The Effects of Land-use, Tillage and Earthworms on Biosolid-Soil Interactions

by
Kirsty Marie Elliott

Faculty of Science

Department of Animal and Plant Sciences

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Declaration

I, Kirsty Marie Elliott, confirm that this Thesis is my own original work. I am aware of the University's Guidance on the Use of Unfair Means (www.sheffield.ac.uk/ssid/unfair-means). This work has not been previously presented for an award at this, or any other, university.

At the time of submission, no work from this thesis has been published.

KMElliott

Kirsty Marie Elliott

March 2021

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Summary

Conservation agricultural practices, including reducing tillage, are becoming more popular across the globe to help combat soil degradation and depletion of organic carbon caused by long term intensive agriculture. At the same time, the reintroduction of organic materials to agricultural land provides an end use for waste products while reportedly having beneficial effects on soil quality. Biosolids are one form of organic material derived from wastewater treatment. Their disposal to land is regulated through assurance schemes and codes of practice that require them to be mechanically incorporated into the soil in most instances, which is incompatible with the increasing use of minimal or no-tillage agriculture.

This thesis aims to assess the possibility of enhanced benefits from applying biosolids under reduced tillage, and to evaluate the possibility of updating regulations to allow for the surface application of biosolids. To do this, a large experiment was conducted on intact soil monoliths from a range of land managements from conventional intensive arable through leys and permanent pasture with biosolids surface applied, including manipulation of earthworm populations. A range of biological, chemical, and physical soil measurements were taken throughout a growing season. To ascertain the incorporation of the biosolids into the monolith soil, a novel method utilising a fluorescent tracer was pioneered and used to study the abundance of biosolids by depth. Biosolids showed a consistent but non-significant trend reducing the level of soil macroaggregation in the monoliths. To gain further insight into this, a pot experiment was conducted to confirm the effect of biosolids on soil aggregation, as well as the contribution of flooding and soil saturation to disaggregation.

The findings provide new insights in demonstrating that although biosolids add organic matter and nutrients to agricultural soils, their effects on soil structure may be slightly detrimental, or give only very modest improvements. Surface applications of biosolids appear to be incorporated more rapidly in soils under leys or grassland cover than ploughed arable soils that are more biologically and structurally degraded. The finding highlights the need for further research on the effects of biosolids processed in different ways on a broader range of soil types, to study the effects of surface applications and their interactions with climate change variables such as more extreme drought and rainfall events.

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Abbreviations

AD	Anaerobic digestion
ADLS	Biosolids that have been anaerobically digested and then lime stabilised
ADTH	Biosolids that have been thermally hydrolysed and then anaerobically digested
AHDB	Agricultural and horticultural development board
CEC	Cation exchange capacity
CN	Carbon and nitrogen
CO_2	Carbon dioxide
CT	Conventional tillage
DEFRA	Department for farming and rural affairs
EA	Environment agency
EEC	European economic commission
EU	European union
HCl	Hydrochloric acid
K	Potassium
LEY	Ley (grass clover mixture)
LEY-CT	Ley converted to conventional tillage arable
LEY-e	Ley (grass clover mixture), treated to kill earthworms and earthworm cocoons
LEY-NoT	Ley converted to no-tillage arable
LS	Lime stabilisation
LTA	Long term arable
LTA-e	Long term arable, treated to kill earthworms and earthworm cocoons
LTP	Long term pasture
M	Molar
MT	Minimum tillage
N	Nitrogen
NoT	No-tillage
NVZ	Nitrate Vulnerable Zone
P	Phosphorous
PTE	Potentially toxic element
SOC	Soil organic carbon
SOM	Soil organic matter
TDS	Total dry solids
THP	Thermal hydrolysis process
UK	United Kingdom
WSA	Water stable aggregates

Chapter 1: General introduction - agricultural land management, climate change, biosolids, and their interactions.

This chapter seeks to give a general introduction to the topics covered in this thesis to provide the reader with a current “state of the art” perspective and sufficient knowledge to understand subsequent chapters' content fully. Starting with a history and current issues in agriculture, moving through agricultural land management and its effect on soils, biosolids their production and use in agriculture and finally changing weather and climate patterns. At the end of this chapter, gaps within the field are drawn together and research questions for the thesis presented, followed by an outline of the thesis and the content of each chapter described in brief.

1.1 Introduction

1.1.1 Background to arable agricultural production

Since humans first started cultivating the land to produce food to sustain themselves, there has been a significant shift in ethos from subsistence and sustainable food production on a small scale to intensified monocultures spanning acres run for maximum output and profit, being driven by industrial development, increasing populations and urbanisation (Godfray & Garnett, 2014). With the global population exponentially growing, it is now estimated that global food demand will require an increase of 69%, 1 billion tonnes, of annual cereal production and an increase of 57%, 200 million tonnes, of annual meat production compared to 2005 production levels (Alexandratos & Bruinsma, 2012) to feed a 79% increase in global population to 9.7 billion by 2050 (United Nations, 2017). A review of major cereal and crop production trends shows that although global supply is increasing in many areas, including Europe, growth trends are declining and beginning to plateau (Brisson *et al.*, 2010; FAO, 2009). Faced with this projection, the agricultural industry and researchers are increasingly concerned about food production systems' capability to sustainably intensify to meet demand (Godfray & Garnett, 2014). Historical intensification of crop production can be attributed to the mechanisation of farming practices since the industrial revolution in the 1800s and the introduction of fertilisers, chemicals, and higher-yielding varieties since the green revolution in the 1950s (Godfray & Garnett, 2014). Since then, agronomic inputs of Nitrogen (N), Phosphorus (P) and Potassium (K) have been increasing year on year to increase yields to meet demand, the latter two fertilizers being obtained from the mining of finite mineral resources (Cordell *et al.*, 2009).

1.1.2 Current issues

Bolstering food production levels to meet future demand is but one solution to feed a growing population. There are issues within the agricultural sector, including nutrient security and widespread soil degradation, which require addressing if even current rates are to be maintained. Current nutrient use efficiencies are very low; Withers *et al.*, (2014) concluded that of all agricultural inputs of P, efficiency from source to end-user is less than 20% with only 15 - 30% of fertilisers (N, P, K) applied being utilised by plants (Roy *et al.*, 2006). There are two major problems with this. Firstly, the long-term sustainability of a production system reliant on finite resources, and secondly, the fate of the excess nutrients in the soil not taken up by crops. These are lost from the soil to watercourses either by leaching (N, K) or by the loss of soil particulates through wind and water erosion (especially P), causing pollution.

Long term supply of essential macronutrients is uncertain. Phosphate rock is the primary source of P used in fertilisers and is a finite resource with current estimates for resource longevity ranging 50 - 400 years depending on the source (Heffer *et al.*, 2006; Hilton, 2006; Smit *et al.*, 2009; USGS, 2010). Similarly to P, although smaller amounts of K are required for plant growth, it is solely sourced from geological materials (Manning, 2010). While K is the 7th most abundant element in the earth's surface (Manning, 2010), it takes a long time to be released from rocks through natural processes. Consequently, it is processed industrially to be applied as potash to agricultural land (Manning, 2010). However, unlike P, there is no evidence to suggest K supply will become a future limiting factor. In contrast to geologically sourced nutrients, the Haber-Bosch process can be used to fix N from the atmosphere and is considered energy efficient (Dawson & Hilton, 2011). Nevertheless, this is reliant upon energy production derived mainly from fossil fuels; 1.1 % of global energy production is used to support half of global food production (Dawson & Hilton, 2011). Any future energy insecurity could threaten this source of N fertiliser. With P sources limited and isolated to a few geographical locations, mainly in N. Africa (USGS, 2010), future political insecurities could threaten their supply.

Exacerbating food production issues further is the ongoing degradation of arable soils all over the world. Continuous cultivation of arable fields and intensification since the green revolution has led to the loss of essential soil constituents, including soil organic matter (SOM) and soil organic carbon (SOC). Soil organic matter is made from the decomposition of plant residues, roots and soil organisms and early research attributed 58% of SOM as carbon, a factor of 1.724 (Van Bemmelen, 1890). More recently, it has been evaluated that SOM/SOC relationships can range from 1.4 to 2.5, depending on the soil type and properties (Pribyl, 2010). Maintaining SOM levels are important for nutrient and water retention within soils and the availability of these nutrients for plant uptake. Intensive mechanical cultivating of soil has been traditionally been used to prepare seedbeds, bury weeds and increase nutrient mineralisation (Kuhn *et al.*, 2016). However, this intensive manipulation of soils has been linked to reduced topsoil depth, degraded soil structure, soil compaction, loss of SOM and SOC, and nutrient depletion. Consequently long term agricultural yields have plateaued and have even begun to decline (Seitz *et al.*, 2019). Furthermore, the increases in agricultural activities has compounded soil compaction leading to reduced pore space and the loss of Water Stable Aggregates (WSA). This leaves soils at an increased risk of erosion, further degradation, and flooding (Tisdall & Oades, 1982). SOM contributes to cation exchange capacity (CEC) and nutrient retention, which in turn encourages organisms that breakdown and recycle nutrients within the soil, enhancing soil structure, improving water holding capacity and infiltration rates (Bot *et al.*, 2005). Healthy soil rich in SOM and organisms can maintain soil structure over seasonal variations in weather and supply crops with the necessary nutrients to maintain crop yields year on year.

1.1.3 Creating sustainable food production systems

The future and resilience of food security, particularly that of arable crops, is dependent on two main factors, (1) securing long term sources of essential macro-nutrients, N, P and K and (2) preventing further soil degradation by prioritising long term sustainable agricultural management practices. With the challenges facing long term nutrient supply, the implementation of conservation measures and closing nutrient cycles in the anthropogenic food chain is key (Childers *et al.*, 2011; Dawson & Hilton, 2011). It is estimated that more than 75% of the worlds land surface is degraded (Gibbs & Salmon, 2015). Preventing further soil degradation by understanding processes that exacerbate it and implementing measures to elevate and prevent it is vital (Dragović *et al.*, 2020). Adding another layer of complexity to providing long term sustainable food production is the impact of climate change and the increasing uncertainty of weather patterns (Howden *et al.*, 2007). Increasing instances of extreme weather are putting further pressure on agricultural production, drought, flooding, and heavy rainfall, require it to become more resilient than ever to secure long term food production (Boardman, 2015; Lal *et al.*, 2012).

In the past decade there has been a shift in how soils are viewed by the public and in policy. Their role in carbon storage and facilitation of ecosystem services is changing how they are managed both 'on the ground' and in policy. In the UK the introduction of the governments 25 year plan (H.M. Government, 2018) for a green future includes "replenishing depleted soils" and "recovering soil fertility". This is being phased into policy, with the traditional Common Agricultural Policy (CAP) farm payments moving away from paying farmers per land area, to paying farmers for the ecosystems services that their farms provide, through the Environmental Land Management Scheme (ELMS) (DEFRA, 2021). This change in policy is likely to influence farmers and land managers to adopting more sustainable management practices.

1.2 Agricultural soil management

The most intensive soil management intervention for arable farming is tillage, the mechanical manipulation of the soil to prepare the seedbed. Tilling the soil aerates it, mineralises nutrients to increase crop availability, reduces weeds and is an opportunity to incorporate organic materials into the soil. However, this intensive manipulation of soils is linked to reduced topsoil depth, degraded soil structure, soil compaction, loss of SOM, nutrient depletion, and as a consequence of all of these things, long term yield plateau and even decline (Seitz *et al.*, 2019).

1.2.1 Tillage and non-tillage systems

Current tillage systems in the UK can be divided into two broad categories based on their manipulation of the soil, inversion, and non-inversion, and then subdivided further based on the degree of disturbance to the soil: conventional, minimum, strip and no-tillage, shown in

Figure 1-1. Conventional Tillage (CT) uses multiple steps, mouldboard ploughing followed by harrowing before sowing, this method causes maximum soil disruption to the top 20-30 cm of soil, but provides good aeration and is considered reliable for maintaining yield with the addition of fertilisers (Morris *et al.*, 2010),. Minimum Tillage (MT) uses fewer field passes to prepare the seedbed, and approximately 30% of crop residue is left on the soil surface, with the rest incorporated (Soane *et al.*, 2012). No-tillage (NoT) also uses fewer passes than CT; seeds are sown directly into previous crop residue using direct drilling. Generally, NoT is seen as better for soil health as it promotes natural undisturbed soil cycles, and the crop residue protects the soil surface from erosion (Kassam *et al.*, 2019). Strip tillage (ST) sits between MT and NoT, only a narrow band of soil is prepared for sowing (less than 1/3 total field area), and the rest is left undisturbed, crop residues are retained and moved onto the untilled strips (Morris *et al.*, 2010).

Although not strictly a tillage system, controlled traffic farming (CTF), which limits vehicle and machine movement to designated tracks, provides similar benefits by reducing soil compaction in the non-trafficked areas. Farm machinery today puts 14-fold stress on soils compared with 100 years ago (Galambošová *et al.*, 2017). The negative impacts associated with soil compaction include increased bulk density, reduced hydraulic conductivity and water holding capacity, reduction in yield and a loss of soil macrofauna (Keller *et al.*, 2019). In regularly trafficked fields the field area that is trafficked can be as much at 80 - 100 % of the area, with this reducing to 30 - 60 % for conservation tillage practices, such as reduced and NoT. In contrast only 10 - 20 % of the field area is trafficked in CTF fields (Gasso *et al.*, 2013), reducing compaction within cropped areas maintaining soil productivity more sustainably.

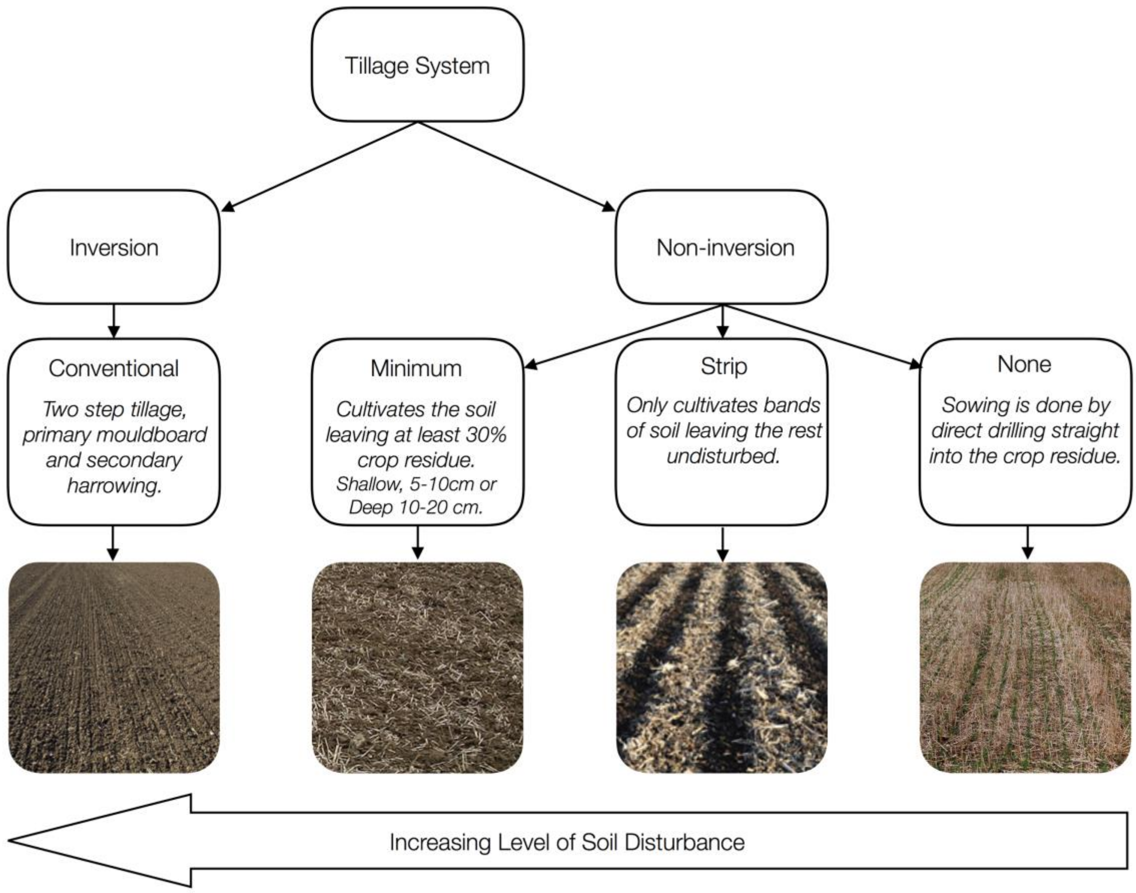

Figure 1-1: Classification of tillage systems in relation to tillage intensity (Modified from Morris et al. 2010).

1.2.2 Advantages and disadvantages of the utilisation of different tillage systems

There are many advantages and disadvantages to using different tillage practices, a summary has been presented in Table 1-1 for CT and NoT. As mentioned previously, the degradation to soils directly linked to intensive tillage is the primary reason that farm managers transition towards no-tillage (Kassam *et al.*, 2019). However, fears of reduced yield are seen as the primary constraint on the uptake of MT and NoT systems. NoT has even come under some criticism for prioritising soil health enhancement over maintaining yields (Pittelkow *et al.*, 2015). Economic drivers, including volatile grain prices, mean overall profitability is a significant advantage of MT and NoT systems. The reduction in field passes and consequently reduced fuel and labour costs can greatly outweigh the additional cost of herbicide and pesticide usage; production costs of NoT are estimated to be 25-35% of that of CT (Morris *et al.*, 2010).

Table 1-1: Advantages and disadvantages for the use of conventional tillage and no-tillage. Compiled from (Bullock & Anon, 2004; Cavigelli et al., 2012; Fullen et al., 2014; Gajri et al., 2002; Kassam et al., 2019; Lal et al., 2007; Morris et al., 2010). Continued onto next page.

	Conventional tillage		No-tillage	
	Advantages	Disadvantages	Advantages	Disadvantages
Physical	Complete burial of weeds and crop residues. Easy incorporation of manures, fertilisers, or lime amendments. Increased porosity and loosened soil allow for increased air exchange and root growth. Looser soil allows for faster warming of the soil in the spring.	Exposing surface soil to wind and rain erosion and loss of soil. Increased aeration of the soil increases moisture loss. High susceptibility of soil re-compaction.	No compaction below plough furrow. Reduced erosion from wind and water due to crop cover and higher infiltration rates. Stones are not brought to the surface. Improves soil water infiltration and percolation. Increase in soil aggregate formation. Increase in soil water retention. Crop residues decrease evaporation.	Risk of topsoil compaction over time. Organic amendments which are good for improving structure and SOC can't be incorporated, this must be done naturally, which is slower.
Chemical	Mixing of nutrients throughout the soil profile. Increased nutrient mineralisation, especially nitrogen.	Increased mineralization of SOC so this decreases. Soil slumping and capping due to weak aggregation. Increased erosion and consequently soil and nutrient loss. SOC contained in smaller soil aggregates, more prone to erosion.	Reduced runoff and loss of particle P. Increased SOC and carbon sequestration. Increase soil N retention, increasing N availability for plants and long-term N mineralisation.	Crop establishment issues can occur in extreme wet/dry conditions. Compaction can cause conditions to become anaerobic. Slower mineralisation of crop residues on the soil surface compared to buried. Increased risk of N2O emissions and loss of dissolved reactive P leaching.

	Conventional tillage		No-tillage	
	Advantages	Disadvantages	Advantages	Disadvantages
Biologica	Increased aeration helps warm the soil surface in the spring and enhances germination. Kill weeds before the sowing of the crop.	Significant decrease in shallow and deep burrowing earthworms. Previously buried weeds can be brought back to the surface. Breaking up of mycorrhizal fungi.	Better soil biological and fungal activity due to more SOM and reduced soil disruption. Break crops encourage surface invertebrates that predate on crop pests. Greater abundance of larger soil organisms, including earthworms. Higher microbial and fungal activity, especially in the topsoil. Higher enzyme activity. Crop residues increase earthworm activity.	Decrease in shallow burrowing earthworm species in continuous arable system. Weed and pest control problems. Generally, a lower crop yield than CT. Initial root development may be delayed due to compaction.
Climatic	Reliable method in all seasons.	High release of CO2 to atmosphere	Reduced emissions of CO2 and nitrogen. Less vulnerable to extreme weather.	Release of more N2O into the atmosphere than CT due to less aerobic conditions.
Ecological		Reduced surface water quality due to loose material. Prone to erosion and runoff.	Reduced run-off of nutrients and amendments due to higher infiltration rates.	Not suitable for all soil types - especially those with a coarse and sandy structure.
Socio-economic	Machinery widely available and cheap. Techniques well known to farmers.	High fuel cost associated with ploughing and multiple passes of the field. Slow work rate due to multiple passes. Increased emissions CO2 (fuel and oxidation of SOC).	Increased use of herbicides/insecticides, supporting the economy.	Machinery available but can be expensive. Can be a learning curve for farmers. Benefits may not be seen straight away. Reduced crop yield reliability.

One of the main benefits of reducing tillage is the beneficial effects on soil biology due to the reduction in the soil's physical movement, specifically earthworms, which have been reported to significantly increase in both biomass and abundance under reduced tillage (Pelosi *et al.*, 2016). This is of particular interest, as earthworms are considered ecosystem engineers due to their major impacts on soil systems, from the recycling of nutrients to the bioturbation of soil, increases in infiltration due to the creation of macropores via burrowing and increasing soil water stable aggregates (Babu Ojha & Devkota, 2014). They are also a good indicator species for soil's overall health in agroecosystems, especially the larger species that are the most prone to disturbance (Fusaro *et al.*, 2018).

1.2.3 Other conservation agriculture management practices

Other conservation agricultural practices which have been highlighted alongside reduced tillage as the priority practices for sustainable intensification by Dicks et. al., (2019) include: applying more organic materials to soils, reintroducing leys and cover crops into rotation, and using more resilient crop varieties. Reintroducing organic matter ensures that nutrients lost are replaced. Still, the organic material applied is valuable for creating soil aggregates that improve soil structure, cation exchange capacity, and increases crop uptake of nutrients (Bhogal *et al.*, 2018). Reintroducing leys and cover crops into rotations allows for the soil to have some rest and recovery from intensive arable cropping (Berdeni *et al.*, 2021). Some cover crops can fix nitrogen into the soil, and often the crop residues are left to provide more organic material for decomposition (Sharma *et al.*, 2018). Choosing crop varieties that are more resilient to drought or have greater water use efficiency can help maintain crop yields. Some varieties are also chosen to have shorter straw height and larger ears so that more of the plant growth efforts are put into the grain, increasing yield per hectare (Hawkesford, 2014). Mismanagement of soils can lead to increased soil loss through erosion, an increase in greenhouse gas emissions. These issues highlight the importance of continual research and implementation of suitable agricultural land management methods to provide sustainable crop yields and soil ecosystem services without further degradation.

1.3 Sewage sludge use in agriculture

1.3.1 History

Human excreta has been used as a fertiliser to replenish N and P in the soil to enhance crop production as long ago as 5000 years (Cordell *et al.*, 2011). Only small proportions of ingested P are retained in the body. Large amounts are lost in excreta and urine, making it an effective source of P and other nutrients (Schröder *et al.*, 2010). Mihelcic *et al.*, (2011) calculated that the quantity of P available from urine and faces, using the 2009 global human population of 6.85 billion, is approximately 3.4 million metric tons and, if collected, could have supplied 22% of total global P demand that year. In Europe, the continued development of urban drainage networks and centralisation of sewage treatment in larger wastewater treatment works, combined with EU legislation 91/271/EEC requiring all significant discharges of sewage (populations >2000) to be treated before reaching watercourses, makes European sewage treatment works a large catchment of P and N which can be utilised in agriculture (Council of European Communities, 1991). Sewage sludge can be applied to agricultural land in both treated and untreated forms. Treatment focuses on reducing pathogens in the material. Since 1991 treated sewage sludge has been termed a "Biosolid". In the UK alone, 2012 saw 3-4 million tonnes of biosolids applied to agricultural land, representing 75% of biosolids produced, applied to 150,000 hectares of primarily arable agricultural land (Water UK *et al.*, 2015). Nizzetto *et al.* (2016) also reported that 50% of sewage sludge is now processed for agricultural use in Europe.

1.3.2 Biosolids, their classes, production, and constituents

Since the ban on discharging sewage to watercourses, sewage treatment has come a long way with a vast network of sewers and wastewater treatment centres all over the UK. On reaching a wastewater treatment centre, sewage is taken through multiple processes. The primary stage is splitting the sewage into a solid and liquid fraction before further treatment (Russell, 2019). See Figure 1-2 for a complete wastewater treatment diagram, including the different stages for both the solid and liquid fractions. The liquid fraction is treated further using microbial and chemical processes to become compliant with regulatory standards. This waste liquid, now termed effluent, is discharged to watercourses (Russell, 2019). The solid fraction, known as sludge, is also treated, and this can be conducted in several ways as outlined in Table 1-2 "examples of treatment processes". This may include a pre-digestion treatment such as thermal hydrolysis or a post-digestion treatment such as lime stabilisation (AHDB, 2017; DEFRA, 2018). Over the past two decades, sludge treatment has become a way for water utilities to not only process sludge but also recover as many marketable materials and costs as possible (Liu *et al.*, 2020). Processes that involve anaerobic digestion produce methane, which can be converted to produce both electricity and recover heat to feed back into the system (Liu *et al.*, 2020). Both conventional and enhanced treatment processes produce biosolids as a by-product, which are suitable for application onto agricultural land, and are rich in organic matter and nutrients (Assured Biosolids, 2021).

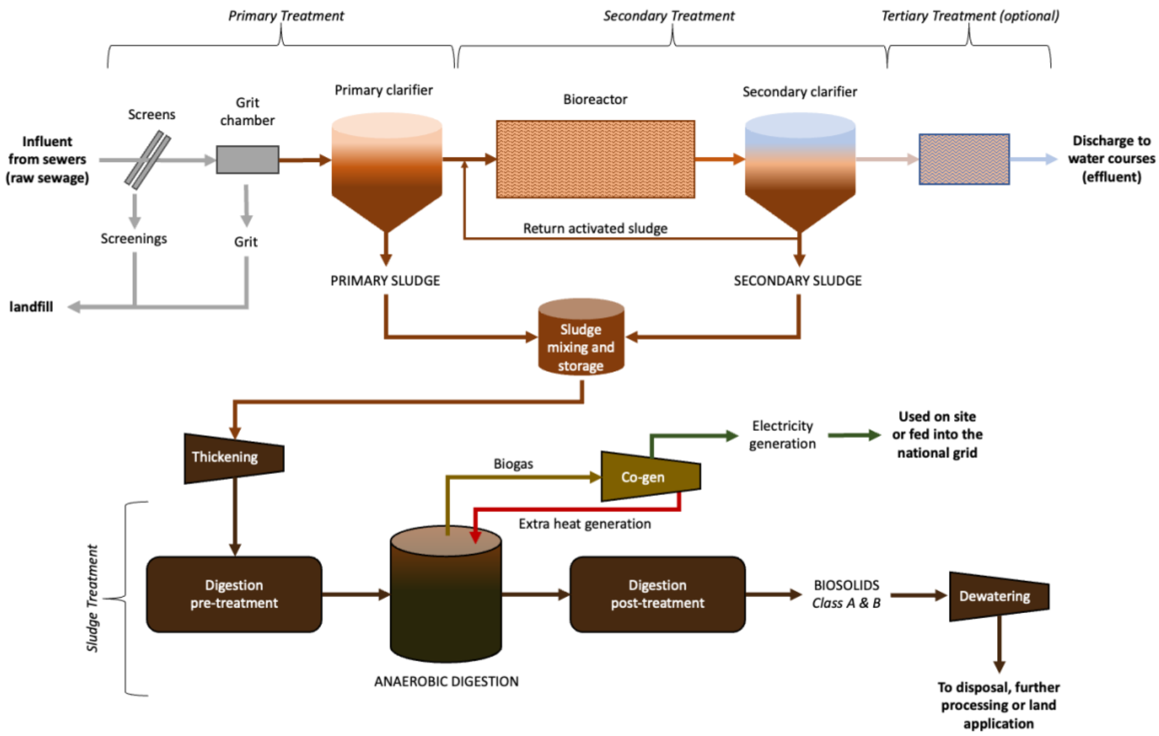

Figure 1-2: Wastewater treatment diagram, including the sludge treatment process and water treatment process. Blocks representing pre and post digestion treatments have been included to generalise this process flow diagram. There is likely to be pre or post-treatment as opposed to both. Depending on the post digestion treatment, dewatering may come before, for example, if the post-treatment is composting. Adapted from (Kor-Bicakci and Eskicioglu, 2019).

Table 1-2: Sludge Treatment methods for Sewage Sludge to procedure different grades of Biosolids and government guidelines for treating sewage sludge to meet minimum standards (AHDB, 2019; DEFRA, 2018). Continued onto next page.

Sludge Category	Description	Examples of Treatment Process	Method
Untreated Sludges (Not a Biosolid)	May include Primary, Secondary and/or Surplus Activated Sludge (SAS).		
Conventionally Treated Sludges (Class B Biosolid)	Sludge that has undergone a defined treatment process to ensure pathogen content is	Mesophilic anaerobic digestion	At least 12 days primary digestion at 35°C ±3°C or at least 20 days at

Sludge Category	Description	Examples of Treatment Process	Method
	decreased by at least 99% (log 2 reduction). Treatment relies on chemical, biological or heat treatment.		25°C ±3°C followed by a secondary stage of at least 14 days
		Thermophilic anaerobic Digestion	Mean retention period of at least 7 days digestion and subjected to a minimum of 55°C for 4 hours
		Lime Stabilisation (of liquid sludge)	Addition of lime to raise pH to above 12 for at least 2 hours
		Composting (windrows or aerated piles)	40°C for at least 5 days including 4 hours at 55°C followed by a period to complete the compost reaction process
		Pasteurisation	Minimum of 30 minutes at 70°C or 4 hours at 55°C (or appropriate intermediate conditions), followed by primary mesophilic anaerobic digestion
Enhanced Treated Sludges (Class A Biosolid, previously Advanced treated)	Sludge that has undergone treatment that virtually eliminated all pathogens present. Contains no Salmonella and 99.9999% of pathogens, using E. Coli as an indicator species, destroyed (log 6 reduction). Treatment relies on a combination of biological, chemical and heat treatments.	Thermal Hydrolysis followed by mesophilic anaerobic digestion	
		Enzyme hydrolysis and pasteurisation followed by mesophilic anaerobic digestion	
		Mesophilic anaerobic digestion followed by Thermal drying, composting or lime stabilisation	

Note: Sludges may be thickened (usually using a coagulant or flocculant and polymer) to increase dry solids content prior to treatment, further treatment, or transportation. For treated sludge this must not introduce more pathogens.

Biosolids have significant potential value for the agricultural industry as they contain large amounts of N and P and smaller amounts of other macronutrients (K, Calcium, Magnesium and Sulphur) and micronutrients (Copper, Zinc, Sodium, Iron, Molybdenum, Manganese, Cobalt, Boron) which are also beneficial in agricultural use. Furthermore, more than half of biosolid mass from municipal sewage is made up of organic material to contribute towards soil organic carbon (Kominko *et al.*, 2017). However, attention must be paid to Potentially Toxic Elements (PTE), elements or substances that could adversely affect soil quality or risk human and ecological health if they enter the food chain, surface, or groundwaters. Main PTE in biosolids are heavy metals (Cadmium, Chromium, Lead, Nickel) and micronutrients that are beneficial for plant growth and human nutrition but pose a risk at elevated levels (Copper and Zinc) (Torri & Corrêa, 2012). Sources of heavy metals which accumulate in sewage sludge include industrial activities and storm runoff from roads (Healy, 2018). Other PTE include inorganic micro-pollutants from domestic human activities include disinfectants, detergents, steroids, hormones and Pharmaceutical and Personal Care Products (PPCPs) (Gonzalez-Gil *et al.*, 2016). There has also been an increasing recent concern for microplastics accumulation in biosolids during wastewater treatment and subsequent disposal onto agricultural soils (Magnusson & Norén, 2014; Nizzetto *et al.*, 2016). Since 2020 there has also been a concern for public health in light of the COVID-19 pandemic, with regards to sludge spreading. However, Gianico *et al.*, (2021) surmised that the risk to public health was greatly diminished through treatment which reduces pathogen count significantly, such as composting, thermal drying, anaerobic digestion and liming. There is a high variability of specific biosolid characteristics depending on the location, catchment geology, source of waste (municipal, industrial or combination) and the time of year (wet or dry). Biosolids used in this thesis were extensively analysed, and results are shown in Chapter 2.

1.3.3 Current use and legislation

The use of sewage sludge in agriculture predates the implementation of the EU Sludge Directive (86/278/EEC) in 1989, where it was used in an unregulated manner. Many regulations have since been put in place to reduce harm to human and environmental health when using sludges and biosolids in agriculture. Table 1-3 summarises the major directives, legislation and codes of practice that have affected their use in England. However, practical guidance is now mainly limited to applying organic manure under Statutory Management Requirement 1 (SMR1): to reduce water pollution in Nitrate Vulnerable Zones (NVZs).

Table 1-3: Regulations and guidance regulating the use of sewage sludge and biosolids in England (ADAS, 2014; AHDB, 2017, 2019; Assured Biosolids, 2021; Environment Agency, 2013; Llewellyn, 2016; The Sludge (Use in Agriculture) Regulations, 1989).

Year	UK Regulations, Codes of Practice & Guides	Description
1989; 1990	The Sludge (Use in Agriculture) Regulations	Implementation of the EU Sludge Directive (86/278/EEC), 1996. Outlines maximum permissible heavy metal concentrations for soil after sludge applications and maximum annual application rates of heavy metals over a 10-year period. Stipulations for testing both the soil and sludge before any applications are made. Does not cover the storage of sewage sludge. Applies to agricultural land only.
1996; 2009; 2017	Codes of Good Agricultural Practice. Including: The Code of Practice for the Agricultural Use of Sewage Sludge – renamed The Sewage Sludge on Farmland Code of Practice. The Code of Good Agricultural Practice.	Compliments "The Sludge Regulations" to ensure applications are in accordance with good agricultural practices to reduce the impact on human and environmental health. Guidance on how to meet cross compliance measures. Guidance for producers, suppliers, and customers of biosolids and sludge products, to ensure that the long-term viability of the soil is protected while limiting the effect and nuisance to human and environmental health.
2001	The ADAS Safe Sludge Matrix	Provides guidance on crop types that can be grown following sludge application and harvesting intervals after applications to ensure food safety.
2008; 2013; 2016	Cross Compliance (The Nitrates Regulations)	Implementation of The EC Nitrates Directive (91/676/EEC) 1991. Aims to protect surface and ground waters from nitrate pollution. Implemented through the designation on Nitrate Vulnerable Zones (NVZs), where surface waters already contained high levels of nitrates. NVZs have additional restrictions and legally binding rules that must be adhered to when applying sludges, including closed spreading periods, proximity to watercourses and maximum N application rates.
2010	Environmental Permitting Regulations and exemptions	Standard Rules 5 & 6 for the use of sludge for non-agricultural land and non-food crops respectively. Standard Rule 4 for the co-compost/ co-digestion of sewage sludge with waste products (as the sludge then becomes a waste product). Exemption certification S3 for the storage of sludge on land (which is not covered by The Sludge Regulations).
2010; 2017	The Fertiliser Manual (RB209) – 8th edition (DEFRA). Updated to: The Nutrient Management Guide (RB209) 9th Edition.	Provides details of nutrient levels in different organic manures, sludges, and fertilisers. Details of crop nutrient requirements and how to calculate required loading rates of fertilisers.
2014	The Biosolids Nutrient Management Matrix (ADAS)	Defines good practice for biosolids application to manage P inputs, considering the nutrient requirements of crops in rotation and is self-limiting.
2015	The Biosolids Assurance Scheme	Currently a voluntary scheme to gain independently audited accreditation. Combines all the legislation, cross compliance, and codes of practice. Includes a HACCP assessment. The purpose of which is to demonstrate that biosolids are recycled to land in a responsible manor, using safe and sustainable practices. May become part of legislation in the future/ a mechanism for end of waste. Only covers agricultural land.
2019	Agriculture and Horticulture Development Board (AHDB) Nutrient Management Guide (RB209). Section 2, Organic Materials.	Latest revision of the RB209 nutrient management guide, revised based on research carried out since previous version published in 2010.

Legislation for Wales, Scotland and Northern Ireland are similar but may differ slightly.
In 1991 treated sewage sludge was termed Biosolids.

In practice, to be surface applied to land, sewage sludge must have been treated to reduce pathogen content (sewage sludge processing is detailed in section 1.3.2). Further restrictions apply for different grades of biosolid and the crop or land that the biosolid is to be used on, outlined in the Safe Sludge Matrix, Figure 1-3. In addition, biosolids are not permitted to be applied to legume crops unless composted due to the levels of readily available nitrogen (AHDB, 2019). According to NVZ guidance and SMR1, when spreading biosolids onto stubble or bare soil, then it must be incorporated into the soil, usually by ploughing, as soon as practicable and within 24hrs at the most for organic manures with high readily available N (>30% total N content). SMR1 also states that "as far as practically possible" organic manure should be spread in the spring. However, this assumes that there is a low autumn crop N requirement and would mean application immediately before sowing a spring crop with little time for the nutrients to mineralise and become crop available.

CROP GROUP	UNTREATED SLUDGES	CONVENTIONALLY TREATED SLUDGES	ENHANCED TREATED SLUDGES
FRUIT	✗	✗	✓ (10 month harvest interval applies)
SALADS	✗	✗ (30 month harvest interval applies)	✓ (10 month harvest interval applies)
VEGETABLES	✗	✗ (12 month harvest interval applies)	✓ (10 month harvest interval applies)
HORTICULTURE	✗	✗	✓ (10 month harvest interval applies)
COMBINABLE & ANIMAL FEED CROPS	✗	✓	✓
GRASS & FORAGE - *GRAZED*	✗	✗ (Deep injected or ploughed down only) 3 week no grazing and harvest interval applies	✓ 3 week no grazing and harvest interval applies
GRASS & FORAGE - *HARVESTED*	✗	✓ (No grazing in season of application) 3 week no grazing and harvest interval applies	✓ 3 week no grazing and harvest interval applies

Figure 1-3: The ADAS Safe Sludge Matrix (ADAS, 2001).All applications must comply with the Sludge (Use in Agriculture) Regulations and The Sewage Sludge on Farmland Code of Practice (✔); Applications not allowed, except where stated conditions apply (✗).

Application rates must consider the crop's nutrient requirements, but this is the one stipulation for quantity in a non NVZ area. AHDB Nutrient Management Guide RB209 (2019) provides estimates for different crops through the growing period and available nutrient content of different manures and fertilisers, which can be used for calculating crop use and additional requirements. Approximately 58% of land in England is designated an NVZ (DEFRA, 2016), and crops grown in an NVZ must say within an N Max limit averaged across the farm. In all NVZ areas, the organic N field limit for applications is 250 kg/ha in any 12-month period. Closed spreading periods apply in NVZs for manures/biosolids with high readily available N (>30% total N content) from 1st October to 31st January (1st August to 31st December on sandy or shallow soils) on arable land and 15th October to 31st January (1st September to 31st December on sandy or shallow soils) on grassland (DEFRA, 2018). Under the standards for Good Agricultural and Environmental Condition (GAEC), non-organic fertiliser and pesticide are not allowed within two metres of the surface of a watercourse, and non-organic manure will are not allowed within ten metres of a watercourse (DEFRA, 2018). Under SMR1, NVZ designated that land spreading of organic manures, including sewage sludges, must not occur: if the ground is waterlogged, flooded, snow-covered or frozen for more than 12h in the previous 24h; less than 50m from a spring, well or borehole; within 10m of surface water (with exceptions for some bird breeding areas); within 6m of surface water if using precision application equipment (Environment Agency, 2017). To reduce the risk of pollution from nutrients leaching to watercourses, the biosolid nutrient management matrix, Table 1-4, is used to ensure biosolids applications adhere to legislation by setting limits on the amount of biosolids that can be applied to soils based on their phosphorous content (ADAS, 2014).

Table 1-4: Biosolids nutrient management matrix, adapted from ADAS (2014) to include concentrations for soil Olsen extractable phosphorous (AHDB, 2017). Continued onto next page.

Soil P index	Olsen extractable P (mg/l)	Maximum potential application of lime stabilised biosolids[a]	Maximum potential application of all other biosolids types
0-2	≤ 25	250 kg/ha total N in any twelve-month period	250 kg/ha total N in any twelve-month period
3	26 – 45	250 kg/ha total N in any twelve-month period – application 1 year in 4 on sandy soils and 1 year in 2 on all other soils	250 kg/ha total N in any twelve-month period – application 1 year in 2 on sandy soils[b]
4	46 – 70	250 kg/ha total N in any	250 kg/ha total N in any

Soil P index	Olsen extractable P (mg/l)	Maximum potential application of lime stabilised biosolids[a]	Maximum potential application of all other biosolids types
		twelve-month period – application 1 year in 5 on sandy soils and 1 year in 3 on all other soils	twelve-month period – application 1 year in 4 on sandy soils[c] and 1 year in 2 on all other soils
5 +	≥ 71	No application	No application

[a] Lime addition rate >5% w/w on a dry solids basis

[b] Composted biosolids can be applied annually and [c] can be applied one year in two.

1.3.4 Effects of biosolids on soil properties

There have been many reported benefits of biosolids application to agricultural land, the main two being: (1) the addition of organic matter and (2) the addition of nutrients important to crop growth. Sharma *et al.*, (2017) published a comprehensive review of the agricultural utilisation of biosolids and summarised the reported effects on soil and crop growth, most measures saw an increase except for bulk density and pH. However, this depended on the initial pH of the soil, and the biosolids applied. Taking this into account and looking at other published studies where biosolids have been applied, observed physical changes to soils that are beneficial include increases in the water holding capacity, infiltration rate and water stable aggregates, and decreases in bulk density (Bhogal *et al.*, 2018; Nicholson *et al.*, 2018; Tsadilas *et al.*, 2005; Yucel *et al.*, 2015). However, many of these observed changes were not significantly different from the controls or were not significant for all treatments. The reported effect on water stable aggregates is mixed; most published studies reported no effect (Nicholson *et al.*, 2018; Petersen *et al.*, 2003). Jin *et al.*, (2015), however, reported a decrease in WSA with increasing levels of biosolids addition on grassland soils, compared to Yucel *et al.*, (2015), who observed an increase in aggregate stability after biosolid application on a corn-soybean rotation, but only in the long term (greater than five year) plots. Many of these changes are associated with the addition of organic material and carbon, which biosolids are high in. Most studies reported a significant increase in SOM after biosolids addition (Mossa *et al.*, 2017; Wu *et al.*, 2010). The increase in organic matter also increased SOC, although this was not always significant (Latare *et al.*, 2014; Urbaniak *et al.*, 2017). The addition of biosolids also increases the abundance of macronutrients, micronutrients, and cation exchange capacity in most studies (Ahmed *et al.*, 2010;

Andrés *et al.*, 2011; Antolín *et al.*, 2005). There was also an increase in heavy metal concentrations of cadmium, copper, lead and zinc in the soil, especially in older studies which is likely linked to industrial activity (Antolín *et al.*, 2005; Hazard *et al.*, 2014; Mossa *et al.*, 2017).

Biological responses to biosolids were generally seen in trends for non-significant increases in microbial biomass and activity (Carbonell *et al.*, 2009; Mossa *et al.*, 2017). Studies on the effect of biosolid application on enzymatic activity are few, with little consensus with regards to the reported effects (Banerjee *et al.*, 1997; Dar, 1996; Kizilkaya & Bayrakli, 2005). The effect on mycorrhiza fungi has received little attention, and in the one report seen it seemed to have no effect (Hazard *et al.*, 2014). One of the best biological indicators of soil health, earthworms, are reported to either be unaffected or to decrease (Barrera *et al.*, 2001; Carbonell *et al.*, 2009; Kiss, 2019; Waterhouse *et al.*, 2014). Crop responses including biomass, plant height and grain yield are well reported to increase after biosolids addition (Latare *et al.*, 2014; Petersen *et al.*, 2003; Tsadilas *et al.*, 2005; Waterhouse *et al.*, 2014). However, some studies also observed increases in crop and grain PTE accumulation, but these were again in older studies (Antolín *et al.*, 2005; Benítez *et al.*, 2001).

The differences in the biosolids' impact on soil are likely due to the different application methods, biosolids type, soil type and climate. It is worth noting that most of these studies applied the biosolids and mixed them with the soil in the field by ploughing or by hand in the lab, as would be done under current regulations in the field. Long term biosolids plots in the UK mainly report either increasing or no effect of biosolids on soil measures (Bhogal *et al.*, 2018; Nicholson *et al.*, 2018; Water UK *et al.*, 2015). There is agreement within the field for many of the metrics which define biosolid efficacy; however, for water stable aggregates and bulk density the reported changes resulting from biosolid application are mixed.

1.4 Combining biosolids use with different agricultural soil managements

Most studies outlined above on the effect of biosolids on soil properties are applying biosolids (of different grades) on conventional tillage agriculture, where the effects of biosolids have been extensively researched. In reduced tillage arable systems, the effect of biosolids has had minimal direct research, likely due to the legislative restrictions regarding the surface application of biosolids to agricultural soils without incorporation via ploughing. Research conducted outside of these restrictions across the globe suggest that biosolid application also increases SOC in no-tillage treatments. However, most reported differences are only seen in the surface soil layer 0 – 2.5 cm depth. Research conducted by Yucel et. al., (2015) in Wisconsin USA on a no-tillage corn soy bean rotation, and using a surface application of lime stabilised anaerobically digested liquid biosolids over annual applications ranging in time from 0 to 25 years. In the surface soil layer 0-15 cm they found a significant increase in SOC (but only in the 5 year treatment) and a significant increase in aggregate mean weight diameter in the 5 and 25 year plots compared to the control. Even historical applications of biosolids, although the type was not specified, reported significant increases in carbon content compared to control under continuous and rotational no-tillage after 3+ years in Virginia USA (Spargo *et al.*, 2008). Furthermore, no-tillage arable cropping of wheat, barley and soybean, found a significant interaction of biosolids in the surface soil layers where carbon content was significantly higher than the control treatment (Stewart *et al.*, 2012). The general consensus was that the longer the biosolids were applied, the greater the benefit to soil structures. However, there was a concern raised regarding loss in runoff of reactive nitrogen and reactive phosphorus, as accumulations in the surface soil layer and evidence of leaching between layers was present (Yucel *et al.*, 2015). The consensus was that biosolids addition under reduced tillage provided many beneficiary changes in soil nutrient levels seen in other tillage systems, but the surface application led to stratification of nutrients and OM within the soil profile and enhanced physical properties in structure and hydrology were seen only in long term experiments greater than 2+ years (Spargo *et al.*, 2008; Stewart *et al.*, 2012). Other land managements that utilise the addition of biosolids include agricultural land used for grass, forage and non-legume cover and break crops. In these systems, biosolids will be surface applied (Silveira *et al.*, 2019). There is little published research into the utilisation of biosolids in these systems; published studies are usually in the USA or Canada; and what there is shows that biosolids are typically beneficial for soil structure, increasing aggregate stability, SOC content and infiltration. Though there is evidence of stratification of the nutrients in the soil (Jin *et al.*, 2015; Wallace *et al.*, 2016). It is also worth mentioning, although not an agricultural use, biosolids are also utilised for land reclamation projects and enhanced treated biosolids are becoming increasingly popular for urban soil improvement (Alvarez-Campos *et al.*, 2018; Antonelli *et al.*, 2018).

