crop demand, an alternative scenario characterises developing countries and emerging economies (Cordell and White, 2011). Within these nations, demand for P is increasing rapidly alongside growing demand for food, because of sub-optimal P availability in many agricultural soils (Cordell and White, 2014). Given that the price of P fertiliser on the global market may prohibit such nations from meeting demand for P through inorganic sources, increasing utilisation of organic amendments to meet the growing demand for P in developing countries and emerging economies may be a viable alternative strategy (Cordell and White, 2014).

In contrast to inorganic fertilisers, increased utilisation of slurry and other organic amendments avoids both use of non-renewable resources, e.g., phosphate rock and fossil fuels, and excessive energy costs for production of inorganic fertilisers (Dawson and Hilton, 2011). Furthermore, the utilisation of organic amendments has the potential to make agricultural production more sustainable, by lowering fossil fuels and energy inputs, as well as reducing economic and geopolitical imbalances (Pimentel et al., 2005).

6.6 Soil acidification following inorganic fertiliser versus slurry application

The application of slurry to soil affected soil pH in the experiments reported in this thesis, for example resulting in a significantly higher pH compared to control and inorganic fertiliser treatments during an 85-day soil incubation as reported in Section 3.4.2. According to Rengel (2003), soil acidification is a globally-distributed phenomenon. Rengel (2003) classified acid topsoils and subsoils of varying intensity for various regions of the world and, globally, between 3.78 and 2.92% of topsoils and

subsoils, respectively, fell into acidic soil classes. Beyond Europe, regions in the eastern USA, the former Soviet Union and large parts of Asia, many parts of South America, as well as West, East and Southern Africa are at risk of soil acidification (Bouwman et al., 2002). Soil acidification occurs under natural conditions through thousands of years, but is accelerated by a number of practices associated with agricultural production (van Breemen et al., 1984, Blake et al., 1999). Acidic precipitation and atmospheric deposition of acidifying gases and particles, such as NH_3, sulphur dioxide, nitric and hydrochloric acids, are the main causes in all unfertilised agricultural, natural and semi-natural lands (Goulding, 2016). In fact, although NH_3 is itself an alkaline compound, it can cause acidification through its deposition from the atmosphere and transformation to NO_3-N (Goulding, 2016).

Among the factors that cause soil acidification in agriculture, the fixation of atmospheric N_2 by legumes, such as clover, has been identified as one of the most common ways in which soil pH is reduced. This occurs through NH_3 formation within root nodules, the uptake of an excess of cations and net H^+ release into the soil to maintain charge balance during the uptake process (Marschner, 2012). The application of inorganic N fertilisers represents another common cause of acidification of agricultural soils, especially under intensive farming practices (Goulding and Annis, 1998, Goulding and Blake, 1998). In particular, the application of ammonium-based and urea fertilisers, followed by subsequent nitrification, can contribute significantly to soil acidification, through the release of protons as defined by Equations 6.1 and 6.2 (DEFRA, 2010, Goulding, 2016):

$$NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \qquad (6.1)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad (6.2)$$

Soil acidification can significantly affect chemical and biological processes within agricultural soils. For example, long-term acidification of grassland and woodland soils can irreversibly compromise the cation exchange capacity of a soil, mobilise Al, Fe and Mn to potentially toxic concentrations, alongside being associated with structural deterioration of soil (Blake and Goulding, 2002). Decreases in soil pH can also reduce crop plant growth through effects on the bioavailability of both major plant nutrients, such as N, P, K and S, but also some micronutrients, such as Ca, Mg, boron, copper, zinc and molybdenum (Goulding, 2016). Therefore, the application of liming materials, including ground limestone, chalk, burnt lime and hydrated lime, is a common agricultural practice to neutralise soil acidity associated with the applications of inorganic N fertilisers, alongside other naturally-occurring soil acidification processes (Hoyt and Hennig, 1982, Lickacz, 2002, Opala et al., 2012). In particular, Bennett et al. (2014) pointed out that liming material improves soil aggregate stability, hydraulic conductivity, total C and N, soil respiration, as well as vegetation cover. Furthermore, more recently lime has received much attention due to its impacts on C sequestration by soil and, thus, on climate change (Goulding, 2016). According to Paradelo et al. (2015), the increase in crop yields and, therefore, residue returns is the reason for the increase in soil C content following lime application. Fornara et al. (2011) also observed a significant increase in net C sequestration in limed compared to unlimed soils, despite the increase in soil respiration rates following liming.