1.5 Biosolids, agriculture and climate change

Adding another level of complexity to sustainable food production is the changes in climate and extreme weather events continuing to increase in intensity and frequency (Jones *et al.*, 2013). Analysis of UK rainfall data from 1961 to 2009 by Jones *et al.*, (2013) confirmed a significant increase of annual maxima in the UK over the study period and an increased frequency of extreme winter rainfall events from 1 in 25 years to 1 in 5 years. They also found that rainfall events in the summer were becoming longer, as are the dry periods. Extreme weather events can have devastating impacts on agriculture. Exposed soil is prone to erosion and crusting, which can increase the chances of flooding; periods of drought can stress and kill crops and cause degradation to soil structures (Holman *et al.*, 2003). Increases in extreme weather combined with soils low in OM from years of intensive agriculture put UK conventionally tilled soils at increased risk. Their low OM content reducing soils resilience to buffer against extreme weather events (Hueso *et al.*, 2011).

Management options for buffering against extreme weather involve increasing SOM content and maintaining crop coverage throughout the year through management strategies such as conservation agriculture (Lal *et al.*, 2012). The change in farm payments may also drive more farmers to uptake more sustainable farming practives add additional organic inputs to their soils. The improved soil hydraulic properties observed after the uptake of NoT and biosolids amendment individually and combined increase infiltration and reduce the chance of flooding compared to CT. UKWIR (2015) found that after 20 years of biosolids application, soil water infiltration and plant available water capacity increased significantly compared to the control, with more water held in the soil for longer periods. Biosolid amendments, a good source of OM, reduced drought stress in barley on Mediterranean soil (Antolín *et al.*, 2010).

1.6 Knowledge gaps and research questions

Biosolids can be a beneficial addition of organic matter and nutrients to soils, and agriculture is the most efficient use of this by-product of wastewater treatment. In line with current regulations, biosolids are either applied in the autumn or spring and ploughed into the soil as soon as practicable when applied to bare earth and stubble in arable systems (Environment Agency, 2017). However, land management is shifting towards a more conservation-based approach, with increasing uptake of reduced tillage (Kassam *et al.*, 2019). Soils under reduced tillage systems are reported to have increased biological activity whereby surface amendments may be integrated into the soil profile in a timely manner as to not cause losses to the environment (Pelosi *et al.*, 2016; Zanon *et al.*, 2020). An assessment is needed for the suitability of utilising biosolids in reduced tillage systems and how changes in climate may affect soil-biosolid interactions. There are mixed reports of biosolids' effects on soil structural properties, most notably water stable aggregates (Jin *et al.*, 2015; Nicholson *et al.*, 2018; Wallace *et al.*, 2016). The need to create resilient arable soils to buffer against extreme weather events is becoming more pertinent. Combining reduced tillage and biosolids application could provide a good option for improving soils resilience to extreme weather and protect crop yields. However, research is needed into the effects that extreme weather has on soils amended with biosolids and if extreme weather events enhance or hinder the effects of biosolids addition.

Hence this thesis aims to answer the following research questions.

1. How does land management affect biosolid-soil interactions when surface applied?
2. How do biotic and abiotic factors contribute to biosolid-soil interactions?
3. Does the type of biosolid, how it has been processed and produced, influence the effect of biosolid-soil interactions?
4. How does flooding affect biosolid-soil interactions?

1.7 Thesis outline

This thesis comprises of six chapters, a general introduction, general methodology, three experimental chapters and a general discussion. Figure 1-4 provides a visual representation of the thesis regarding the main research questions, experimental chapters, and the interconnectivity of each thesis component. Each chapter is briefly outlined below.

Chapter 1: General introduction and literature review. This chapter has provided a general introduction with reference to relevant literature that is pertinent to understanding the major themes that run within this thesis as well as the knowledge gaps that exist and subsequent research questions that this thesis aims to answer.

Chapter 2: General methodology. Methodologies, sites, and materials utilised in more than one part of the thesis have been outlined here. Where chapters have used experimental methods with a methodology unique to a single chapter, they have not been included here.

Chapter 3: Surface application of biosolids under ambient UK winter weather conditions, the effect on biological-chemical-physical properties of soil and crop production. Large intact soil monoliths from a range of 5 different land management practices were extracted in pairs from the field. Each pair consisted of a control and a biosolids applied treatment. A subset of monoliths was treated to remove earthworms. Arable land management treatments were planted with winter wheat in November 2017, and vegetative land management treatments were trimmed at regular intervals. Intensive monitoring of soil physical-chemical and biological parameters was conducted throughout and after harvest in August 2018. A range of results are presented with a discussion on the effect of biosolids-soil interactions and this effect on soil and crop parameters.

Chapter 4: Tracing and quantifying the movement of biosolids through a soil matrix using low-cost fluorescent particles; a method development. Run in conjunction with the monolith experiment in chapter 3, fluorescent tracer particles were added to biosolids and analysed post-harvest. Particles were successfully re-identified within the soil matrix and quantified based on image processing in conjunction with a range of fluorescent particle-soil mixture standards. Results presented give greater insight into biosolids' movement within the soil matrix and add depth to weather biotic or abiotic factor control the movement of biosolids.

Chapter 5: The effect of biosolids on water stable aggregate distribution of agricultural soil under flooded and unflooded conditions. Investigating unpredicted results from chapter 3, this chapter explores the interactions between soil and biosolid interactions on water stable aggregate distributions and their carbon and nitrogen composition. As part of the research questions addressing biosolids and soil interactions in the presence of abiotic factors and climate change, a treatment with flooded pots was also conducted.

Chapter 6: Drawing together results and observations from experimental chapters 3, 4 & 5 along with reference to the literature reviewed in Chapter 1 and new publications. This chapter discusses the themes of the thesis in reference to the specific research questions (as outlined above) and draws conclusions from the work of the thesis and suggests further work that could be conducted to advance the research further.

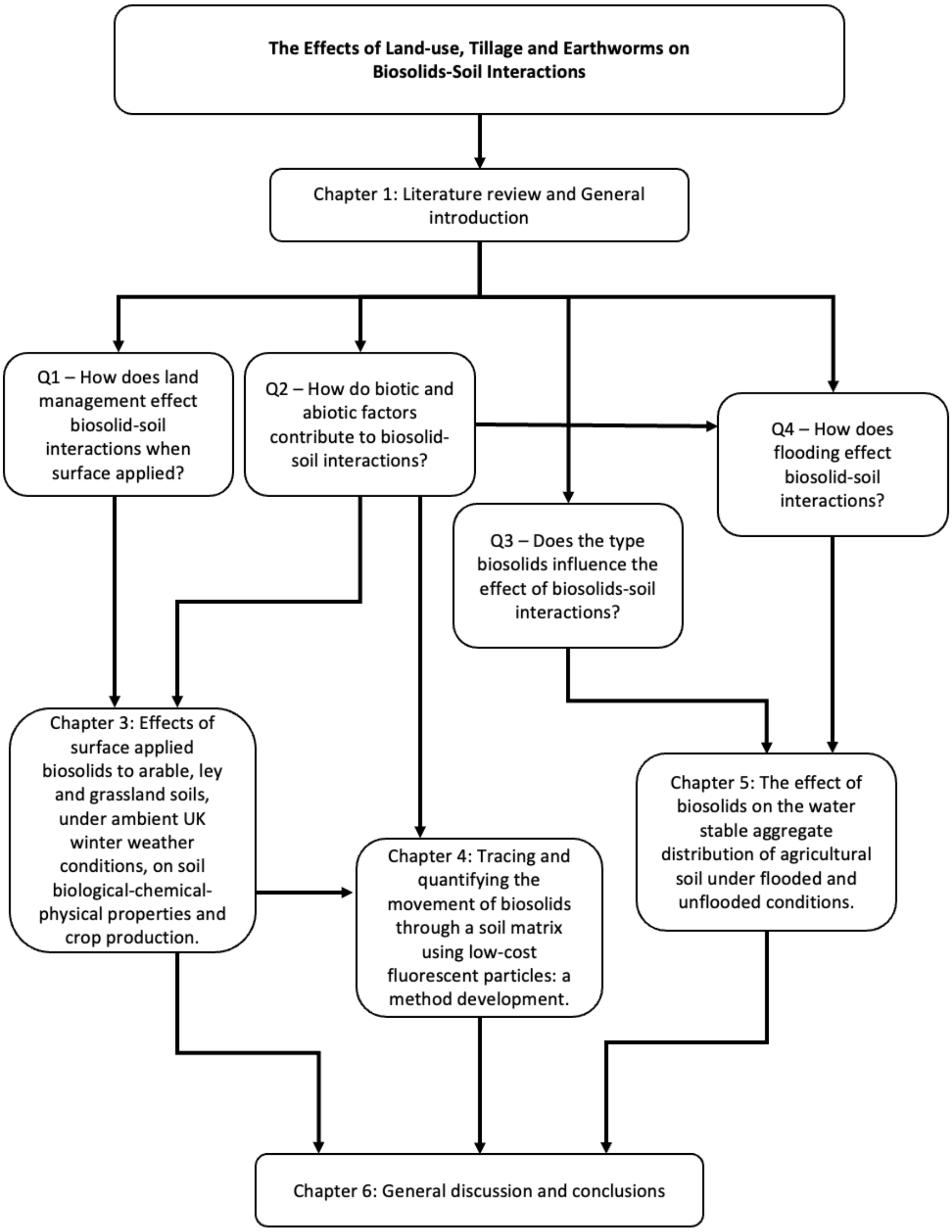

Figure 1-4: Visual representation of this thesis with the interconnectivity of chapters and research questions.

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Chapter 2: General Methodology

2.1 Introduction

This chapter outlines the general methods, sites and materials that were used in this thesis. Experimental chapters with unique and specific (non-general) methodologies have not been included here and shall remain included in their relevant methods section.

2.2 Soil

2.2.1 Details of the field sites (Chapters 3, 4 & 5)

The field site chosen for soil extraction was Leeds University Farm, also known as Spen Farm, Tadcaster (53°52'20.3"N 1°19'46.0"W), which is a commercial mixed farm in northern UK (see Figure 2-1). Within the farm the experimental site consisted of four different arable fields and three different grassland fields, with the soil comprising mainly of Tickenham and Aberford soil series, which are both calcareous brown earth loams (Calcaric Endoleptic Cambisols; Cranfield University, 2020). See Figure 2-2 for a historic soil type map of the whole site; Figure 2-3 shows a satellite view of the fields used in this experiment with field names. The experimental fields are outlined in red. The soils at the site are underlain with Permian Magnesian limestone of the Cadeby formation (Lott & Cooper, 2005), which generates a high magnesium content, causing the soils to swell and shrink (depending on the moisture content), and causes surface disaggregation and structural problems in the absence of adequate organic matter. The main soil type at the site is the Aberford series, an important regional soil type primarily used for arable farming on gently sloping land; Table 2-1 outlines the main soil types within the experimental area with the corresponding modern soil types and a brief description. Reported in Holden *et al.*, (2019) soil depths ranged from 50 – 90 cm and the site has a mean annual precipitation of 647 mm and mean annual temperature of 9.2 °C.

Figure 2-1: Map of England with site locations. Spen farm, Tadcaster (53°52'20.3"N 1°19'46.0"W) where soil was collected, and the Arthur Willis Environment Centre (AWEC), Sheffield (53°22'52.8"N 1°29'55.3"W), where the experiment was conducted.

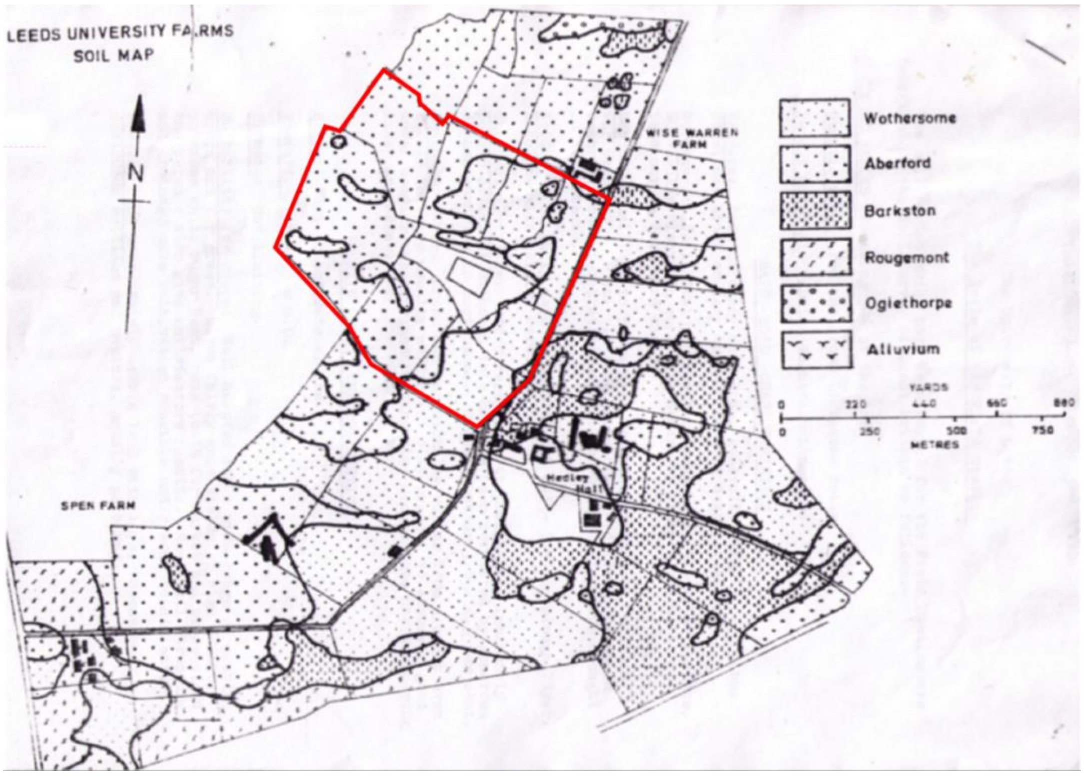

Figure 2-2: Soil type map of study fields at Spen Farm, Tadcaster, UK (53°52'20.3"N 1°19'46.0"W). The region outlined in red is the study area, where Aberford and Wothersome are dominant with one region of alluvium. Modern soil type comparisons are outlined in Table 2-1.

Table 2-1: Soil types within the research area. Old and new soil types are described (Cranfield University, 2020).

Old	New	Major Soil Group	Soil Group	Soil subgroup	Soil Series
Wothersome	Tickenham	05, brown soils	7, argillic brown earths	1, typical argillic brown earths	Reddish medium loamy over clayey drift with limestones
Aberford	Aberford	05, brown soils	1, brown calcareous earths	1, typical brown calcareous earths	Medium loamy material over lithoskeletal limestone

This site was chosen due to a unique experimental set up for the SoilBioHedge project, part of the NERC funded Soil Security Programme, where ley strips connected and unconnected to the hedgerow were sown into arable fields to investigate the possibility of harnessing hedgerow soil biodiversity for improving arable soils. As described by Holden *et al.,* (2019), Hallam *et al.,* (2020) and Berdeni *et al.,* (2021), four arable fields at Spen farm had pairs of strips 70 m long and 3 m wide, perpendicular to the field edge, cultivated in April 2015 and sown with a grass-clover mixture in May 2015. This was described by Berdeni *et al.*, (2021), comprising of two varieties of tetraploid *Lolium x boucheanum* (12% and 16%), diploid and tetraploid *Lolium perenne* (20%, and 16%, respectively), *Festulolium* spp., 16%, *Trifolium repens* 5%, and *Trifolium pratense* 15%, at an application rate of 4.2 g, equivalent to 178 m^{-2}. Figure 2-3 shows a satellite image of the experimental site at Spen farm with field names, the four arable fields with ley strips sown: BSSE, BSSW, Copse and Hillside and the grassland fields: Warren Paddock, Sub Paddock, and Valley. As the site is a research farm the cropping history for the past 20 + years is known; see Table 2-2.

Figure 2-3: Monolith extraction locations for Chapters 3, 4 & 5, at Spen farm, Tadcaster, UK. For Chapters 3 & 4, Long Term Pasture (LTP), ley (LEY) and Long Term Arable (LTA) were represented, no-tillage and ley to conventional tillage were simulated from monoliths extracted from the ley strips (LEY-NoT and LEY-CT respectively). A subset of LTA and LEY monoliths were extracted from BSSE & BSSW for treatment for earthworm removal. For Chapter 5, LEY soil from BSSE only was used. Source of photo: Google Earth (Pro 7.3.3.7786) image© 2020 Google Earth. http://earth.google.com.

Table 2-2: Land use history for fields used for soil collection in Chapters 3, 4 & 5. Ley strips 3 m wide by 70 m long were sown into the 4 arable fields in 2015. (PP = permanent pasture, SB = Spring Barley, WB = Winter Barley, SW = Spring Wheat, WW = Winter Wheat, WW2 = Winter Wheat year 2, POTS = potatoes, BEET = sugar beet, VPEAS = vining peas). Continued onto the next page

Field Name	Year									
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
BSSE & BSSW	WW	WW	POTS	WW	OSR	WW	BEET	WW	WW	POTS
Copse	WW	WW	POTS	WW	WW	VPEAS	WW	OSR	WW	POTS
Hillside	PP	PP	PP	PP	PP	PP	PP	PP	PP	WW
Warren Paddock	LEY	LEY	MZ	WW	WW	POTS	WW	WW	OSR	WW
Sub Paddock	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP
Valley	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP

Field Name	Year

	2010	2011	2012	2013	2014	2015	2016	2017
BSSE & BSSW	WW	OSR	WW	VPEAS	WW	WW2	SB	WB
Copse	WW	OSR	WW	VPEAS	WW	WW2	SB	WB
Hillside	OSR	WW	WW2	WB	OSR	WW	WW2	WB
Warren Paddock	WW2	OSR	PP	PP	PP	PP	PP	PP
Sub Paddock	PP	PP	PP	PP	PP	PP	PP	PP
Valley	PP	PP	PP	PP	PP	PP	PP	PP

2.2.2 Extraction of intact soil monoliths from different land managements (Chapters 3 & 4)

For the experiments in Chapters 3 & 4, intact soil monoliths were required so that all soil structure and biodiversity were preserved; this provides as close to a field-experimental soil as possible. A total of 54 intact soil blocks, measuring 27 x 37 x 20 cm (length x width x depth), were extracted in pairs at 64 m from the edge of each field, as shown in Figure 2-3, over a period of 3 weeks in October and November 2017. To extract intact monoliths, a method was developed by the SoilBioHedge team, whereby one of the monolith boxes had the bottom removed and a trench was cut in the field around the box's footprint down to the depth of the box, see Figure 2-4. The bottomless box was then slid over the intact block, undercut, and the whole intact block transferred to a new box with a bottom. The new box had nine drainage holes 10 mm in diameter in a three-by-three grid and 250 µm mesh glued on the inside to the bottom to stop earthworms from escaping and limit soil from washing through the drainage holes.

Figure 2-4: Intact monolith extraction in the field. BSSW long term arable monolith extraction. Note the ley strip in the middle background, with two recently extracted monoliths in boxes.

The soil monoliths came from 3 different soil managements, Long Term Pasture (LTP, taken from 3 fields), Long Term Arable (LTA, taken from 4 fields) and Ley (LEY, taken from 4 fields). Further monoliths were extracted from LEY soils for simulation of no-tillage arable cropping (LEY-NoT) and simulation of conventional mouldboard ploughing and arable cropping (LEY-CT) (see below for detail of the methods involved).

The first 16 monoliths (4 from the ley strip and 4 from the arable soil, in 2 fields BSSE and BSSW) were frozen to remove earthworms and earthworm cocoons, detailed below in 2.2.3. The remaining 38 monoliths were dug up while the first 16 were being frozen. After extraction, they were stored outdoors at Spen Farm. The 16 ley monoliths for conversion to LEY-NoT and LEY-CT were separated and sprayed with the herbicide glyphosate (Round Up Pro) at 20 ml in 1 l using a knapsack sprayer. The 38 monoliths were transported to the Arthur Willis Environment Centre (AWEC), Sheffield (53°22’52.8”N 1°29’55.3”W) in November 2017. See Figure 2-1 for location. All fieldwork was completed in October 2017, 2 years and 5 months after ley establishment. Figure 2-5 shows exposed soil profiles from LTA and LEY sites at the time of monolith extraction in 2017. Monoliths extracted for the LTA and LTA with earthworms removed (LTA-e) treatment had already been planted with winter wheat, wheat plants were small at the time of extraction and weeding out of the wheat seedlings did not cause much soil disturbance.

Figure 2-5: Exposed soil profile from BSSE field, (left) long term arable with winter wheat, (right) ley strip after 2 years 5 months of establishment (Note: there is a shadow across the soil profile). Photos, and fieldwork October 2017.

Table 2-3: Monolith treatment outline. LTP field replicates, Valley, Sub Paddock and Warren Paddock. LTA and LEY replicates from Hillside, Copse, BSSW and BSSE. LEY and LTA subset for earthworm removal treatment field replicates from BSSE & BSSW.

Agricultural System	Control	Biosolids	Field Repeats	Total	No worms control	No worms Biosolids	Field Repeats	Total
Long term pasture (LTP)	1	1	3	6				
Long term arable, conventionally ploughed (LTA)	1	1	4	8	2	2	2	8
Ley (LEY)	1	1	4	8	2	2	2	8
No-tillage (Ley-NoT)	1	1	4	8				
Conventional till (LEY-CT)	1	1	4	8				
Total: 54				38				16

2.2.3 Earthworm and earthworm cocoon defaunation (Chapters 3 & 4)

For the subset of de-faunated monoliths with earthworms removed (LEY-e and LTA-e), the first 16 monoliths extracted which were from ley strips in BSSE & BSSW were frozen at – 20 ^{o}C for 21 days to kill earthworms and earthworm cocoons, a method described by Barley (1961) and Bruckner *et al.,* (1995). Hallam *et al.,* (2020) had employed the same approach to monoliths of arable soil before conversion to ley in the same fields where the LEY monoliths in this experiment were extracted to investigate the effects of earthworms in the leys on soil structure and functions. In the present study, after a 21-day treatment of deep-freezing at -20 ^{o}C, the monoliths were transported to AWEC in November 2017, where they could thaw under ambient conditions outdoors before experimental set up. The freezing treatment was sufficient to kill all the vegetation in the LTA and LEY monoliths. For the LEY monoliths, which had been frozen to maintain an equal comparison to the LEY monoliths that had not been frozen, vegetation from the ley strips on the corresponding fields was extracted at the same size as the monoliths to 2.5 cm rooting depth in November 2017. Vegetation was meticulously washed to remove all soil, earthworms, and earthworm cocoons before transplanting into the de-faunated LEY monoliths.

2.2.4 Experimental treatments and monolith set-up to study the effects of surface-applied biosolids (Chapters 3 & 4)

At AWEC, the LEY monoliths that were treated with herbicide were converted to no-tillage (LEY-NoT) with no additional treatment, and simulated conventional inversion ploughing and harrowing tillage (LEY-CT) by turning out the soil block, inverting the top 50 % by depth and breaking up the surface with a hand harrow tool. The full design of the experiment and the different treatments are shown schematically in Figure 2-6. Each monolith was set up with a leachate collection tray, a mini rhizon soil solution sampler (rhizon soil moisture sampler, 10-micron porous membrane, 9 cm long x 4.5 mm wide, product code 19.21.01, Van Walt, Ltd.) installed 2 – 10 cm below the soil surface, at an angle of 45 degrees, in the centre of each monolith. An iButton temperature logger with waterproof wrap (one in every third monolith, representing a range of treatments) was installed at the monoliths' centre at a depth of 10 cm. This was done by removing a 1 cm wide by 10 cm deep core of soil, inserting the iButton and replacing the intact soil core. The iButton loggers were set to measure the temperature every 3 hours, at 0.5 °C increments for a period of 10 months. All arable treatments, LTA, LTA-e, LEY-NoT and LEY-CT, were planted in early November 2017 with winter wheat, Skyfall, in a 2 cm deep slit created in the soil and at twice the field planting density, 60 seeds per monolith, in 2 rows equidistant from the centre and edge of the monolith. This slot-sowing simulated the soil disturbance of direct drilling with a slot-drill in the monoliths without simulated ploughing and harrowing. Seedlings were thinned or supplemented with seedlings up to 30 plants per monolith (equivalent to field density), 15 in each row in early March 2018. Figure 2-7 shows a schematic of the monolith boxes set up.

Nitrogen fertiliser (Nitram®, formula is $NH_4.NO_3$, containing 34.5% N, CF Fertilisers UK Ltd.) was applied to all pots growing wheat (LTA, LTA-e, LEY-NoT and LEY-CT) at the field recommended rates for wheat of 0.5 g per monolith on two applications, 31st May 2018 when the wheat was tillering and 19th June 2018 when the grain was filling, total equivalent to 34.5 kg N ha^{-1}. The total N application rate for the control (non-biosolid amended) pots was 34.5 kg N ha^{-1} and the total N application rate for the biosolid amended pots was 202.5 kg N ha^{-1}.

Due to the lack of rainfall and drought conditions in summer 2018, all monoliths were given supplementary water in May, June, and July, totalling 16.5 litres per monolith (equivalent to 167 mm of rainfall), before wheat and vegetation was harvested on 31st July 2018.

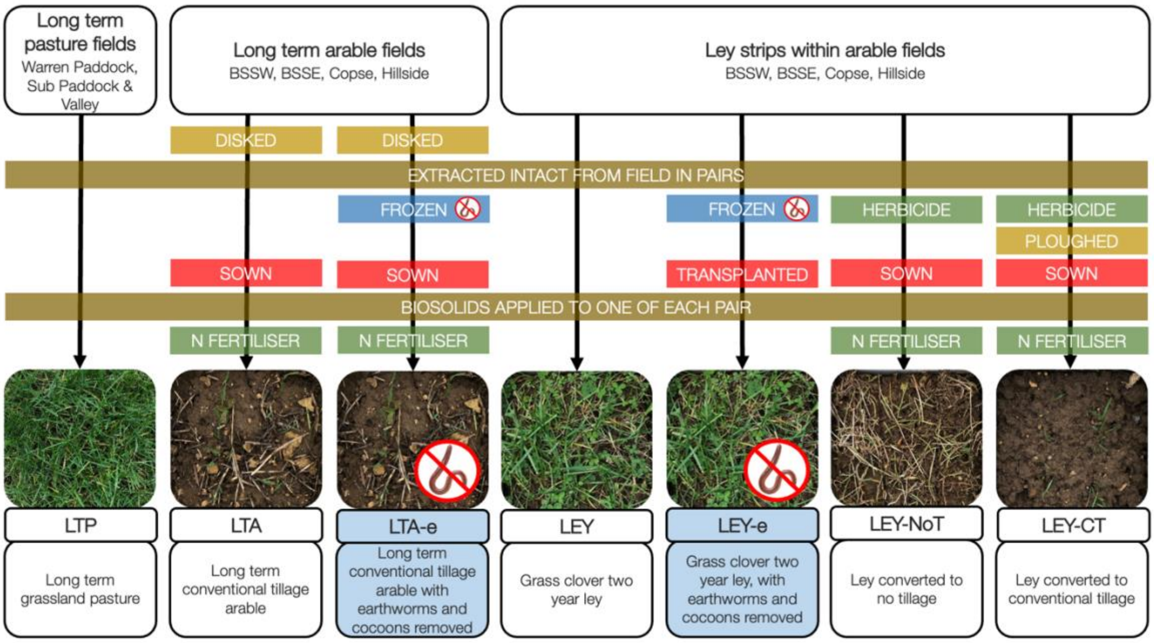

Figure 2-6: Monolith treatment outline, 2 biosolid treatments and 5 agricultural land management systems. Monoliths were extracted in pairs, one of each pair was applied with biosolids at a rate of 100 g per monolith (field equivalent to 10 t ha^{-1} or 168 kg N ha^{-1}), the other acted as a control with no biosolids applied. The 5 agricultural systems included long term pasture (LTP), ley (LEY), ley converted to no-tillage (LEY-NoT), ley converted to conventional tillage (LEY-CT) and Long term arable (LTA), with an additional subset of defaunated monoliths (earthworms and earthworm cocoons removed) for LEY and LTA, denoted as LEY-e and LTA-e respectively.

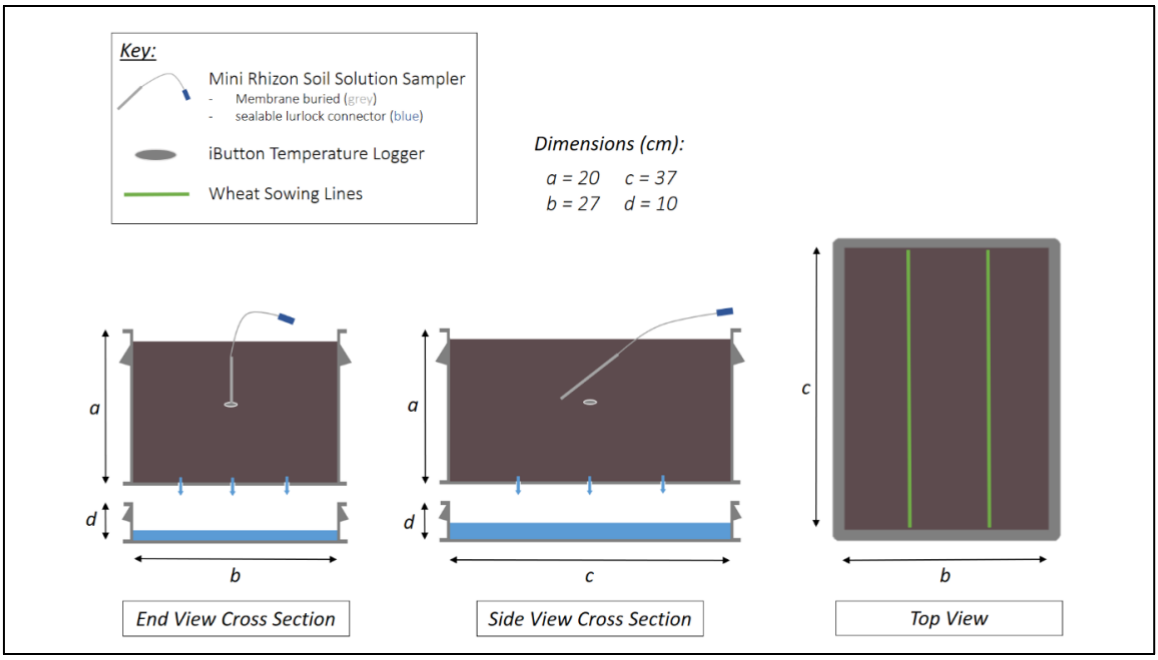

Figure 2-7: Schematic of monolith box set up. All monoliths had a leachate collection tray and mini rhizon soil solution sampler. One in every three monoliths had an iButton temperature logger representing a range of treatment. All arable cropping monoliths had wheat sown at twice the field density and thinned or made up to field density in March 2018.

2.2.4.1 Soil Solution and Leachate Analysis

Selected soil solution and leachate samples (as described further in Chapter 3) were analysed at an external laboratory (Kroto Institute, Sheffield), methods as follows (Kroto Institute, 2021). Liquid ion chromatography was conducted using a Dionex™ UltiMate™ 3000 (Thermo Scientific) to analyse the fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulphate, lithium, sodium, ammonium, potassium, magnesium and calcium levels of the samples. The samples were filtered to 0.45 µm; 10 µl of which was used for chromatography diluted with eluent (34 mM potassium Hydroxide at 0.2 ml/min used for the anions, 28 mM Methane Sulphonic Acid at 0.4 ml/mi is used on the Cations). Standards of known concentrations were run alongside the experimental samples to determine accurate quantities. Dissolved organic carbon was analysed using a Shimadzu VCPH/CPN Total Organic Carbon Analyser. Total Organic carbon is the total carbon minus the inorganic carbon. To determine total carbon, the sample is combusted in the presence of oxygen and the carbon is converted to carbon dioxide. The gas is then it passes through a halogen scrubber before it reaches the cell of a non-dispersive infrared NDIR gas analyser, where the carbon dioxide is detected. To determine inorganic carbon: the sample is acidified with a small amount of hydrochloric acid to obtain a pH less than 3, converting all of the carbonate into carbon dioxide. The carbon dioxide and dissolved carbon dioxide in the sample are volatilized by sparging zero grade air through the sample. Only the inorganic carbon of the sample, injected into the reaction vessel, is converted to carbon dioxide and detected by the NDIR.

2.2.5 Extraction of ley soil to determine the effects of biosolids on soil structure (Chapter 5)

For the experiment in Chapter 5, a site at Spen farm was chosen based on accessibility and results from Chapter 3, as explained in Chapter 5.2. Soil was extracted from the ley strip in BSSE field, see Figure 2-3, in January 2020, 4 years and 8 months after ley strip establishment. Approximately 0.5 t of soil was extracted at between 60 and 65 m along the ley strip from the field edge, which was a part of the ley strips that had not been extensively sampled in the SoilBioHedge studies, so the soil was undisturbed. The soil was extracted in approximately 5 kg intact blocks by four vertical-spade cuts of 15 cm width to about 15 cm depth, to give blocks of approximately 3.3 litres. Vegetation was kept at the time of extraction. All soil was collected in one day and transported to AWEC, where it was stored outdoors under shelter in ambient weather conditions to acclimatise for 3 weeks, until the experiment was set up.

2.3 Biosolids

2.3.1 Sources of biosolids

Biosolids were collected from sludge treatment centres near the field site. Biosolids from Esholt sludge treatment centre (53°51'06.1"N 1°43'03.4"W) were used for experiments in all chapters and biosolids from Knostrop sludge treatment centre (53°46’48.9”N 1°29’18.3”W) in Chapter 5 only. Figure 2-8 shows the locations of Esholt and Knostrop in the north of England, UK. The utility responsible for both sites is Yorkshire Water Ltd. Both sites consist of indigenous wastewater treatment and sludge treatment centre, treating indigenous sludge and imported sludge from smaller wastewater treatment works nearby. Table 2-4 outlines the site-specific details, including location, biosolid processing technique and grade of biosolid produced. Esholt produces enhanced treated biosolids, treating the sludge through a combination of thermal hydrolysis followed by mesophilic anaerobic digestion. Thermal hydrolysis is a process whereby the sludge is held at temperatures and pressures above that of an autoclave for a defined period of time prior to anaerobic digestion (Barber, 2016). Knostrop produces enhanced treated biosolids, treating sludge through a combination of mesophilic anaerobic digestion followed by lime stabilisation, where lime is added to raise the pH of the biosolids to above pH 12 and for a minimum period of 2 hours (DEFRA, 2018). Details of the different sludge processing techniques were described in detail in Chapter 1 and are discussed again in Chapter 5.

Figure 2-8: Map of biosolids sites sampled, both Yorkshire Water Ltd. wastewater treatment sites with sludge treatment centres. Esholt in Bradford and Knostrop in Leeds. Esholt biosolids only were used in Chapters 3 & 4, both Esholt and Knostrop biosolids were used in Chapter 5.

Table 2-4: Esholt and Knostrop biosolids production site specific details. These details were collected from the site managers around the time of sampling, Esholt details were checked again but had not changed at the time of the second sampling.

Site name	Esholt	Knostrop
Site location	53°51'06.1"N 1°43'03.4"W Bradford, England.	53°46'48.9"N 1°29'18.3"W Leeds, England.
Site details	90 TDS/day output capacity, working at 80 TDS/day 17,028 TDS annual throughput 2019 60/40 primary to secondary sludge ratio	130 TDS/day output capacity, working at 90 TDS/day 24,339 TDS annual throughput 2019 70/30 primary to secondary sludge ratio
Biosolid processing technique	Thermal hydrolysis	Lime stabilisation
Biosolid processing grade	Enhanced	Enhanced
Biosolid sampling date	For Chapters 3&4, November 2017. For Chapter 5, January 2020.	For Chapter 5, January 2020.

2.3.2 Fieldwork, biosolids sampling

In all instances, biosolids were collected from the biosolid cake export bay. This is where the biosolids that are ready to go out to fields are stored. Esholt biosolids were used in Chapters 3 & 4 sampled in November 2017. Both Esholt and Knostrop biosolids were used in Chapter 5 were sampled in January 2020. Figure 2-9 shows biosolids in situ at Esholt and in the lab. All samples were transported back to the University of Sheffield on the day of sampling and stored at 5 °C until used.

Figure 2-9: Photographs of biosolids, (left) in situ at Esholt wastewater treatment works, and (right) in the lab after sampling before analysis.

2.3.3 Laboratory analyses of biosolids

As soon as possible after biosolids collection fieldwork, the same or next day, a 500 g sample was sent to NRM laboratories, which specialise in analysing agricultural samples, for a suite of analyses. Results are shown in Table 2-5 with the industry typical biosolids values from the AHDB (2019) RB209 nutrient management guide. These nutrients are those that are used while calculating crop requirements and application rates. The maximum amount of biosolids that can be applied is equivalent to 250 kg N ha^{-1}. Biosolids were also analysed for some potentially toxic elements (PTE) as well as elements of interest, including Iron, Sodium and Calcium.

The NRM laboratory methods used for biosolids analysis were completed as follows (NRM Laboratories, 2021). Determination of pH measured potentiometrically of a solution prepared with 1:6 of biosolids: dH_2O undertaken in a controlled temperature environment. Dry matter as mass loss on ignition determined gravimetrically after drying at 105°C until no weight change. Calcium, lead, phosphorus, zinc, potassium, cobalt, magnesium, copper, manganese, iron, molybdenum and nickel using oven dried (105°C) sample and an aqua regis digest on a hot block, followed by determination by ICP-MS. Total carbon and nitrogen using the Dumas method, samples are totally combusted in an oxygen enriched atmosphere in a reaction tube, combustion products are passed by carrier gas through various absorption / reduction tubes, traps and splitters to result in nitrogen and carbon dioxide. The nitrogen and carbon content is then measured by the signal from either a thermal conductivity detector (TCD) or Infrared detector (IR). N species (Ammonium ,Nitrate and Nitrite) determined on an aqueous suspension of the sample using flow injection with spectrophotometric detection as described in Methods for Chemical Analysis of Water and Wastes (EPA, 1984).

Table 2-5: Laboratory analysis of biosolids, analysis conducted by NRM ltd. Results reported as the quantity on a 'dry matter' basis. Typical values reported in AHDB RB209 nutrient management guide (converted from fresh to dry matter) are also reported for reference (AHDB, 2019) as well as the average annual maximum permissible PTE additions over 10 years (DEFRA, 2018).

Site		Esholt	Esholt	Knostrop	Typical digested cake values RB209	Maximum PTE addition (kg ha^{-1})
Date sampled		Nov. 17	Jan. 20	Jan. 20		
pH		7.7	8.12	8.24		
Dry matter	%	25.7	26.8	26.4	25	
Total nitrogen	% w/w	6.53	5.23	4.61	4.28	
Total Carbon (C)	% w/w	39.8	33.9	31.1		
Nitrate N	mg/kg	<10	<10	<10		
Ammonium N	mg/kg	8,230	6,446	6,387		
Total Phosphorus (P)	mg/kg	26,312	20,131	21,951	4,801	
Total Potassium (K)	mg/kg	1,058	1,015	971	498	
Total Magnesium (Mg)	mg/kg	2,873	2,972	3,978	965	
Total Sulphur (S)	mg/kg	10,647	6,905	9,617		7.5
Total Copper (Cu)	mg/kg	225	154	170		15
Total Zinc (Zn)	mg/kg	603	485	565		
Total Sodium (Na)	mg/kg	703	515	467		
Total Calcium (Ca)	mg/kg	24,803	20,100	49,572		
Total Iron (Fe)	mg/kg	43,712	42,465	31,458		0.2
Total Molybdenum (Mo)	mg/kg	5.95	4.98	7.16		
Total Manganese (Mn)	mg/kg	920	462	797		
Total Cobolt (Co)	mg/kg	7.48	10.6	8.45		
Total Boron (B)	mg/kg	9.3	7.2	17.8		

2.4 Analytical Methods

2.4.1 Water Stable Aggregates

Since biosolids were surface applied to the monoliths, the analysis was done on a 5 x 5 x 5 cm cube of soil from the surface of each monolith and air-dried. Biosolids were mixed into the pots, so a 10 x 5 cm depth x diameter cylinder was sampled from the surface and air-dried. For both experiments, the air-dried soil was passed through a 1 cm sieve to remove large stones and followed by the wet sieving method by Cambardella & Elliott (1993). This is where a 50 g sample of air-dried soil is placed onto a 2 mm sieve submerged in water by 15 mm above the mesh for 5 minutes before moving the sieve in strokes above and below the waterline with stroke lengths of 3 cm for 50 strokes over 1 minute 30 seconds. Floating litter and stones were removed, and the soil aggregates transferred into tin boats and oven-dried at 105 °C for 24 hours. The water and soil that passed through the first sieve was poured into the next sized sequential sieve. For the monolith experiment in Chapter 3 the process was repeated for 1 mm, 250 µm and 53 µm sieves with 40 stokes over 1 min 10 sec, 30 strokes over 1 min 20 sec and 10 strokes over the time necessary to drain, respectively. These size fractions were chosen as they are standard soil fractions based on the Tisdall and Oades (1982) model for the formation of soil aggregates and distribution of organic matter fractions in soil by Cambardella & Elliott (1993), of < 53, 53-250, 250-1000, 1000-2000 and >2000 µm. For the pot experiment in Chapter 5 the process was repeated for the 1 mm sieve only to save time and as the > 1 mm size fractions showed the greatest change in Chapter 3. The analysis was repeated in duplicate. The weights of each size fraction were converted into proportions of the total weight of soil (with stones and litter removed). Mean weight diameter (MWD) of soil water stable aggregates was calculated using the equation and method described by Nimmo and Perkins (Nimmo & Perkins, 2002), see

Equation 2-1 and description below.

Equation 2-1: Calculation for mean weight diameter (MWD) of water stable aggregates (Nimmo & Perkins, 2002).

$$MWD = \sum_{i=1}^{n} wi \ \bar{X}i$$

Where n is the total number of different aggregate size fractions, i is the number of the size fraction between 1 and n, wi is the proportion of the total weight of water stable aggregates in that corresponding size fraction, and $\bar{X}i$ is the arithmetic mean of the size fraction. Calculations were done for each data point and then summed together for each pot before averaging for each treatment.

2.4.2 Soil Carbon and Nitrogen

Organic carbon and nitrogen content were analysed in the largest WSA fraction, > 2 mm. Each oven-dried soil sample was ground in an agate ball-mill (Fristch Pulverisette). Inorganic carbon was removed by adding 800 µl of 6 M HCl to 60 mg of soil for 24 hrs, then heated at 105 °C for 24 hours to evaporate excess acid and cooled. For each duplicate sample, 20 mg +/- 5 mg was sealed in tin boats and analysed using a CN elemental analyser (Elementar Vario EL Cube). Standards of acetanilide (C_8H_9NO) and blanks were run at regular intervals during each batch of analysis. At the end of the run, the soil sample results were standardised using the results from the acetanilide samples, which have a known carbon and nitrogen content of 71.09 % and 10.36 % respectively.

2.5 Software

Data analysis and figures were produced for all chapters using the open-source software R, version 4.0.3 (R Core Team, 2020) and R studio, version 1.3.1093 (RStudio Team, 2020). All maps were created using the open-source software QGIS, version 3.16.1 (QGIS Development Team, 2020).

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Chapter 3: Effects of surface applied biosolids to arable, ley and grassland soils, under ambient UK winter weather conditions, on soil biological-chemical-physical properties and crop production.

3.1 Introduction

Although the term 'sustainable intensification' has become somewhat of a controversial topic both politically and within the scientific community (Struik *et al.*, 2014). Producing sufficient quantities of food for human consumption to meet population expansion without negatively impacting the environment is an important, if not the most important, challenge that we face today (Fróna *et al.*, 2019). However, the current concept of intensive agriculture is flawed; it is degrading to both soil quality and quantity and is reliant upon the use of finite resources of mineral-based fertilizers such as P derived from high-grade rock-phosphate (apatite) and causes widespread environmental damage from excess nutrient release into the air and water (Admundsen *et al.*, 2015). There is a clear consensus in the scientific community that there is an urgent need to improve the practices that are currently used to produce a greater amount of food in a sustainable manner. Evans *et al.*, (2020) estimated the impact land management can have on soil erosion rates. They concluded that one-third of conventionally managed soils may only have a lifespan of < 200 years, 16% with < 100 years, comparing this to conservation agriculture methods that were shown to have substantially longer life spans, with 39% at > 10,000 years.

Dicks *et al.*, (2019) published an interdisciplinary review of the top priorities for sustainable intensification of agriculture, highlighting the top three priorities as: (1) grow varieties with increased tolerance to stress; (2) reduce tillage to minimum or no till; and (3) incorporate cover crops, green manures and other sources of organic matter to improve soil structure. This will also recycle nutrients and so reduce dependence on external inputs from finite sources such as rock phosphate (Elser & Bennett 2011). Priority (1) applies to livestock and arable farming, however, (2) and (3) apply to arable farming only, focusing on conserving and improving soil health which is the key to sustainable intensification for long term global food security.

3.1.1 Reduced tillage

Reducing tillage through the implementation of conservation agricultural practices has been shown to have a significant impact on improving soil health parameters. Studies have reported improved soil structure (Abdollahi & Munkholm, 2014), greater mean weight diameter of water stable aggregates (Samson *et al.*, 2020), increases in soil carbon (Bhogal *et al.*, 2008; Loaiza Puerta *et al.*, 2018) and increases in earthworm populations (Briones & Schmidt, 2017; Pelosi *et al.*, 2014), Improvements in these soil properties are also reflected in long term sustainable crop yield improvements (Kuhn *et al.*, 2016). Traditional tillage methods, most commonly inversion mouldboard ploughing, which turns and breaks up the soil and has been widely used globally, promotes nutrient mineralisation and the short-term creation of loose soil to form a good seed bed, but at the expense of a degrading soil structure and soil carbon loss. Buchi *et al.*, (2017) found that yields from reduced tillage plots were the same long term compared to those of conventionally ploughed plots; however the soil properties were significantly different. Soil organic carbon decreased significantly in the conventionally managed plots with no change in the reduced tillage plots. Some issues with no-tillage were reported by Schluter *et al.*, (2018), who evaluated the long term effects of conventional and reduced tillage on a range of soil health parameters, and found that bulk density increased, with the loss of air-filled pore space, there was greater compaction at the surface, and vertical stratification of nutrients in the no till plots. However, overall benefits to long term soil fertility through the adoption of reduced tillage have been found in many studies (Morris *et al.*, 2010) especially linked to soil aggregation and carbon sequestration. For example, Samson *et al.*, (2020) found that reduced tillage management practices, favoured particulate organic matter carbon accumulation and consequently are particularly efficient in increasing soil organic carbon stocks in the surface soil layer, irrespective of soil texture.

No-tillage strongly promotes earthworm populations, especially for the larger species such as the anecic *Lumbricus terrestris*, which are essential for generating large macropores in soil, increasing surface infiltration rates during rainstorm events (Holden *et al.*, 2019). A global meta-analysis conducted by Briones & Schmitt (2017) of earthworm abundance in soils under different tillage managements showed that all conservation tillage methods, when compared to conventional ploughing, significantly increased earthworm abundance by an average of 132% and biomass by 148%, with longer-term studies giving the greatest increases.

3.1.2 Cover crops and organic materials

Incorporating cover crops, green manures, and other sources of organic matter to improve soil structure are long- and well-established methods of reintroducing carbon and nutrients into soils. These practices are currently used in organic farming systems, but are now being integrated into conventional agriculture to improve soil health parameters, including soil organic matter content and soil water retention (Berdeni *et al.*, 2021). The improved soil structure results in greater aeration for microbes and fresh organic matter allowing soil microbial processes to thrive, including decomposition, organic matter cycling, nutrient mineralisation, and the formation and stabilisation of soil aggregates. However, given the extent of soil organic matter depletion from intensively cultivated arable soils in the UK, and the modest additions of organic carbon from cover crops, there is a need to explore using as many available sources of organic matter as possible. For this reason, farmers have been keen to utilise biosolids, the by-product of wastewater treatment. Although a 'waste' product, the main route for disposal, or in this instance, reuse, is its application on agricultural land. In the UK 78% of all biosolids are applied to agricultural land as its main destination (Collivignarelli *et al.*, 2019). Biosolids are rich in organic matter as well as macro- and micro-nutrients. These characteristics make biosolids a potentially beneficial amendment for agricultural fields while being a useful end-use of the by-product, especially considering the reported improvements to soil quality (Water UK *et al.*, 2015). The most noted effect of biosolids application on soils was the increase in soil organic matter, with short term increases of 0.2% after a single application (Wu *et* *al.*, 2010) and up to 3.7% increase after three years of annual applications (Tsadilas *et al.*, 2005). Fresh organic matter helps bind soil mineral particles and carbon into aggregates, improving resistance to erosive forces. However, in biosolids, the organic matter has been highly chemically or biochemically degraded, so the effect on soil structure may not be so beneficial. Indeed, in UK long term biosolids trials plots, Nicholson *et al.*, (2018) found 20 years of application of low metal biosolids, comparable to the biosolids produced today, significantly increased soil organic matter content but did not affect aggregate stability compared to untreated control plots. However, several other studies have reported that biosolids additions decrease soil bulk density (Nicholson *et al.*, 2018; Tsadilas *et al.*, 2005; Yucel *et al.*, 2015), and increases water holding capacity (Jin *et al.*, 2015), and water infiltration rates (Tsadilas *et al.*, 2005), which suggests improvements to soil structure and function that are typically associated with improved water-stable macroaggregation.

Other reports of the effects of biosolids application include those on soil chemistry, including decreasing pH (Antolín *et al.*, 2005; Benítez *et al.*, 2001; Tsadilas *et al.*, 2005), and increased heavy metal concentrations. These include cadmium, chromium, lead, nickel, together with the micronutrients copper and zinc that are beneficial for plant growth and human nutrition but pose a risk at elevated levels (Antolín *et al.*, 2005; Latare *et al.*, 2014; Mantovi *et al.*, 2005; Mossa *et al.*, 2017). The nutrient elements can be beneficial to plants and crop production (Andrés *et al.*, 2011; Latare *et al.*, 2014; Petersen *et al.*, 2003).

Effects of biosolids on soil biology include those on microbial biomass (Mossa *et al.*, 2017; Parat *et al.*, 2005; Yucel *et al.*, 2015). It can also affect microbial activity (Albiach *et al.*, 2001; Carbonell *et al.*, 2009; Mossa *et al.*, 2017), fungi (Hazard *et al.*, 2014; Mossa *et al.*, 2017), bacterial populations (Mossa *et al.*, 2017) and enzyme activities (Albiach *et al.*, 2001; Antolín *et al.*, 2005; Kizilkaya & Bayrakli, 2005). Biosolids can also affect earthworm biomass (Barrera *et al.*, 2001; Carbonell *et al.*, 2009; Waterhouse *et al.*, 2014), although the reported effects varied widely depending on the study.

Overall, the physical, chemical and biological effects of biosolids applications to arable land have generally been considered to be favourable, and the effects on crops beneficial, including increases in biomass (Petersen *et al.*, 2003; Tsadilas *et al.*, 2005; Waterhouse *et al.*, 2014), and grain yields (Antolín *et al.*, 2005; Latare *et al.*, 2014). However, there remain some concerns about the potential effects of contaminants in sludges, and the short-term and long-term impacts of current and emerging contaminants are being actively researched. Contaminants of concern include heavy metals, microplastics, persistent organic pollutants which were banned but persist (PCBs. PCDD / Fs) and recently banned (PBDEs, PFOS, PFOA). Some pathogens, including SARS-CoV-2, have been found in human excrement which may result in re-exposure via biosolids, (Gianico *et al.*, 2021), particularly for those chemicals or biological agents that resist degradation. In addition, there are concerns about complex mixtures of pharmaceutical products, including synthetic hormones and other bioactive compounds in personal care products that may be biologically active at very low concentrations (Ivanová *et al.*, 2018).

3.1.3 Combining biosolids and reduced tillage agriculture

As detailed in Chapter 1, legislation covering biosolids and other sludges to land requires them to be incorporated, usually via ploughing, as soon as practicable on bare soil and stubble where fields are within Nitrate Vulnerable Zones (NVZs). The Biosolids Assurance Scheme (BAS) (Environment Agency, 2013; Llewellyn, 2016), which all of the main water utilities in the UK now follow, requires the incorporation by ploughing which can have a negative impact on soil structure and biology (Morris *et al.*, 2010). Current guidance for biosolids disposal to land (only permissible by plough-based incorporation) would exclude the possibility of its disposal onto arable land that is under no-tillage or minimal disturbance arable cropping (e.g. shallow disk cultivation), which are starting to be more widely adopted by UK farmers seeking to reduce soil disturbance and improve soil quality (Kassam *et al.*, 2019). Importantly, the evidence shows that land which has undergone conversion to reduced tillage practices experience substantial increases in biological activity, such as earthworm population increases, especially in the surface soil layers (Busari *et al.*, 2015). This generates improved soil aggregation and macroporosity, suggesting that more sustainable forms of soil management may enhance the biological incorporation of surface-applied biosolids.

The possibility of combining reduced tillage and biosolids amendments via surface applications without incorporation could provide a route to apply biosolids to cropland to provide the additional benefits of nutrients and organic matter to agricultural fields, whilst minimising soil disturbance. There is little research on this approach, and it may have effects, both positive and negative. Surface application will leave the material open to possible physical erosion from rainfall and wind but also the possibility of nutrient loss through runoff, which has the potential to pollute watercourses. The past few years have seen a surge in research into how earthworms help support soil ecosystems and how they can be used to indicate soil health (Hallam *et al.*, 2020). Very little research has been done on how biosolids affect earthworms, or if earthworms influence how biosolids interact with soil ecosystems. The UK long term sludge plots found the low metal biosolids plots, similar to those produced today, significantly increased earthworm biomass (Nicholson *et al.*, 2018). However, Waterhouse *et al.*, (2014) observed all earthworms disappearing in biosolid amended treatments. Possible positive effects on soil structure could be hypothesised based on research showing improved soil structure under reduced tillage, and where surface additions have been made of organic matter, and these effects appear to be additive when both approaches are combined (Loaiza Puerta *et al.*, 2018).

Organic residue inputs have been found to increase aggregate stability, with the less easily decomposable materials increasing it the most (Al-Maliki & Scullion, 2013). Earthworms interacting with these residues also affected the soil microbial communities, as they preferentially ingested more palatable and readily degradable materials, consequently increasing aggregation in the short term, but with unclear effects in the long term (Al-Maliki & Scullion, 2013). In terms of crop production, organic matter applications can reduce nitrogen mineralisation, and therefore availability, compared to inorganic fertilisers. As a result, crop production may be negatively affected by the unavailability of readily available nitrogen during key growing periods if only organic materials are applied (AHDB, 2019).

3.1.4 Aims, objectives and hypothesis

The effects of biosolids on soil properties and functions remain incompletely understood due to the development of a wide range of biosolid production methods, soil types, climates, crops, and cultivation methods used. Current and previous research has focused on the effects on soil, crops, and losses in runoff after the materials have been incorporated via ploughing (Nicholson *et al.*, 2018; Sharma *et al.*, 2017). To move towards more sustainable agricultural practices, research is needed into the effects of biosolids on soil quality and crop production under conservation tillage methods, and soil health-promoting management practices (such as the introduction of legume-rich leys into arable rotations), which can be combined with no-tillage cropping. This chapter aims to address the knowledge gap regarding how biosolids that are surface applied affect soil biological, physical, and chemical properties and how this compares to experiments reported in the scientific literature of biosolid incorporation.

It is thought that soils under conservation management methods will exhibit a wider variety of biological activity, such as larger earthworm populations, which will generate the greatest soil-biosolid interactions. Consequently, crop production should increase, and soil health parameters will be enhanced. Therefore, this work aims to evaluate the effect of biosolids, as an addition of organic matter and nutrients, on soil quality and crop production under a range of different soil management strategies, which are in line with the priority practices for sustainable intensification of agriculture. Developing an overview of how different land management types function and how surface applied biosolids may affect that functioning. To do this, intact soil monoliths will be used to allow for as close to field conditions as possible, retaining the structure and biota present in the field while facilitating close and comprehensive monitoring of the whole system. Monoliths will be extracted intact from soils of different land managements in pairs. One of each pair will have no biosolids applied to act as a control, and the other will have surface applied biosolids. A comprehensive array of biological, chemical, and physical measurements will be taken during one cropping cycle, sowing to harvest. This work aims to shed light on the drivers that regulate how biosolids interact with soils when surface applied and the consequential effects on soil quality and crop production.

3.2 Methodology

3.2.1 Experimental Set Up

3.2.1.1 Fieldwork

Intact soil monoliths fitting in boxes of 37 x 27 x 20 cm (approximately 19.5 litres of soil) were extracted from Leeds University Research Farm, Spen Farm, Tadcaster, UK, consisting of 3 different soil managements; Long Term Pasture (LTP), Long Term Arable (LTA) and grass-clover leys, sown into the arable fields (LEY), with additional LEY monoliths extracted to simulated no-tillage (LEY-NoT) and conventional mouldboard plough (LEY-CT). An additional subset of LTA and LEY monoliths were extracted and treated to remove earthworms and earthworm cocoons (LTA-e and LEY-e respectively). Figure 3-1 outlines the parent soil and simulations for each experimental treatment. Further details of the site-specific characteristics, fields, and management, and how the monoliths were extracted are detailed in Chapter 2.

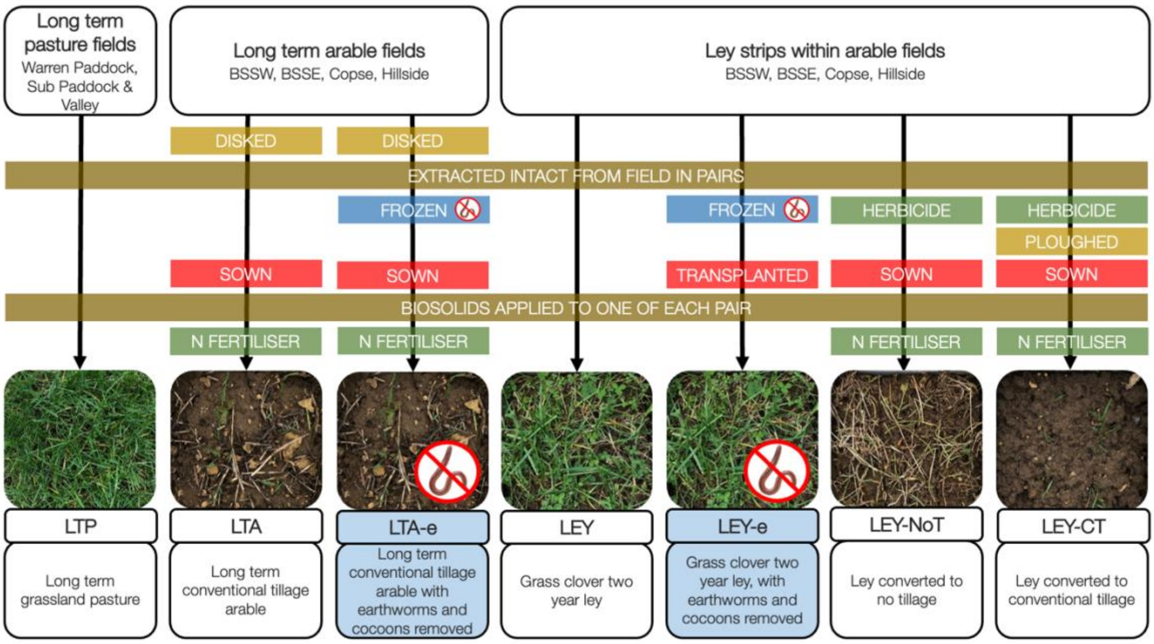

Figure 3-1: Monolith treatment diagram. There were five different agricultural management systems; long term pasture (LTP), long term arable (LTA), Ley (LEY), ley to simulated no-tillage (LEY-NoT), and ley to simulated conventional mouldboard ploughed tillage (LEY-CT). In a subset of two treatments monoliths were deep frozen to remove earthworms and earthworm cocoons. These were long term arable with earthworm removal treatment (LTA-e) and ley with earthworm removal treatment (LEY-e). Monoliths were extracted in pairs with biosolids applied to one of each pair. Replication of n=4 fields, with the following exceptions: LTP n=3 fields, LTA-e and LEY-e n=2 fields with 2 within field replicates.

3.2.1.2 Installation at ex-situ experimental site

Soil monoliths were installed at the Arthur Willis Environment Centre (AWEC), Sheffield, UK (coordinates 53.381391, -1.498775) on raised benches approximately 1 m above the ground, with 15 cm insulated boarding placed on all four sides and under the monoliths. Each monolith was set up with a leachate collection tray, temperature logger and soil solution sampler. The monoliths were arranged in two blocks; those that had earthworms and those that had been frozen to remove earthworms. This was done to reduce the risk of earthworm movements between monoliths (into those that had previously been frozen). Within each block, monoliths were randomised. An earthworm exclusion 'fence' was set up between the two blocks by erecting a steel mesh, of less than 1 mm pore size, from the base of the leachate tray to a height of 30 cm from the top of the monolith boxes. Winter wheat was drilled at twice field density in early November 2017 and thinned or made up to field density of 30 plants per pot in March 2018. Figure 3-2 shows a schematic of the monolith set up and a photograph of the installation at AWEC. Wheat was sown into LTA, LTA-e, LEY-NoT and LEY-CT monoliths only. Further details of the site-specific characteristics and treatment simulation and set up are detailed in Chapter 2, Section 2.2.2 to 2.2.4. Monoliths were installed under ambient weather conditions in a fenced compound covered by netting to exclude birds and squirrels.

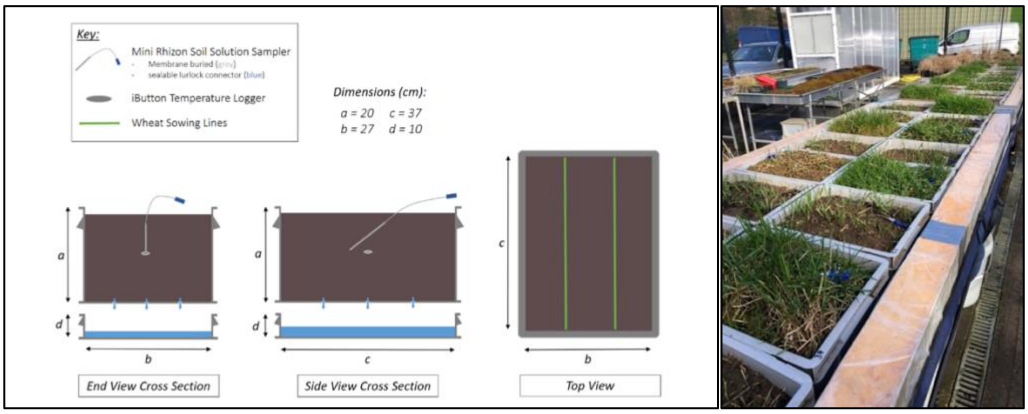

Figure 3-2: Monoliths set up, (left) schematic and (right) in situ photograph of monoliths at the University of Sheffield Arthur Willis Environment Centre, Sheffield, UK, taken January 2018.

3.2.2 Biosolids

The biosolids used in this experiment are enhanced treated, sourced from Esholt Wastewater Treatment Plant Bradford, UK. A silica-based fluorescent particle which was matched to the size distribution of the biosolid particles, was mixed at 1:99 with the biosolid. The rationale and method development regarding the fluorescent particle additions are explained further in Chapter 4. The biosolid-particle mixture was thoroughly homogenised and passed through a 1 cm riddle to create a uniform sized 'crumb'. The biosolid-particle mixture was applied by hand over the surface of one of each pair of monoliths from each replicate field / treatment, at a rate of 100 g per monolith, equivalent to 10 t ha^{-1} or 168 kg N ha^{-1}, which is slightly below field application limits to allow for edge effect due to soil expansion and shrinkage within the boxes. The biosolids were surface applied on 25th November 2017, designated day 0 of the experiment. Further details of the wastewater treatment site-specific characteristics and biosolids collection are detailed in Chapter 2. A subset of soil surface images after biosolids application are shown in Figure 3-3.

Figure 3-3: Surface images of monoliths on 22/12/17, 4 weeks post biosolid application. One of each biosolids applied treatment pictured. Left to right; long term pasture (LTP), long term arable (LTA), long term arable with earthworms removed (LTA-e), ley (LEY), ley with earthworms removed (LEY-e), ley to simulated no-tillage (LEY-NoT) and ley to simulated conventional tillage (LEY-CT).

3.2.3 Measurements and Analysis

3.2.3.1 Soil hydrological and hydro-chemical monitoring

Soil temperatures at 10 cm depth were measured 4 times per day +/- 0.5 ℃ and recorded with a real-time clock using buried iButtons (DS1921G-F5 thermochrons). Data was recorded from 3rd November 2017 to 17th July 2018 and downloaded after iButton recovery during monolith dismantling.

Soil leachate volumes were measured, and a sub sample collected, from the drip trays after accumulation over 72 hrs every 3 weeks during the 'wet' season and over the full period of 3 weeks in the 'dry' season. Soil Solutions were collected simultaneously as the soil leachates but for a period of 24 hrs from microrhizon soil solution samplers installed in the monoliths (Van Walt SMS 10) using 10 ml luer-lock syringes and inducing a vacuum in the syringes. All leachate and soil solution samples were stored at -20 ℃ until further analysis. Throughout the growing season each monolith was weighed to +/- 10 g every 2-3 weeks using an engine hoist, straps, and digital scales. Chemical analysis was conducted on one set of soil solutions and leachates, those sampled 06 and 07 January 2018 respectively, these were the most complete set of samples, with 53 soil solutions and 54 leachates. Samples were analysed externally at the University of Sheffield Kroto Institute. All samples were analysed for nitrate (NO_3), nitrite (NO_2^-), ammonium (NH_4^+), orthophosphate (PO_4^-), dissolved organic phosphate and total organic carbon.

Post-harvest infiltration rate was measured for each monolith using mini-disk tension infiltrometers (Decagon Devices, Inc). The 4.5 cm instrument was placed on a thin layer of fine sand on the soil's surface and moistened for optimum contact. Infiltration rate was measured through three pore sizes, at -0.5, -3 and -6 cm tensions which excludes flow through pore sizes of > 6 mm, > 1 mm and > 0.5 mm respectively. Infiltration rate was calculated using the method described by Reynolds and Elrick (1991) from steady-state flow rates, the starting volume was recorded, and the volume remaining recorded at set time intervals for each tension and until at least 15 ml of water had infiltrated.

Post-harvest soil moisture samples were taken during monolith dismantling; each monolith was weighed, all contents passed through a 1 cm sieve to remove stones. Stones were washed, dried, and weighed. A 1 kg sample of homogenized sieved bulk soil was taken for soil moisture analysis. Approximately 100 g of moist soil was weighed, dried at 105 ℃ for 72 hours, reweighed (repeated in triplicate) and the soil moisture calculated from the difference. This final soil moisture and dry-stone weight, in combination with the soil moisture content of all samples removed prior to dismantling, was used to back-calculate soil moisture throughout the experiment.

3.2.3.2 Biomass harvest

Throughout the growing season, the vegetation on the LEY and LTP monoliths was trimmed periodically, approximately every 4 – 6 weeks with most frequent in the spring summer, to 7 cm height as was done in the field. At the end of the growing season, vegetation was again trimmed to 7 cm before all the above ground biomass was removed by trimming to the soil surface level using scissors. Wheat shoots were harvested with 2 cm stubble remaining at the soil surface, as it would be in the field. Wheat was split into straw and head, dried at 80 ℃ until constant weight and weight recorded. Grain was separated from the chaff, every grain counted, dried at 80 ℃ until constant weight and weight recorded. Chaff weight was calculated from the difference between head and grain weights after separation. The number of wheat grains per monolith was counted.

3.2.3.3 Post-harvest soil analysis

Bulk density was measured for each monolith by moistening with 1 litre of water to soften the soil. Three 100 cm^3 cores for bulk density were taken at sequential depths down the soil profile. Cores were emptied into individual tin boats and oven-dried at 105 ℃ for 72 hrs. Three intact cores spanning the full depth of the monoliths (to a maximum of 25 cm), were taken using a split corer from each monolith, split into 2 cm fractions by depth and each depth was pooled, one core was left intact and stored at 5 ℃ for the re-identification of fluorescent particles, detailed in Chapter 4.

Water stable aggregate (WSA) analysis was done on a 5 cm^3 block of soil sampled from the surface of each monolith and air-dried. The air-dried soil was passed through a 1 cm sieve to remove large stones. Following the wet sieving method by Cambardella & Elliott (1993), a 50 g sample of air-dried soil was placed onto a 2 mm sieve submerged in water by 15 mm above the mesh for 5 minutes before moving the sieve in strokes above and below the waterline with stroke lengths of 3 cm for 50 strokes over 1 minute 30 seconds. Floating litter and stones were removed, and the soil aggregates transferred into tin boats and oven-dried at 105 ℃ for 24 hours. The water that passes through the sieve was poured through the next sequential sieve and the process was repeated for 1 mm, 250 µm and 53 µm sieves with 40 strokes over 1 min 10 sec, 30 strokes over 1 min 20 sec and 10 strokes over the time necessary to drain, respectively. The analysis was repeated in duplicate. The weights of each size fraction were converted into proportions of the soil's total weight (with stones and litter removed). Mean weight diameter (MWD) of soil water stable aggregates was calculated using the equation and method described by Nimmo and Perkins (Nimmo & Perkins, 2002).

Organic carbon and nitrogen content were analysed in the largest WSA fraction, > 2 mm. Each sample of oven-dried soil was ground in an agate ball-mill (Fristch Pulverisette). Inorganic carbon was removed by adding 800 µl of 6 M HCl to 60 mg of soil for 24 hrs then heated at 105 ℃ for 24 hours to evaporate excess acid and cooled. For each duplicate sample, 20 mg +/- 5mg was analysed using a CN elemental analyser (Elementar Vario EL Cube).

Earthworms were collected during monolith dismantling. They were counted, weighed fresh and preserved in 80% ethanol at 5 ℃ until identified in the lab. Adult and juvenile earthworms were separated and the adults identified to species level using the AIDGAP Earthworms Identification Guide (Sherlock, 2018).

3.2.3.4 Statistical analysis

Before conducting statistical analysis the data was assessed for normal distribution to meet the assumptions of the tests. If the data was not normally distributed, for instance proportional data, it was transformed. To test the differences between groups, where results comprising of continuous and count data for a single time point, the difference between means was analysed using an analysis of variance (ANOVA) test. Where data sets comprised of time series data, a repeated measure ANOVA was used. The data was tested for the effects of biosolids, earthworm removal treatment and soil management, and the interactions between groups. Proportional data was arcsine square root transformed before analysis. Post hoc Tukey HSD tests were conducted to identify differences between the control and the effect of sludge addition within soil management treatments.

To assess for the major contributions and effects of the treatments, a principal component analysis (PCA) was conducted on a data set including measures from a range of biological, chemical, and physical measurements and presented with a 95% confidence interval. Where error bars are presented on figures, this is the standard error calculated from the standard deviation of the results and the number of replicates. Where statistics are presented on figures, this is a Tukey HSD test comparing the control to the biosolids amended treatments for each soil management, unless otherwise specified. The results of the analysis of variance performed to test for treatments between groups are presented in the text and summarised in Table 3-2, 3 & 4 at the end of the results section. Three main groupings were used for analysis, (1) all soil management treatments, including those which had been treated to kill earthworms (LTP, LTA, LTA-e, LEY, LEY-e, LEY-NoT and LEY-CT), (2) a subset consisting of all treatments with earthworms (LTP, LTA, LEY, LEY-NoT and LEY-CT), and (3) a subset comparing LTA and LEY treatments with and without earthworms (LTA, LTA-e, LEY and LEY-e).

3.3 Results

3.3.1 Observations on changes to the surface applied biosolids

Visual observations of the changes to biosolids dispersal and distribution, a selection of which are shown in Figure 3-4, revealed that where biosolids were surface applied to land managements with permanent vegetation cover, the biosolids become quickly engulfed and covered by the vegetation. For surface applied biosolids in the arable rotation, we see a faster disappearance of biosolids, particularly in the LEY-NoT, followed by the LEY-CT and finally the LTA. There was evidence that earthworms increased rates of biosolids incorporation as in the LEY and LTA monoliths biosolids disappeared from the surface to a greater extent and faster than in the corresponding LEY-e and LTA-e monoliths. At the end of the experiment (post-harvest 08/08/1028 after all biomass had been removed), LEY, LEY-NoT and LEY-CT showed no visual evidence of biosolid addition. LTP and LTA had similar amounts of biosolids still visible on the soil surface, followed by LTA-e. Finally, LEY-e had the most remaining (Figure 3-4, bottom row).

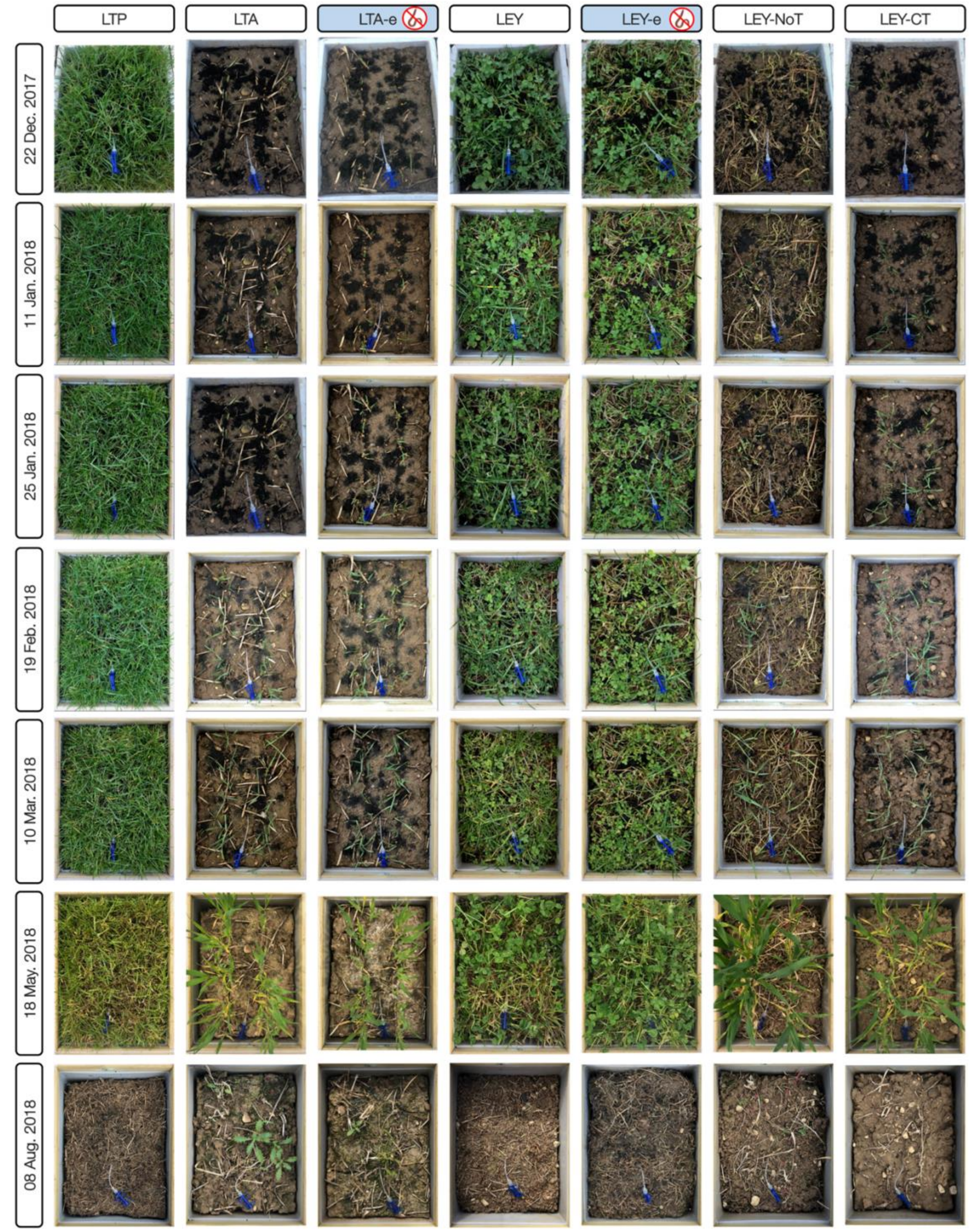

Figure 3-4: Monolith surface images for visual observations over time. These were taken from 22 December, one month after application to 08 August post-harvest, giving 7 images in total. The blue objects are the attachments for the microrhizon soil solution samplers.

3.3.2 Soil moisture content and thermal properties

3.3.2.1 Soil moisture

Monolith soil moisture results are presented in Figure 3-5. From the ANOVA on the mean soil moisture, there was no overall effect of biosolids, and the Tukey HSD test (presented in Figure 3-5C), showed no significant difference between the control and biosolids amendment, except for LTA (p <0.01) where the biosolids amended treatment had significantly higher soil moisture than the control. The effect of earthworms was also significant (p <0.001), where treatments with earthworms had higher soil moisture than those without. The overall effect of management was significant (p <0.001) with LTP having significantly higher mean soil moisture than all other treatments, followed by LEY-CT and LTA. During the winter, the LTA-e had the lowest soil moisture. However, this changed to LEY and LEY-e during the summer. From the repeated measure ANOVA, there was a significant effect of biosolids, with the biosolids amended treatments showing a higher soil moisture than the control for all treatment groups ($p < 0.05$) overall, and for treatments including earthworms, and ($p < 0.001$) for LEY and LTA comparing with and without earthworms.

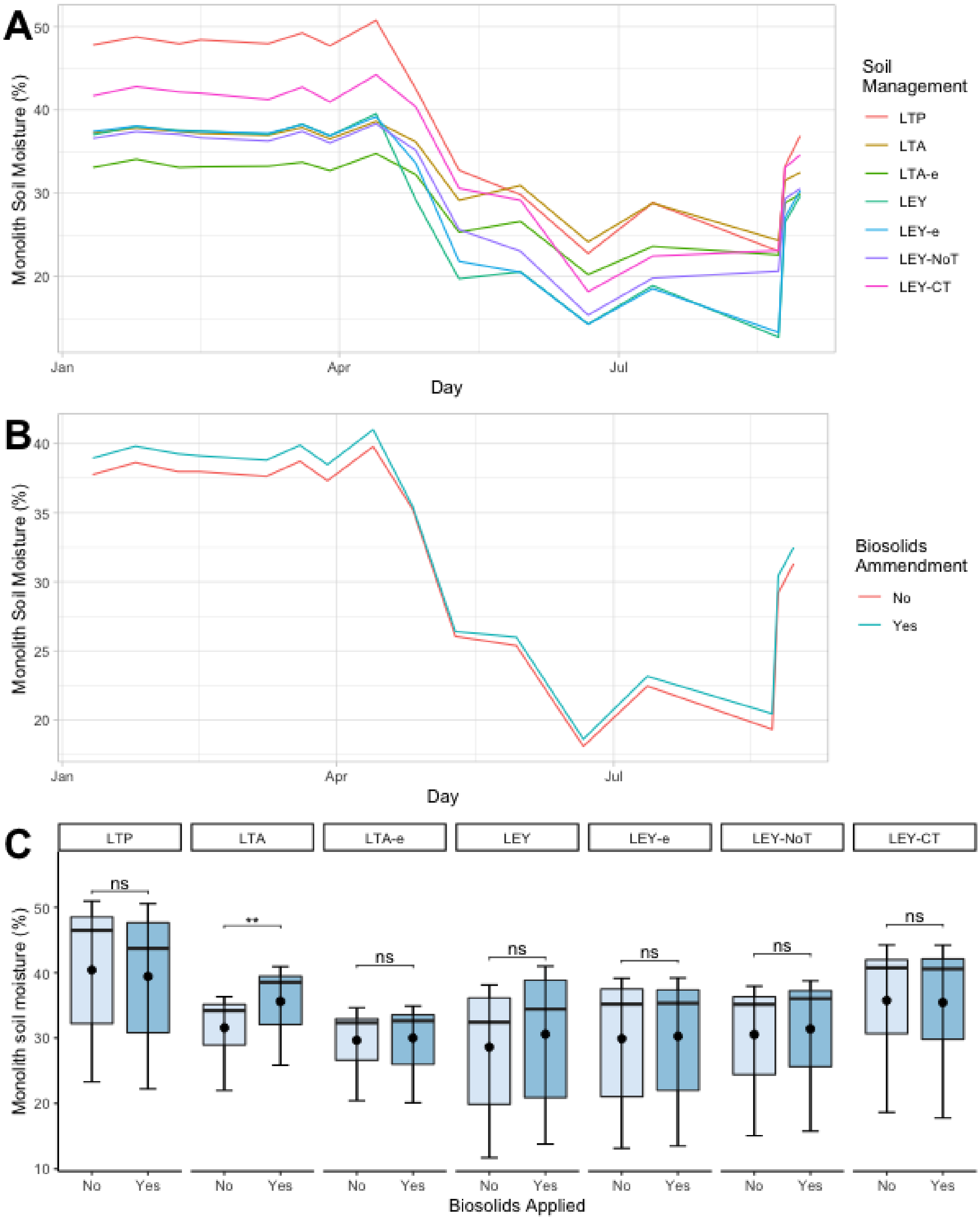

Figure 3-5: Soil Moisture throughout the year. (A) grouped by soil management, (B) grouped by biosolids amendment, and (C) summary of monolith soil moisture. Weights were only measured from day 48 in January 2018 due to equipment procurement and the last measurement taken at day 278, which was pre-harvest. In (C) which provides summary statistics for soil moisture over the growing period, the horizontal line is the median, the central black dot represents the mean, and the whiskers are the maximum and minimum soil moistures observed and the boxes represent the interquartile range.

3.3.2.2 Soil temperature

Monolith soil temperature at 10 cm depth is shown in Figure 3-6, with time series data for the growing period and cumulative summary of the daily minimum and maximum soil temperature. Replication was not sufficient for statistical analysis as n = 1 or 2.

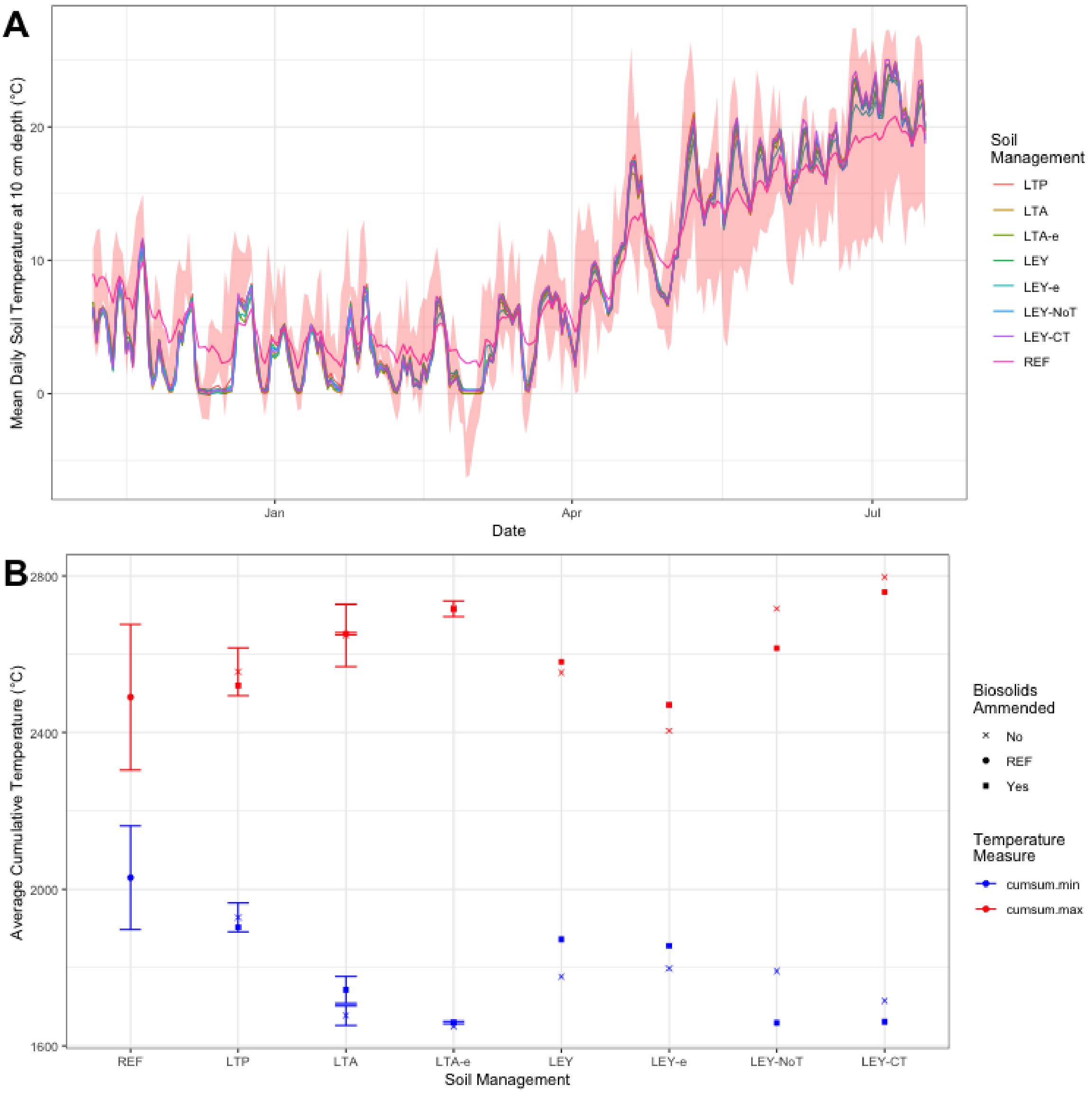

Figure 3-6: Monolith soil temperature at 10 cm depth. (A) temperature change over the experimental period, red band is the air temperature daily min/max (provided by Sheffield Museums from the Western Park weather station) and REF is the soil temperature at 10 cm depth at the front and back of the AWEC site. (B) Monolith soil temperature at 10 cm depth, cumulative sum of daily minimum and maximum, replication not sufficient for statistical analysis (n=1 or 2) where error bars are present, n=2, where they are absent n=1.

3.3.3 Soil Physical Properties

3.3.3.1 Bulk density

Bulk density was measured for three horizons, (A) surface soil 1 - 6 cm depth, (B) subsurface soil 8-13 cm depth, and (C) deep subsurface soil 15-20 cm depth. Results are presented in Figure 3-7, showing the surface addition of biosolids had no significant effect compared to the control for any treatment, soil horizon or overall. Soil management treatment had a significant effect on bulk density for all horizons ($p < 0.001$ for A and C and $p < 0.05$ for B horizon). In horizon A, LTP has the lowest bulk density, and this is significantly lower than all other treatments. LTA and LTA-e had the highest bulk density, but this was not significantly different from most other treatments. This trend follows though horizon B and in horizon C the bulk density of all soil management treatments is similar, except LEY-CT has a much lower bulk density. The effect of earthworms, comparing LEY and LTA with and without earthworms, had a significant effect on bulk density in the surface soil horizon ($p < 0.01$), where LTA-e and LEY-e had higher bulk densities than LTA and LEY treatments respectively. This was not observed in any other horizon.

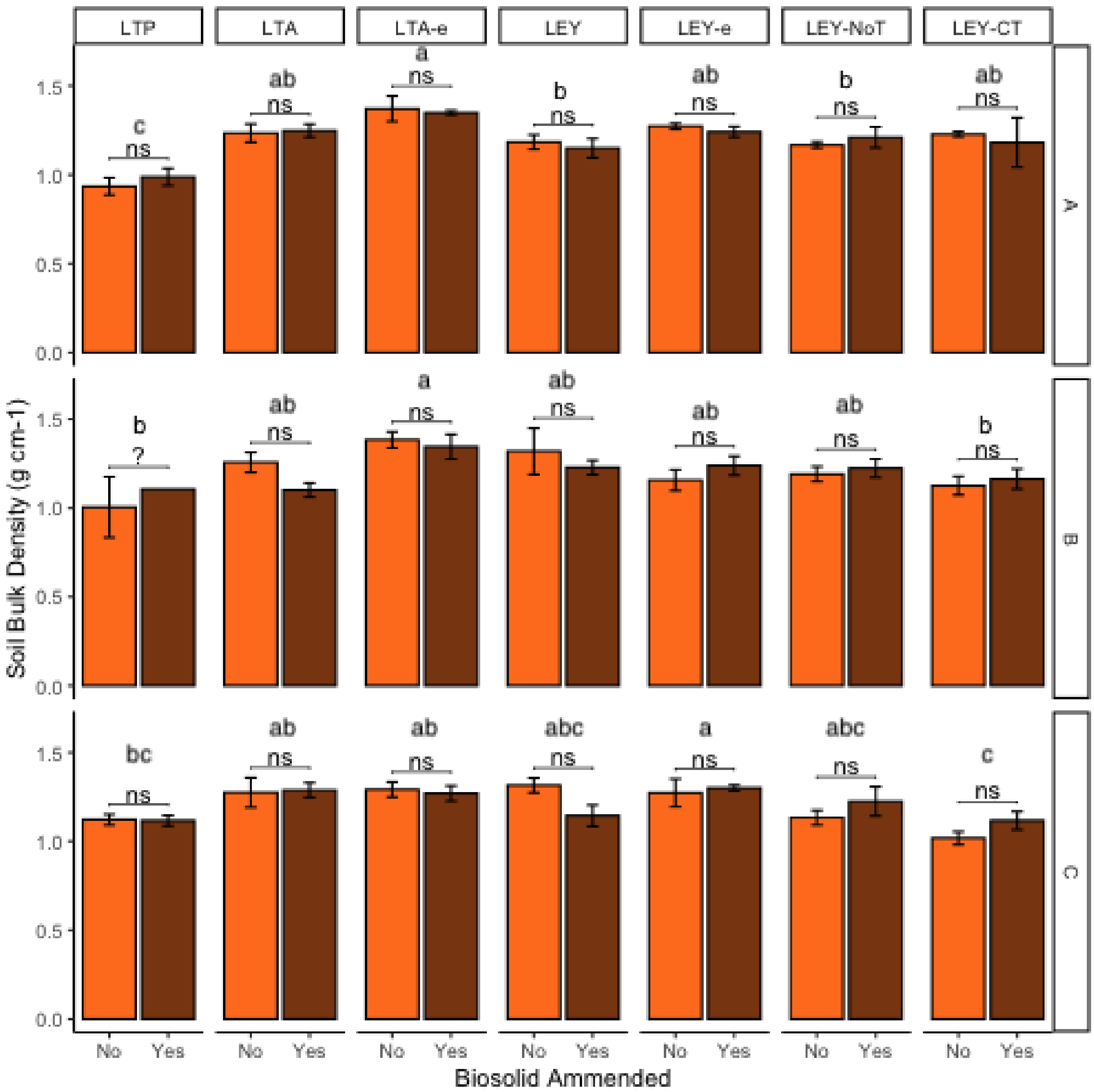

Figure 3-7: Monolith bulk density for three depth horizons (A, B & C at 1-6, 8-13 and 15-20 cm depth respectively). The "?" denotes inadequate replication for statistical analysis.

3.3.3.2 Soil water stable aggregates and carbon and nitrogen analysis

Soil water stable aggregate (WSA) fraction results are presented as proportions of the total weight of each soil samples in Figure 3-8, with the larger diameter fractions in darker colours at the bottom of the stacked bar chart and the smaller fractions in lighter colours at the top of the chart. From the Tukey HSD tests, the difference between the control and biosolids amended treatment was not significant within treatment groups and size fractions, except for LEY-NoT 1000-2000 µm ($p = 0.0108$). However, as seen in Figure 3-8, there is a trend showing biosolids may have reduced the proportion of aggregates in the >2000 µm fraction for all treatments except LEY-e. Results of the analysis of variance between treatment groups for each size fraction is shown in Table 3-1, where p < 0.001 for all treatment groups within each size fraction.

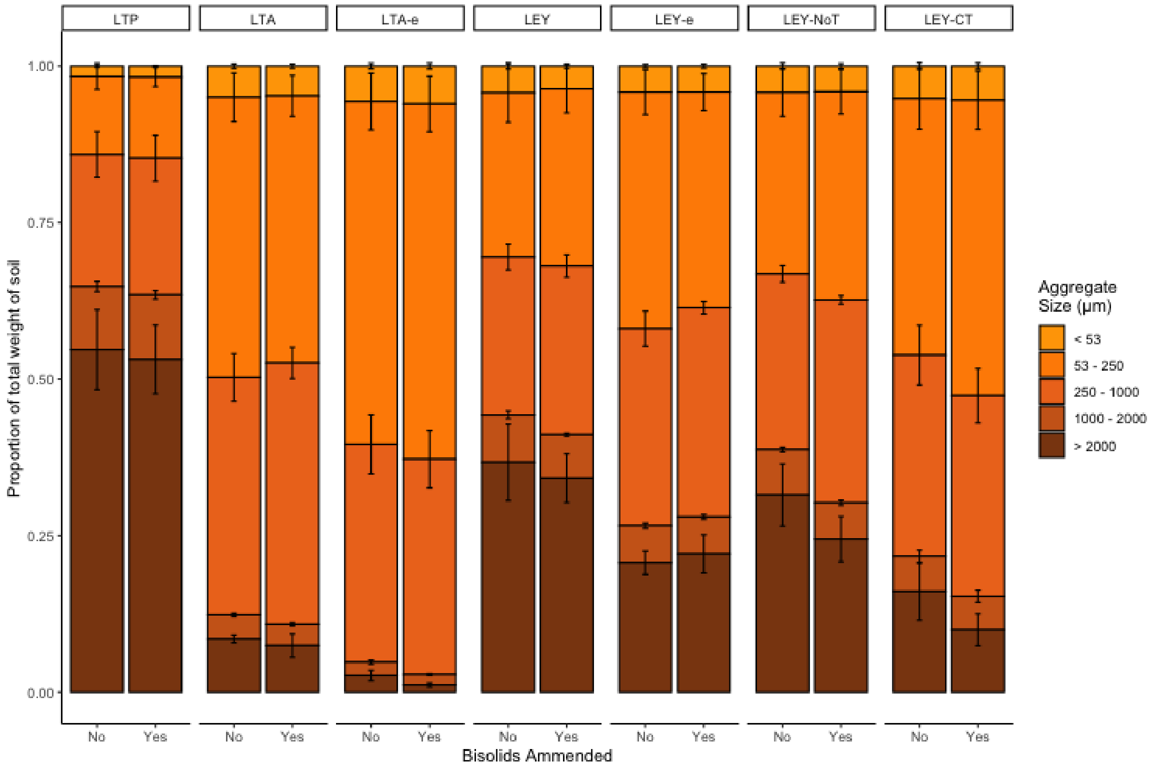

Figure 3-8: Water stable aggregate proportional fractions of monolith soil. All control vs biosolids Tukey tests within soil management and size fraction were not significant, except for LEY-NoT 1000-2000 µm where p = 0.0108, due to reduced macroaggregates on biosolids addition.