However, due to the high cost and lack of availability of liming material in many areas, particularly in developing countries, attention has been diverted to plausible alternatives to address soil acidification without the need to apply liming materials (Wang et al., 2012). Notably, application of organic amendments, such as chicken and cattle manure, as well as sewage sludge, has been shown to positively affect soil pH

(Hue, 1992, Lupwayi et al., 2014). According to Eghball (1999), changes in the pH of cattle-manured soil are due to buffering from $CaCO_3$, because excess $CaCO_3$ provided in cattle diets may be excreted in manure and subsequently applied to soil via manure. However, Whalen et al. (2000) found significantly higher soil pH following manure application compared to control soil, even though only large quantities of bicarbonate but no carbonate were observed in fresh manure and soil treated with manure. Therefore, due to the half reactivity of bicarbonate compared to carbonate, it was hypothesised that compounds other than bicarbonates and carbonates, such as carboxyl and phenolic hydroxyl groups, have a significant role in buffering soil pH and lowering the acidity of soils treated with manure (Whalen et al., 2000). Therefore, the potential positive effects of organic amendments on soil pH can be summarised as: the potential to reduce the input of inorganic N fertilisers that may drive soil acidification during nitrification; the potential for input of pH buffering compounds to soil directly within the slurry; through both these effects, the potential to help to mitigate the adverse impacts of soil acidification on nutrient availability and the need to rely on liming materials to manage pH in agricultural soils.

6.7 Effects of slurry application to soil on phosphine emission from agriculture

In Chapter 3, the possible release of phosphine (PH_3) from slurry during storage was hypothesised as an explanation to account for the lower TP content in control slurry compared to slurry that had received the SlurryBugs additive during storage (see Section 3.4.1). Phosphine is a reactive and reduced P compound that has been recognised as a gaseous P carrier in global biogeochemical cycles in two distinct

forms: free gaseous PH_3 and matrix-bound PH_3 (Eismann et al., 1997b, Glindemann et al., 2005). According to Han et al. (2011), a seasonal distribution of PH_3 has been observed in marsh and paddy fields with significant association with high temperatures and increased vegetation. A number of studies have highlighted PH_3 emissions from manure, slurry, marsh gas, sewage treatment plants and municipal solid waste, as well as from anaerobic sediments, sludge and soils, where PH_3 is present in matrix-bound form (Feldmann and Hirner, 1995, Glindemann and Bergmann, 1995, Eismann et al., 1997a, Eismann et al., 1997b, Cao et al., 2000, Roels and Verstraete, 2004). Phosphine has received much attention due to lethal effects in humans through inhibition of aerobic respiration, as well as because of genotoxic effects to lymphocytes (Garry et al., 1989, Jenkins et al., 2000). Phosphine has also shown harmful behaviour in the environment, competing in the atmosphere with some GHGs for hydroxyl radicals and, therefore, enhancing an indirect greenhouse effect (Prinn, 1994).

Phosphine release has been shown as the reduced gaseous end product of manure and slurry fermentation from obligate anaerobic bacteria (Glindemann et al., 1996, Jenkins et al., 2000). The emission is estimated to occur not only during slurry storage and transport but also after application of slurry to soil (Eismann et al., 1997a). According to Glindemann and Bergmann (1995), the PH_3 released by pig slurry is greater than that produced by cattle slurry, with lytic processes involved in PH_3 release from both slurries. Eismann et al. (1997a) hypothesised that grain fumigation could represent a further source of PH_3 in manure/slurry before animal manure enters manure treatment plants, due to matrix-bound PH_3 residues observed in the feed. However, Jenkins et al. (2000) proposed that PH_3 is generated by some microorganisms through reduction of phosphates during slurry storage. Several

fermentative bacteria, such as mixed acid fermenters (*Escherichia coli* and *Salmonella* spp.) and solvent fermenters (*Clostridium* spp.) were hypothesised as the microorganisms able to generate PH_3. In addition, a possible correlation between PH_3 production and methanogenesis has been proposed, due to concurrent emission of these gases in natural sediments, such as harbour mud, as well as in sewage sludge digesters (Dévai et al., 1988, Gassmann and Glindemann, 1993, Gassmann and Schorn, 1993, Jenkins et al., 2000) and during anaerobic fermentation of swine manure (Eismann et al., 1997a). In addition, the central role of PH_3-generating fermentative bacteria in the multi-stage process of methanogenesis can account for correlation between PH_3 and CH_4 production, although Eismann et al. (1997a) claim that no evidence has been produced for a causal connection between the metabolic activity of methanogenic bacteria and PH_3 production.

Phosphine has also been found to be released after manure/slurry application to soil (Eismann et al., 1997b). In particular, PH_3 emission into the atmosphere is the result of a sequence of slow processes in soil, PH_3 desorption from soil particles, its diffusion through the soil and/or soil water, and its release into the atmosphere (Cao et al., 2000). Furthermore, it was observed that the addition into sediments of P-containing biogenic materials, such as sterilised and dried chicken faeces and animal bone powder, resulted in a significant increase in PH_3 emission compared to a control treatment, thus showing that matrix-bound phosphine in sediments serves as a PH_3 pool (Cao et al., 2000). It was hypothesised that emissions of PH_3 differed between the two slurry treatments reported in Chapter 3, and specifically that the SB additive likely reduced the emission of PH_3 from slurry. The dominance of specific microbial groups in SB-amended slurry, such as *Bacillus* spp., alongside *Bacillus* spp. in the additive, may account for reduced PH_3 emission compared to the control slurry. It is

possible that more widespread treatments of slurry with additives such as SB could lower PH_3 emissions, both from storage and from soil following application, although further research would be required to test this hypothesis. If proven, treatment of slurry with biological additives such as SB could help to maintain the TP 'value' of slurry whilst also reducing the adverse environmental impacts of PH_3 emissions to the atmosphere.

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