Considering all the WSA size fraction proportions into a single measure, the mean weight diameter (MWD) of the WSA was calculated with the results presented in Figure 3-9. The effect of biosolids amendment on MWD was not significant compared to the control within soil management treatments or overall. However, the trend of a reduction in MWD where biosolids have been applied is again present, although not significant. Soil management treatment had a significant effect (p <0.001) for all groups of treatments, Tukey HSD results are shown in Table 3-1. LTP had significantly higher MWD than all other treatments, followed by LEY and LEY-NoT. LTA and LTA-e had the lowest MWD, which was significantly lower than most other treatments. The effect of earthworms had no overall significant effect, but LEY-e had a significantly lower MWD than LEY treatment when considering earthworms and management.

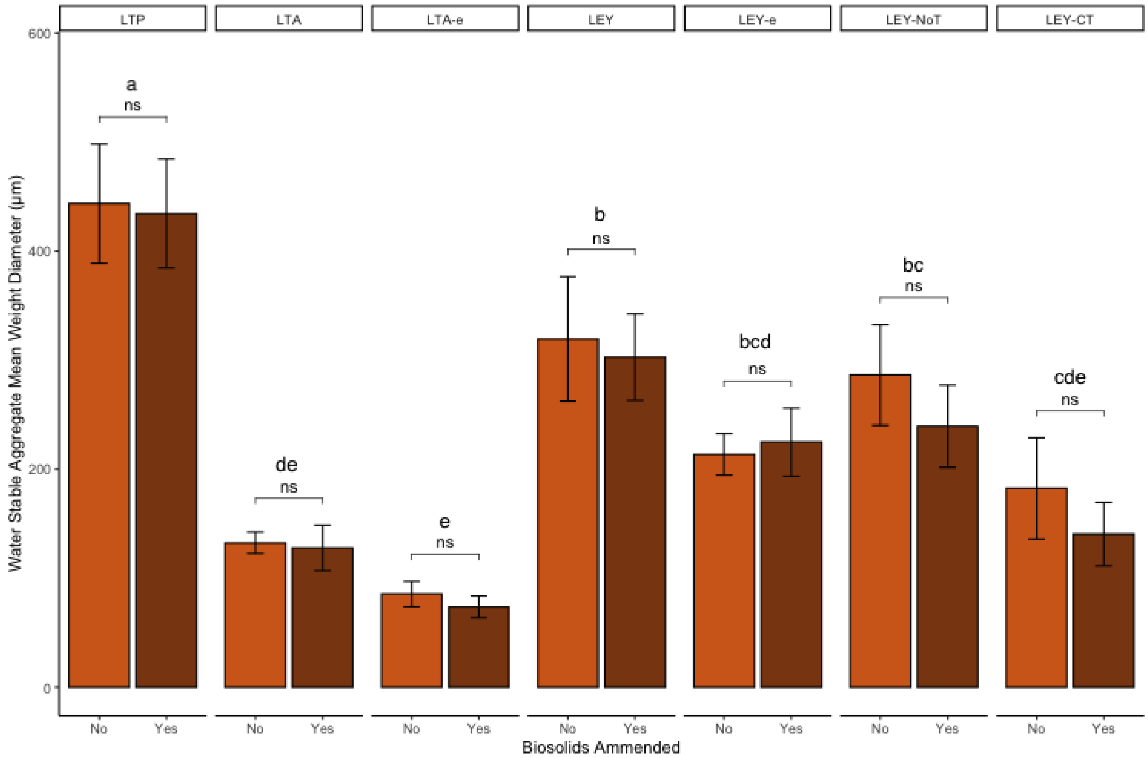

Figure 3-9: Mean weight diameter of soil water stable aggregates, showing no beneficial effect of biosolids additions but substantial effects of land management practices.

Table 3-1: Water stable aggregate proportional fractions of monolith soil and overall mean weight diameter of aggregates, results from Tukey HSD test for the effect of soil management treatment on water stable aggregates.

Size fraction (µm)	LTP	LTA	LTA-e	LEY	LEY-e	LEY-NoT	LEY-CT	p value
< 53	0.017	0.049	0.059	0.040	0.042	0.042	0.053	
	d	abc	a	c	bc	bc	ab	<0.001
	c	ab		b		b	a	<0.001
		ab	a	bc	c			<0.001
53 – 250	0.127	0.437	0.557	0.273	0.361	0.311	0.441	
	d	b	a	c	bc	c	b	<0.001
	c	a		b		b	a	<0.001
		b	a	c	bc			<0.001
250 - 1000	0.215	0.400	0.346	0.261	0.325	0.302	0.321	
	c	a	ab	bc	ab	abc	abc	<0.001
	c	a		bc		abc	ab	<0.001
		a	a	b	ab			<0.001
1000-2000	0.102	0.036	0.019	0.073	0.059	0.065	0.055	
	a	d	e	b	bc	bc	c	<0.001
	a	d		b		bc	c	<0.001
		c	d	a	b			<0.001
>2000	0.539	0.080	0.019	0.354	0.214	0.280	0.130	
	a	d	e	b	c	bc	d	<0.001
	a	c		b		b	c	<0.001
		c	d	a	b			<0.001
Mean weight	439	130	79	311	219	263	161	
diameter	a	de	e	b	bcd	bc	cde	<0.001
(µm)	a	d		b		bc	cd	<0.001
		c	c	a	b			<0.001

The carbon and nitrogen content and CN ratio of WSA in the > 2 mm fraction is presented in Figure 3-10. The effect of biosolids addition showed no significant effect within soil management treatments for all 3 measures. LTP had a significantly higher carbon and nitrogen content when compared to all other treatments, however considering the CN ratio, there was also no significant effect of management or earthworm treatment overall.

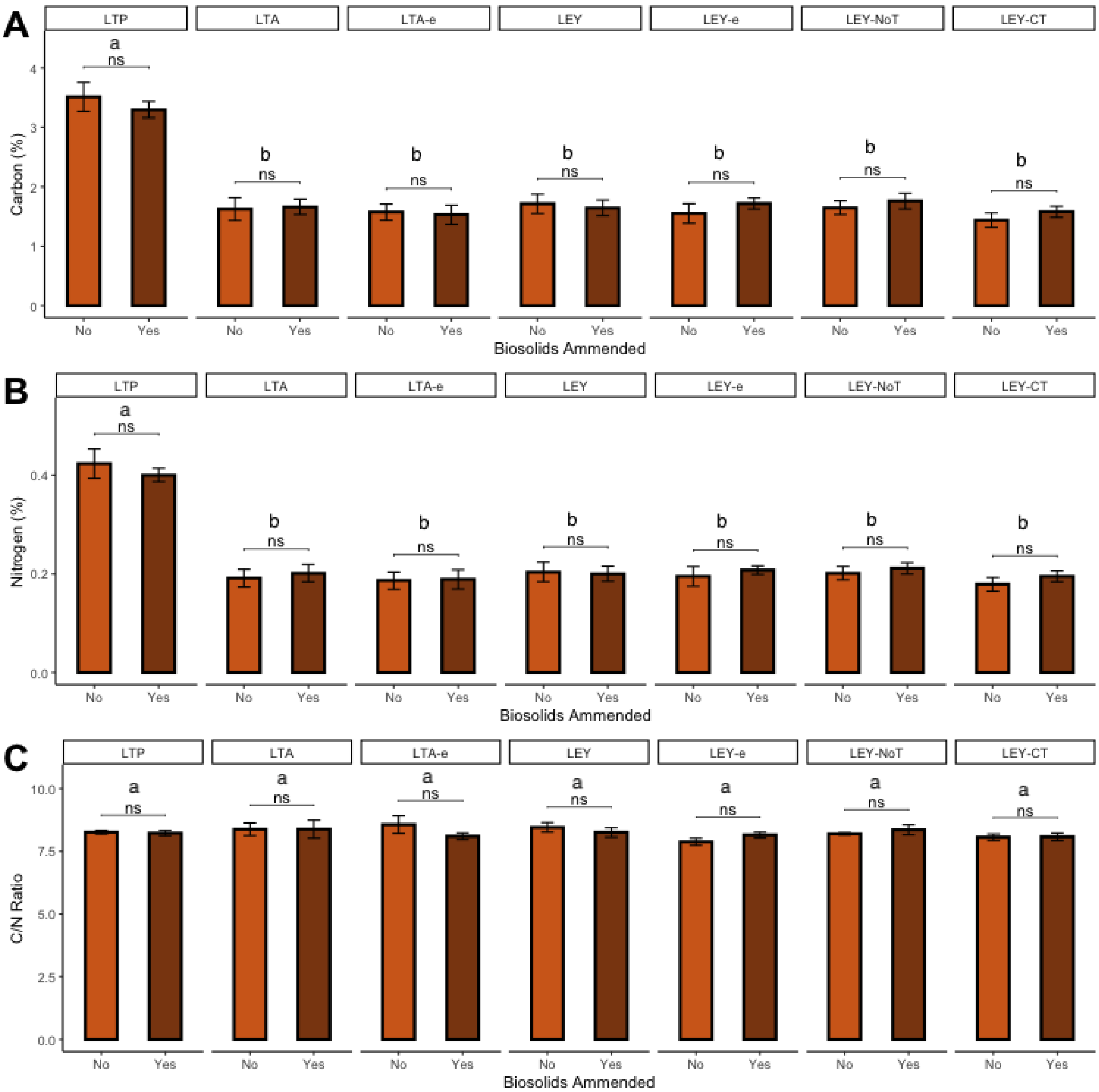

Figure 3-10: Water stable aggregate, >2mm fraction, (A) soil carbon, (B) soil nitrogen and (C) soil CN ratio.

3.3.4 Soil hydrology & water chemistry

3.3.4.1 Infiltration rate

Results for soil infiltration are presented in Figure 3-11, where the tensions 6, 3 and 0.5 represent flow through 0.5 mm, 0.5 mm - 1 mm and > 1-6 mm pore sizes respectively. Combining the flow through all pore sizes gives the total infiltration rate. The effect of biosolids amendment compared to the control within each soil management treatment showed no significant difference, except for the LEY-e treatment ($p < 0.05$), which had a significant reduction after biosolids were applied. There was not one overall trend for biosolids' effect on infiltration rate; LTP, LTA, LEY-e and LEY-NoT all saw reductions in total infiltration rate, whereas LTA-e, LEY and LEY-CT saw increases where biosolids were applied. Considering all treatments, there was no significant effect of any treatment on flow at tension 6 or 0.5 (0.5 mm and >1-6 mm pore sizes respectively), but a trend towards significance for the interaction of earthworm treatment and soil management at tension 6 ($p = 0.0963$) with LTA and LEY treatments that had been treated for earthworm removal trending towards lower infiltration rates. As shown in Figure 3-11, the management treatments with vegetative cover, LTP, LEY and LEY-e, have a slightly higher infiltration rate than the arable treatments. However, this was not significant.

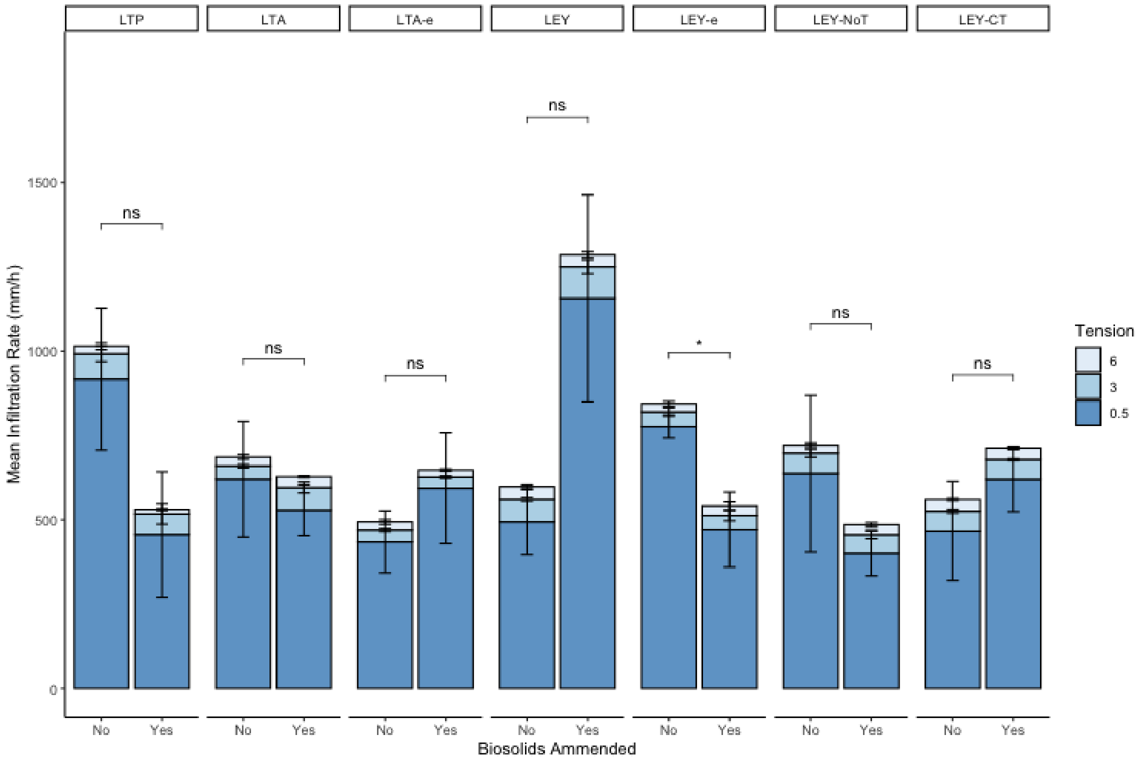

Figure 3-11: Monolith infiltration rates. Tensions 6, 3 and 0.5 represent flow through 0.5 mm, 0.5 mm - 1 mm and > 1-6 mm pore sizes respectively and combined to give total infiltration rate.

3.3.4.2 Soil water flow

Monolith hydrology, including total water volume in the soil (from rainfall and manual watering in the summer, this was the same for all monoliths), average soil moisture (presented again here) and total leachate volume out of the monoliths are presented in Figure 3-12. Considering leachate volumes only, there was no significant effect of biosolid amendment compared to the control within any of the treatments and no overall trend. Soil management had an overall significant effect on leachate volumes (p <0.001) for all groups of treatments, with the vegetation covered monoliths being significantly lower than the arable treatment monoliths. There was no significant effect of earthworm treatment on the leachate volume, and volumes out were similar between LTA and LTA-e and between LEY and LEY-e.

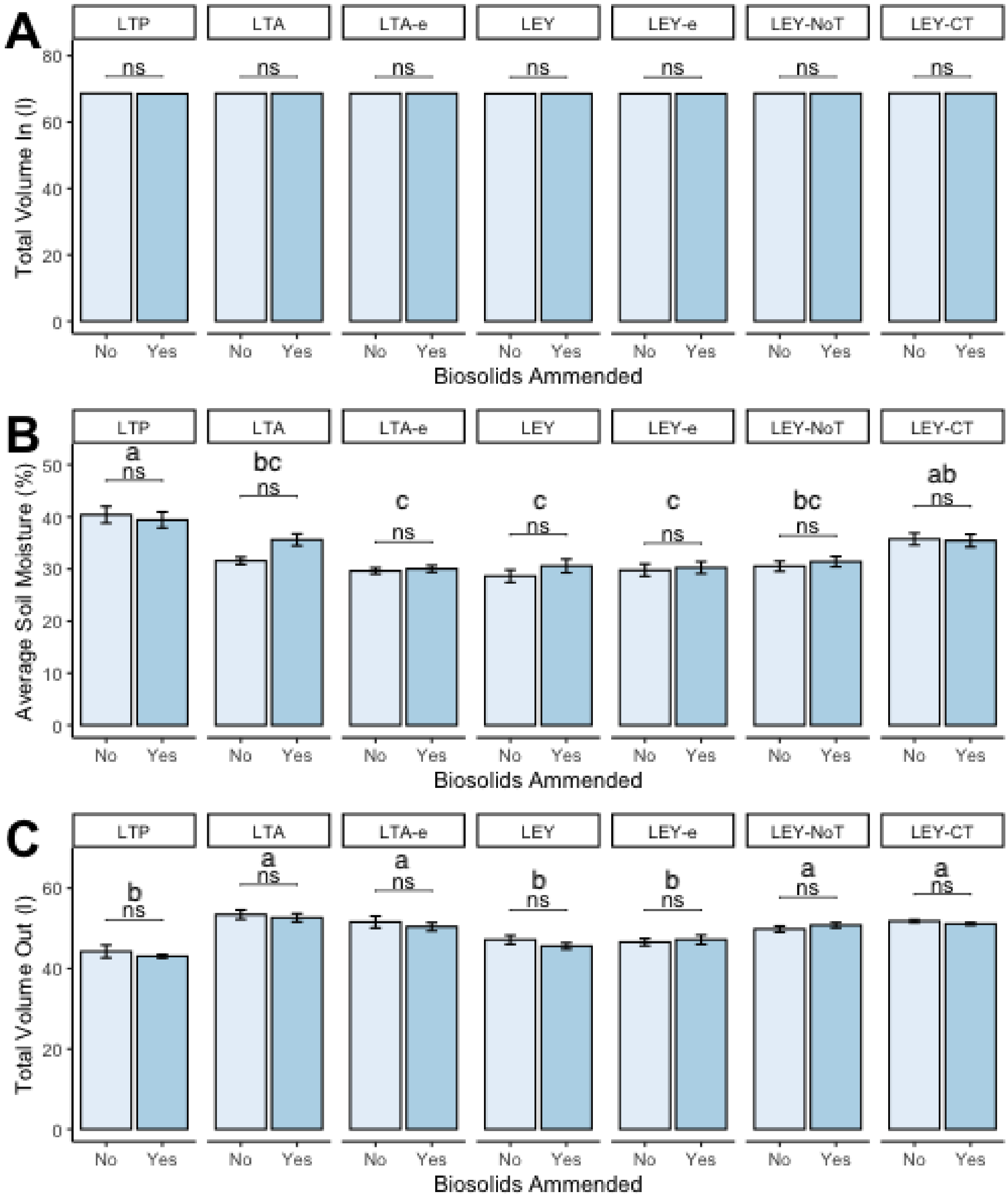

Figure 3-12: Monolith hydrology, total volume in (rainfall and added irrigation water), average soil moisture, and total moisture drained out under gravity as leachate.

3.3.4.3 Soil solution and leachate chemistry

Results from the liquid chemical analysis of the soil solutions leachates, where the majority of the dataset fell below detectable limits of analysis, are presented in Appendix A. Orthophosphate and ammonium are analytes of particular interest in this study. Analytes with sufficient data (within the limits of detection) are presented here and include nitrite, nitrate, and particulate inorganic and organic carbon.

3.3.4.3.1 Carbon

Figure 3-13 presents the inorganic and organic carbon content of soil solutions and leachates. In general, there was no significant effect of biosolids addition within soil management treatments, with two exceptions; LTA-e leachate had a significantly lower concentration of inorganic carbon where biosolids were added, and LEY-CT leachate had a significantly lower ($p < 0.05$) concentration of organic carbon where biosolids were applied. There was however, no consistent trend in the effect of biosolids amendment compared to the control in the leachate or soil solution.

Soil management had no significant effect on leachate inorganic carbon concentration but did have a significant effect on leachate organic carbon and soil solution inorganic and organic carbon ($p < 0.001$). LTP had the highest inorganic and organic carbon levels in soil solutions, followed by the LEY and LEY-e treatments. The lowest concentrations of carbon in the soil solutions were observed in the LEY-CT treatment. Organic carbon in the leachate showed a different trend to the soil solution, with LTP significantly higher than most of the other treatments, however this was followed by LEY-CT and the other treatments were not significantly different from each other.

Considering the effect of earthworms, there was no overall significant effect. There was no significant difference between LTA and LTA-e and between LEY and LEY-e; however, there was a trend towards carbon increasing in soil solutions where earthworms were removed and decreasing in leachate where earthworms were removed.

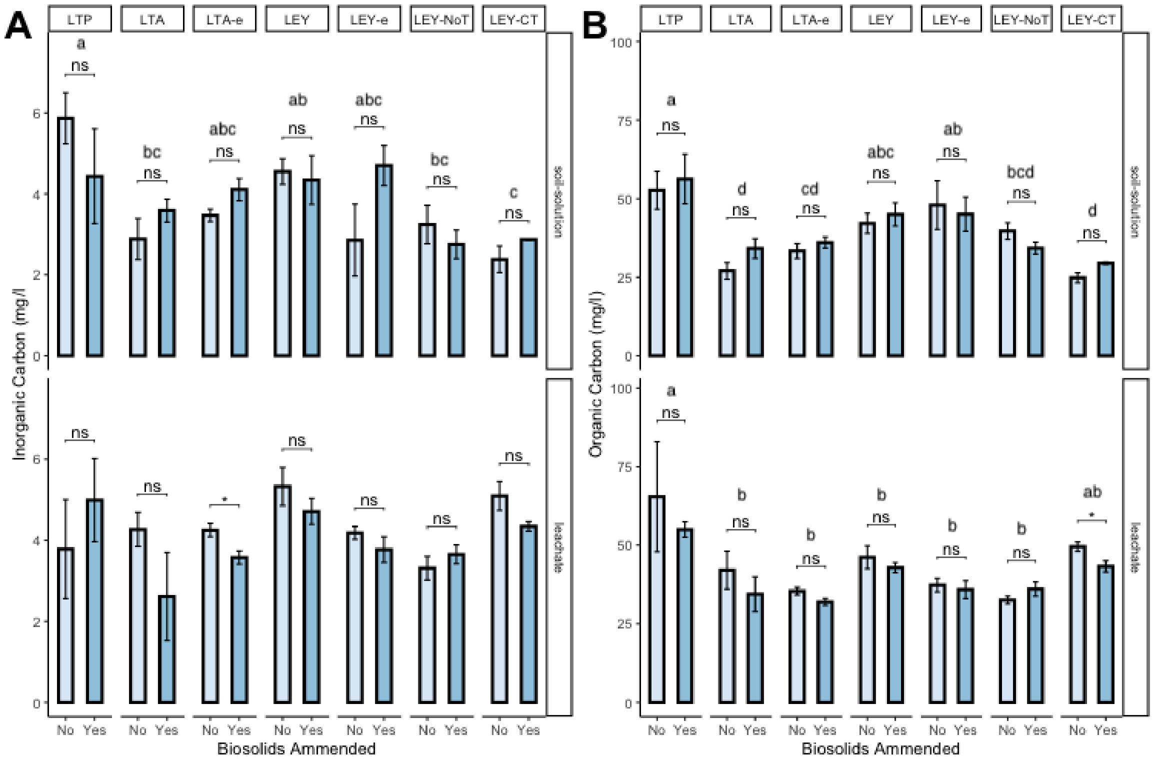

Figure 3-13: Monolith liquid chemistry, carbon. Soil solution and leachate inorganic and organic carbon content.

3.3.4.3.2 Nitrogen

Nitrate and nitrite results are presented in Figure 3-14. Nitrite showed no significant effect of biosolids compared to control for soil solutions and leachates, and no overall effect of earthworm treatment. There was a significant effect of management ($p < 0.05$) and a trend towards significance ($p = 0.07$) of biosolids amendment increasing the nitrite in both soil solution and leachate.

Biosolids had a significant effect ($p < 0.05$) on nitrate compared to the controls for some treatments in both the soil solution and in the leachate. These were mainly in the arable treatments where nitrate was significantly higher after biosolids amendment. There was also an overall significant increase due to biosolids ($p < 0.001$). Management had an overall significant effect ($p < 0.05$) on nitrate in the soil solution and leachate ($p < 0.001$). The full range of analysis that was conducted is presented in the summary tables at the end of this section.

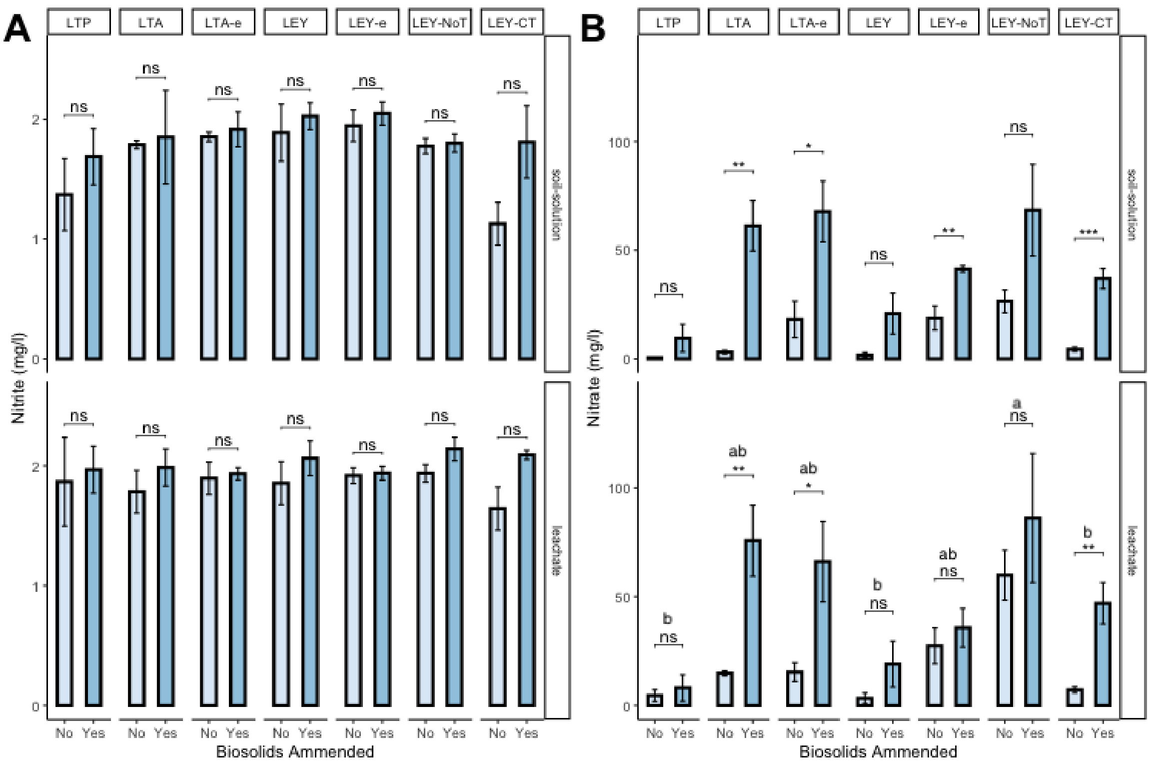

Figure 3-14: Monolith liquid chemistry, nitrogen. Soil solution (upper panels) and leachate (lower panels) for (A) nitrite and (B) nitrate concentrations. The ammonium concentration was below detectable limits in 95% of samples so is not presented here.

3.3.5 Soil Biological Properties

The results of the earthworm analysis pre-identification are presented in Figure 3-15, and post-identification in Appendix A. There was generally no significant effect of sludge addition compared to the control, except for fresh biomass where a significant increase (p <0.05) in mass was observed after biosolids addition in the LTP, LTA and LTA-e treatments, there was also a trend observed that earthworm biomass increased where biosolids had been applied, as this was the trend for all treatments except LEY-e. Management had a significant effect on fresh earthworm biomass, where LEY and LEY-CT had the highest biomass and LTA-e with the lowest observed biomass. The earthworm removal treatment was not 100 % effective, but there was a significant effect (p <0.05) of earthworm treatment on earthworm fresh biomass. The number of earthworms followed a similar trend to that of the fresh biomass; however, LEY-CT had the highest number followed by LEY-NoT, with LTA-e having the lowest of any treatment. Again, there was no significant effect of biosolids amendments compared to the controls within treatments, and no overall significant effect on earthworm number. Earthworm weight to number ratio, or weight per earthworm showed almost the opposite trend, with LTP having the highest average mass per earthworm, and LTA-e, LEY-NoT and LEY-CT the lowest. There was no overall significant effect of biosolids on earthworm weight, but there was a trend towards significance (p = 0.091) with biosolid amendment increasing the weight of earthworms, although within treatments, this was only a significant increase (p <0.05) for LTA.

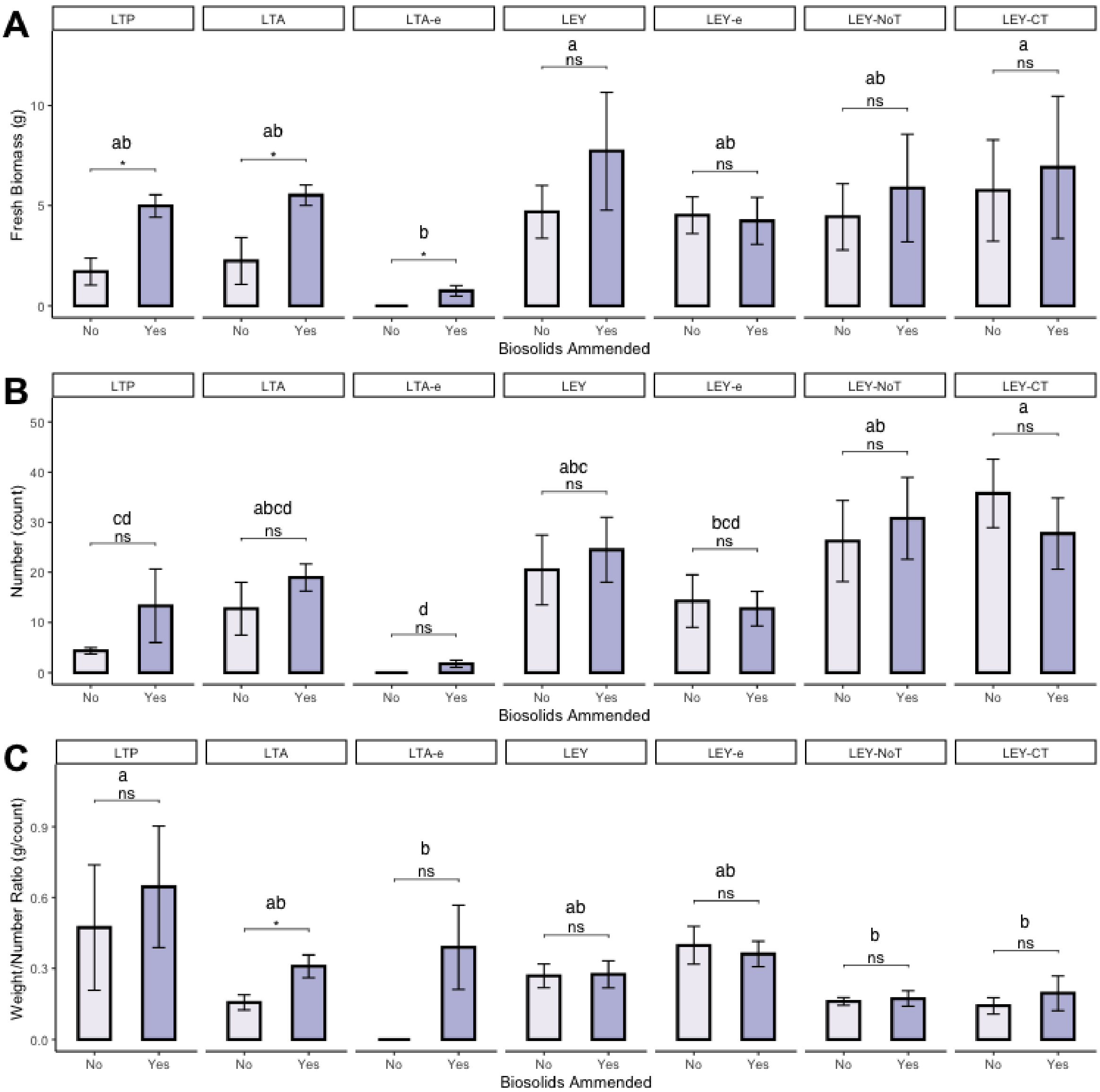

Figure 3-15: Monolith earthworm results pre identification. (A) fresh mass, (B) total number and (C) weight ratio.

3.3.6 Crop Production

Results of wheat production are presented in Figure 3-16, where grain and vegetative biomass have been separated and grain mass adjusted to 15 % moisture to be comparable to national reporting yield data.

For all biomass, the effect of biosolids amendment compared to the control showed no significant effect within any soil management treatment and had no overall significant effect on biomass production. There is, however, a trend in all biomass measures for biosolids amendment to increase crop production. For wheat biomass, soil management and earthworms had a significant effect ($p < 0.001$), with treatments derived from LEY having significantly higher crop production. Comparing LTA and LTA-e there was approximately 20 % reduction in the crop production in the LTA-e; however, the difference was not significant. Grain yield at 15% moisture showed the same trends as the wheat total biomass, with no significant difference between the control and biosolids within treatments and a significant effect of management on grain yield ($p < 0.05$). Again, the trend of the data showed that biosolids amendments did increase grain yield, although this was not significant. LEY derived treatments had a significantly higher, and almost double, the grain yield of permanent arable and there was no significant difference between LTA and LTA-e. There was no overall significant effect of biosolids or earthworms for pasture and ley vegetation, but soil management had an overall significant effect ($p < 0.05$). Although there was no significant effect of biosolids amendment within soil management treatments, there was a strong trend towards significance of biomass increasing after biosolids amendment. Soil management significantly affected biomass production ($p < 0.05$), with LEY producing the highest weight of biomass and LEY-e and LTP producing similar quantities.

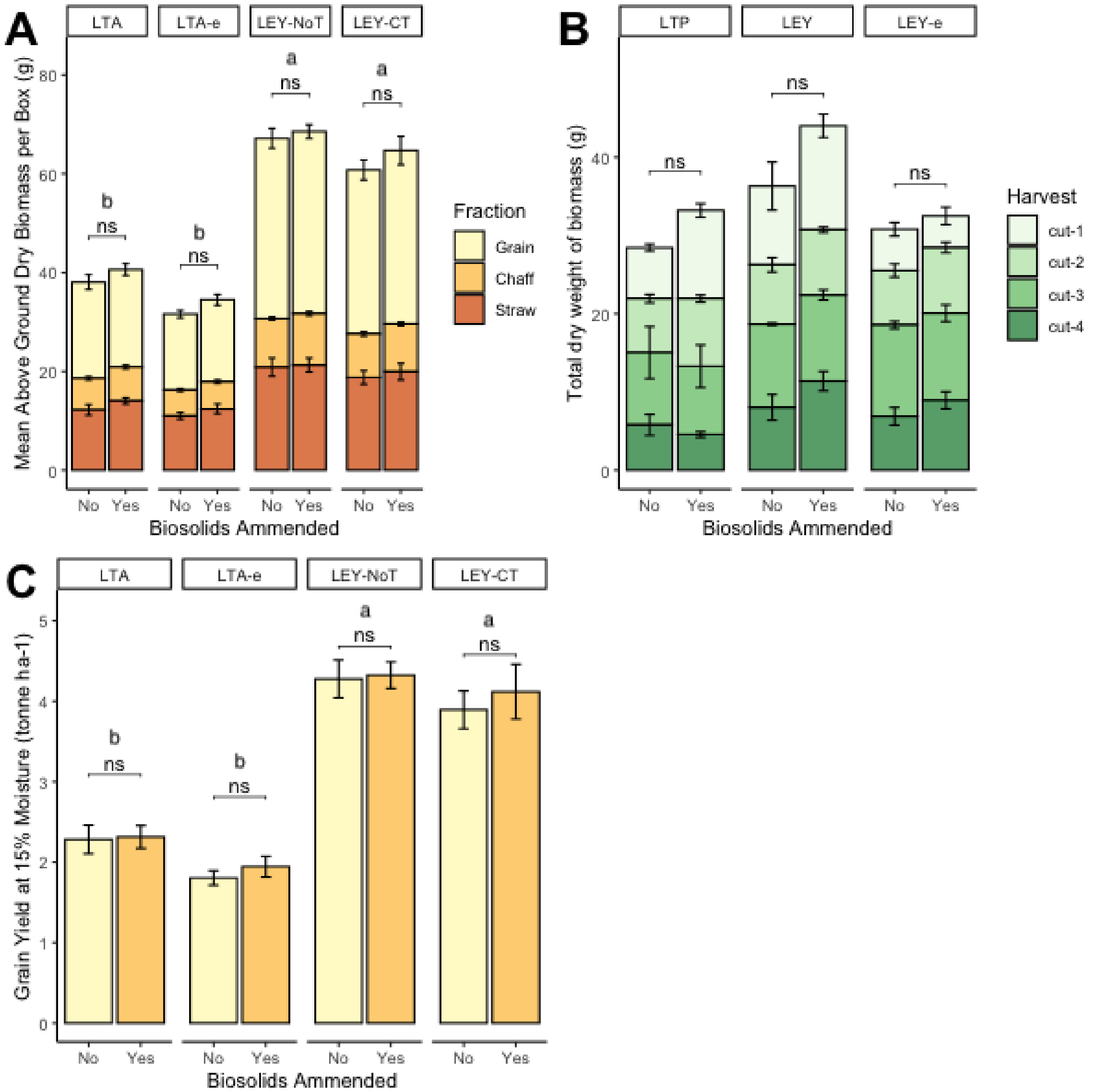

Figure 3-16: Monolith biomass results. (A) wheat above ground dry weight biomass, (B) vegetation dry weight biomass, and (C) wheat grain yield adjusted to 15% moisture.

3.3.7 Interactions between variables

The PCA conducted included as many of the determinants that were measured as possible, the results of which are presented in Figure 3-17. The figure shows the results of the PCA, grouped by the different treatments, (A) soil management, (B) biosolids amended and (C) earthworm treatment. A further group (D) is displayed to assess the contribution of field variation on the results. Where ellipses are separate, this suggests significantly different results between groups.

The x-axis, Dim 1, represents 24.6% of the variation within the results and comprises of mainly soil physical characteristics (bulk density, MWD, soil C and N content, and soil moisture). The y-axis, Dim 2, represents 21.9% of the variation in the results and comprises mainly of the soil biological characteristics (fresh earthworm biomass, total earthworm number and adult/juvenile number, and total crop/vegetation biomass). Combined Dim 3 - 5 represent a further 25% of the samples' variation and comprises of mainly chemical characteristics of the soil (soil moisture, soil solution and leachate chemistry, infiltration rate and C:N ratio).

In Figure 3-17A, the LTP and LTA-e treatments are separate from the other treatments and are therefore significantly different. LEY-e is shifting away from LEY and towards LTA-e but they still overlap. Interestingly LEY-NoT and LEY-CT (which were derived from LEY) do not overlap LEY, suggesting that they are now significantly different and have shifted more to LTA after just one cropping cycle. In Figure 3-17B, biosolid amendment (Yes) is still overlapping the control (No), suggesting there is no overall significant difference between the two. In Figure 3-17C there is complete separation between the treatments with and without earthworms, suggesting that their functioning is now significantly different. In Figure 3-17D, there is separation between the LTP fields. However, there is also a wide spread of the arable fields, where the ellipses only overlap one or two of the other fields, showing that there are significant differences between field results in this experiment.

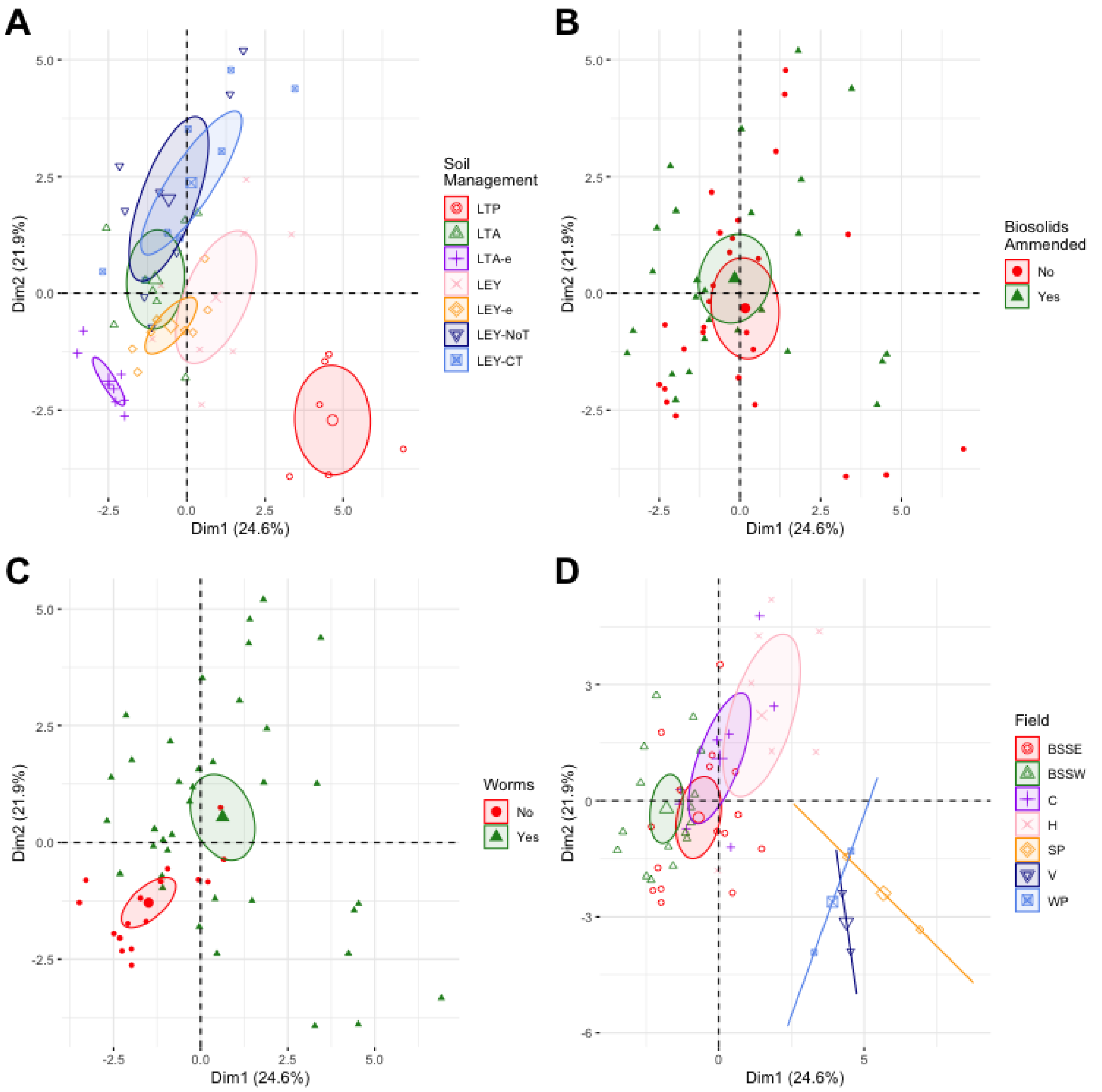

Figure 3-17: Monolith experiment principal component analysis. Ellipses represent 95% confidence intervals. Measures included: bulk density (g/cm2), total infiltration rate (mm/hr), fresh earthworm mass (g), earthworm number, earthworm adult number, earthworm juvenile number, earthworm species diversity, water stable aggregate mean weight diameter, macroaggregate carbon content (%), macroaggregate nitrogen content (%), macroaggregate C/N ratio, dry weight of biomass (g), average soil moisture (%), leachate chemistry (nitrate, nitrite, inorganic carbon and organic carbon, in mg/l), and soil solution chemistry (nitrate, nitrite, inorganic carbon and organic carbon in mg/l).

3.3.8 Summary ANOVA *p* values tables

A summary of statistical tests conducted for each subgroup of analysis are presented below, group (1) all soil management treatments in Table 3-2, group (2) a subset consisting of all treatments with earthworms in Table 3-3, and group (3) a subset comparing LTA and LEY treatments with and without earthworms in Table 3-4.

Table 3-2: Statistical summary table, considering all treatments. Values presented are p values with significant or almost significant results.

Soil chemical, structural and hydrological variables All treatments	Biosolids	Earthworms	Management	Biosolids X earthworms	Biosolids X management	Earthworms X management	Biosolids X Earthworms X Management
Soil moisture and thermal properties							
Average soil moisture	ns	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Soil moisture (repeated measure)	0.011*	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Soil physical properties							
Bulk Density 1 - 6 cm depth	ns	<0.001***	<0.001***	0.006**	<0.001***	<0.001***	<0.001***
Bulk Density 8 – 13 cm depth	ns	0.020*	0.025*	ns	ns	0.003**	0.032*
Bulk Density 15 – 20 cm depth	ns	0.001**	<0.001***	0.015*	<0.001***	<0.001***	0.003**
Bulk Density all depths	ns	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
WSA Macroaggregate (>2mm) proportional weight	ns	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
WSA mean weight diameter	ns	0.005**	<0.001***	0.0424*	<0.001***	<0.001***	<0.001***
WSA Macroaggregate (>2mm) CN ratio	ns	ns	ns	ns	ns	ns	ns
Soil infiltration and water chemistry properties							
Soil infiltration rate 6	ns	ns	ns	ns	ns	0.096^	ns
Soil infiltration rate 3	ns	0.002**	ns	0.013*	ns	0.0168*	ns
Soil infiltration rate 0.5	ns	ns	ns	ns	ns	ns	0.079^
Soil infiltration rate total	ns	ns	ns	ns	ns	ns	0.049*
Total leachate through monoliths	ns	ns	<0.001***	ns	<0.001***	<0.001***	<0.001***
Soil solution chemistry - Nitrite	0.071^	0.063^	0.009**	0.064*	0.012*	0.036*	0.086^
Soil solution chemistry - Nitrate	<0.001***	ns	0.018*	<0.001***	<0.001***	0.025*	<0.001***
Soil solution chemistry – Organic Carbon	ns	ns	<0.001***	ns	<0.001***	<0.001***	<0.001***
Soil solution chemistry – Inorganic Carbon	ns	ns	<0.001***	ns	<0.001***	<0.001***	0.001**
Soil Leachate chemistry - Nitrite	0.018*	ns	ns	0.061^	ns	ns	ns
Soil Leachate chemistry - Nitrate	<0.001***	ns	<0.001***	0.008**	<0.001***	0.002**	<0.001***
Soil Leachate chemistry – Organic Carbon	ns	0.010**	<0.001***	0.039*	<0.001***	<0.001***	<0.001***
Soil Leachate chemistry – Inorganic Carbon	ns	ns	0.05*	ns	0.034*	0.030*	0.034*
Soil biological properties							
Earthworm total fresh biomass	0.095^	0.017*	0.050*	0.022*	ns	0.027*	ns
Earthworm total number	ns	<0.001***	<0.001***	0.004**	0.002**	<0.001***	<0.001***
Earthworm weight/number ratio	0.091^	ns	0.002**	ns	0.003**	0.008**	0.014 *
Crop production*							
Wheat total harvested biomass	ns	<0.001***	<0.001***	0.001**	<0.001***	<0.001***	<0.001***
Wheat grain yield at 15% moisture (tonne/ha)	ns	<0.001***	<0.001***	0.001**	<0.001***	<0.001***	<0.001***
Vegetation total harvested biomass	ns	ns	0.015*	ns	0.025*	0.056^	ns

* Wheat straw and grain biomass (LTA, LTA-e, LEY-NoT and LEY-CT only), Vegetation biomass (LTP, LEY and LEY-e only).
Significance codes: *p* <0.001 (***), p<0.01 (**), p<0.05(*), p<0.1(^), p>0.1 (ns).

Table 3-3: Statistical summary table, considering all treatments with earthworms only (LTP, LTA, LEY, LEY-NoT, LEY-CT). Values presented are p values with significant or almost significant results.

Soil chemical, structural and hydrological variables All +e treatments	Biosolids	Earthworms	Management	Biosolids X earthworms	Biosolids X management	Earthworms X management	Biosolids X Earthworms X Management
Soil moisture and thermal properties							
Average soil moisture	ns		<0.001***		<0.001***		
Soil moisture (repeated measure)	0.012*		<0.001***		<0.001***		
Soil physical properties							
Bulk Density 1 - 6 cm depth	ns		<0.001***		0.032*		
Bulk Density 8 – 13 cm depth	ns		0.098^		ns		
Bulk Density 15 – 20 cm depth	ns		0.005**		0.005**		
Bulk Density all depths	ns		<0.001***		<0.001***		
WSA Macroaggregate (>2mm) proportional weight	ns		<0.001***		<0.001***		
WSA mean weight diameter	ns		<0.001***		<0.001***		
WSA Macroaggregate (>2mm) CN ratio	ns		ns		ns		
Soil infiltration and water chemistry properties							
Soil infiltration rate 6	ns		0.042*		ns		
Soil infiltration rate 3	ns		ns		ns		
Soil infiltration rate 0.5	ns		ns		ns		
Soil infiltration rate total	ns		ns		0.085^		
Total leachate through monoliths	ns		<0.001***		<0.001***		
Soil solution chemistry - Nitrite	0.0941^		ns		ns		
Soil solution chemistry - Nitrate	<0.001***		0.026*		<0.001***		
Soil solution chemistry – Organic Carbon	ns		<0.001***		<0.001***		
Soil solution chemistry – Inorganic Carbon	ns		<0.001***		0.003**		
Soil Leachate chemistry - Nitrite	0.0179*		ns		ns		
Soil Leachate chemistry - Nitrate	0.00639**		0.001**		<0.001***		
Soil Leachate chemistry – Organic Carbon	ns		<0.001***		0.009**		
Soil Leachate chemistry – Inorganic Carbon	ns		0.042*		0.064^		
Soil biological properties							
Earthworm total fresh biomass	0.0691^		ns		ns		
Earthworm total number	ns		0.009**		0.089^		
Earthworm weight/number ratio	ns		0.003**		0.040*		
Crop production*							
Wheat total harvested biomass	ns		<0.001***		<0.001***		
Wheat grain yield at 15% moisture (tonne/ha)	ns		<0.001***		<0.001***		
Vegetation total harvested biomass	ns		0.090^		ns		

* Wheat straw and grain biomass (LTA, LTA-e, LEY-NoT and LEY-CT only), Vegetation biomass (LTP, LEY and LEY-e only).
Significance. codes: *p* <0.001 (***), *p* <0.01 (**), *p* <0.05(*), *p* <0.1(^), *p* >0.1 (ns).

Table 3-4: Statistical summary table, considering treatments to compare the effect of earthworms (LTA, LTA-e, LEY, LEY-e). Values presented are p values with significant or almost significant results.

Soil chemical, structural and hydrological variables LTA and LEY + & - e	Biosolids	Earthworms	Management	Biosolids X earthworms	Biosolids X management	Earthworms X management	Biosolids X Earthworms X Management
Soil moisture and thermal properties							
Average soil moisture	0.020*	0.025*	0.0101*	0.004**	0.006**	<0.001***	<0.001***
Soil moisture (repeated measure)	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Soil physical properties							
Bulk Density 1 - 6 cm depth	ns	0.003**	0.014*	0.031*	0.097^	<0.001***	0.021*
Bulk Density 8 – 13 cm depth	ns	ns	ns	ns	ns	0.031*	0.084^
Bulk Density 15 – 20 cm depth	ns	ns	ns	ns	ns	ns	ns
Bulk Density all depths	ns	0.006**	0.018*	0.014*	0.072^	0.002**	0.005**
WSA Macroaggregate (>2mm) proportional weight	ns	0.004**	<0.001***	0.037*	<0.001***	<0.001***	<0.001***
WSA mean weight diameter	ns	0.051^	<0.001***	ns	<0.001***	<0.001***	<0.001***
WSA Macroaggregate (>2mm) CN ratio	ns	ns	ns	ns	ns	ns	ns
Soil infiltration and water chemistry properties							
Soil infiltration rate 6	ns	0.080^	ns	ns	ns	ns	ns
Soil infiltration rate 3	ns	0.004**	0.095^	0.005**	ns	0.005**	0.017*
Soil infiltration rate 0.5	ns	ns	ns	ns	ns	ns	0.052^
Soil infiltration rate total	ns	ns	ns	ns	ns	ns	0.021*
Total leachate through monoliths	ns	ns	<0.001***	ns	<0.001***	<0.001***	<0.001***
Soil solution chemistry - Nitrite	ns	ns	ns	ns	ns	ns	ns
Soil solution chemistry - Nitrate	<0.001***	ns	0.085^	<0.001***	<0.001***	ns	<0.001***
Soil solution chemistry – Organic Carbon	ns	ns	0.085^	ns	0.085^	0.085^	0.011*
Soil solution chemistry – Inorganic Carbon	0.057^	ns	0.065^	ns	0.072^	ns	0.052^
Soil Leachate chemistry - Nitrite	ns	ns	ns	ns	ns	ns	ns
Soil Leachate chemistry - Nitrate	<0.001***	ns	0.045*	0.009**	<0.001***	ns	<0.001***
Soil Leachate chemistry – Organic Carbon	ns	0.019*	0.094^	0.044*	ns	0.028*	ns
Soil Leachate chemistry – Inorganic Carbon	0.035*	ns	0.040*	ns	0.020*	0.034*	0.029*
Soil biological properties							
Earthworm total fresh biomass	ns	0.023*	0.006**	0.006**	0.020*	0.002**	0.008**
Earthworm total number	ns	0.002**	0.015*	0.015*	0.097^	<0.001***	0.009**
Earthworm weight/number ratio	0.053^	ns	0.094^	ns	0.0057**	ns	0.025*
Crop production*							
Wheat total harvested biomass		ns					
Wheat grain yield at 15% moisture (tonne/ha)		ns					
Vegetation total harvested biomass		ns					

* Wheat straw and grain biomass (LTA, LTA-e, LEY-NoT and LEY-CT only), Vegetation biomass (LTP, LEY and LEY-e only).
Significance. codes: *p* <0.001 (***), p <0.01 (**), *p* <0.05(*), *p* <0.1(^), *p* >0.1 (ns).

3.4 Discussion

The increasing uptake of reduced tillage and conservation agricultural practises, including the addition of organic matter, provides an opportunity to improve the quality of agricultural soils in the UK. Biosolids are one form of organic material; however, the regulations through assurance schemes and statutory management requirements mean the physical incorporation of the material within 24 hours of application is currently necessary. An assessment needs to be made to determine the extent to which the surface application of biosolids interacts with soil systems and how this interaction compares to previous studies in which biosolids are ploughed in. In particular, this study has assessed whether biosolids are incorporated into the soil, through biotic and abiotic factors, in a timely enough manner when the soil has previously been undisturbed or under management that maintains larger populations of earthworms than conventional annual cropping with tillage. In this discussion the utility of applying typical biosolids to a soils surface are discussed based on the results of this experiment.

3.4.1 Observations from monolith surface images

The surface images of the biosolids amended plots reveal some interesting differences between soil managements and the biosolids and rainfall interactions. The treatments with the most biosolids remaining on the surface at the end of the experiment include those from the most degraded soil treatments, LTA, LTA-e and LEY-e. LTP, which would be considered biologically active, also had regions with biosolids remaining at the end of the experiment. This could be due to the soil surface cover by grass; however, this was not observed for LEY. This suggests that in this treatment, earthworms were either not choosing the biosolids as food choice or that the earthworms were not as active at the surface. The treatments with vegetation cover did provide a quick coverage of the biosolids, providing protection from erosive forces. Interestingly the LEY-NoT had the fastest disappearance of biosolids from the soil surface of all the arable treatments. This is particularly interesting, as it shows that when biosolids are surface applied to biologically active soils, the material is incorporated/disappeared from the surface faster than for conventionally managed and more biologically, structurally and organic-matter depleted, degraded soils. In the field, studies of the earthworm populations at Leeds University Farm in the arable-to-ley conversions have found higher numbers of earthworms in the leys than in permanent grassland after 2-3 years, possibly due to the palatability of clover litter (Leake personal communication, 2021). Another interesting observation was that LEY monoliths with and without earthworms are at opposite ends of the incorporation/disappearance spectrum. This is an indication of the important role that earthworms play in soil systems to incorporate biosolids and other organic materials. The fastest incorporation of biosolids still took 4-5 months over winter, which could pose a threat for runoff and erosion of the biosolids material in the field. The incorporation became faster in March-May after the spring rewetting when the earthworm's activity increases as the soils warm (Eggleton *et al.*, 2009; Potvin & Lilleskov, 2017). This could indicate that spring application of biosolids may be more appropriate where biosolids are surface applied, making use of the increased earthworm activity for bioturbation. However, this may cause a delay in the mineralisation of nutrients delaying their availability for crops during vital stages in crop growth.

3.4.2 Soil moisture and thermal properties

The difference in soil moisture is related to air and soil temperature, with an increase in evaporation and evapotranspiration during warmer periods. During the winter season, there were periods of sub-zero temperatures, however, the soil at 10 cm depth did not reach temperatures of less than zero degrees Celsius. The soil moisture during this time was held at approximately 40% with differences between treatments likely due to the limit of water holding capacity of the soils and the variation in soil weight due to stones in each monolith. During the summer, there was a heatwave and prolonged period of drought. Even with supplementary watering to account for the lack of subsoil water reserves, the monoliths succumbed to a great reduction in moisture content (to approximately 20%) as the soil temperatures by May reached consistently above 10 degrees. During this period, treatments with vegetation (LTP, LEY and LEY-e) lost the most soil moisture, likely due to increased levels of evapotranspiration. Interestingly treatments that had the highest variability in soil moisture did not always have the highest variability in temperature. The vegetation covered monoliths appeared to be the most insulated from temperature fluctuations, with the arable monoliths seeing the most variability in temperature. The effect of biosolids on soil moisture were modest increases, although the lack of effect on soil physical structure indicates this is solely due to the water holding capacity of the biosolids material and not a result of the biosolids affecting the soil structure.

3.4.3 Soil physical properties

Although there was generally no significant effect of biosolids amendment on soil properties, for WSA there was a strong trend towards significant negative effects, especially in the largest size fraction of macroaggregates which showed a consistent trend of reduction in six out of the seven treatments. This is an important finding, as although not significant, may suggest a negative impact of the biosolids on soil physical properties which has not been reported previously and should be investigated further.

Soil management had a significant effect on soil physical properties across bulk density and water stable aggregates. The bulk density at the soil surface is the most variable, as expected. It is the most manipulated and vulnerable layer to management practices and the effects of weather patterns. The WSA and MWD results also highlight the impact that management can have on soil properties. The LTP treatment had over 50% of aggregates in the largest aggregate fraction, however, years of continuous arable cropping reduced this to less than 10% in the LTA treatment. The role of leys in restoring soil properties can then be seen in the significant increase in large WSA back up to approximately 35% after 3 years. Following the restorative ley period, immediate cultivation through conventional ploughing instantly reduced this proportion of large WSA by more than half in the LEY-CT treatment, whereas converting ley to no-tillage only reduced the proportion of macroaggregates by 20% in the LEY-NoT treatment. This highlights the importance of responsible soil management, where years of soil restoration can be undone almost immediately. This is consistent with the study of Low (1972), which showed that grassland soils with 75% of the soil mass as water-table macroaggregates lost more than 50% of these in the first year of ploughing and arable cropping. The larger proportion of largest water stable aggregates will help with water holding capacity, nutrient retention, and resilience during extreme wet and dry periods.

The role of earthworms in soil aggregation has been strongly linked (Al-Maliki & Scullion, 2013) and with the inclusion/exclusion of earthworms, this is clearly seen with a 75% reduction in >2 mm aggregates between LTA to LTA-e and 33% reduction from LEY to LEY-e. Again, supporting the importance of creating an environment where earthworms' populations are promoted. Reduced tillage is the obvious management practice that promotes earthworm populations, but even more so is the use of leys in arable rotations and the application of organic materials to the soil, replacing what is removed and providing sufficient material for earthworms to feed on.

The carbon and nitrogen analysis of the >2 mm WSA fraction, which is the most important for storing carbon and nitrogen long term (Wright & Hons, 2005), showed no significant difference between most treatments. The LTP treatment, which is the most undisturbed long-term treatment, was significantly higher, showing the importance of long-term soil management. The addition of biosolids did not have a significant effect on these variables, but there were slight increases in carbon %, which may compound to become significant after subsequent years of biosolids amendment.

3.4.4 Soil hydrology and soil solution and leachate chemistry

Unsurprisingly the largest proportion of flow occurred through the > 1 mm pore sizes, with flow through the < 1 mm pores making up only about 10 % of the flow. Within the LEY-e treatment, there was a significant effect of biosolids, where a reduction in infiltration rate was seen. This interaction is hard to explain as there was no overall trend in the effect of biosolids on the infiltration rate. It may be due to disaggregated biosolids particles blocking drainage channels or reduced earthworm activity at the surface, creating fewer channels for drainage but could also be caused by the relatively high variability in the measurements. The effect of soil management was not significant, and there were no patterns in the infiltration rate data, meaning that there is either no effect or the variation in the data is masking any effect. The trend towards significance for the effect of earthworms in the smallest pore size and a significant effect of earthworms in the medium pore sizes, suggests that earthworms greatly influence the infiltration rate of the soil, especially in the smaller pore size. Although it was not significant, there is a reduction in the total infiltration from LTA to LTA-e and LEY to LEY-e, suggesting that earthworms play a vital role in the water infiltration between soil managements, which is supported by the literature (Abid & Lal, 2009) including, at the field site at Spen farm from which the monoliths were obtained (Hallam *et al.*, 2020).

The monoliths' total leachate was higher for the arable treatments, but not significantly different between them. This suggests that it is the vegetation cover that was the primary influencer on leachate volume. This is expected, as the water balance will be driven by rainfall inputs, soil moisture and water outputs, weather this be through evapotranspiration or leachate.

Changes in the dissolved organic and inorganic carbon within the soil solutions and leachates did not see any general effects of biosolids amendment. The main changes were due to the changes in soil management. The increase in inorganic and organic carbon seen in the soil solutions for the vegetative samples are likely an effect of the soil management system as increases in dissolved organic and inorganic carbon are linked to turnover of leaf and root biomass (Hussain *et al.*, 2020), the lower concentrations in the arable treatments seen in this experiment supports this. Higher concentrations in leachates may provide a pathway for carbon from the agricultural system, reducing their contribution to long term soil stocks and may contribute to pollution of surface and groundwater by dissolved organic carbon and co-associated nutrients and other chemicals.

Nitrite and ammonium were found in low concentrations, and for nitrite there was no significant effect of management or biosolids addition, making nitrate the main source of readily available nitrogen in the system. The higher levels of nitrate in the biosolids amended treatments will be directly from the biosolids amended on the surface, but interestingly management also had a significant effect on the nitrate concentration. This may indicate that the management is having an effect on the mineralisation of nutrients from the biosolids, may be storing more/less of it bound to soils, or more nitrate is available from the soil itself. From the biosolids analysis, results of which were presented in Chapter 2, there was a high amount of ammonium in the biosolids at 8200 mg/kg, and a low amount of nitrate and nitrite, <10 mg/kg. This suggests that the ammonium from the biosolids has been converted to nitrite and then further to nitrate, ready for uptake by plants. The lower concentrations of nitrate in the vegetative treatments suggest that plants had already took up more of this nitrate, or there was a greater interaction between the soil nitrifying bacteria and the biosolids through rainfall where the biosolids in the arable treatments were more exposed on the surface. As the soil solution and leachate samples analysed here were from January 2018, where there was little plant growth, this suggests that the latter is true. Smith and Chalk (2020) determined through a review of published studies that N mineralisation was primarily influenced by soil moisture, followed by soil temperature. However, comparing the nitrate concentrations to soil temperature, there was no real difference between the soil temperatures and where soil moistures were higher in LTP, LEY-CT and LTA, these were not the same treatments that had the highest nitrate concentration.

3.4.5 Soil biology

There was some evidence to suggest that biosolids amendment significantly increased earthworm biomass and this was also reflected in the number of earthworms present, however, this was not seen in all treatments. It does suggest that the earthworms were interacting with the biosolids material. As described by Lavelle *et al.*, (2007), earthworms consume increasingly richer substrates (containing more organic matter and nutrients) as temperatures decrease. Therefore, the biosolids may have helped support earthworm populations over the winter. This is supported by Doube *et al.*, (1997), who reported most earthworm species preferring consuming a mixture of organic and mineral sources compared to each individually. The treatments with the highest earthworm biomasses also had high earthworm numbers, many of which were juveniles, suggesting that biosolids were not inhibitory to earthworm reproduction. Treatments with the lowest biomass and number had fewer but larger earthworms. Endogenic earthworms were the most common type in the monoliths, and this ecotype is characteristic of earthworms that are smaller in size and form shallow burrows throughout the surface soil layer (Sherlock, 2018). The lack of subsoil and relatively shallow depth of the monoliths may have prevented deeper burrowing species from thriving.

The significant effect of soil management on earthworm biomass and number, where LEY derived monoliths had significantly larger populations than LTA derived monoliths, provides additional evidence that management practices that are more disruptive and provide less carbon inputs to the soils have a significant negative impact on earthworm populations.

3.4.6 Crop production

The effect of biosolid addition on wheat production was not significant overall but did contribute to a trend of increased biomass production compared to the control seen in all treatments, likely due to the addition of nutrients rather than any effects on soil properties as these were mainly not significant. The increase may not have been significant due to the relatively low proportion of available nutrients added from the biosolids compared to the nitrogen fertiliser applied to the wheat monoliths. Despite the nutrient additions from the biosolids and fertilisers, the LEY-NoT and LEY-CT had significantly higher biomass and grain yields to that of the LTA and LTA-e, suggesting that the time the soil spent in ley contributed to the soil nutrient pool with legume N fixation, and the improvements in soil structure that supported a better environment for crop growth.

Although not reflected in the statistics, there was a reduction in the biomass production in treatments where earthworms had been removed compared to where earthworms were still present, LEY to LEY-e and LTA to LTA-e. This reinforces the idea that earthworms act as vital ecosystem engineers and are likely contributing to the movement of nutrients within soils, improving soil nutrient availability and water regulation. Hallam *et al.*, (2020) also found that wheat biomass production was significantly increased in the same soils at Leeds University Farm, when earthworms were added back into monoliths from which they had been removed by the same deep-freezing treatment used in the present study.

3.4.7 Interactions between treatments assessed by multivariate analysis

Soils are complex systems with numerous interactions ranging in magnitude and effect. Principal component analysis was the obvious multivariate analysis technique to quantify the overall effects of biosolids amendment, earthworm inclusion/exclusion and soil management treatment in an unsupervised manner. Assessing the effects of biosolids, the results of the PCA supported the findings that biosolids had mostly no significant effect on measured parameters in this study. Although it was hypothesised, the PCA nicely summarises the substantial effect that soil management has on soil parameters and functioning with most treatments significantly different to about half of the other treatments. However, the PCA results support the hypothesis that earthworms have a significant impact on soil function. Due to the availability of soil at the study site, treatment replicates were extracted from different fields. Although the fields were very similar in characteristics, the PCA confirmed that there were differences between fields. This, in combination with the low monolith replication used in the study may have masked significant results. However, by designing the study to include replicates from different fields, the results are generalizable to more than a single field location on the regionally important Aberford soil type.

A surprising result of the PCA was the contribution of parameters to the main dimensions in the analysis. Dim 1 comprised mainly of soil physical characteristics, Dim 2 soil biological characteristics and Dim 3-5 soil chemical characteristics, the separation into biological, chemical, and physical traits was not expected. Although this will be influenced by the soil parameters that were measured, it also summarises nicely the importance of soil physical structure, earthworms, crops on overall soil functionality.

3.4.8 Answers to research questions

Returning to relate the results and discussion to the hypothesis and aims of this chapter. There was a successful evaluation of the effect of biosolids on soil physical, biological, and chemical parameters, including crop production. The outcome of which showed there was an overall negligible effect of biosolids, however if larger quantities were applied some of the trends seen may become significant. As expected, the contribution of soil management to soil functioning and crop production was in most cases significant and highlights the importance of choosing the most appropriate soil management method to reduce the impact of agriculture on soils and promote their functioning long term. The effect of earthworms had a greater influence on many soil parameters than initially expected, and their status as ecosystem engineers is well earned. Promoting earthworm populations within agricultural soils and valuing their importance will be critical for long term food security, consistent with other recent studies (Hallam *et al.*, 2020). In terms of the interactions between biosolids and soil management, the contribution of biosolids was low in comparison to the effect of soil management.

The hypothesis that soils under conservation management methods will exhibit a greater array of biological activity which will allow for the greatest soil-biosolid interaction and consequently crop production will be increased and soil health parameters will be enhanced, was mostly correct, and the difference in LEY, LEY-e and LTA, LTA-e was the greatest support for the acceptance of this hypothesis.

3.4.9 Critical evaluation of the study

Overall, the experiment was successfully delivered and met all the objectives and the original aims. There were a few areas where improvements could have been made. The earthworm removal technique, although very successful in the LTA treatment, was less successful in the LEY treatment. This may have been due to a number of reasons, including refugia in these soils, especially for the killing of earthworm cocoons. The freezing methodology was used by Hallam *et al.* (2020) who also found the freezing process was inadequate to completely remove all earthworms and cocoons in the LEY soil. In a similar monolith experiment, Berdeni *et al.*, (2021) found some evidence that earthworms moved between boxes despite measures in place to prevent them. If this was also the case in this experiment, it would be hard to quantify, but the presence of earthworms in higher numbers in some treatments could be an indicator of preferential choice of habitat. During the initial set up of the experiment, the quantity of biosolids applied was calculated based on values that had been supplied in the wrong unit, and hence the application rate was only about two-thirds of the maximum that could have been applied under regulations. This may have limited any effect of the biosolids, however, the non-significant effects shown and the trends towards significance for crop growth are positive indicators for applying more of the biosolids up the maximum permissible with possibly no or a positive effect. On the other hand, the almost negative impact on the largest WSA fraction may indicate that greater quantities may exacerbate this further.

The monolith boxes did provide an excellent ex-situ system for simulating how the soil in the field would react to the biosolids amendment and soil management treatments, the significant differences between physical soil properties of the soil management treatments reflect this. There are however limitations of this method, and this was particularly seen in the summer where the lack of subsoil during a period of drought really affected the soil moisture. This box and drainage set up may also have restricted the flow of water through the monoliths during the wet winter causing the soil in the lower sections of the monolith to become saturated. Although this is not necessarily different to what happens in the field, if it was uneven between treatments, it may have had an uneven effect on soil properties. For example, there is evidence that suggest continued cycles of wetting and drying, as well as periods of saturation, can cause disaggregation of soil aggregates through the dissolving of cohesive binding agents between soil particles (Ponnamperuma, 1984). The monolith box set up was, however, quite effective at providing an intact soil environment; the insulation boarding helped to maintain more steady temperatures in the soil blocks. However, they did succumb to more extreme temperature variations than the reference soil temperatures in the ground at the site.

3.5 Conclusions

This chapter's main aim was to provide a comprehensive evaluation of the effects of surface applied biosolids under different soil management systems. This aim was successfully met, and the main findings included the following: The application of biosolids, in most cases, did not cause an effect that was significant to the control. For MWD there was trend towards significance and disaggregation of water stable aggregates in the largest size fraction, the variations in the field replicates may have masked a significant effect. The effect of soil management (in most cases) had a significant effect on the soil physical and biological properties, and crop production, highlighting the importance of choosing the correct soil management practices. When evaluating the effect of earthworms in the subset of LEY and LTA plots, there were significant differences between the treatments with earthworms and the corresponding treatments without, often without earthworms having a negative impact on the property/measure. This is novel evidence that supports the importance of earthworms as ecosystem engineers and the value of promoting earthworm populations through management practices that do not negatively impact them. Monoliths with high infiltration rates and good earthworm populations saw a relatively faster disappearance of biosolids from the soil surface, this was seen most effectively in the LEY-NoT treatment and supports the conclusion that where earthworms are present, soil surfaces are exposed, and there is good infiltration, the combination of rainfall and earthworms incorporate the biosolids into the soil the fastest.

Considering the research questions of the thesis, the nutrients for biosolids are released and the conversion of the ammonium to nitrate in the soil solution has shown that they are available for plant uptake. The evidence from this experiment is that biosolids do contribute to the carbon and nitrogen in larger WSA fractions, but the non-significant increases suggest that this process is slow and long-term applications may be needed. The overall effects of biosolids on soil physical properties were mostly negligible, however, there is evidence to suggest that they may contribute to the inhibition of soil aggregation or even contribute to disaggregation of WSA. In this experiment, earthworms had a greater beneficial effect that biosolids amendment. However, there was evidence to suggest that biosolids increase earthworm populations, which should be considered. The main drivers regulating biosolids-soil interactions from the work in this chapter were soil management, that encompassing vegetation cover and earthworm populations and the associated differences in functioning.

3.5.1 Suggested further work

Based on this chapter's results, recommendations for further work would enhance and build upon the knowledge gained here. This should include an assessment of the contribution of biosolids to soil aggregation or disaggregation as published results provided no overall trend on the effect given the range of biosolids, soil types and management strategies used. In relation to this, as the results here showed that biosolids did disappear from the soil surface within the LEY-NoT treatment faster than that of the other arable treatments, further research (that was outside the scope of this thesis) to confirm these results on a wider range of soil types and ley systems is needed to help aid policy change. This could include application at the maximum permissible application rate, on a range of soil types, and an assessment will need to be done on the possible contribution to readily available nutrients in runoff during and after storm events that may cause pollution, and the effects of slope angles on this as in the present experiment the soil surface was level.

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Chapter 4: Tracing and quantifying the movement of biosolids through a soil matrix using low-cost fluorescent particles: a method development.

4.1 Introduction

Current environmental regulations for the application of biosolids to agricultural land, comprising of the Nitrate Vulnerable Zone guidance (Environment Agency, 2013), Statutory Management Requirements 1 (Environment Agency, 2017) and The Biosolids Assurance Scheme (Assured Biosolids, 2021), require biosolids to be incorporated as soon as practicable when spread on bare soil or stubble. However, research has highlighted the negative effects of ploughing, including subsoil compaction, loss of water-stable aggregates (leading to soil slumping), increased bulk density and reduced infiltration rate and earthworm abundance (Baumhardt *et al.*, 2015; Soane *et al.*, 2012). In the 21st Century, there has been much progress in the development of minimum tillage and no-tillage, which have become standard practice in Canada and seen major uptake in the USA (Baumhardt *et al.*, 2015). Conservation tillage methods including no-tillage have seen a remarkable increase in uptake over the past decade. Kassam *et al.*, (2019) reported a 69% global increase in the adoption of conservation agriculture practices between 2008/9 to 2015/16. Dicks *et al.*, (2019) also highlighted that reduced tillage was one of the priority practices for sustainable intensification, along with the use of organic matter sources to improve soil structure. Combining these practices to promote a more circular economy is feasible and has the potential to further enhance the productivity, resilience, and sustainability of farming systems. This combination of reduced tillage with additions of organic materials has been taking place in some areas with published long term experiments in China, India and Brazil (Zanon *et al.*, 2020). However, reported increases in nutrient losses during extreme rainfall events have limited widespread adoption.

There are challenges associated with combining organic amendments to soils under reduced tillage, including reports of stratification of nutrients and carbon in the topsoil layer of the soil profile, which is the most prone to gaseous emissions, wind and water erosion, causing losses and potential environmental pollution, for example, of watercourses (Morris *et al.*, 2010). In arable fields where organic amendments, such as biosolids, are applied to the surface, their incorporation or dispersal will be controlled by a combination of biotic and abiotic factors, which will be influenced by specific management practices, such as the extent of stubble, crop residues or living vegetation in the case of cover-crops or leys in rotations. It is important to understand how these land management practices, and biotic and abiotic processes interact with the natural incorporation of biosolids into soil to evaluate whether ploughing or physical incorporation is always necessary, and if not to inform possible updates to regulations to be more compatible with the increasing adoption of more sustainable soil management practices.

4.1.1 Factors effecting biosolid-soil interactions

Where biosolids are applied to the surface of agricultural soils, rainfall and wind are the abiotic factors which provide kinetic energy that would impact biosolid particle dispersal and subsequent flow through pathways into or across soil surfaces. Scott Van Pelt *et al.*, (2017) showed that although both wind and rainfall erosion can occur in any climatic region, rainfall will be the predominant erosive driver in humid and semi-humid environments, whilst the effect of wind will be increasingly significant in arid and semi-arid climates. From the Rose model (Hairsine & Rose, 1991) for raindrop-impact erosion and follow on experiments such as Walker *et al.*, (2007), as well as the revised universal soil loss equation (Renard *et al.*, 1991), we can understand that soil surface erosion is directly related to the rainfall rate, drop size, soil detachability, slope angle and vegetation cover. Practically, this means that during rainfall events fine-textured soils, and soils with poor aggregation and low porosity on slopes, result in greater water run-off, which can carry particles away. Conversely, soils with similar characteristics but on low lying and flatter areas will be more prone to flooding. Specific properties of rainfall events with different intensity and duration influence whether the materials on the surface are broken up, washed away or similarly drawn into the soil to that of disaggregated soil particles. As climate change drives increasing instances of extreme seasonal weather events, this may cause a shift in rainfall-soil and rainfall-soil-amendments interactions. For example, Boardman (2015) summarised the relationship between extreme rainfall on cultivated landscapes, where increasing instances of extreme rainfall events, > 50 mm day^{-1}, may increase the frequency of surface crusting, resulting in increased surface run-off and erosion. However, prolonged periods of low intensity rainfall can also lead to soil erosion after the soil becomes saturated. In both cases where erosion could be a risk, although soil type and topographical characteristics can contribute to risk, it is widely acknowledged that modern land-use management practices lead to increased surface run-off and erosion (O'Connell *et al.*, 2007).

Earthworms have been described as ecosystem engineers because of their substantial influence on soil structure and functionality and consequently formed a life-long interest of Charles Darwin. He published his final book summarising his observations and experiments on their effects in drawing vegetable matter into soil and causing stones to settle through their effects moving soil (Darwin, 1881). He was the first to quantify the amount of plant and soil material ingested and excreted in casts on Britain's soil surface, finding it amounted to 10 - 41 t ha^{-1} y^{-1} (Darwin 1881). However, surface castings have since been estimated to comprise only 1.7 – 3.5 % of the total amount of soil ingested, suggesting an even greater role of these organisms (Feller *et al.*, 2003; Lavelle *et al.*, 2015; Blouin *et al.*, 2013). Earthworm body size and ecotype are important controls on macroporosity at the soil surface, with the vertical burrowing long-lived anecic species like *Lumbricus terrestris* generating some of the largest pores with deep channels, whilst endogeic species such as *Allolobophora chlorotica*, an earthworm common to intensively cultivated fine-textured agricultural soils, form shallow burrows throughout the surface layers of the soil (Sherlock, 2018). Epigeic species are surface dwellers feeding directly on decaying matter and consume little to no soil. Consequently, they are not as influential to soil pore regulation but will play a larger role in the recycling of organic residues on soil surfaces, and hence are not as abundant in more disturbed and degraded soils with frequent manipulation, such as those in arable fields compared to grasslands (Sherlock, 2018). In a review of the feeding ecology of earthworms, Curry & Schmidt (2007) reported that although variable between species, the size of material ingested by earthworms is directly related to their body size, and the particle size of organic materials is known to strongly influence growth rates and fecundity. They also noted the early work of Doube *et al.*, (1997) who found that most species seem to prefer ingesting organic-mineral mixtures to pure organic or pure mineral food sources. As temperature decreases earthworms feed on progressively richer substrates with more organic matter and higher microbial activity (Lavelle *et al.*, 2007). Consequently, the timing of biosolids application, autumn, or spring, could play a role in the selectivity of earthworms to biosolids over other substrates. The critical role that earthworms play in moving organic and mineral materials within the soil makes them the most dominant biotic factor in soil systems that will contribute to the movement of materials, like biosolids, as they bury as much as 90 – 100% of surface litter amounting to several tonnes per hectare per year (Feller *et al.*, 2003).

Combining the potential effects of both rainfall and earthworms on the movement of surface applied materials, it could be expected for materials to become concentrated within regions of the soil which play key roles for both structures of porosity (macropores, earthworm channels and other bio-pores which are important for the movement of earthworms) and for water infiltration. Mechanisms controlling the abiotic movement of biosolids down and within the soil will relate to water infiltration into macropores during rainfall events (Luo *et al.*, 2010), transporting entrained particles and solutes into the soil. Therefore, the macroporosity of soil at the surface becomes a potentially critical determinant of the rate of incorporation of biosolids into the soil matrix. This macroporosity is known to be generated by earthworms and other biological agents that tend to be much more active under zero tillage and rotations, including leys (Pelosi *et al.*, 2014). Hence, land management likely plays a key role in regulating the incorporation of surface applied biosolids into soil. Although it will not contribute to physical movement, the choice of land management may cause facilitation or limitations on the degree to which rainfall and earthworms can interact with biosolids. The characteristics of the soil surface (bare, stubble, or vegetation cover etc.) will affect the impact of rainfall on the soil surface but also provide different food choices for earthworms, who may not prefer biosolids. Below ground effects of land management may also limit the degree to which materials can be drawn in by earthworms or washed down by rainfall where plough pans may be present. In soils that have become structurally degraded due to continuous arable cropping and over-exploitative management with low carbon inputs, declining soil carbon can lead to a loss of soil structure, leading to slumping and compaction, which is likely to inhibit the movement of materials. On the other hand, well managed soils with a good structure, such as in well-managed permanent grasslands (Holden *et al.*, 2019; Kodesova *et al.*, 2011), may see an increase in movement of materials with more earthworm channels and enhanced movement of water through and deeper into the soil.

4.1.2 Aims and hypothesis

Despite the risk of erosion, run-off and nutrient loss from surface applied biosolids, the reported increases in biological activity, specifically increases in earthworm numbers (Pelosi *et al.*, 2016), that coincide conservation agriculture and their effects on soil hydrology could provide an enhanced means for the sequestration of surface applied organic amendments through bioturbation and hydrology in a timely manner. For autumn applied biosolids, earthworms are at peak activity after the rewetting of soils post summer, which may lead to enhanced interaction, however, there will be fresh crop residues providing a choice of substrates for earthworms to feed on. It was hypothesised that the end fate of biosolids (depth of incorporation) applied to the surface of agricultural soils under different tillage and land-use managements, with associated differences in soil hydrological properties, biological activity, and vegetation cover, would be significantly different. In addition, soils with greater hydrological connectivity and earthworm numbers would incorporate biosolids to a greater depth through the soil profile. For a comprehensive assessment of the contribution of rainfall and earthworms to the incorporation of the biosolids, a comparison of land managements treatments with a range of vegetation covers, tillage systems and earthworm activity will be vital. The aims of this chapter are to:

- Assess the contribution of earthworms to the incorporation of biosolids that are surface applied through earthworm inclusion and exclusion.

- Assess the interactive effect of land management, with ambient weather and earthworms on the incorporation of biosolids that are surface applied by measuring hydrological indicators and earthworm numbers.

The aims and hypothesis outlined above require a new methodology that can trace how surface applied materials, in this case biosolids, are physically dispersed and move through a soil system. Physical changes within soil systems take time, and the methodology ideally needs to be applicable to a full cropping cycle or full calendar year duration to encompass seasonal variations in weather, plant, and earthworm activities. Identification of a suitable tracer with scope for tracking and quantitative tracing will be required. Tracers can be used to follow or identify a substance within a matrix. A tracer can be a native substance to the material, a substance that is added to the material (Glaser *et al.*, 2020), or radioactive isotopes of certain elements can be used (Zapata, 2003). Native substances or components of the material can be used when the substance is sufficiently abundant and unique from the matrix it moves within. If the material and the matrix are too similar, a substance or isotope can be added to the material, which can then be traced. Previous studies have been done on identifying the end fate of specific components of biosolids in the environment, most work has been done on pharmaceuticals however, whose methods are expensive for extensive analysis (Fu *et al.*, 2019). Previous tracing of the physical constituents of biosolids through a soil matrix has not been done. A low cost, high throughput method tailored to the soil environment would be highly advantageous for evaluating the physical and biological incorporation of biosolids applied to the soil surface under different land managements.

4.2 Identifying a suitable tracer

4.2.1 Characterising the biosolids

A full characterisation of the biosolids was carried out to evaluate tracer suitability. A sample of biosolids (full details outlined in Chapter 2) was sent for a suite of analysis and full characterisation (NRM laboratories). More in-depth analysis was considered but not proceeded with due to cost.

Results of biosolid characterisation are shown in Table 4-1. The biosolids are held together with a water-soluble polymer. To analyse the biosolids particle size distribution, 50 g of biosolids were mixed with 500 ml dH_2O with a metal stirrer at 500 rpm for 6 hours until the polymer had completely dissolved and the biosolids particles were suspended in solution. The solution was passed through a 2 mm sieve and analysed in a liquid matrix particle size analyser (Malvern Panalytical Mastersizer/E laser particle size analyser). The analysis was repeated in triplicate, and the averaged results are presented in Figure 4-1.

Table 4-1: Chemical constituents of Biosolids collected from Esholt Wastewater Treatment centre, November 2017, analysed by NRM Laboratories. Results presented on a 'dry matter' basis.

Analyte	Unit	Result	Analyte	Unit	Result
ph		7.7	Total Copper (Cu)	mg/kg	225
dry matter	%	25.7	Total Zinc (Zn)	mg/kg	603
Total nitrogen	% w/w	6.53	Total Sodium (Na)	mg/kg	703
Total Carbon (C)	% w/w	39.8	Total Calcium (Ca)	mg/kg	24,803
Nitrate N	mg/kg	<10	Total Iron (Fe)	mg/kg	43,712
ammonium N	mg/kg	8,230	Total Molybdenum (Mo)	mg/kg	5.95
Total Phosphorus (p)	mg/kg	26,312	Total Manganese (Mn)	mg/kg	920
Total Potassium (K)	mg/kg	1,058	Total Cobolt (Co)	mg/kg	7.48
Total Magnesium (Mg)	mg/kg	2,873	Total Boron (B)	mg/kg	9.3
Total Sulphur (S)	mg/kg	10,647			

* % w/w is the percentage by weight

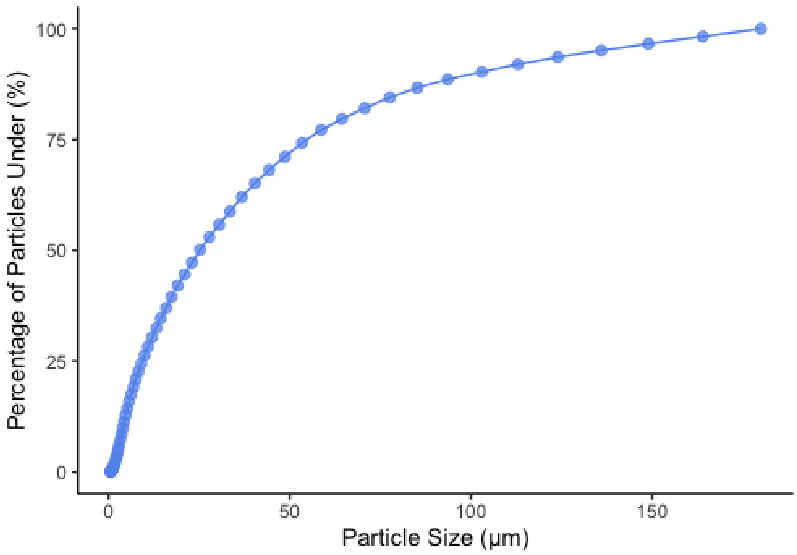

Figure 4-1: Biosolid particle size distribution. Biosolid and dH_20 solution, passed through a 2 mm sieve and analysed in a liquid matrix particle size analyser (Malvern Panalytical Mastersizer/E laser particle size analyser).

4.2.2 Choosing a suitable tracer

Native, radioactive isotopes and substance addition were all considered to trace the biosolids, shown in Table 4-2. From the chemical analysis of the biosolids (Table 4-1), there were no native substances that could be used as a tracer that were sufficiently different from soil. Radioactive labelling presents challenges for incorporating a long-lived radioisotope to into the material that would remain bound to the particles as dosing at the sewage treatment plant is not an option. An alternative would be to add the radioisotope bound to another substance into the biosolids mix, for example bound in lignin. Although this could allow for the tracing of carbon/nitrogen/phosphorus through the soil profile, the lignin being of a different origin to the biosolids would most likely behave differently as it would be part of a 'younger' and less processed material. Adding a substance or material that would behave like the biosolid was therefore the logical choice. As the method development was for the physical incorporation of the biosolids, again, it was logical to use a substance that reflected the physical aspects of the biosolids rather than chemically. Of all the methods considered, the one that stood out most prominently for the length of the study, chemical signatures, inert properties, safety, and ease of utilization was fluorescent particles, which were chosen as the tracer material.

Table 4-2: Materials and substances assessed for suitability for tracing biosolids within a soil matrix.

Tracer material or substance	Advantages	Disadvantages	Selected?
Chemical constituent of the biosolids	Accurate, quantitative.	Cost for analysis, signature of different sludges may be different, not different from the soil of the study.	No
Radioactive labelling	Accurate, could trace through soil and plant material, could specifically trace carbon.	Cost for materials and analysis, potentially hazardous to health, difficult to add radioisotopes to biosolids, adding bound in another material may behave differently.	No
Fluorescent Particles	Add known quantity, low cost, inert.	Time for sample processing and analysis, method for quantification needed.	Yes

4.2.3 Customising the tracer

After selecting fluorescent particles as the material for tracing the biosolids, different options were considered. Fluorescent plastic beads, fluorescent coated minerals, fluorescent liquids. Fluorescent coated minerals from Partrac™ were the final choice due to their customizability. Particles were selected that were as close to a match to the size of the biosolids particles, from the particle size distribution in Figure 4-1, as possible and fluorescent coated in an inert substance that was non-hazardous and of known excitation wavelength of 532 nm and an emission wavelength of 485 nm. Partrac™ particles have been used in previous studies in both the marine and terrestrial environments, mostly focusing on sediment movement into or within aquatic environments (Collins *et al.*, 2013) with some more recent studies on sediment movement (average 250 µm diameter) during rainfall events, with successful re-identification in the lab and field (Hardy *et al.*, 2019; Hardy *et al.*, 2017). Whilst there was an option to add magnetic components to the particles, it was decided to focus on the use of fluorescent tracers only in this study, as we did not have a straightforward method to extract magnetic particles from large volumes of soil. The complete characteristics of the particles used are shown in Table 4-3.

Table 4-3: Partrac™ tracer particles specific characteristics.

Characteristic	Specifications
Colour	Chartreuse (visually green)
Constituents (% of total)	Natural Sand/Silt grains (silicon dioxide, or SiO2) = 85 %. Dye Pigment (8 %). Polyester Resin Binding/Coating agent (7 %).
Size	(d50) = 30 - 50 µm
Density (specific gravity)	2650 kgm-3
Signature	Fluorescence. Excitation wavelength at 485 nm. Emission wavelength at 523 nm.

4.3 Sample Processing Trials

To optimise sample processing and fluorescent particle quantification methods, a pot trial was set up to facilitate method development and testing for the purposes of this chapter.

4.3.1 Trial pots

Two treatments were set up with the fluorescent particles (FP) at an abundance of 1% of the total mixture: (1) arable soil from Spen Farm mixed with FP (S+FP), (2) biosolids mixed with FP (B+FP). The treatments were applied by spreading on the surface of each pot which contained a 2 cm stone layer topped with a 15 cm layer of field moist soil that had been sieved to 1 cm (Figure 4-2). Application rate matched that of the monolith experiment (Details outlined in Chapter 3), giving a field equivalent rate of 10 t ha^{-1} or 168 kg N ha^{-1}. Pots were left for 8 months (November 2017 – June 2018) and exposed to ambient weather conditions outdoors at the Arthur Willis Environment Centre. Pots were re-wetted prior to sampling, with the addition of 5 litres of tap water over a 5-minute period. A single core per pot was taken from the centre using a split soil corer, which allows for the removal of the soil core intact. Each core was further split into depth fractions of 2.5 cm. The soil was oven-dried at 105 °C for 72 hours and stored in airtight bags until analysed further.

Figure 4-2: Images of pilot pots of soil applied with (Left) a soil and fluorescent particles mixture and (Right) a Biosolids and Fluorescent Particles mixture. Photograph from the day of application.

4.3.2 Initial sample processing and quantification research

The aim was to be able to identify the presence and abundance of fluorescent particles in a way that allowed for the comparison between samples. After reviewing the literature, assessing time, equipment, and budget availability, this was narrowed down to one method of identification, fluorescent microscopy and one method of quantification, image analysis in ImageJ. Considering these methods, the samples needed to be prepared in a way appropriate for further analysis.

4.3.3 Sample preparation trials

Two methods for sample processing were tested: (1) sieving and (2) ground and homogenised (Table 4-4). The rationale for these approaches is explained below.

Table 4-4: Possible combinations of processing, re-identification, and quantification techniques.

Sample Processing	Particle Identification	Abundance Quantification
Sieved into fractions (<53, 53-250, 250-1000, 1000-2000 µm)	Microscopy	ImageJ
Ball milled	Microscopy	ImageJ

4.3.3.1 Sieving

Sieving was chosen as a method to separate the soil into fractions where the fluorescent particles could potentially be more abundant in the 0 - 53 or 53 – 250 µm fractions, as the fluorescent particle sizes are below 250 µm and new materials are more likely to be bound into smaller microaggregates before these are sequentially bound together as macroaggregates (Tisdall & Oades, 1982). A standard set of 5 sieves for soil aggregate sieving (53, 250, 1000 and 2000 µm) were used to separate the soil into 4 fractions. A 1 g sample of each treatment and fraction was examined under the fluorescent microscope at 5 % luminosity (the point at which the particles fluoresce but were not illuminated by the light) and an image captured. This was repeated twice for the B+FP and S+FP trial plots. Figure 4-3 shows the results from the B+FP plots at 0 – 2.5 cm, S+FP plots showed similar trends. Figure 4-4 shows the B+FP samples in the top 10 cm of soil, fractioned at 2.5 cm intervals.

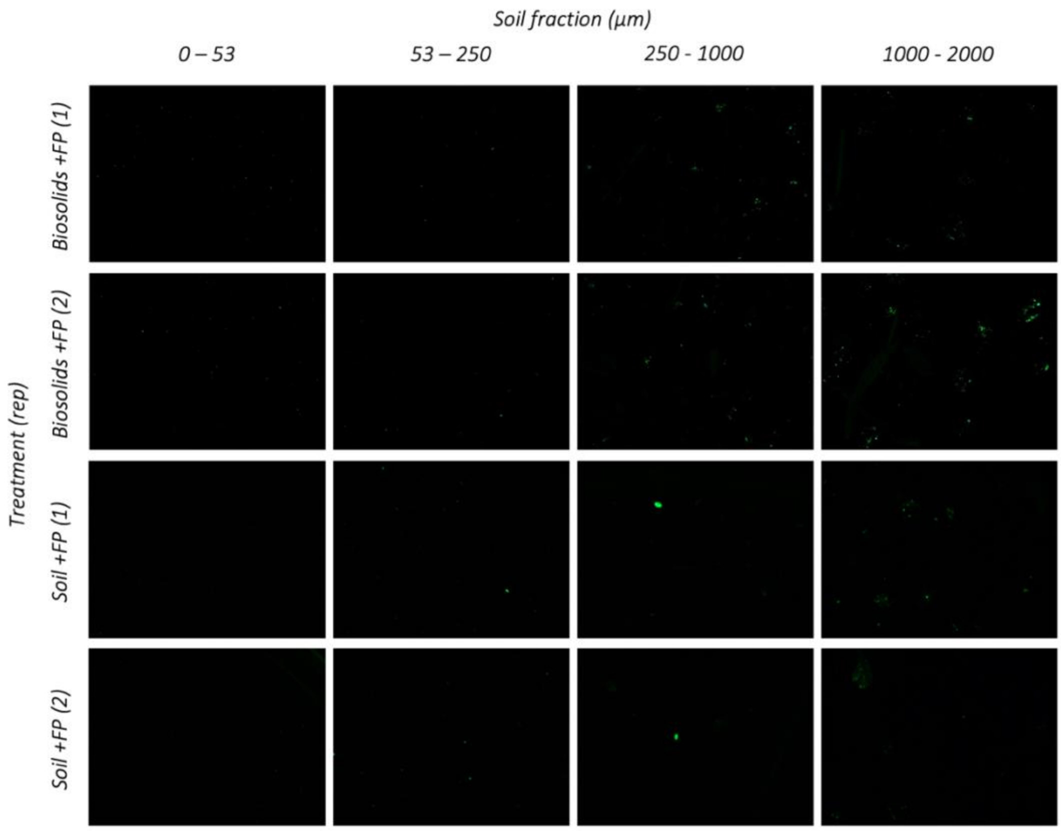

Figure 4-3: Example of the images from fluorescent microscopy from the 0 - 2.5 cm soil core from the Biosolids + FP applied and Soil + FP applied pot, 2 reps of each. Sample sieved to 4 aggregate size fractions and examined under fluorescence.

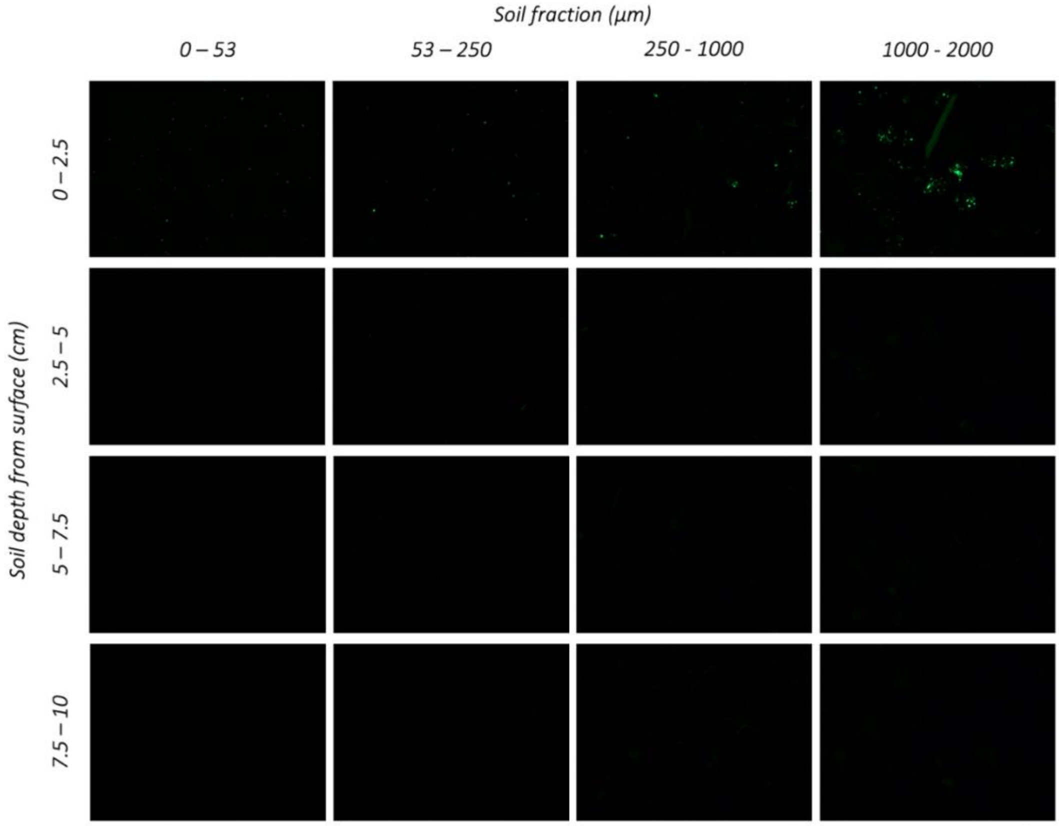

Figure 4-4: Example of the images from fluorescent microscopy from the surface 10 cm soil core from the Biosolids + FP applied plot. Sample sieved to 4 aggregate size fractions and examined under fluorescence.

4.3.3.2 Ground and homogenised

To provide a more representative sample of the whole soil to more easily be able to process, store and compare concentrations between samples, a trial of ground and homogenised whole soil was done. A ball mill was used to grind the soil samples into a homogeneous mixture of particles less than 250 µm. Figure 4-5 shows the results from the trial pots after grinding and homogenising the whole sample, by depth, with an agate ball-mill (Fristch Pulverisette). Although it is hard to see in the small images, it is possible to identify the fluorescent particles in the surface layer (see Figure 4-6 for a larger view), and intermittently down the soil profile.

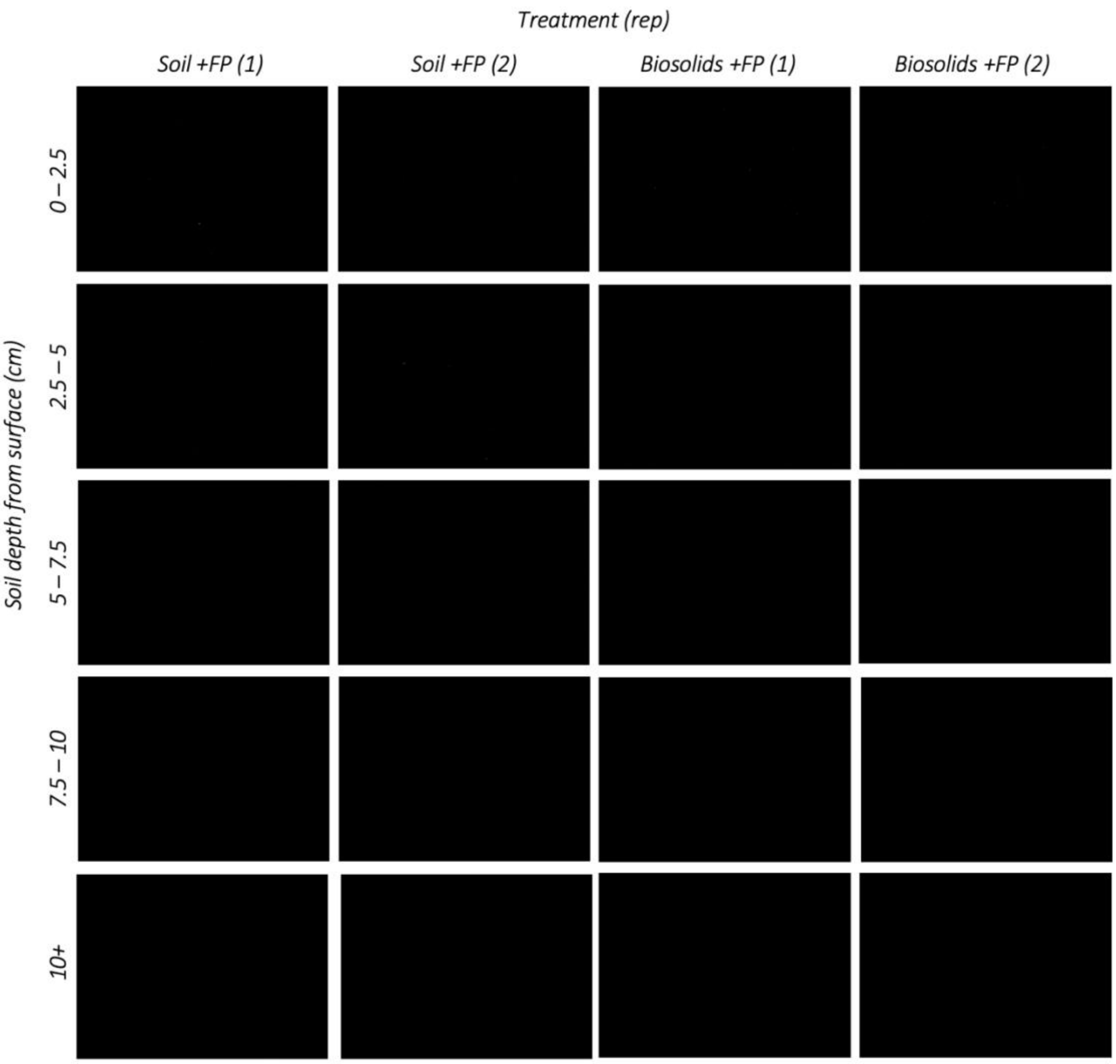

Figure 4-5: Trial pots, whole sample, ball milled sub-sampled and imaged. Images by depth and trial plot replicate.

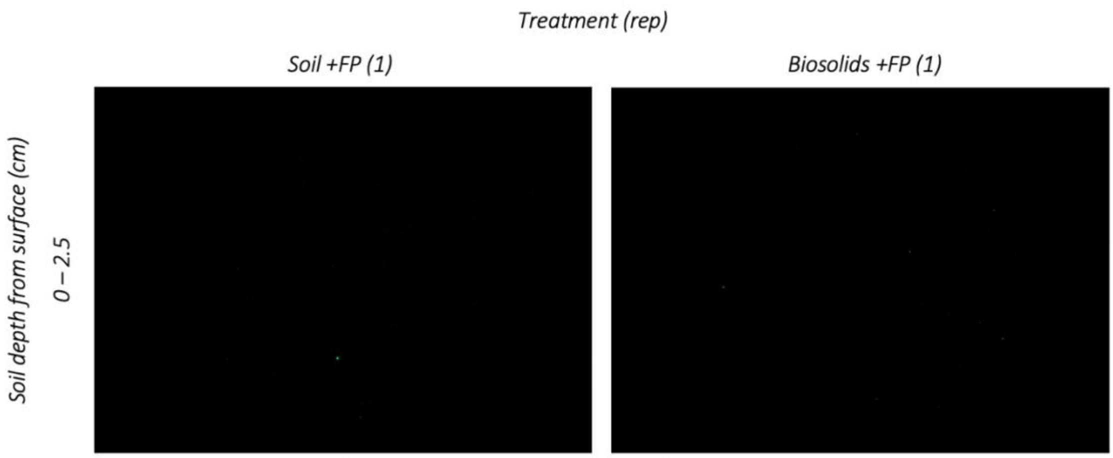

Figure 4-6: Trial pots, whole sample, ball milled sub-sampled and imaged.

4.3.4 Sample processing conclusions

The microscope images from the sieving trial showed the fluorescent particles were bound in larger aggregates. These larger aggregates were also harder to view evenly under the microscope due to the uneven surface of the sample. Following these trials, sieving was decided not to be used further as there was not a clear size fraction where the particles resided, especially at depth. Another observation in Figure 4-4, 0 – 2.5 cm, 1000-2000 µm sample, a piece of vegetation is visible in the image; it will be essential to ensure complete vegetation removal as it also has fluorescent properties. The results from the ball mill trial showed that fluorescent particles are still identifiable, and with the whole sample homogenised and in view, it provides a more representative view of the whole sample. From the trials, the most efficient method was ball milling the samples. It allowed for the fluorescent particles to be broken apart from the larger soil aggregates providing a cleaner image view and homogenised sample, and unlike sieving, meant that only one sample per treatment and depth fraction was required. However, the small abundance of particles in the soil will require multiple sub-samples or fields of view for analysis.

4.4 Particle re-identification and quantification trials

One method was trialled for the re-identification and quantification of the fluorescent particles: image acquisition from fluorescent microscopy followed by image analysis in ImageJ.

4.4.1 Sample preparation for microscopy

Samples were prepared for imaging by microscopy through trial and improvement, where the size of the field of view under the microscope defined the optimal size of the petri dish used to mount the samples. Using a small petri dish allowed most of the dish area to be included in the field of view, ensuring representative samples were observed. The size of the petri dish determined the required soil mass; the aim was to have as thin a layer as possible without empty patches.

4.4.2 Fluorescent microscopy image acquisition

A Leica fluorescence stereomicroscope (M165 FC) was used. An excitation wavelength of 523 nm and a fluorescence emission wavelength of 485 nm was observed using a GFP filter. A petri dish measuring 32 mm in diameter was filled with 1 g of processed sample, the soil was shaken so that the sample was spread evenly over the dish in a thin layer approx. 1.5 mm. Various light intensities were trialled in line with the data sheet that came with the fluorescent particles for optimal fluorescence from the particles without additional illumination of the sample. In a dark room with a side lamp for sample preparation, a 5 % brightness of the cool LED fluorescent lamp was optimal. A rectangular image of the sample was taken within the microscopes field of view using the Leica LAS X software. Exposure time was set at 1 second, and contrast was set at 1. The same settings were used for all image captures. The size of all images was 17.3 by 19.97 mm.

4.4.2.1 Unresolvable issues during image acquisition

During image processing in ImageJ it was observed that samples at very low fluorescent particle concentration gained noise typical to overexposure of an image. This was not obvious at the time of image acquisition. Subsequent image acquisition techniques were trialled to resolve the issue but were unsuccessful. One technique was image capture in black and white as opposed to colour, but this exacerbated the issue as shown in Figure 4-7. During image processing in ImageJ, a workflow was trialled to reduce the noise but was not successful in providing samples that could be directly compared without significantly large error. Unfortunately, this issue was unresolvable, however as it only occurred at very low concentrations, the method development continued taking this into consideration.

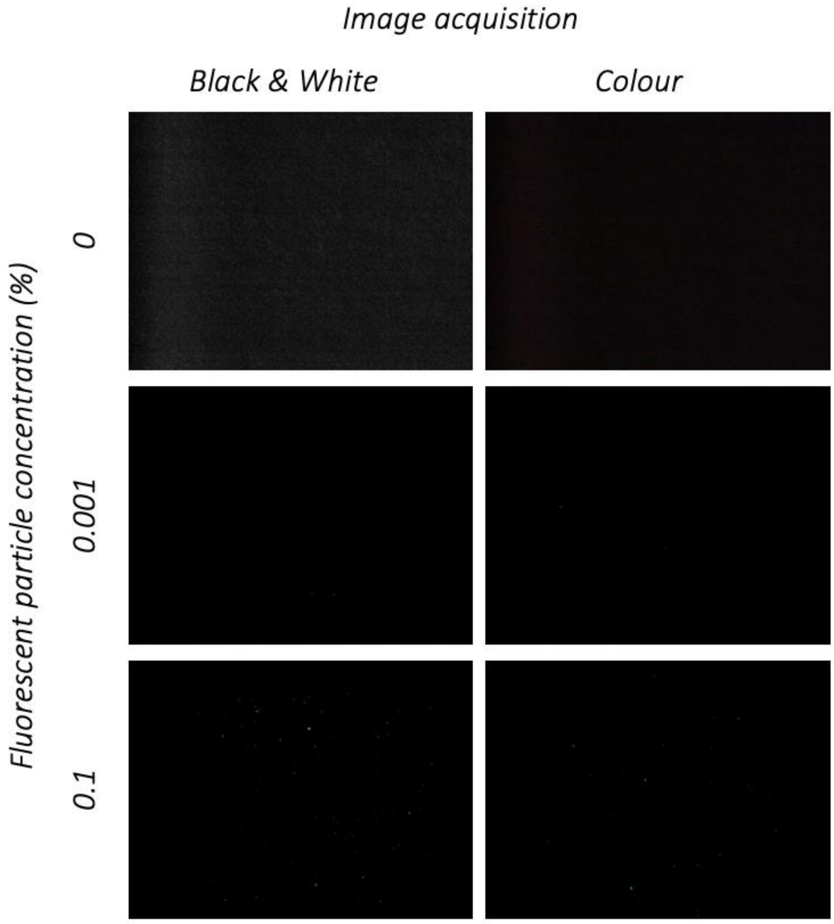

Figure 4-7: Image acquisition trials. Black & white vs colour. Artificial noise can be seen in the black and white 0% fluorescent particle image. This was also seen in the colour capture of the same sample, although it is difficult to see in the figure.

4.4.3 ImageJ particle Quantification

ImageJ (version 1.52k) was used to process the images. A variety of tools were trialled to quantify the overall abundance of the fluorescent colour in the samples. Due to the high proportion of dark colour, primarily black, in images with low fluorescent particle abundance, some attempts to count fluorescence were ineffective. The initial method trialled utilised the count tool on ImageJ and required manually clicking on every particle in the sample image using the count tool. Due to the manual nature of the method, smaller fragments were easily missed, and the method was very time intensive. A more standardised and quantitative approach was needed. Another method trialled (which is used for counting cells for biological purposes) was a workflow which made the image binary, inverted the image, and counted the white areas. Although this method works well for cells, the size of fluorescent particles in the images was too small and all the area was counted as one group. The most successful, repeatable, and consistent methods were the "Find Maxima" (FM) tool (part of the ImageJ package) and the "Colour Pixel Counter" (CPC) tool (a plug-in by Pichette (2010)).

The FM tool counted the areas where the colour was different to the background, in this instance black. The tool has a changeable threshold which can be changed to provide a size of the maxima to be counted, reducing the noise of any background fluorescence. For the fluorescent particles a noise tolerance of 10 was adequate to count the particles in the sample. This also allowed for the larger clumps of particles to be counted more than once, representing its size. The output is a count of maxima meeting the set criteria.

The CPC tool was customisable to the colour of pixels, in this instance green, and counted the number of pixels of this colour. Similarly, to the count maxima tool, this tool allowed for a changeable threshold for the green colour, allowing for brighter green to be counted while excluding darker green, and vice versa. For the fluorescent particles, setting the CPC at color=Green, cells=20, pixels=0.2100, and minimum=15 was optimal for these samples. Where there were larger clumps of particles the tool counted all the pixels, considering the size of the fluorescent particle to a greater degree than that of the find maxima tool. The output is a count of pixels meeting the set criteria.

For both tools producing a macro for the workflow allowed for multiple images to be analysed sequentially and compile the data into a table. This method (once optimised for the fluorescent particles used in this study) was relatively quick and accurate for quantification. Once standardised for the area of each image, it also provided the capability for direct comparison between samples. However, the tool that was ultimately chosen to progress with was the colour pixel counter. This was due to the less selective nature of the counting process. Rather than counting local points with high contrast to the background, it counted pixels within a specific colour band, meaning that it was more accurate at lower concentrations than the count maxima tool.

Unfortunately, the presence of noise in images of samples with very low concentrations of fluorescent particles meant that there was a lower limit on the accuracy of both methods. For the count maxima tool this was 0.01 %, and for the colour pixel counter this was 0.001%. The relationship between the FM tool or the CPC tool and the concentration of fluorescent particles is slightly different, see Figure 4-8 for raw data plots of samples with known concentrations. While the FM maintains a more linear relationship, the CPC tool has a slightly quadratic relationship. Given that the concentrations of fluorescent particles in the trial pots were low, the colour pixel counter tool was selected as the most appropriate for the images and data acquisition and is further explained below.

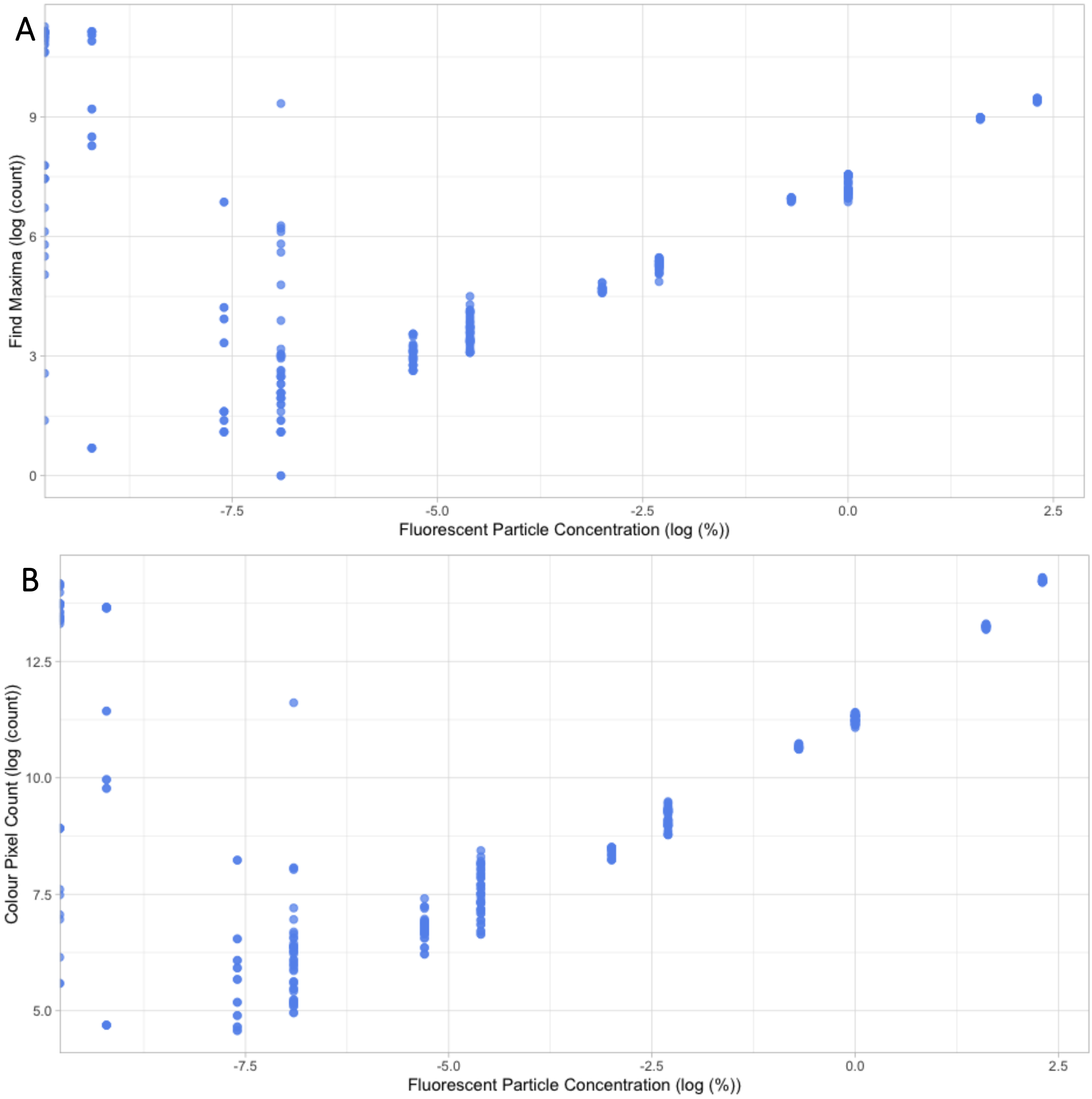

Figure 4-8: Count data from different methods of image analysis using ImageJ to analyse the concentration of fluorescent particles from standard samples. Plot A) Count data from the Find Maxima tool, showing a more linear relationship before it dissolves into noise at the lowest concentrations. Plot B) Count data from the Colour Pixel Counter tool, the relationship appears to be quadratic and there is less noise towards at lower end of the concentration compared to that of plot A.

4.4.4 ImageJ calibration curve

Following this method development, it was hypothesised that as the fluorescent particles had a standard luminescent output that a calibration curve for pixels mm^{-2} and therefore particle concentration could be created. A calibration curve was produced as follows. Virgin soil that was left over from the trial plots and that had not been in contact with the fluorescent particles was spiked at a range of concentrations from 0.0001 - 10 % with the fluorescent tracer particles. Samples were prepared as outlined in 4.4.1 and images were taken outlined in 4.4.2 for each concentration. Three sample replicates were prepared for each concentration, and three analytical reps were run on each sample by shaking, re-levelling, and re-imaging on the fluorescent microscope. All images were processed in ImageJ using the colour pixel counter tool and analysed in R, using RStudio. A calibration curve was created for fluorescent particle concentration (%) and pixel count, Figure 4-9.

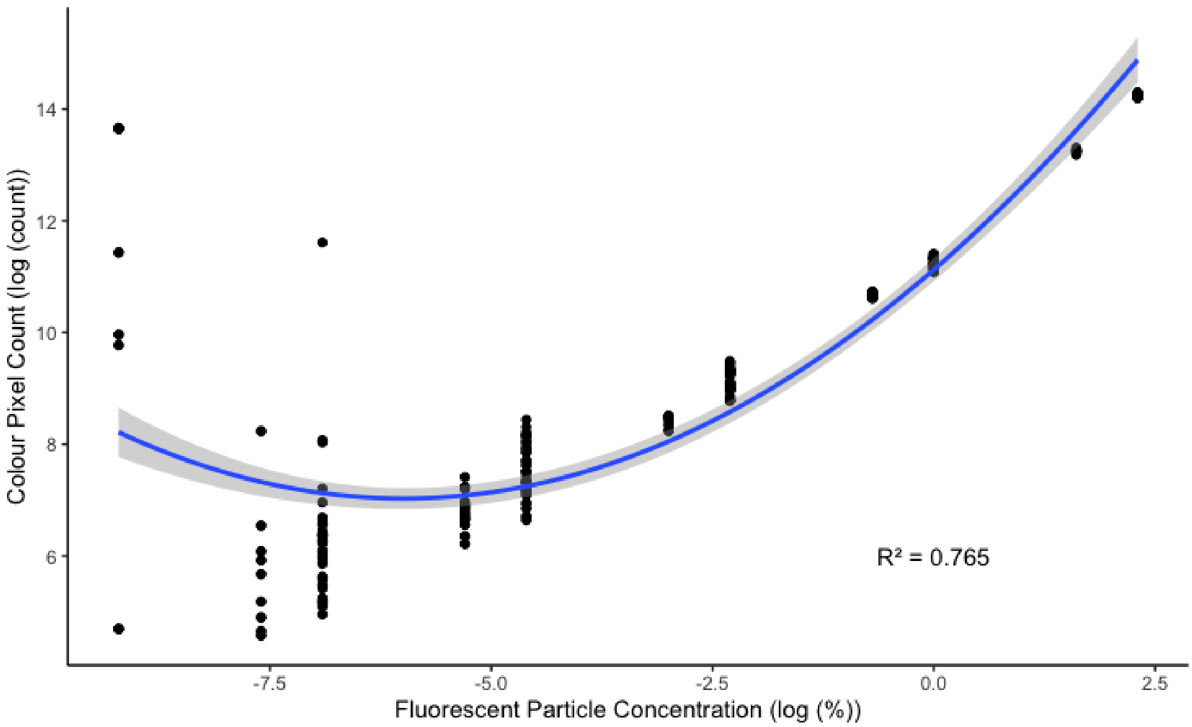

Figure 4-9: Calibration curve with zero samples removed (as log 0 = ∞) but all other samples included ($R2 = 0.765$, $y = 11.12 + 1.37\ x + 0.11\ x^2$ where y = log(colour pixel count) and x = log(Fluorescent Particle Concentration), $p < 0.001$).

4.4.4.1 Overcoming noise in low concentration samples

As image acquisition at very low concentrations contained unexpected noise and was unfortunately unresolvable, a workflow to eliminate samples subject to the noise was developed.

Starting with the calibration curve, all 0 % and 0.0001 % samples were removed from the dataset, following this, major outliers were removed from concentrations of 0.0005 and 0.001 % at a threshold of 1000 pixel count and 1500 pixel count respectively, based on observations made on the graphical representation of the dataset. Of 358 standard samples, 297 were left in the dataset to produce the calibration curve in Figure 4-10, producing an equation representative of the true CPC to concentration relationship, see Equation 4-1 and Equation 4-2.

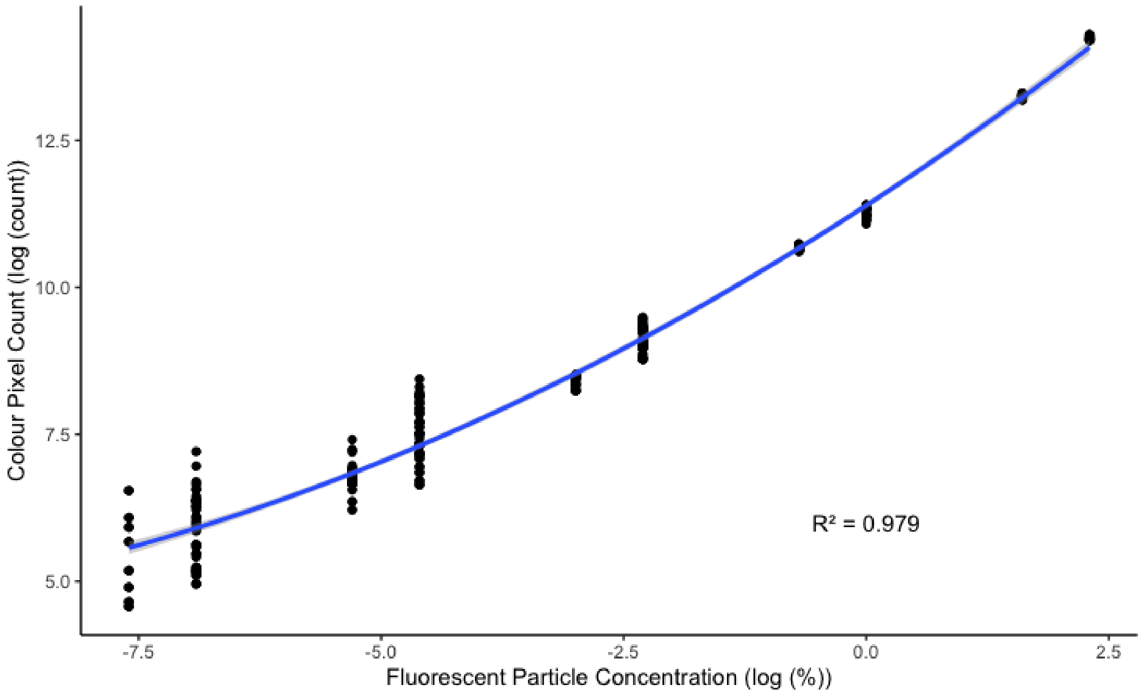

Figure 4-10: Calibration curve with samples containing noise removed, this included all the 0 % and 0.0001 % samples from the dataset, concentration, and the far outliers from 0.0005 % and 0.001 % concentrations. (R2 = 0.979, y = 11.39 + 1.07 x + 0.04 x^2 where y = log(Colour Pixel Count) and x = log(Fluorescent Particle Concentration), $p < 0.001$).

Equation 4-1: Calibration curve equation for the relationship between fluorescent particle count, as counted by the number of pixels (CPC) and the concentration of fluorescent particles in standard samples (FPC).

$$\log(CPC) = 11.39 + 1.07 \log(FPC) + 0.04 \log{(FPC)}^2$$

Equation 4-2: Calibration curve equation for the relationship between fluorescent particle count, as counted by the number of pixels (CPC) and the concentration of fluorescent particles in standard samples (FPC). Rearranged for FPC.

$$FCP = \ e^{-13.375+0.625\sqrt{-271+64(\log(CPC))}}$$

4.5 Final Method Workflow

The final methodological workflow is shown in Figure 4-11. Stage 1 included choosing a tracer, mixing with the biosolids, and applying it to the soil's surface. Stage 2 included the sampling of the soil, soil processing and storage. Stage 3 includes the particle re-identification and quantification using fluorescent microscopy, ImageJ, and R. This workflow produces a count by proxy of fluorescent particles for each sample that can then be converted into the concentration of biosolids in the sample.

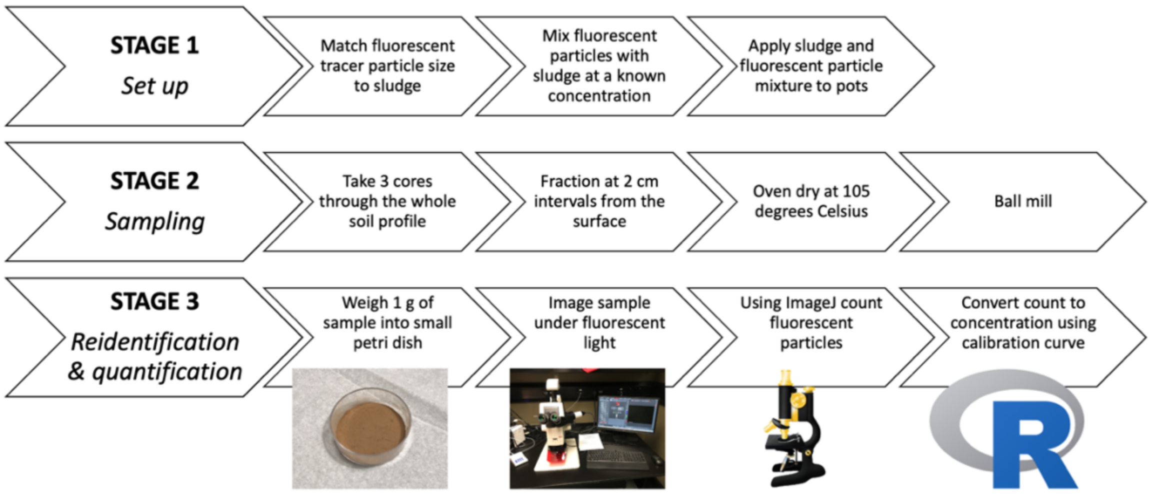

Figure 4-11: Workflow of the method development for the re-identification and quantification of fluorescent particles within a soil matrix.

4.6 Implementation in an Experiment

4.6.1 Monolith experiment Introduction & Methodology

During the initial method development, the fluorescent particles were utilised in a large experiment that was set up at the same time as the trial pots. This large experiment, detailed in Chapter 3, was the driving force for the method development. The aim of the fluorescent tracers was to help answer the hypothesis from Chapter 3. When biosolids are surfaced applied to soils under different managements are they incorporated to different depths and are the mechanisms for incorporation different between managements. Details of the experimental set up are outlined in Chapter 2. The large monolith experiment was set up to investigate the interaction between surface applied biosolids and different soil managements. Intact monoliths of soil were extracted from Long Term Pasture (LTP), Long Term Arable (LTA) and Ley (LEY) fields. A subset of LEY monoliths was extracted and simulated to No-tillage (LEY-NoT) and Conventional Tillage (LEY-CT). Parallel monoliths of LTA and LEY with 2 field replicates were extracted and frozen at -20 °C for 21 days to remove earthworms and large soil macrofauna (LTA-e, LEY-e). For all soil managements (apart from LTP), there were 4 field replicates, LTP had 3 field replicates. Each soil management and field had two treatments, control and biosolids surface applied. Figure 4-12 shows detailed flow of how each treatment was set up.

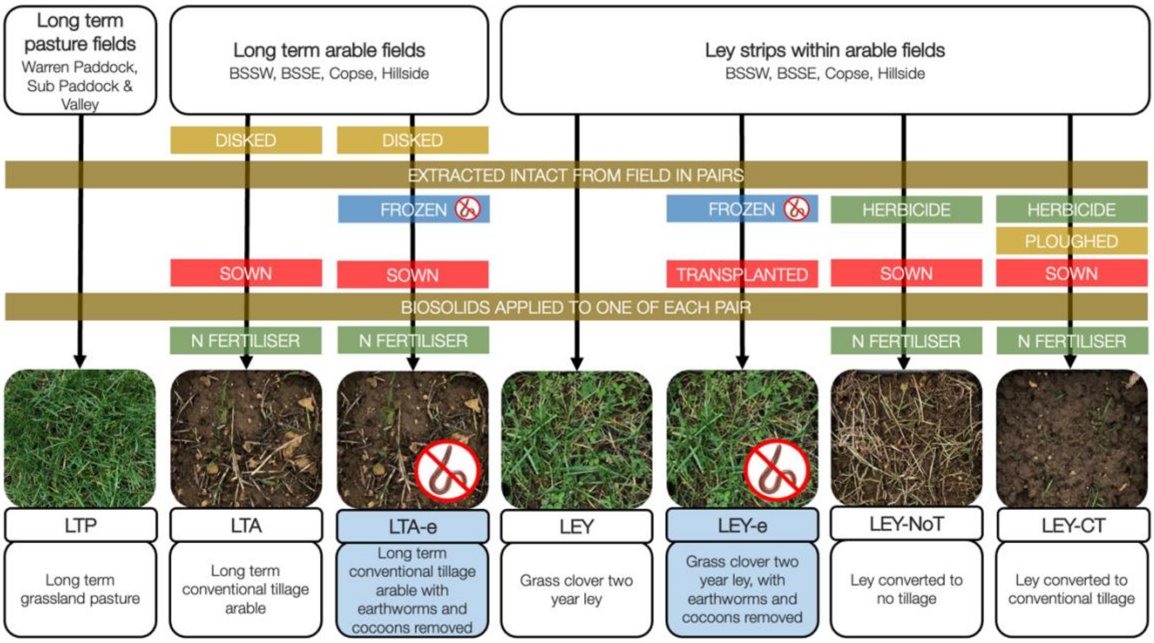

Figure 4-12: Monolith treatment diagram. There were five different agricultural management systems; long term pasture (LTP), long term arable (LTA), Ley (LEY), ley to simulated no-tillage (LEY-NoT), and ley to simulated conventional mouldboard ploughed tillage (LEY-CT). In a subset of two treatments monoliths were deep frozen to remove earthworms and earthworm cocoons. These were long term arable with earthworm removal treatment (LTA-e) and ley with earthworm removal treatment (LEY-e). Monoliths were extracted in pairs with biosolids applied to one of each pair. Replication of n=4 fields, with the following exceptions: LTP n=3 fields, LTA-e and LEY-e n=2 fields with 2 within field replicates.

The monolith experiment ran from October 2017 to July 2018, and the soil was sampled in August 2018. For analysis of the fluorescent particles, 4 intact soil cores were taken through the depth of the soil (between 18 and 20 cm deep), 1 remained intact and was stored, the other 3 were fractionated into 2 cm portions by depth and the three corresponding depth fractions pooled, sieved at 1 cm to remove large stones and oven-dried at 105 °C for 72 hrs and stored in airtight bags in the dark until further analysis took place. The samples were processed in line with the method produced in this method development, through sample processing, image processing and analysis. Due to the large number of samples, standards were run for every batch of samples run through the microscope. A calibration equation was produced for all samples based on the results of all standard samples. As outlined in 4.4.4.1, monolith samples without pixel count data outside of the standardised thresholds were excluded from the dataset to accurately assess the quantity of particles in the samples given the noise on low concentration images from image acquisition.

4.6.1.1 Overcoming noise in low concentration samples

Monolith samples were affected by the noise more than the standard samples as they appeared to fall towards the lower concentration region; see Figure 4-13A for a raw data plot, including samples affected by noise. As concentrations above approximately 0.5 % were not visually observed in any monolith samples during image acquisition, processing, or method development, these were all excluded from the data set by calculating an average value for CPC at 0.5 % from the standard samples and thresholding the monolith samples to below this value (mean CPC at 0.5 % FP concentration = 42811 count = 10.66455 log(count)). From the total number of sample data points, including all sample reps and analytical reps of 1219, after thresholding 182 data points remained in the data set, Figure 4-13B shows the raw data after thresholding, as described here, was completed.

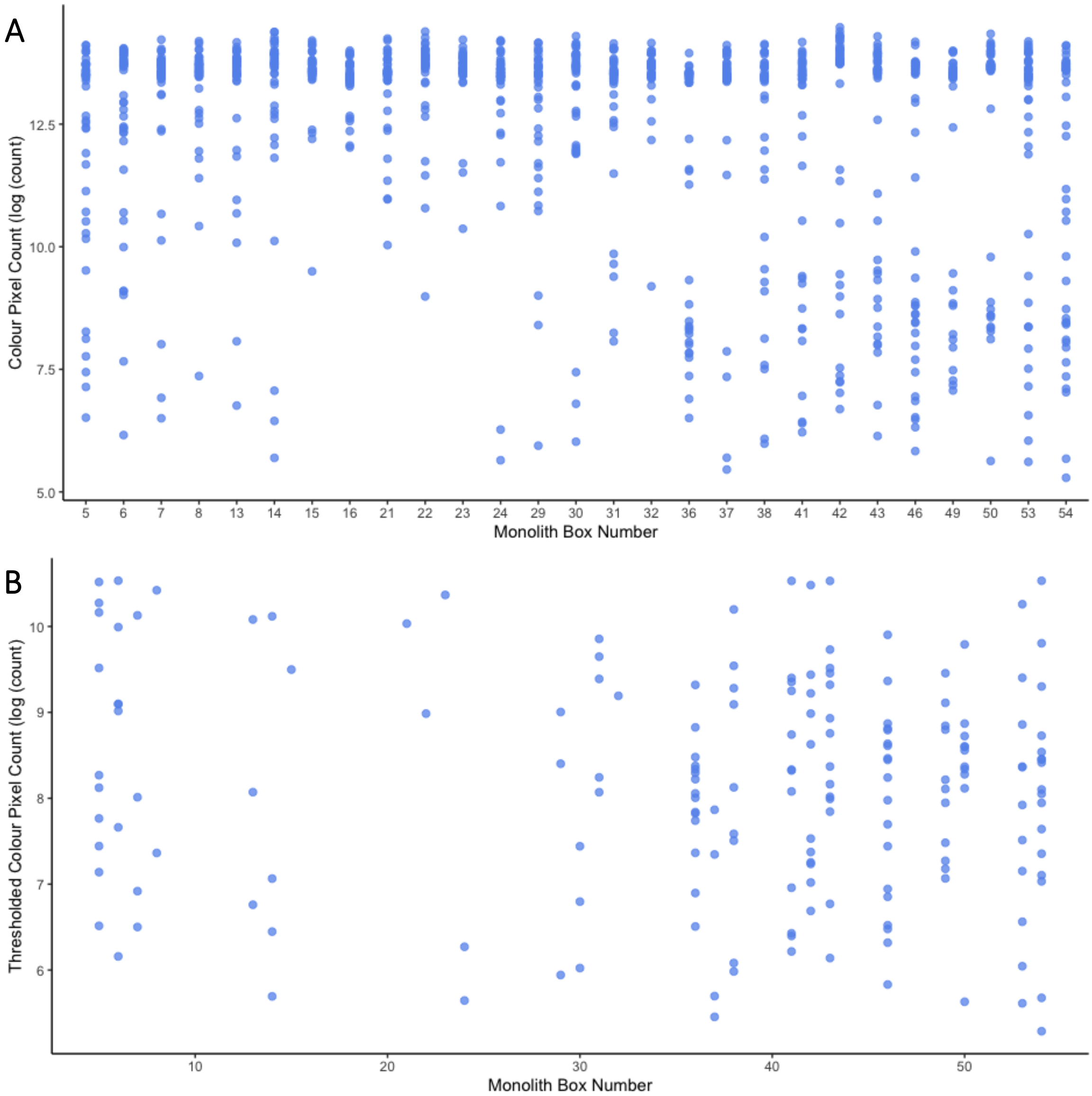

Figure 4-13: Colour Pixel Count (CPC) data for the monolith samples. Plot A) showing the pre thresholding data containing 1219 data points, the high values are samples that have been affected by noise. Plot B) showing the remaining data after thresholding containing 182 data points. Thresholding was done based on the average log(CPC) at 0.5 % Fluorescent Particle Concentration (FPC) of 10.66455 based on visual observations of the images, none of which appeared over 0.5 % (FPC).

4.6.1.2 Calculating the concentration of fluorescent particles and biosolids in the monolith samples

The calibration curve (Equation 4-2) was used to calculate the concentration of fluorescent particles in the monolith samples. The concentration of biosolids in the samples can then be calculated by compensating for the proportion of fluorescent particles mixed with biosolids at the start of the experiment. In this case, that was 1:99 (fluorescent particles to biosolids). Therefore, the concentration of the fluorescent particles as a percentage can be converted to g/kg (1 % equates to

10 g/kg) and then multiplied by 100 to obtain the concentration of biosolids within the monolith samples as g/kg. Results for the fluorescent particle concentration and the concentration of biosolids are shown below.

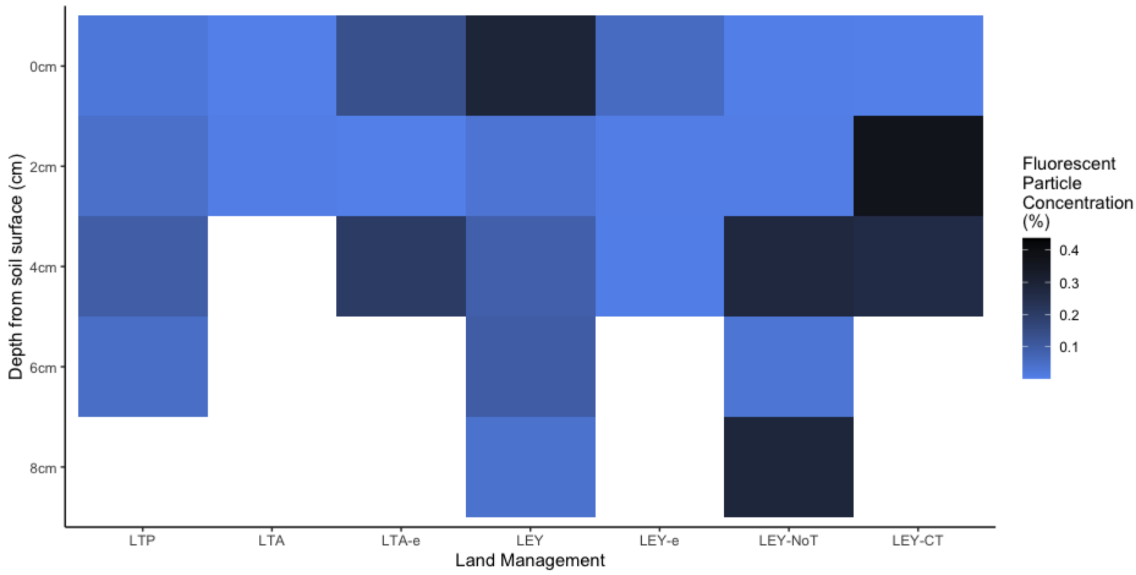

Figure 4-14: Concentration of fluorescent particles in monolith samples based on fluorescent particle method workflow, white areas are where no data after thresholding remains and the concentration can be assumed as < 0.0005 %.

4.6.2 Results

The concentration of biosolids within the monolith soils analysed using the method developed in this chapter are shown in Figure 4-15. Due to the sample thresholding, there was insufficient replication remaining to perform statistical analysis of the results. From the figure, not all treatments at depth have a concentration within detectable limits, particularly after 6 cm depth. Looking at the surface soil layer, 0 – 2 cm, LTA-e, LEY and LEY-e treatments have the highest levels of biosolids present at > 150 g/kg. Moving down the soil profile, the LEY-CT treatment has the highest concentration of biosolids at approximately 250 - 400 g/kg of soil. The LTP treatment showed similar concentrations of biosolids throughout the depth of soil at approximately 100 g/kg, up until the 8-10 cm layer which was below detectable limits. Several treatments including LTA, LTA-e, LEY-e, and LEY-CT saw concentrations of biosolids at depth fall below detectable limits at around the 5cm depth mark. The LEY and LEY-NoT treatments had biosolids concentrations detectable in the full depth of the soil profile ranging from 50 – 300 and 0 – 350 g/kg respectively. Comparing between treatments where earthworm removal was done, LTA-e has a higher concentration of biosolids present compared to that of the LTA with earthworms; this was observed in the surface layer and at 4 – 6 cm depth. Conversely, the LEY treatment saw higher concentrations than LEY-e throughout the depth of the soil, and the LEY-e fell to below detectable limits after a depth of 6 cm.

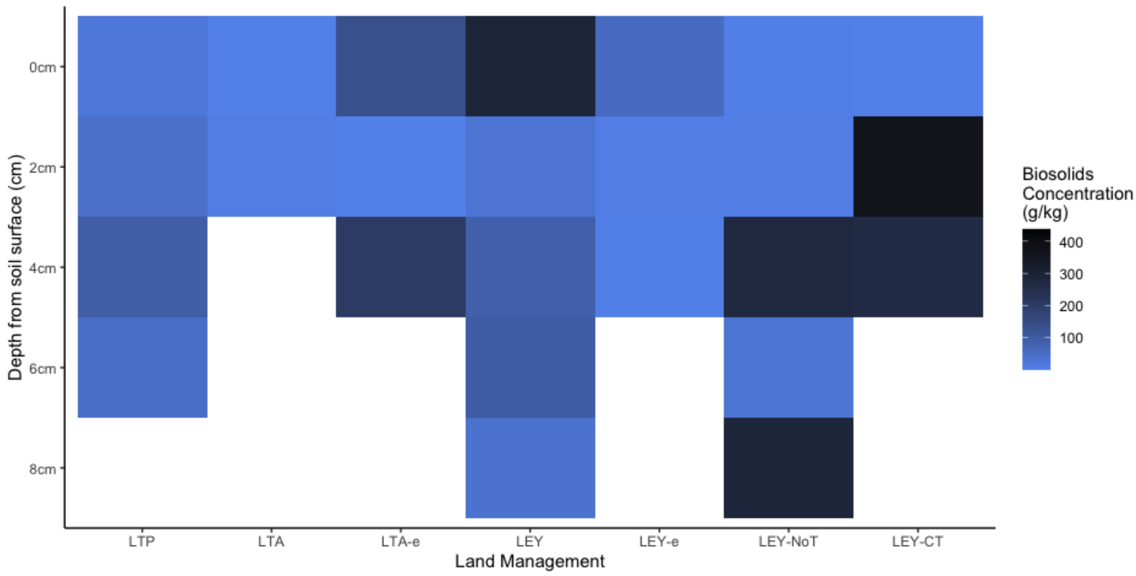

Figure 4-15: Concentration of biosolids in monolith samples based on fluorescent particle method workflow.

To complement the results from this method development utilised in the monolith experiment, the surface images of the monoliths over time provide an extended view of the fate of the surface applied biosolids throughout the duration of the experiment. The figure was previously shown in Chapter 3 and is repeated in Figure 4-16 for reference as it is discussed in relation to the results from this chapter in the discussion below.

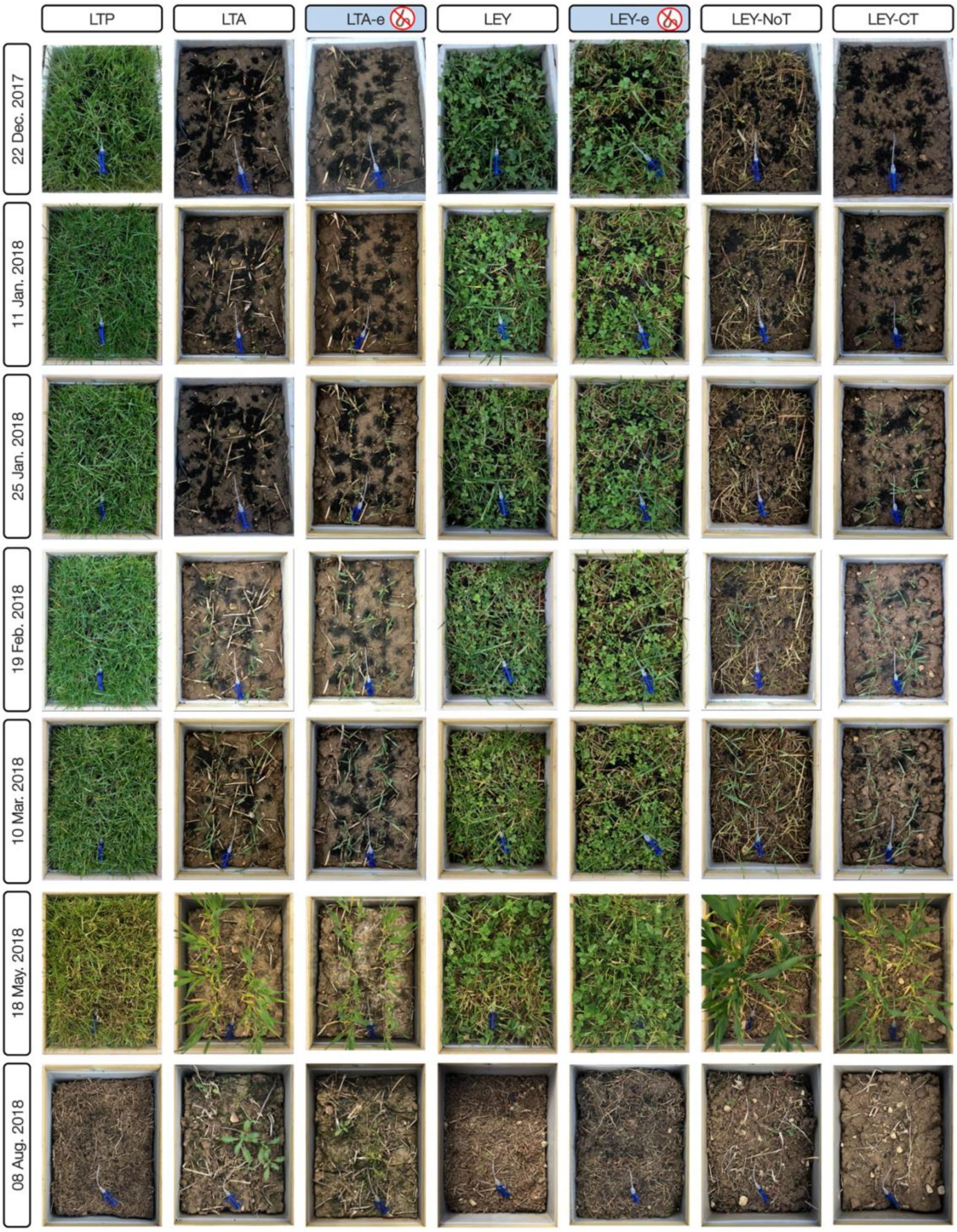

Figure 4-16: Monolith surface images for visual observations over time. From 22 December, one month after application to 08 August post-harvest, 7 images total. This figure also appears in Chapter 3.

4.7 Discussion

Surface applications of biosolids has the potential to encourage the combination of utilising conservation agricultural methods, such as reduced tillage, with surface applications of organic materials. However, it is important that biosolids are incorporated within the soil profile in a timely manner through abiotic and biotic drivers so that the risk of material loss through run-off and erosion is limited. At present, the contribution of rainfall, earthworms, and land management to the incorporation of surface applied biosolids has limited research, likely due to current legislative restrictions, the complex interactions and difficult re-identification of biosolids from within a soil matrix. This method development has helped to quantify the level of incorporation of surface applied biosolids, informing the suitability of biosolids in reduced tillage environments and can provide a basis to guide further research to evaluate current regulations and restrictions for its use.

4.7.1 Evaluation of method

In this study, a method was developed to identify the depth of incorporation of biosolids within a soil profile. Through a combination of fluorescent microscopy and image analysis, the biosolids tracer was successfully re-identified and quantified from within a soil matrix, and the concentration of biosolids calculated. Strengths of the method developed include the low cost for extensive analysis compared to other methods; the fluorescent particles were £100 per kg of material; microscope time was £2 per hour. A total of approximately 250 g of fluorescent material was used during the method development and trial, in total 40 hours of fluorescent microscopy was done. The use of standards of known concentrations within each run (to produce a concentration curve), allows for batch-to-batch standardisation and for a direct comparison of sample concentrations to be made. The find maxima and colour pixel counter tools in ImageJ had a strong and consistent relationships with the concentration of fluorescent particles, over a wide range of concentrations. Where the colour pixel counter was particularly effective in the lower concentration samples, were samples to have a higher concentration the find maxima tool could be utilised for greater accuracy. The method was relatively cheap to run, however there were great time costs, from extracting the cores, fractioning, drying, grinding, weighing, and imaging. There were a large number of samples analysed during this method development, but time may only really be reduced through the processing of fewer samples or changing the core fractioning depth from 2 cm increments to larger fractions. The biggest weakness of this method was the lower limit of detection, which did succumb to extensive noise within the samples and degradation of the count analysis method and the fluorescent particle concentration relationship. Unfortunately, as the method was being developed alongside the running of the monolith experiment, a larger proportion of fluorescent particles could not be added but would be recommended for future experiments. Hardy *et al.*, (2019; 2017) ran plot experiments utilising the same type of fluorescent particles and used fluorescent imaging at night and image analysis to assess the movement of surface soils during rainfall events, they also found that the relationship between concentration and fluorescence degraded at low concentrations.

During the development of this method, the issue regarding low noise concentrations was unable to be resolved, however, this could be improved by having an area with fluorescence and no fluorescence in each image alongside the sample as a way of standardising the lens exposure of each sample to reduce the noise in low concentrations. The greatest limitation of this method was the tracer chosen, although it was a good match in terms of particle size, and being silica based is likely to act in a very similar way in the soil, as it was not an indigenous part of the biosolids, its behaviour will likely have varied slightly to that of the biosolids due to the inherent differences between the materials but serves as a good indicator. It was encouraging to find that in the trial pots, the fluorescent particles were seen to be incorporated within micro and macro aggregates, however as the particles did not contain organic matter, they will have behaved differently and may not have degraded or assimilated into the soil as the biosolids would have. During the method development it was decided to ball mill the soil samples, to create a more uniform surface of the material for imaging and to have a representative sub sample of the whole sample that was homogenised. This was a logical progression of the method, however, as the fluorescent particles were not concentrated through size fractioning of the material, the sampled did fall into lower concentrations, which then succumbed to noise during image analysis. Finally, the application of the material on the surface was not done in a uniform layer and was instead spread at random as may occur during surface broadcast spreading in the field. Cores that were taken for analysis using this method may not have been taken from areas with less or no material applied to the surface. Taking multiple cores which were pooled was the option to overcome this, however, it may still have further reduced the possibility of re-identification.

4.7.2 Monolith samples

The utilisation of the method developed in this chapter within an experiment was largely successful for determining the concentration of biosolids within a soil sample. The samples did succumb to noise in low concentrations and many samples did consequently fall below detectable limits which meant that the remaining results did not allow for statistical analysis, however, the concentrations observed can allow for discussion of the hypothesis.

The results do support the hypothesis that more degraded soils with less earthworms retain biosolids at the surface for longer. This is seen clearly with the LTA and LTA-e treatments, where the concentration is much higher in the LTA-e treatment. The absence of particles in the arable treatments at depths of more than 4 - 6 cm, as the ground is mostly uncovered by vegetation. This supports the hypothesis and literature which states that rainfall is the main driver of erosion and subsequent movement of biosolids in these treatments and climate (Van Pelt *et al.*, 2017). That, when combined with the effect of earthworm channels and macropores (Luo *et al.*, 2010) has drawn the biosolids material down the soil profile to below detectable limits.

The results also support the hypothesis that between biosolids and other food sources, for instance grass or clover vegetation, biosolids were not the preference. This can be observed in the higher concentrations of biosolids in the surface of the LTP, LEY and LEY-e treatments compared to those that were arable cropped and contained earthworms. This was not supported by the literature, where Doube *et al.*, (1997) found that earthworms preferred a mineral-organic mixture for ingestion and Lavelle *et al.*, (2007) who observes earthworms to ingest increasingly richer substrates as temperatures decreased. Interestingly the LEY treatment saw a high concentration remaining on the surface, as well as high concentrations down the soil profile, this may be due to the continued high level of cover from the vegetation on the soil surface which did not stimulate rainfall driven movement. This combined with the earthworm activity, preferentially choosing to consume vegetation over biosolids, may have instead caused the biosolids to be indirectly dispersed through the soil profile as a result of earthworm movement through and around the monolith.

Exceptionally high concentrations of biosolids (notably over 350 g/kg), could be residual noise from samples at low concentrations that were not removed from the dataset during thresholding. However, it may also indicate samples that contain regions where biosolids were concentrated, such as in earthworm burrows which are multi-functional, for the movement of soil through earthworm ingestion and casting but also with associated increases in infiltration.

Relating the results from this chapter to those in Chapter 3, the treatments with the highest number of earthworms, LEY, LEY-NoT and LEY-CT, see the highest concentration of biosolids at depth despite the range of levels of vegetation cover, suggesting that earthworms have been the main driver of biosolids movement. Treatments with the greatest difference between the cumulative highest and lowest daily temperature, LTA, LTA-e and LEY-CT, have results that are below detectable values from approximately 4-6 cm depth, suggesting that greatest temperature fluctuations than the other treatments could have caused reduced incorporation. However, LEY-e showed a similar trend in concentration but had the lowest difference in daily min-max temperature, suggesting that temperature did not have an overall effect on the concentration and distribution of biosolids.

Comparing the concentration of biosolids from this chapter with the infiltration rates and total leachate for each treatment from Chapter 3, treatments with higher infiltration rates LEY and LTP saw some of the highest concentrations of biosolids at depth, suggesting that water moving through the soil profile is aiding the movement of the biosolids, even in vegetation covered treatments. Fluorescent particles were not measured in the leachate from the monoliths due to the sheer volume over the course of the year. However, the treatments which had some of the highest leachate volumes, LTA, LTA-e and LEY-CT, showed some of the shallowest samples where biosolids concentration was below detectable limits. This could indicate that the movement of water through the soil profile has been sufficient to cause the biosolids/fluorescent particles to pass through the monoliths and into the leachate. However, LTA and LTA-e also had the highest surface bulk density which may have inhibited movement.

Evaluating the effectiveness of biosolid incorporation in a timely manner by relating the results here to the figure of the monolith surface images in Chapter 3, repeated in this chapter, Figure 4-16. After the surface application of the biosolids in the November of 2017 they were visibly present on the surface of the soil in most treatments until at least march. The non-arable treatments, LTP, LEY and LEY-e provided the biosolids with surface coverage almost immediately, and biosolids were almost not visible after February 2018. The LTA-e treatment saw biosolids persist on the soil surface throughout the experiment and were still present at harvest. This further reiterates that the reduced infiltration rate and smaller earthworm populations negatively affect and reduce the incorporation of the biosolids material. The most interesting observational result was that the LEY-NoT treatment saw the fastest disappearance of the biosolids from the soil surface, the majority of which was gone by February 2018. This is reflected in the lower concentration of biosolids seen at the surface for this treatment and the higher concentrations seen at depth. Although the abundances of biosolids on the soil surface did decrease in all treatments over the growing season, there were still biosolids present on the surface throughout the winter season, which is a concern for erosion, especially during heavy and prolonged winter rainfall events.

4.8 Conclusion

The fluorescent particles and method developed in this chapter were a good proxy for the end fate of the biosolids despite the limitations of the method. Further analysis to determine the reliability of the method should be done, perhaps utilising a more expensive but extensive analysis method that could validate this method for the quantification of biosolids with a soil matrix on samples of known biosolid concentrations, over known fluorescent particle concentrations. The utilisation of this method in the future should consider the lower limits of detection when deciding on an appropriate quantity of fluorescent particles to use. The method developed here is not limited to the quantification of biosolids only but could be used to trace other substances with similar properties through a soil matrix by customising the fluorescent particle properties, for instance, microplastics. Although the particles have been used in studies for tracing sediments within watercourses (Collins *et al.*, 2013) and the datasheet suggests the materials used for the tracers are stable, a long term assessment to quantify any long term degradation in fluorescence due to prolonged exposure to moisture and heat should be conducted.

Following on from this method development and in conjunction with the idea of utilising the fluorescent particles in the field and imaging plots under fluorescence at night by Hardy *et al.*, (2017, 2019), a continuation of this research could be conducted to determine the timeliness of the disappearance of the biosolids from the soil surface. In this approach, the surface images taken would be done at night under fluorescence to determine how quickly the biosolids material are incorporated into the soil, rather than how deeply they are incorporated. This approach may be a better determinant of whether biosolids applied to the soil surface under reduced tillage are incorporated in a timely enough manner by rainfall and biological agents to sufficiently reduce the risk of nutrient and material losses through erosion and would allow for the tracking of the dispersal and movement of the material on the soil surface.

Overall, the method was successful in the re-identification and quantification of fluorescent particles and consequently, biosolids within a soil matrix. Although there are still improvements to be made to improve the method's effectiveness, the results have helped answer research questions 1 and 2 of this thesis. Showing that good soil management and elevated earthworm populations each substantially affect biosolids' movement within agricultural systems. The combined effect of good soil and land management with high earthworm numbers seen in the LEY-NoT treatment saw some of the fastest and deepest levels of incorporation of biosolids out of any of the treatments, showing that surface applying biosolids onto arable soils can incorporate biosolids within the soil matrix in a timelier manner than conventionally manage soils. However, the timeliness may not be sufficient to eliminate the risk of losses in run-off over the winter as biosolids were still present. This may be anecdotal but suggests that further work should be done to evaluate the potential to utilise biosolids surfaced applied under reduced tillage systems and update regulations and restrictions as appropriate.

4.9 Acknowledgements

Along with the acknowledgement made in the preface of this thesis the author would like to acknowledge the assistance of Marion Bauch for assistance with the fluorescent microscope and Steve Rolfe for discussion and assistance with the use of ImageJ for the processing of images of fluorescent samples.

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Chapter 5: The effect of biosolids on the water stable aggregate distribution of agricultural soil under flooded and unflooded conditions.

5.1 Introduction

A by-product of wastewater treatment, biosolids are rich in organic matter and contain lots of micro and macro nutrients, this can be seen in the biosolid constituents table in Chapter 2. The organic matter content of digested cake biosolids is usually around 50% (AHDB, 2019). During wastewater treatment, liquids are separated from solids and each fraction is further treated in different ways, as outlined in Chapter 1. This includes treating sludges with mesophilic and thermophilic anaerobic digestion, pasteurisation, incineration, and pyrolysis. Anaerobic digestion is the main route for most sludge treatment as it also produced methane, used for the cogeneration of heat and energy for use on site, and excess energy can be injected into the national grid (Gooding & Booth, 2017). This is often complemented with pre- or post-treatments, such as thermal hydrolysis or lime stabilisation. Some methods can enhance the conversion to methane and / or produce higher-grade biosolids as a by-product (Environment Agency, 2017). In the UK, 87% of biosolids produced are recycled to agricultural land (Assured Biosolids, 2021), and as outlined in Chapters 1 and 3, there are many reported studies of biosolids having both beneficial and unfavourable effects on the soils to which they are applied. There are many possible reasons for this, including different soil types, climates and varied biosolids constituents.

5.1.1 Soil aggregates and their importance in soil functioning

Soil aggregates are an essential part of soil structure, holding particles together and maintaining pore spaces between them through which roots can grow, water infiltrates, and gasses exchange with the atmosphere (Churchman, 2010). The variety of sizes, strengths and porosity of aggregates makes them vital in the movement of water, associated solutes and gases, and consequently the ability of the soil to supply these to plants, and for soils to function in water storage and filtration (Churchman, 2010).

Water stable aggregates (WSA) gives a measure of a soil's resilience to disruptive forces and how established and durable a soil is. Aggregates that can remain stable when wetted are important for good soil structure, as they maintain pore spaces, and are of particular importance to soils as they provide a vital role in accumulating, storing, and protecting soil organic carbon (Churchman, 2010; Tisdall & Oades, 1982). Disaggregation causes detached particles to block pore spaces and for soils to slump under their own mass and become compacted when wet. The formation of medium and larger soil aggregates improves soil structure, fertility and can increase crop yields (Tisdall & Oades, 1982).

The organic carbon stored within WSA has been shown to be more resistant to microbial degradation and contribute to long term carbon sequestration. Concentrations of carbon are highest in the largest soil aggregate fractions, > 1 mm; therefore, soils with a larger proportion of these macro-aggregates have the largest organic carbon pool (Yu *et al.*, 2015). The application of organic materials to soils has been shown to increase the soil organic carbon content and contribute to improved soil aggregation (Nimmo & Perkins, 2002). Since biosolids are rich in organic carbon, we might expect them to improve soil aggregation. However, the processes of soil aggregation are strongly linked to the activities of organisms, roots, mycorrhizas, other fungi, bacteria and earthworms (Berdeni *et al.*, 2021), and since the organic matter in biosolids can be relatively stable because of its prior extensive microbial decomposition, its effects may be less apparent than adding more labile forms of C to soils, such as plant residues.

5.1.2 Biosolids, water stable aggregates and soil organic carbon

An intrigue of Chapter 3 was that where biosolids were applied to soil, there was a clear trend for either disaggregation or inhibition of the formation of WSA compared to control treatments. This was observed for almost all land management treatments. Bolan *et al.*, (2013) reported an increase in carbon sequestration from biosolids to soils higher than that of conservation tillage alone.

However, measuring the carbon content of the larger soil aggregates in Chapter 3 revealed that there was no significant differences between soil management treatments or between the biosolids and control within treatments.

From the literature, studies where biosolids were applied and WSA were measured, there was no consensus of the resulting effects (see Table 5-1). Increases in the mean weight diameter (MWD) of WSA was seen by Wallace *et al.*, (2016) and Yucel *et al.*, (2015), although not all significantly different from the controls. Jin *et al.*, (2015) used a biosolid with similar processing to that of the biosolids used in Chapter 3, whereby the sewage sludge was dewatered, anaerobically digested and dewatered again before application to soil, and found WSA to decrease with an increasing biosolids application rate. Nicholson *et al.*, (2018) published a long term study, the closest in soil type, crop and climate to that used in Chapter 3, from four UK arable cropping sites. They found a slight increase at one site; however, there was no significant difference between the control and low metal biosolids (which is the closest to those produced today) in terms of soil aggregation at all four sites. Comparing studies, differences could be due to the varying methods of mean weight diameter (MWD) and WSA used for analysis (Anon, 1982; Kemper & Rosenau, 1986; Nimmo & Perkins, 2002). However, the trends within studies still showed variable effects of the biosolids on soil aggregates. In each of the studies, biosolids with different processing methods were used. Although some did not specify, those with dewatered biosolid cake saw no significant effect or a non-significant decrease in soil aggregation, suggesting that biosolids may be less beneficial for soils than is widely assumed by farmers and agricultural advisors promoting the use of biosolids.

Table 5-1: Details of results from studies where biosolids have been applied and soil aggregate stability analysed. Continued onto next page.

Source	Site Details	Biosolid Details	Aggregate stability method	Effect on soil aggregates	Effect on Soil carbon & Nitrogen in aggregates
(Wallace *et al.*, 2009, 2016)	Canada, grassland, silt loam, pH 8.5	Not specified. Surface applied as a one-off application.	Nimmo and Perkins, 2002. Expressed as MWD.	Significant increase in MWD compared to the control. Biggest difference seen in the >2 mm fraction.	Significant effect of treatment in >2 mm fraction, lower CN ratio for all biosolids treatments compared to control.

Source	Site Details	Biosolid Details	Aggregate stability method	Effect on soil aggregates	Effect on Soil carbon & Nitrogen in aggregates
(Jin *et al.*, 2015)	USA, grassland, silt loam and silt clay loam, pH 7.1	Anaerobically digested and dewatered cake. Surface applied annually.	Kemper and Rosenau, 1986. 1-2mm fraction only.	1-2 mm fraction decreased with increasing biosolid application rate. In the topsoil (0-5 cm), 1-2 mm fraction was lower in long-term treated fields compared to mid-term treated fields.	SOC increased with biosolids addition, C:N ratio decreased with increasing application rate.
(Yucel *et al.*, 2015)	USA, alternate year corn-soy bean rotation, silt loam, pH 7.1.	Lime stabilised anaerobically digested liquid. Surface applied bi-annually.	Nimmo and Perkins, 2002. Expressed as MWD.	MWD was increased after biosolids addition, significantly increased in 5- & 25-year treatments.	No effect significantly different for the control in any treatment.
(Nicholson *et al.*, 2018)	UK, arable ley rotation. Last crop before sampling was either wheat or barley. Four sites including sandy loam, silt loam and clay loam.	Digested cake. Surface applied annually and then incorporated with a spading machine.	Anon, 1982. Dispersion ratio technique.	No significant difference between control and BS1 (equivalent to modern biosolids) treatment at any site.	Significant increase in N for all treatments.

The effect of different processing methods on soil aggregate dynamics and carbon storage could affect organic matter cycling within the soil due to the state of the biosolids materials across a gradient of 'activeness / bioavailability / level of degradation'. The least active and most degraded organic matter profiles will be found where particularly intense pre- and or post-treatments of biosolids have been applied. The biosolids used in Chapter 3 were subjected to dewatering, thermal hydrolysis, mesophilic anaerobic digestion, and dewatering. Thermal hydrolysis is a process whereby the sludge is held at temperatures and pressures above that of an autoclave for a defined period prior to anaerobic digestion (Barber, 2016). The thermal hydrolysis process is also reported to increase the sludge's biodegradability during anaerobic digestion and reduce its volume by 50% (Barber, 2016).

Another sludge processing treatment is mesophilic anaerobic digestion followed by lime stabilisation; lime is added to raise the pH of the biosolids (> pH 12) for a minimum period of 2 hours (DEFRA, 2018). Lime stabilisation creates a biosolid product that also has agricultural value as a liming material (ADAS, 2014). Ferreira *et al.*, (2019) reported an increase in aggregate MWD after surface lime additions to a silty-clay textured soil. This suggests that lime-stabilised biosolids could have an increased aggregating effect on soils, further supporting that different biosolids processing techniques may affect soil structures differently, especially if lime-stabilised. The study using lime-stabilised biosolids by Yucel *et al.*, (2015) observed a long-term increase in aggregate MWD over 5 and 25 years. Lime is recognised as a "cementing agent" that can help to bind soil particles together chemically, especially if the soil pH is neutral or alkaline so that the $CaCO_3$ experiences low rates of acid hydrolysis (Holland *et al.*, 2018; Quirk & Schofield, 1955).

5.1.3 Flooding and soil aggregate stability

Another important consideration for how biosolids effect soils is the weather and climate of the region. From reported studies on weather patterns and climate change, there are an ever-increasing amount of "low probability, high impact" weather events that can cause flooding (Blöschl *et al.*, 2019). During the last century, there is evidence of increases in severe flood events, with mean annual floodwater discharge increasing by 12% in the UK between 1960 and 2010 (Blöschl *et al.*, 2019). The UK has seen extensive flooding in recent years, particularly on agricultural lands in Somerset and during spring 2012 (Morris & Brewin, 2014). The Ouse catchment, where the site for soil collected for this thesis is situated, has a significant threat of flooding from tidal, rainfall and surface water flows. This includes housing, commercial and retail properties and extensive areas of farmland (Environment Agency, 2010). Studies of the 2000 floods by (Holman *et al.*, 2003) highlighted the adverse effects of flooding on agricultural soils, leading to soil structural degradation at 30 – 35 % of the Yorkshire Ouse and UK catchments. The soil taken from Leeds University Farm used in the present studies lies in the Ouse catchment, where poor soil quality is implicated in increasing surface run-off, exacerbating flood risk (Holman *et al.*, 2003).

In Chapter 3, it was discussed that reduced drainage in the monolith boxes might have contributed to disaggregation. The effects of saturation and flooding on agricultural soils most notably cause a breakdown in soil structure. Flooding can dissolve cohesive agents that bind soil particles together into aggregates. Pressure from trapped air within soils can disrupt soil aggregates by causing swelling of soil particles (Ponnamperuma, 1984). Most freely draining agricultural soils experience high-frequency shrink-swell cycles, and those with a higher percentage of clay and organic matter, have a greater swelling rate. Soils that are subjected to high-frequency wetting and drying can be structurally degraded through a loss in soil shear strength, which in turn reduces the resistance of the soil to disruptive forces (Ponnamperuma, 1984). Riparian zones (soils subjected to gradients of flooding on a regular basis) were studied by Liu *et al.* (2021), who found that aggregate stability was extensively governed by the number of flooded days, an increasing amount of which caused aggregate stability to decrease. They also found that increasing soil water content also contributed to decreased aggregate stability, whereas higher proportions of soil organic matter and total nitrogen contributed to increased aggregation. From reviewing the literature, there appears to be a significant gap in knowledge of the effects of biosolids applications followed by flooding on soil structure.

5.1.4 Aims, objectives and hypothesis

The lack of consensus in the literature as to the effect of biosolids on soil water stable aggregates, as well as the trend towards disaggregation after the application of biosolids. The results presented in Chapter 3 highlight a potential emerging concern about the effects of biosolids applications on the structure of agricultural soils. Variation presented in the literature could result from differences in climate, soil type and amount of biosolids used but may also be due to the different biosolids used and how they have been produced. To thoroughly test the changes in aggregate stability, particularly with respect to changing climates and increased flood risk after biosolids addition, it is important to investigate the effect of biosolids addition under flooded and unflooded conditions.

This chapter and experiment aim to follow on from the work presented in Chapter 3 to discern any significant effects of biosolids addition on soil WSA that were not found to be statistically significant, but showed trends, possibly due to low replication and inter-field variability in the studies presented in Chapter 3. The present chapter will also investigate two different biosolid production methods on WSA of agricultural soils after application. Finally, this chapter will investigate the impact of flooding on WSA of agricultural soils, with and without biosolids addition. Collectively, these aims will allow for a thorough investigation of the contributions of biosolids to WSA. This chapter will investigate the following hypotheses:

- The application of biosolids to agricultural soils will contribute to the disaggregation of WSA in the larger size fractions compared to the control.
- Biosolids treated in different ways will experience different changes to WSA distribution.
- Lime-stabilised biosolids will increase soil aggregation to a greater extent than those without lime stabilisation.
- Flooding will contribute to the disaggregation of soil WSA to a greater degree where biosolids have been applied.

To investigate these hypotheses and meet these aims, a pot experiment was set up with agricultural soils and subjected to a range of treatments, including a control, two different biosolids and flooded and unflooded treatments.

5.2 Methodology

This experiment consisted of five phases shown in Figure 5-1:(1) fieldwork and experimental preparation, (2) short term soil acclimatisation to biosolids addition to pots, (3) crop establishment, (4) flood and unflooded treatment period, and (5) soil recovery post-flooding.

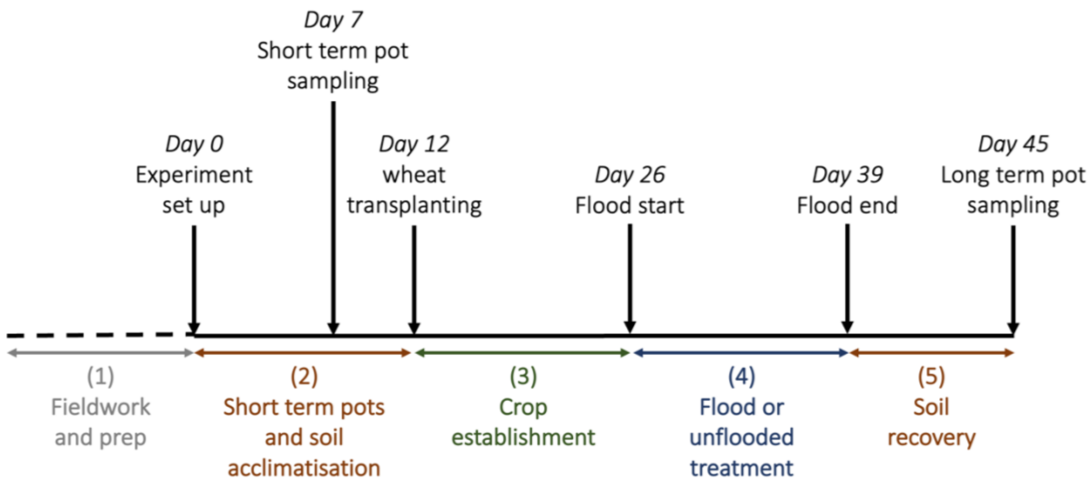

Figure 5-1: Experimental timeline. Spring climate, large pot experiment on field soil with incorporated biosolids, to investigate the effect of flooding and biosolid type on soil water stable aggregate distribution.

5.2.1 Experimental set-up and maintenance

5.2.1.1 Fieldwork

Details of fieldwork for this chapter are provided in Chapter 2. In brief, soil was collected from Spen Farm, Tadcaster, in January 2020 from the ley strip in the BSSE field. All the soil was brought back to the Arthur Willis Environment Centre (AWEC). Biosolids of two treatment types were collected from Esholt and Knostrop in January 2020, providing thermally-hydrolysed and lime-stabilised biosolids respectively. Table 5-2 provides biosolid site details. A sub-sample of biosolids were sent for analysis; the results of which were presented in Chapter 2 and compared with industry standards and limits.

Table 5-2: Biosolid site and constituent characteristics.

Site name	Esholt	Knostrop
Site location	53°51'05.7"N 1°43'01.1"W Bradford, England.	53°46'48.9"N 1°29'18.4"W Leeds, England.
Site details	90 TDS/day output capacity, working at 80 TDS/day 17,028 TDS annual throughput 2019 60/40 primary to secondary sludge ratio	130 TDS/day output capacity, working at 80 TDS/day 24,339 TDS annual throughput 2019 70/30 primary to secondary sludge ratio
Biosolid processing technique	Thermal hydrolysis	Lime stabilisation
Biosolid processing grade	Enhanced	Enhanced

5.2.1.2 Pot set-up

The collected field soil was coarsely riddled (5 cm), see Figure 5-2, so that all green vegetation was removed, and soil aggregates were less than 5 cm in diameter. Large stones were also removed. Square pots of 11 litres in volume (22 cm x 22 cm x 23 cm, width x depth x height respectively) with square saucers (top 29 cm^2, Bottom 22.5 cm^2, Height 5.5 cm) were set up with 7.00 kg of the riddled soil. Biosolids were prepared by sieving to 1 cm to create a uniform crumb size and weighed out for each pot based on an application rate by weight of 92 g per pot, equivalent to 19 t ha^{-1} in the field. For treatments with biosolids, the soil from each pot was individually emptied onto a tray in a layer. The biosolids were then sprinkled over the surface and roughly mixed while returning the soil and biosolids mixture to the pot. In total, 45 pots were set up in line with the treatments shown in Table 5-3.

Figure 5-2: Photograph of soil preparation using a coarse 5 cm sieve to separate soil from vegetation. Prepared soil can be seen in the foreground.

Table 5-3: Pot treatments with a no biosolid addition control, two biosolids treatments. A set of short term 7-day pots and flooded and unflooded long-term (45-day) pots.

Treatment	Without flooding	With flooding	7-day pots
No biosolid addition	n=5	n=5	n=5
With thermally hydrolysed then anaerobically digested biosolids (ADTH)	n=5	n=5	n=5
With anaerobically digested then lime stabilised biosolids (ADLS)	n=5	n=5	n=5

All pots were situated in a controlled environment chamber at AWEC on a raised bench. The chamber had continuous airflow and was set up with a 10-hour day length at 15 °C and 70% humidity and night conditions of 10 °C and 90% humidity. Light length was set at 11 hours. These parameters were chosen to simulate spring temperatures in the field. Figure 5-3 shows the pots *in situ* during the experiment. Each pot was labelled with a randomly assigned number between 1 and 45. Each treatment was assigned a colour code, white – 7-day pots, blue – 45-day flooded treatment, yellow – 45-day unflooded treatment. Throughout the experiment, the pots were cycled on the bench during weighing and watering by moving the far-right row to the far left and moving the front pots through the middle and the back.

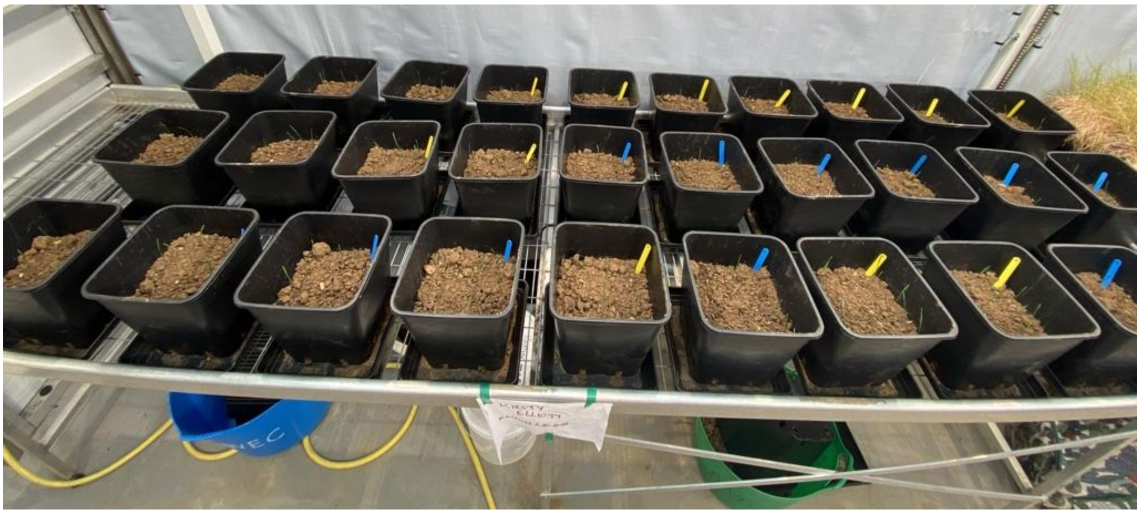

Figure 5-3: Photograph of pots in situ in greenhouse chamber after the short-term pot harvest before flood or unflooded treatment.

5.2.1.3 Experimental Maintenance – watering

After the initial pot set up, the soil was saturated with 1 litre of tap water and allowed to drain for 3 days to give the soil water holding capacity. Pots were then maintained at the average water holding capacity throughout the experiment by weighing all pots and watering with the average of the water lost. Pots were initially watered with 0.5 litres of tap water, and subsequent watering was based on the average water lost measured by weight change. All watering was administered from a watering can to the soil's surface to replicate rainfall as much as possible in a greenhouse experiment.

5.2.1.4 Wheat

Winter wheat variety Skyfall (provided by RAGT Seeds Ltd Ickleton), were germinated in a thin layer of moist native soil and transplanted to the pots at a rate of 12 plants per pot, in 3 rows of 4 seedlings, which corresponds to field density by area. At the end of the experiment, all wheat was harvested from each pot by counting the number of surviving plants and cutting off the surface vegetation at the soil level.

5.2.1.5 Flooded treatment

Flooded treatments (n=15) were set up by lining an empty pot with a double layer of polythene before placing the soil-filled pot within. Water was then added to submerge the pots with a 5 cm flood level. Flooded pots were also weighed twice a week and topped up to the flood level with the average volume lost over the flooded pots. At the end of the flood, the soil pots were removed from the waterproofed container and sat back on the saucer. They were not watered again before sampling as they had not returned to the pre-flood water holding capacity when the experiment was ended. Figure 5-4 shows a photograph of the flooded and non-flooded treated pots in situ.

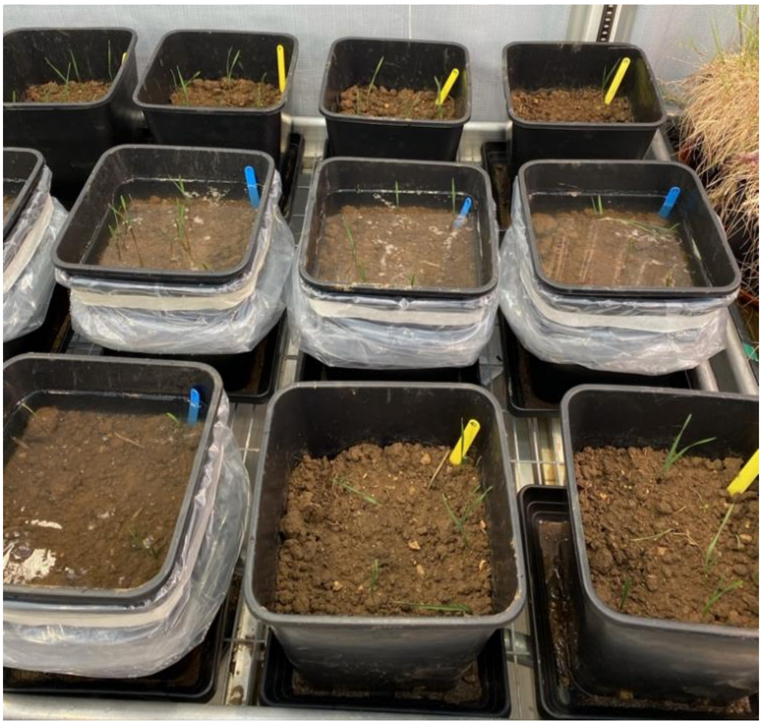

Figure 5-4: Photograph of flooded an unflooded pot set up in situ. An earthworm can be seen on the soil surface in the front-left flooded pot.

5.2.1.6 Earthworms and soil fauna

During soil sieving for pot set up, earthworms were left within the soil matrix. However, slugs and leather jackets were removed when seen to limit crop damage. During the flooded period, it was observed that leather jackets and slugs that were not extracted in the soil preparation stage were coming to the soil's surface and destroying the wheat seedlings. It was therefore decided to remove them from the pots; they were identified and counted.

5.2.2 Experiment harvest – day 7 and day 45

On day 7, 15 pots were harvested, in line with the treatments in Table 5-3. Pots were weighed and then sampled for WSA by taking a 10 cm deep and 5 cm wide core weighing approximately 500 g. On day 45, the remaining 30 pots had the wheat harvested (as described in 5.2.1.4), the soil was sampled in the same way as the 7-day pots. All soil samples from day 7 and day 45 were subsampled for soil moisture and left to air dry at room temperature (20 °C) until analysed for WSA.

5.2.3 Further laboratory analysis

Soil moisture was calculated based on weight lost after drying the subsample at 105 °C until no weight change. Details of water stable aggregate analysis and CN analysis are described in Chapter 2. In brief, for WSA analysis air-dried soil was sequentially wet sieved into > 2 mm, > 1 mm and < 1 mm fractions. Fractions above 1 mm had stones and vegetation removed. Each fraction was oven-dried at 105 °C for at least 24 hrs until no weight change. Aluminium dishes were used to dry the samples; these were pre-weighed, and soil and dish dry weights were recorded. Soil weights were then calculated for each size fraction. All samples were ground into a fine powder for CN analysis. Samples for organic analysis were treated to remove carbonates using 6 M HCl and dried at 105 °C. These acid-stripped samples were weighed out at 20 mg +/- 5 mg, sealed into tin boats and analysed using a CN elemental analyser. This method is described in further detail in Chapter 2.

5.2.4 Statistical analysis

Before conducting statistical analysis, the data was assessed for normal distribution to meet the assumptions of the tests. If the data was not normally distributed, for instance proportional data, it was transformed. To test the differences between groups, where results comprising of continuous and count data for a single time point, the difference between means was analysed using an analysis of variance (ANOVA) test. The data was tested for the effects of biosolids, flooding, and the interactions between groups. Proportional data was arcsine square root transformed before analysis. Post hoc Tukey HSD tests were conducted to identify differences between the control and the effect of sludge addition within soil management treatments.

5.3 Results

For conciseness and clarity, the following notations have been used to represent each treatment: (1) Sampling interval as either 7-day (7D) or 45-day (45D). (2) Biosolid treatment as either control (CONT) with no biosolid, thermally-hydrolysed then anaerobically-digested biosolids (AD-

TH), or anaerobically-digested then lime-stabilised biosolids (AD-LS). (3) Flood treatment as either unflooded (NO) or flooded (YES).

5.3.1 Water stable aggregates

Soil water stable aggregate (WSA) fraction results are presented as proportions of the total weight of each soil samples in Figure 3-8, with the larger diameter fractions in darker colours at the bottom of the stacked bar chart and the smaller fractions in lighter colours at the top of the chart. Data was arcsine square root transformed before analysis.

From the results in Figure 3-8 compared to soil fresh from the field, there is an immediate decline in the proportion of aggregates sized >2 mm after handling and processing by day 7 in the experiment, consequently increasing the proportion of <1 mm aggregate. After 7 days, the biosolids amended treatments maintained a larger proportion of >2 mm aggregates compared to the control, with ADTH having the largest proportion of the two biosolids treatments and the effect of biosolids was significant (p = 0.0423). After 45 days, the unflooded pots see a recovery and an increase in the >2 mm size fraction for all treatments. The biosolids amended treatments again have the highest proportion of >2 mm aggregates; however, the ADLS treatment had the highest proportion. The effect of biosolids treatment was significant (p < 0.01). ADLS unflooded also has the largest proportion of >2 mm aggregates in all treatments during the experiment and is significantly different from all 7-day and 45-day-flooded treatments. After 45 days, the flooded treatment did not see the same level of recovery as the 45-day unflooded treatment. Biosolids application had no significant effect on the proportion of >2 mm aggregates which was at the same level as the corresponding biosolids treatment in the 7-day pots.

The 1-2 mm size fraction for all treatments was a similar size. However, there was an overall significant effect of biosolids (p <0.005), with 45-day ADTH unflooded having the highest proportion of aggregates in this fraction and this was significantly different from the lowest seen in the 45-day control flooded treatment. All other treatments fell in between and were not significantly different from either the highest of the lowest. As the results are proportional, the <1 mm fraction saw similar, but opposing, changes to the >2 mm fraction so have not been described here. A statistical summary for WSA is presented in Table 3-1, and a summary ANOVA is presented at the end of the results section in Table 3-2.

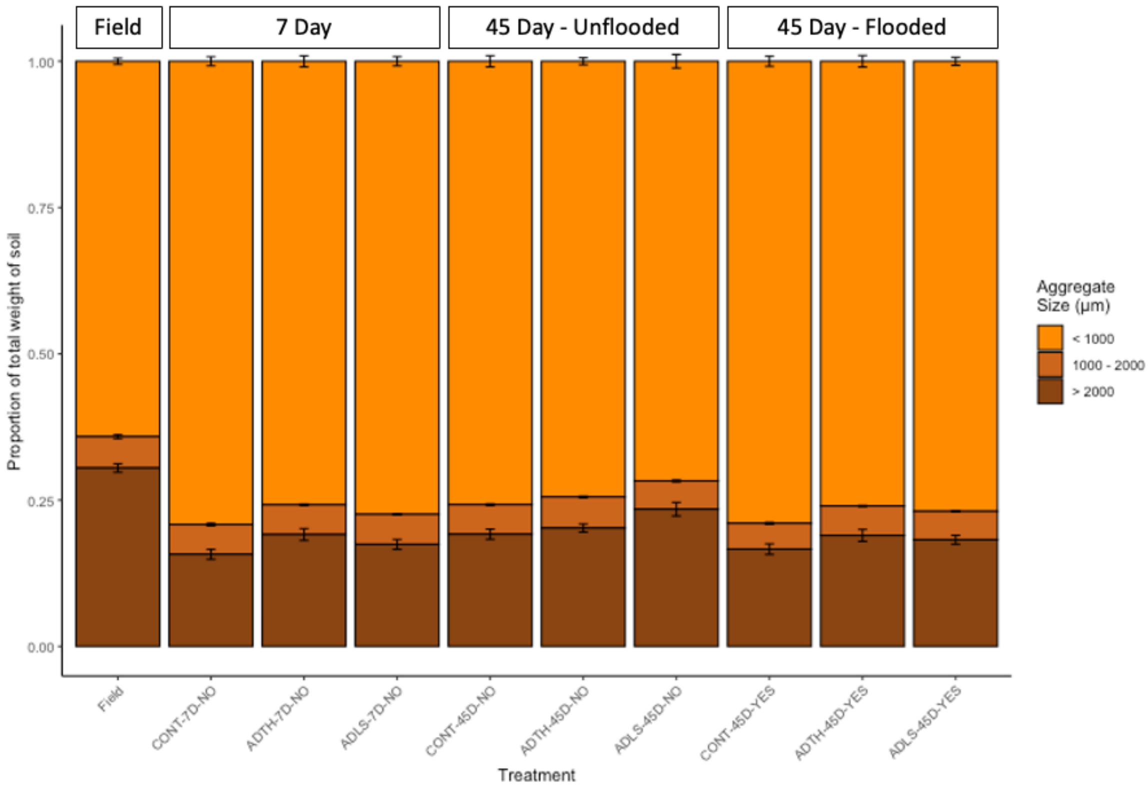

Figure 5-5: Water stable aggregate proportional fractions of pot soil.

The MWD of soil aggregates is presented in Figure 3-9, showing similar trends to that of the >2 mm WSA fractions. The overall effect of biosolids treatment on WSA (as summarised as MWD) was significant within the 45-day unflooded treatment (p <0.05). There was a trend towards significance within the 7-day pots (p = 0.08), and there was no significant difference within the 45-day flooded treatment. The pot with the highest MWD was 45-day ADLS unflooded treatment, which was significantly higher than the 45-day ADLS flooded treatment. Comparing all treatments within the 45-day flooded and unflooded pots, there was a significant difference in flood treatment (p <0.005) and a trend towards significance of biosolid treatment (p = 0.082). A statistical summary for

MWD is presented in Table 3-1 and summary ANOVA presented at the end of the results section in Table 3-2.

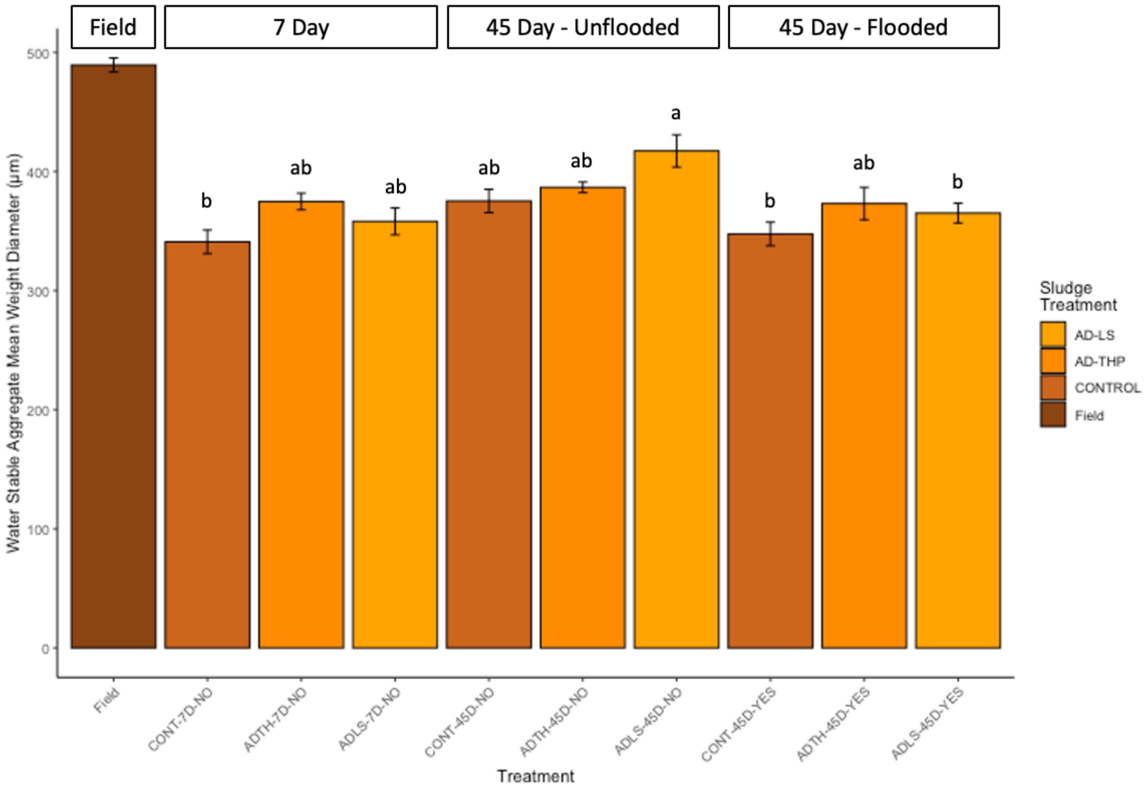

Figure 5-6: Mean weight diameter of soil water stable aggregates, showing no effect of biosolids additions but substantial effects of land management practices. Statistics shown do not include field soil and are a Tukey HSD test.

Table 5-4: Water stable aggregate proportional fractions of pot soil and overall mean weight diameter of aggregates, p values from analysis of variance and significance codes (abc etc.) from Tukey HSD test for the effect of treatment on water stable aggregates.

Size fraction (µm)	7-Day Control	7-Day AD-TH	7-Day AD-LS	45-Day Unflooded Control	45-Day Unflooded AD-TH	45-Day Unflooded AD-LS	45-Day Flooded Control	45-Day Flooded AD-TH	45-Day Flooded AD-LS	*p* value
< 1000	0.792	0.758	0.774	0.758	0.745	0.717	0.790	0.760	0.769	
	a	*ab*	*ab*	*abc*	*bc*	*c*	*a*	*ab*	*ab*	<0.001***
	a	*b*	*ab*							0.027*
				a	*ab*	*b*				0.017*
							a	*a*	*a*	0.055^
				ab	*bc*	*c*	*a*	*ab*	*ab*	<0.001***
1000-2000	0.051	0.051	0.051	0.051	0.053	0.048	0.044	0.050	0.049	
	a	*a*	*a*	*ab*	*a*	*ab*	*b*	*ab*	*ab*	0.005**
	a	*a*	*a*							ns
				a	*a*	*a*				0.086^
							b	*a*	*ab*	0.006**
				a	*a*	*ab*	*b*	*a*	*ab*	0.001**
>2000	0.157	0.191	0.174	0.192	0.202	0.235	0.116	0.190	0.182	
	c	*bc*	*bc*	*abc*	*ab*	*a*	*bc*	*bc*	*bc*	<0.001***
	b	*a*	*ab*							0.042*
				b	*ab*	*a*				0.01*
							a	*a*	*a*	ns
				b	*ab*	*a*	*b*	*b*	*b*	<0.001***
Mean weight diameter (µm)	341	375	358	375	387	417	348	373	365	
	b	*ab*	*ab*	*ab*	*ab*	*a*	*b*	*ab*	*b*	<0.001***
	a	*a*	*a*							0.08^
				b	*ab*	*a*				0.031*
							a	*a*	*a*	ns
				ab	*ab*	*a*	*b*	*ab*	*b*	0.003**

5.3.2 Water stable aggregates carbon and nitrogen content

The organic carbon, nitrogen, and CN ratio results for the biosolids amendments are detailed in Table 5-5, followed by the results of the organic carbon and nitrogen analysis of the 45-day WSA fractions are presented in Figure 5-7. The biosolids results show that both biosolids are rich in carbon, with over 20 % total carbon content, the ADTH had the highest total and organic carbon content of both the biosolids, as well as the highest nitrogen content. The organic CN ratios of both sludges are the same at 7.1.

Table 5-5: Biosolid carbon and nitrogen analysis results. n = 2.

Biosolid	Carbon type	Carbon (%)	se	Nitrogen (%)	se	CN ratio	se
ADLS	total	25.4	0.050	4.0	0.009	6.3	0.014
	organic	21.8	0.121	3.1	0.003	7.1	0.041
ADTH	total	28.1	0.030	4.2	0.010	6.7	0.021
	organic	23.8	0.156	3.3	0.012	7.1	0.026

There was no significant effect of any treatment in the <1 mm fraction with carbon, nitrogen and CN ratio showing similar results between treatments. In the 1-2 mm fractions, there was no significant effect of any treatment on CN ratio, however, there was a significant effect of biosolids treatment for both organic carbon (p <0.05) and nitrogen (p <0.05), where biosolids amended treatments were higher than the control treatments for both flooded and unflooded treatment. There was no significant effect of flood treatment on carbon, nitrogen, or CN ratio. The >2 mm fraction showed the most variation, with a significant effect of both biosolids treatment (p <0.05) and flood treatment (p <0.05) on organic carbon. The biosolids amended pots had a higher organic carbon content, and between flooded and unflooded treatments, there was a higher carbon content in flooded treatments, although the differences were not significant from a Tukey HSD test. For the >2 mm aggregates, there was also a significant effect of biosolids on nitrogen content, again with biosolid treatment having a significant effect (p <0.05), where biosolids amended pots having a slightly higher concentration of nitrogen. There was no effect of biosolid treatment on CN ratio, however, flood treatment was significant (p <0.001). A summary ANOVA presented at the end of the results section in Table 5-6: Analysis of variance summary table, considering all treatments. Values presented are p values with significant or near significant results.Table 5-6.

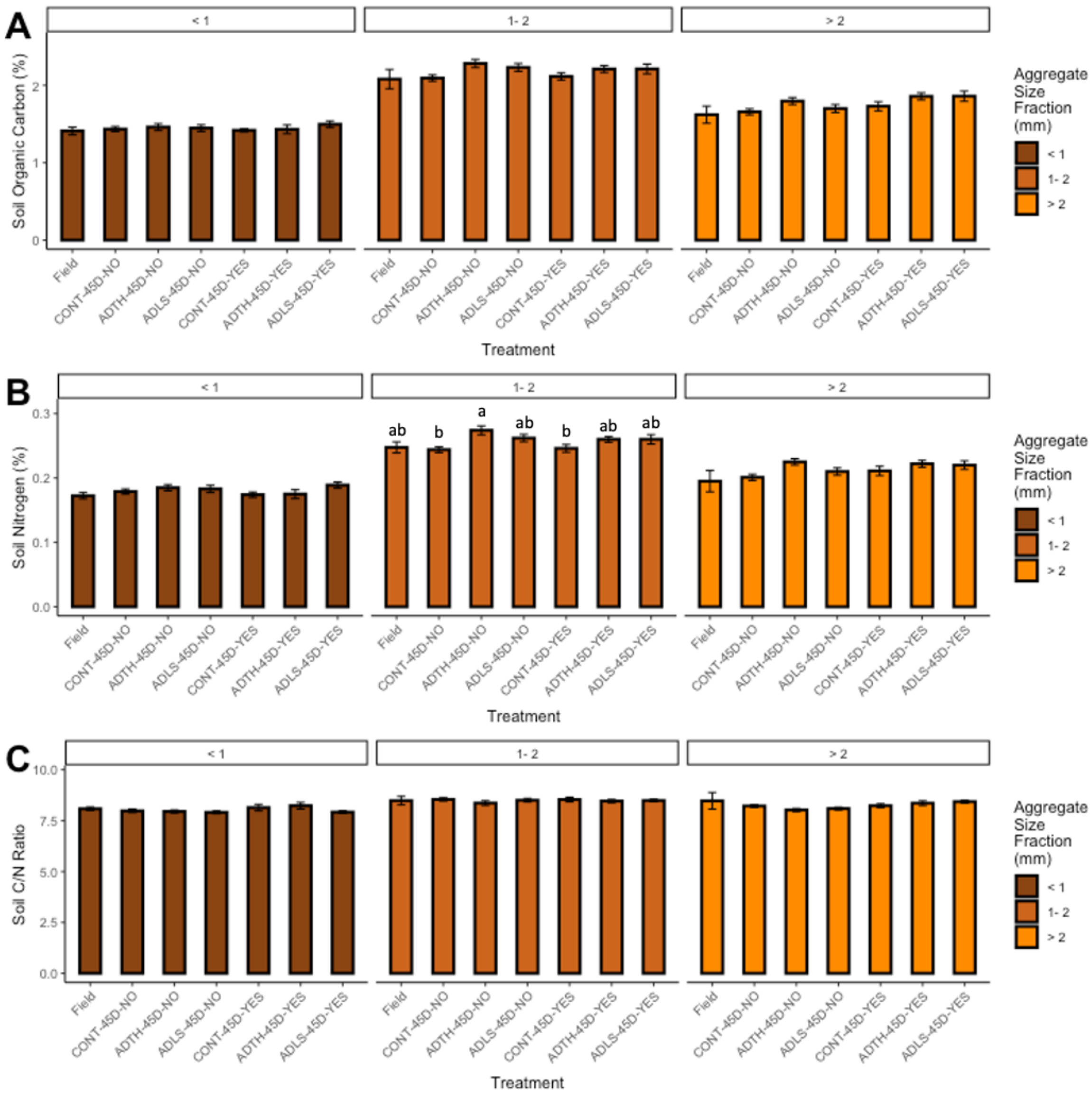

Figure 5-7: Water stable aggregate carbon and nitrogen content. Statistics shown are a Tukey HSD test where there is no notation the outcome was not significant (n = 5).

5.3.3 Soil moisture and hydrology

Soil moisture results are presented in Figure 5-8, which includes all 45-day treatments. The results are presented as additional information rather than for statistical testing, however, before the start of the flooded period, there was a significant difference in soil moisture between the control and both biosolids amended treatments (p <0.001). During the flooded period, there was an increase of approximately 200% in the flooded treatments soil moisture compared to the unflooded treatments. Overall, there was no significant effect of biosolids treatment on soil moisture when all the available data is considered. After the flood event, the flooded pots took approximately 7 days to return to unflooded moisture levels.

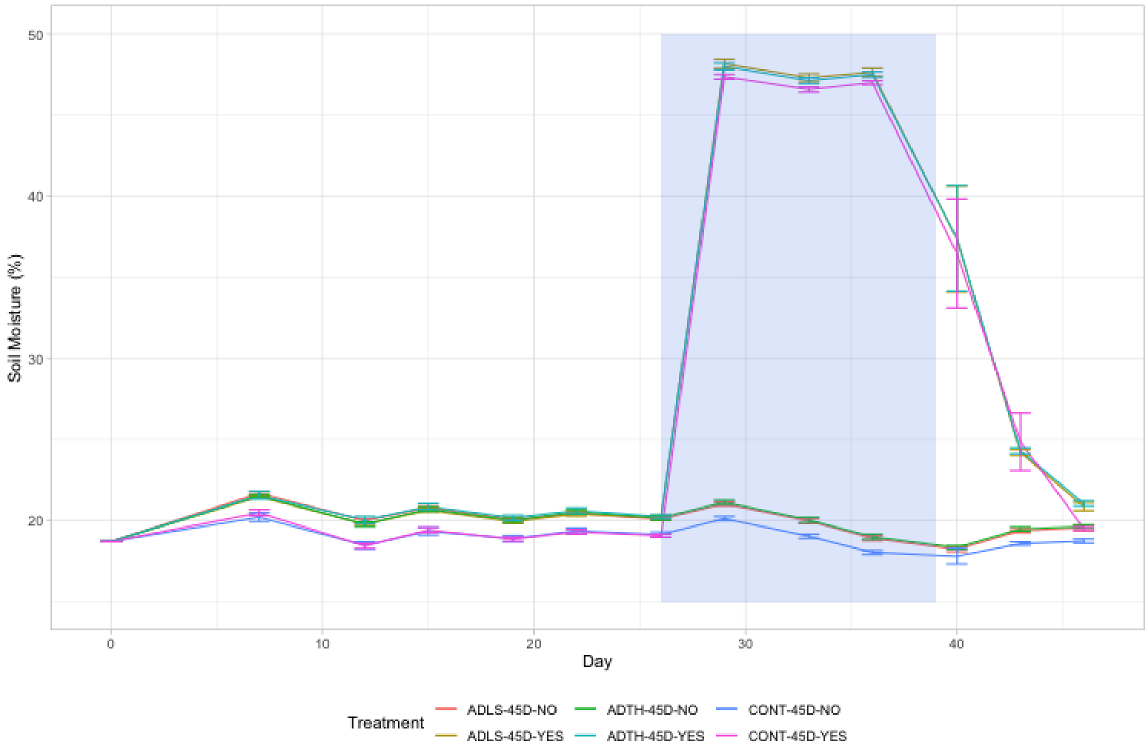

Figure 5-8: Average pot soil moisture (wt %) of all the pots the 45-day experiment, with the blue shaded area signifying the flooded period.

5.3.4 Wheat production and soil fauna

Wheat seedlings survival is presented in Figure 5-9. There was less than 50% survival in all treatments, with unflooded and biosolids amended treatments appearing to have the lowest seedling survival. However, there were no significant differences between individual treatments and no significant effect of biosolids, or flood treatment. Anecdotal results of pests that are detrimental to wheat and which were removed from the flooded pots if observed, are presented in Figure 5-10, although no statistical tests have been conducted.

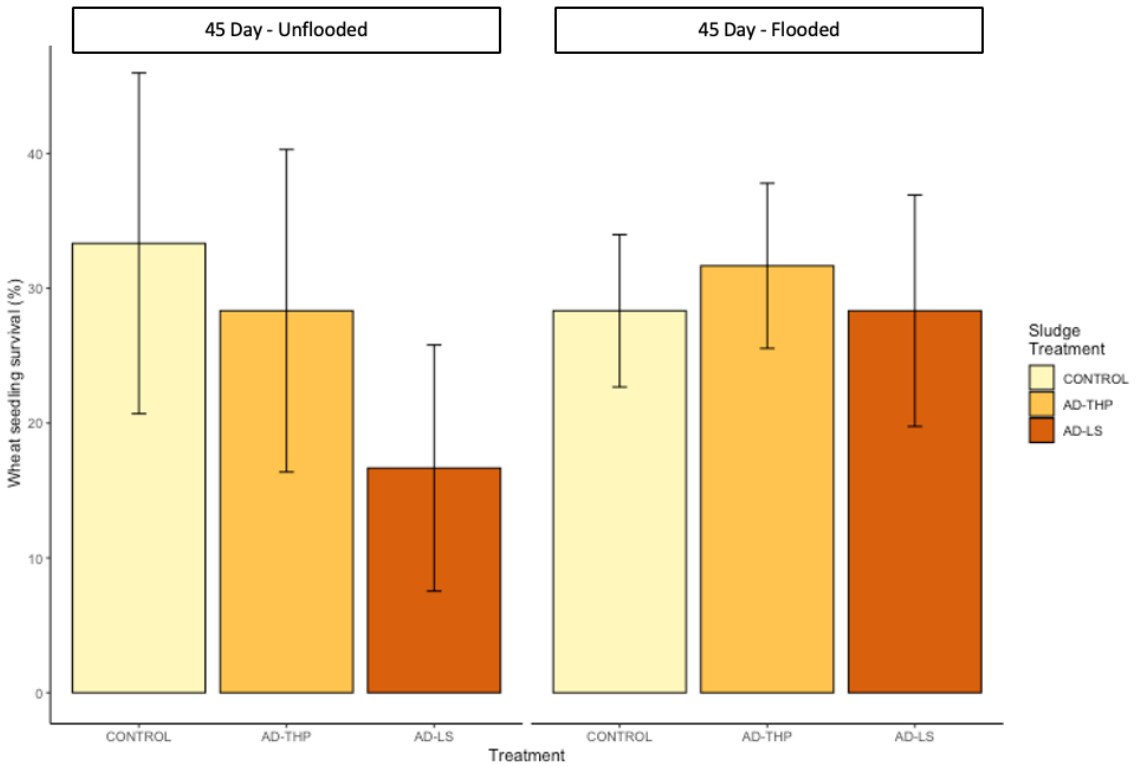

Figure 5-9: Wheat seedling survival represented as a percentage at harvest on day 45.

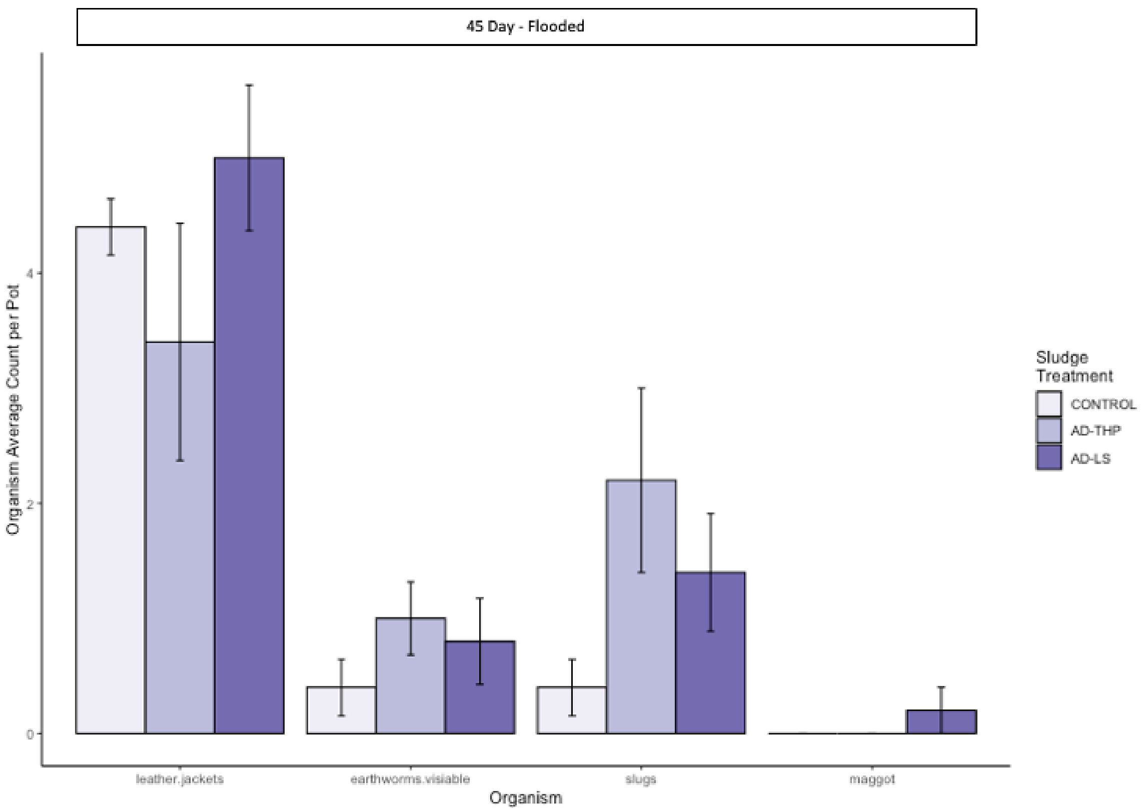

Figure 5-10: Visible earthworms and pests detrimental to wheat from the 45-day flooded pots. From left to right, organisms are leather jackets, visible earthworms, slugs, and maggots.

5.3.5 Summary of statistics

A summary of statistical ANOVA tests conducted for each subgroup of analysis are presented below in Table 3-2: group (1) all unflooded treatments and group (2) 45-day treatments.

Table 5-6: Analysis of variance summary table, considering all treatments. Values presented are p values with significant or near significant results.

Measurements	(1) 7D and 45D Unflooded			(2) 45D Unflooded and 45D Flooded		
	Biosolid treatment	Sample interval	Biosolids treatment x Sample interval	Biosolid treatment	Flood treatment	Biosolids treatment x Flood treatment
WSA proportion >2mm	0.0253*	<0.001***	<0.001***	0.026*	<0.001***	<0.001***
WSA proportion 1-2 mm	ns	ns	ns	0.014*	0.022*	0.001**
WSA proportion <1 mm	0.0201*	<0.001***	<0.001***	0.015*	<0.001***	<0.001***
WSA MWD (µm)	0.0819^	<0.001***	<0.001***	0.082^	0.003**	0.003**
Organic Carbon >2mm (%)				0.048*	0.030*	0.034*
Organic Carbon 1-2 mm (%)				0.014*	ns	0.088^
Organic Carbon <1 mm (%)				ns	ns	ns
Nitrogen >2mm (%)				0.016*	ns	0.050*
Nitrogen 1-2 mm (%)				0.001**	ns	0.008**
Nitrogen <1 mm (%)				ns	ns	ns
CN ratio >2mm				ns	0.002**	0.012*
CN ratio 1-2 mm				ns	ns	ns
CN ratio <1 mm				ns	0.098^	ns
Wheat Seedling survival (%)				ns	ns	ns

Signifiance codes: p <0.001 (***), p <0.01 (**), p <0.05(*), p <0.1(^), p >0.1 (ns).

5.4 Discussion

In Chapter 3 the results of the WSA analysis suggested that there was a trend towards reduced aggregation, particularly in the largest size fraction, after the application of biosolids. However, a significant effect was not seen, and this may have been due to the variability between field replicates and the relatively low, single application of biosolids. The literature reporting the effects of biosolids on WSA (summarised in Table 5-1) is limited and the results inconclusive as to a consensus on the effect under reduced or conventional tillage. Wallace *et al.* (2009) reported a significant increase in MWD compared to the control 4 years after a one-off surface biosolids application. The biosolid type was not reported, but the significant increase was only seen in the highest biosolid loading of 60 dry Mg ha^{-1} and not in the 20 dry Mg ha^{-1} (similar to this experiment). Jin *et al.*, (2015) surface applied biosolids at 20, 40 and 60 Mg $ha^{-1}yr^{-1}$, after 8 years there was no significant difference between the control and lowest application treatment, however, the higher application rates showed a significant decrease in the 1-2 mm WSA fraction by approximately 50%. The lack of consensus is likely due to several factors; climates, soil types and biosolids used differed to varying degrees in each experiment. Therefore, it is important to understand the possible impacts of biosolids on agricultural soil structure, especially a component as critical for soil function as macroaggregation. In particular, this study has targeted an assessment of the effect of biosolids on soil aggregation using two biosolids of different processing methods. Additionally, an assessment of the effect a flood event has on soil aggregation has been conducted, which gives an important insight given the changing climate and increasing frequency of extreme weather events. In this discussion the effect of biosolids and flooding on soil aggregation are discussed based on the results presented here.

5.4.1 Assessment of the differences in biosolid characteristics

To fully understand the effects of the different biosolids, an assessment of the properties of the biosolids is essential. From Table 5-2, the site characteristics of the biosolids are similar; Knostrop (ADLS) has a higher annual throughput and a slightly higher primary to secondary sludge ratio than Esholt (ADTH). From the laboratory analysis of the biosolids sampled in January 2020 (presented in Chapter 2, Table 2-5), both sludges have similar profiles, with a pH of approximately 8.2 and a dry matter content of approximately 26%. The differences are seen in the micro- and macronutrients; in the ADTH biosolids, total N and iron is 15% and 25% higher than ADLS respectively. However, there is almost 2.5 times the amount of calcium and 8% higher phosphorus content in the ADLS biosolid compared to ADTH. The difference in these components of the biosolids is two-fold; the N and P differences will be mostly affected by the sludge chemical characteristics prior to sludge processing and anaerobic digestion which may be affected by primary to secondary sludge ratio. The differences in iron may be due to the input of iron from industrial wastewater that ends up in the sludge. Still, they may also be due to iron dosing into the anaerobic digesters, which can improve anaerobic digestion performance, including increased methane production and facilitation of organic matter solubilisation (Romero-Güiza *et al.*, 2016). The high proportion of calcium in the ADLS biosolid is undoubtedly due to the lime stabilisation treatment where calcium oxide (CaO) or calcium hydroxide ($Ca(OH)_2$) is dosed into the anaerobic digestate liquid. The CN analysis of the biosolids, confirm the proportions of carbon reported as dry matter from the original biosolid analysis in Chapter 2. Additionally, a change of the total to organic carbon reveals that in the ADLS biosolid 86% of the carbon is organic, and for ADTH this is 85%. The organic C:N ratio of the biosolids was the same for ADLS and ADTH at 7.1.

The quantities of biosolid applied in this experiment were based on weight in order to input similar amounts of carbon into each pot, which may aggregate with the soil. In the field, and this experiment, the application rate is limited by the total N content of the material, a maximum of 250 kg N ha^{-1} (AHDB, 2019). ADTH biosolid had the highest N content limiting the application of both biosolids to 92 g per pot, equivalent to 19 t ha^{-1}. In the field, if applying the biosolids separately based on N content, the ADLS biosolid would be permitted to be applied in a higher quantity (by weight of biosolid), meaning more carbon and calcium would be applied compared to ADTH.

5.4.2 Water stable aggregates

The immediate decline in >2 mm WSA fraction observed between the field soil and the 7-day pot experiment is not unexpected and again highlights the effect that disruptive forces, such as ploughing or in this experiment handling of the soil while preparing the experiment, can have on the degradation of macroaggregates and soil structure. The proportion of remaining >2 mm soil aggregates was approximately 50% of that of the field soil after 7 days in situ post-experimental set up, and the results of the WSA analysis provide good evidence for the effect of both biosolids and flooding on soil aggregate distribution.

In the 7-day pots, there were already small, but not significant, short-term differences in the macroaggregation of the soil between treatments, which was unexpected as soil aggregation is usually attributed as a longer-term process. Both biosolids types showed an increased the proportion of >2 mm aggregates and MWD compared to the control. Although this was not significant, it suggests that there is a property of the biosolids that is either aiding the rapid recovery of macroaggregation in this field soil or is protecting the aggregates from disaggregation. The ADTH had higher levels of aggregation than that of the ADLS, suggesting that there was not an effect of the higher calcium content in the ADLS biosolid aiding this short-term increase in aggregation.

In the 45-day unflooded pots, all pots had higher macroaggregation than the 7-day pots, again with both the biosolids amended pots being higher than the control. This builds the evidence that the biosolids are having a positive effect on macroaggregation. Unlike the 7-day pots, the ADLS has the highest proportion of >2 mm aggregates and MWD, which does suggest that the increased calcium content of the biosolids may now be acting as a cementing agent and helping bind soil particles together. Both Holland *et al.*, (2018) and Bashir *et al.*, (2016) found that soil structural improvements are dependent on the carbonaceous composition of organic sources which act as binding agents for soil particles. This is reflected in the significant difference in MWD between the 45-day biosolids treatments, where ADLS is significantly higher than the control. The soil used in this experiment (from the BSSE aka BSE field) has a high silt content (Hallam *et al.*, 2020), and the addition of lime to silty-clay soils by Ferreira *et al.*, (2019) increased soil aggregation, supporting the results seen here.

Comparing the 7-day and 45-day unflooded pots, there was a significant effect of sampling interval, likely due to the increase in the proportion of larger macroaggregates from the 7-day to 45-day pots in all biosolids treatments. This increase will be due to the formation of larger aggregates likely resulting from earthworm and microbial activity, and wheat root exudates.

In the 45-day flooded pots, the control still had the lowest proportion of >2 mm aggregates. Both biosolids treatments have higher proportions, again suggesting that there is a component of the biosolids that maintains or improves the aggregation of the soil. The ADTH had the highest proportion of >2 mm aggregates, although this was at very similar levels to that of ADLS which suggests that there was no effect of the extra calcium aiding aggregation. Comparing the 45-day flooded pots to the unflooded pots, the aggregation in all biosolids treatments is very similar to that of the 7-day pots. This may be due to the flood event inhibiting soil aggregation or the flood event causing the disaggregation of any >2 mm aggregates that built up between day 7 and the start of the flood event. The literature supports the theory that the flood event aided in the disaggregation of soil in flooded pots (Ponnamperuma, 1984; Holman *et al.*, 2003; Liu *et al.*, 2021), which when comparing the MWD of each 45-day flooded pot to the 7-day pots, that of the ADTH was lower at day 45 than day 7, and the control and ADLS were just higher than their corresponding 7-day pots.

The CN analysis results showed that there was no effect of biosolids on soil C:N ratio, and although most of the carbon and nitrogen (apart from nitrogen % in the 1-2 mm aggregates) was not significantly different between treatments, the trends shown in the figure reflect the trends seen in the MWD. There appears to be an increase, even though small, in the carbon and nitrogen content of the biosolids amended treatments. This suggests that the addition of the carbon and nitrogen from the biosolids is a possible factor aiding soil aggregation. Interestingly, the differences seen in the 1-2 mm aggregate fractions are larger than those seen in the >2 and <1 mm fraction; this supports the literature that the assimilation of carbon from organic manures accumulates within the microaggregates within macroaggregate rather than microaggregates alone (Kong *et al.*, 2005).

5.4.3 Soil hydrology, wheat, and fauna

Soil moisture data was presented to provide additional detail about the changes in soil moisture during the flood event. It also showed a significant difference between the control and the biosolids amended treatments soil moisture. This suggests that there is a water holding capacity effect of the biosolids. The moisture changes in the flooded treatments compared to the unflooded treatments reflects the substantial effect of floodwater not only on soil moisture, but on the below-ground ecosystem.

During this experiment, wheat was grown with soil fauna left undisturbed in order to approximate a ‘whole ecosystem’ environment, which best assesses the biosolid and flood treatment effects on soil aggregation in an arable management system. Although the wheat seedling transplanting was successful, the seedling survival was low due to herbivore predation. Even through the removal of pests (shown in Figure 5-10), there was no significant difference in seedling survival between treatments due to the large variability. The soil used in this experiment was from an almost 5-year ley; at this length the populations of pests such as leather jackets and slugs often increase to a detrimental level for crop establishment without the use of insecticide or molluscicide (Blackshaw & Coll, 1999).

As shown in Figure 5-10, the visible earthworms on the soil surface were counted. Observations of the pots during the flood treatment revealed that some earthworms did die during the flooded treatment, some of which were seen on the soil's surface in the flood water and some at the bottom of the floodwater between pots after the flooded period ended. There were also earthworms alive at the end of the flooded period, which were observed in all flooded treatments. Research by Kiss *et al.*, (2021) reported the difference between earthworm species' oxygen requirements and some species' ability to aestivate and survive in low oxygen environments for longer. The earthworm response to flooding highlights the detrimental effects that prolonged flooded soils can have on earthworm populations, indeed ‘ecosystem engineers’, which are critical for maintaining and improving soil structures.

5.4.4 Answers to research questions

A successful experiment was conducted to evaluate the effect of different biosolids and flooding on soil aggregation. There was no evidence to suggest that biosolids contributed to the inhibition or disaggregation of soil macroaggregates. Compared to the control in the unflooded treatments, there is however evidence to suggest that the surface application of biosolids aided soil aggregation, with the ADLS 45-day treatment having significantly higher MWD compared to the 7-day control. The differences in the magnitude of effect of the two biosolids did prove that biosolids processed using different methods do affect soil structural properties to different degrees. This effect is also affected by flooding, where there is almost no effect of biosolids on soil aggregation. The lime-stabilised biosolids did improve soil aggregation to a greater effect than the thermally-hydrolysed biosolids, although this was only seen in the 45-day unflooded treatment and the flood event inhibited this. The flood event did contribute to the inhibition and/or disaggregation of WSA, although the results were not conclusive to whether this effect was enhanced where biosolids were applied.

5.4.5 Critical evaluation of the study

This experiment was largely successful having met most of the original objectives of the study elucidating upon the questions raised in Chapter 3, and this is reflected in the quality results that were produced. However, upon reflection, some factors could have been improved. Extracting soil from the same ley strips as used in Chapter 3, but extracted 2 and a half years later, was good in terms of evaluating the results in Chapter 3 to that of Chapter 5; however, the build-up of pest populations in the ley was detrimental to the production of wheat in this experiment. Outside of the experiment's control, the outbreak of a global pandemic of COVID-19 and subsequent government-enforced restrictions meant that the investigation was cut short by 15 days, which were planned as a recovery period for the flooded pots. Looking at the results now, they highlight the effect flood events can have on soil structure, and if the recovery period was to have taken place, this difference might not have been so pronounced.

5.5 Conclusions

This chapter's main aim was to provide an evaluation of varied biosolids and flooding application on soil aggregation, independently and combined. This aim was met successfully, and the main findings include the following: The biosolids processing method does affect soil aggregation differently, which is primarily due to the change in the biosolids' chemical constituents. In a non-flooded environment, lime-stabilised biosolids increased aggregation more than the thermally-hydrolysed biosolid. This is an important discovery, and it highlights that not all biosolids have the same effects on soil systems. Still, in the majority of cases, their use in agriculture is controlled through the same regulations. In terms of biosolid production, it is a by-product of a process that seeks to extract as much energy and commercial material as possible. Although there have not been any significant detrimental effects of biosolids on agricultural land, not all biosolids provide the same benefits or level of benefits. The effect of flooding on soil aggregation confirmed all the literature that saturated soils are prone to disaggregation. The proportional changes between the 45-day flooded and unflooded pots were higher in the presence of biosolids, showing their combined effect was greater in the disaggregation of the soil.

Considering the research questions of this thesis, again, the effect of an abiotic factor, here being flooding, was more influential on soil aggregation than biosolids. There was no overall combined effect of flooding and biosolids. This chapter highlights that the type of biosolid, through being processed differently, does influence biosolid-soil interactions which is likely due to significant differences in the biosolids' chemical composition. In this experiment the main difference between the two biosolids used was the calcium content, as the ADLS had 2.5 times higher calcium content than the ADTH. The flood event in this chapter had a significant effect on soil aggregation and carbon content in the >2 mm fraction. This supports the literature on flooding and disaggregation but sheds new light on the interaction of flooding and biosolids, where there was no difference between the control and biosolid amended.

5.5.1 Suggested further work

Following on from the result of this chapter, recommendations for further work that would enhance the knowledge gained here should include the following: An assessment of the effects of more types of biosolids on soil aggregation and on a broader range of soil types. To further assess the contribution of flooding to soil aggregation, a similar experiment with a flood event with an extended recovery period, particularly setting up more replicates that can be sacrificially sampled which could provide a timeline for the effect of biosolids and flooding on soil aggregation. This would be particularly interesting, as the increases pre-flooding in aggregation with biosolids, especially ADLS, could provide post-flooding acceleration of the recovery of soil aggregation where biosolids have been applied.

5.6 Acknowledgements

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Chapter 6: General discussion and conclusions

6.1 Introduction

With regulations such as the EU water framework directive (91/271/EEC) requiring the treatment of wastewater from populations >2000 to be treated before reaching watercourses, the opportunity to capture unutilised carbon, nitrogen, phosphorous and other micronutrients for reuse is increasing. At wastewater treatment centres, the waste product of sludge treatment, biosolids, contains all such nutrients and is suitably processed for use on agricultural land. Biosolids production is increasing with rising global populations, and large population centres in cities provide the perfect regional centralisation of wastewater treatment (Collivignarelli et al., 2019). The potential for reuse of biosolids is also changing. They are increasingly seen as a commodity with growing marketability for reuse, spurred on by mounting volatility in traditional fertiliser sources, energy and other commodity markets (Goh et al., 2018). Additionally, consumers and governments are becoming increasingly concerned and involved with the mitigation of the effects of global warming and climate change. Globally the market for biosolids is strong, with biosolids production increasing and many sludge treatment centres have extra capacity built in to meet future treatment demand. North America is projected to continue occupying the major market share of biosolids in 2020-2025, with their biosolids recycled to land rate at approximately 50% of what they produce (Mordor Intelligence, 2021). In Europe, 50% of sewage sludge is processed for agricultural use (Nizzetto et al., 2016), and in the UK, this value is much higher at 87% (Assured Biosolids, 2021). The biosolids market in the UK accounts for approximately 5% of organic materials applied to agricultural land, with farm manures at 90% and industrial 'wastes' comprising the remaining 5% (Bhogal *et al.*, 2008). These biosolids are applied to 1.3% of agricultural land in the UK (Water UK et al., 2015).

The importance of recycling biosolids to agricultural land is evident in the provision of nutrients valued at £25 million per annum to agriculture in the UK, equivalent to £170 per hectare (Assured Biosolids, 2021). However, this value does not include the benefits to soil accompanying carbon inputs, which, when assimilated into soils, increases soil organic carbon providing numerous beneficial effects, including improved soil fertility and functioning (Bhogal *et al.*, 2009).

At present, the utility of biosolids in agriculture is limited by regulations and assurance schemes requiring it to be manually incorporated into the soil when spread on bare earth and stubbles, usually through ploughing (Assured Biosolids Ltd., 2020). This requirement for incorporation may be appropriate on degraded soils which are at increased risk to erosion and nutrient losses, but conflicts with management systems that promote increased soil biological activity and soil functioning through reducing tillage. With the biosolids market projected to increase, exploring the opportunity for biosolids reuse in agricultural systems with low-disturbance soil management, including the use of leys to in arable rotations, could expand and protect land bank opportunities for biosolids use for the future. The improvement to soil characteristics (such as the reduction in soil compaction and formation of water stable aggregates), is one of the most beneficial and prominent reasons to uptake a reduced tillage land management practice (Kassam et al., 2019). A key part of research into biosolids use in reduced tillage systems should include an evaluation of the effects on soil properties and should not become limited by focusing upon crop yields and nutrition. Ultimately, soil health will dictate the long-term viability of crop growth; short-term gains that may be seen from conventional tillage-fertiliser models will not be able to sustain increased demand without properly caring for the soil.

6.2 Discussion

This thesis aimed to assess the possibility of enhanced benefits from applying biosolids under reduced tillage and to evaluate the possibility of updating regulations to allow for the surface application of biosolids. To do this, a large experiment was conducted on intact soil monoliths using a range of land managements from conventional intensive arable, leys and permanent pasture with biosolids surface applied, including manipulation of earthworm populations. A range of biological, chemical, and physical soil measurements were taken throughout a growing season. To ascertain the incorporation of the biosolids into the monolith soil, a novel method utilising a fluorescent tracer was pioneered and used to study the abundance of biosolids by depth. Biosolids showed a consistent but non-significant trend reducing the level of soil macroaggregation in the monoliths. To gain further insight into this, a pot experiment was conducted to confirm the effect of biosolids on soil aggregation, as well as the contribution of flooding and soil saturation to disaggregation.

In this chapter, the main findings from Chapter 3, 4 & 5 will be reviewed in tandem to discern definitive answers to the research questions posed in Chapter 1 (repeated below) and give an overall assessment of the effects of biosolids on agricultural soils under reduced tillage and the effect of biosolids and flooding on soil aggregation. Finally, a comprehensive evaluation of findings in view of the suitability of updating regulations regarding the surface application of biosolids to agricultural soils will be made.

From Chapter 1, the research questions for this thesis were:

1. How does soil management effect biosolid-soil interactions when surface applied?
2. How do biotic and abiotic factors contribute to biosolid-soil interactions?
3. Does the type of biosolid, how it has been processed and made, influence the effect of biosolid-soil interactions?
4. How does flooding effect biosolid-soil interactions?

To aid in the discussion of these research questions, a schematic diagram is presented in Figure 6-1 visually summarising the key factors of land-use, earthworms, flooding, and rainfall that effect biosolid-soil interactions in the context of the experiments undertaken in this thesis.

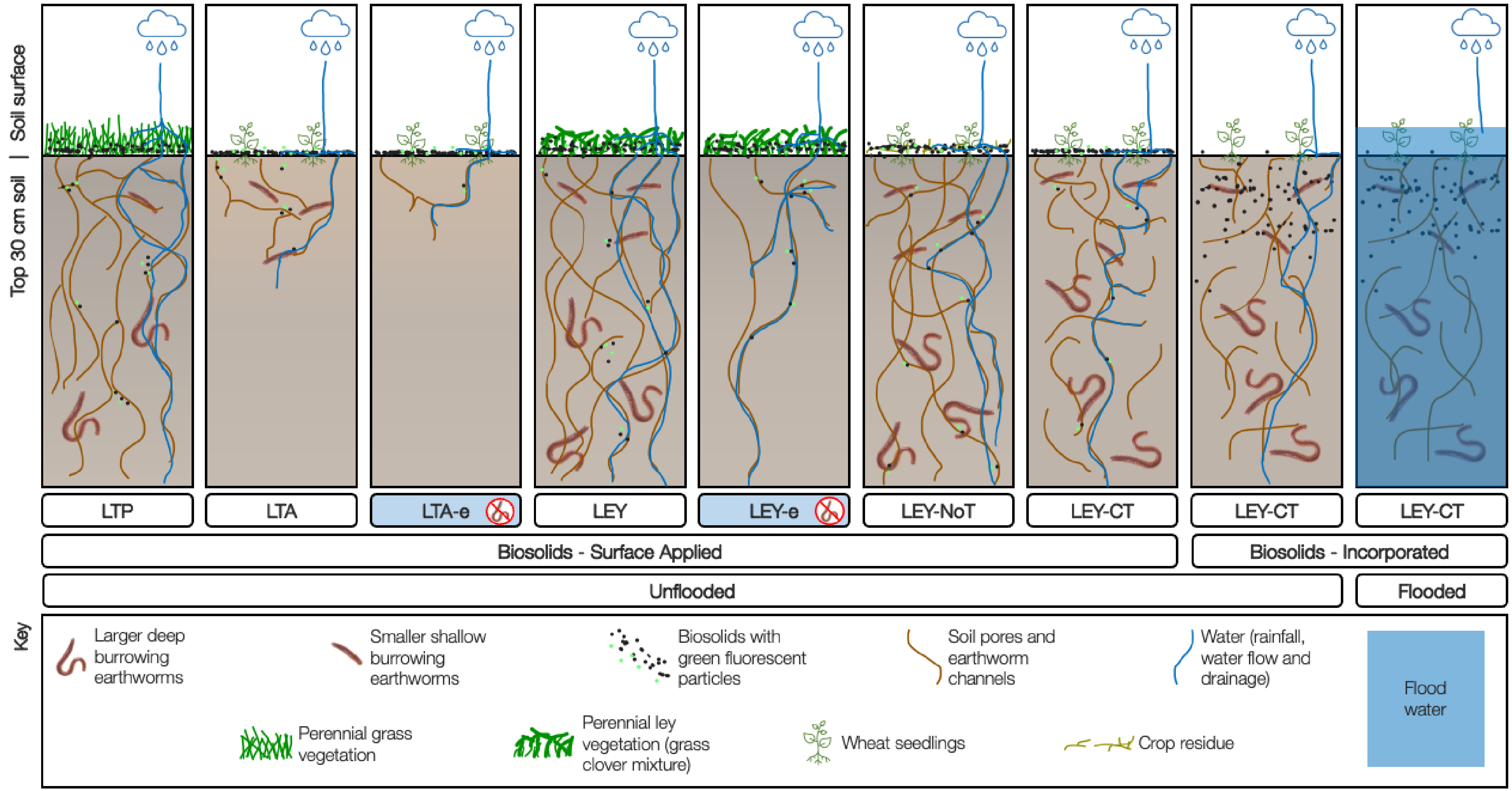

Figure 6-1: Schematic diagram of soil systems from this thesis with land-management, earthworms, rainfall and/or flooding interactions. Long term pasture (LTP), long term arable (LTA), Ley (LEY), ley to simulated no-tillage (LEY-NoT), and ley to simulated conventional ploughed tillage (LEY-CT). A subset of treatments were deep frozen to remove earthworms and earthworm cocoons, long term arable with earthworm removal treatment (LTA-e) and ley with earthworm removal treatment (LEY-e). From Chapter 3 & 4 where biosolids were surface applied with a biosolid and fluorescent particle mixture, and from Chapter 5, where biosolids of different types were incorporated into the soil and subjected to a flooded or unflooded period.

6.2.1 Soil management

The results of this thesis, in particular Chapter 3, provided a critical assessment of the effect of surface applied biosolids in combination with different soil managements, including a range of tillage intensities, soil quality and perennial evergreen cover in the form of leys or permanent grassland. Overall, there were very few instances when biosolids had a significant effect compared to the control within each soil management. It was instead the method of soil management where most of the significant effects were seen. The effects of surface biosolid application in short term no-tillage plots were mostly insignificant, a finding also seen by Yucel *et al.*, (2015). Although, they did see a 15% increase in moisture content and a 13% increase in active carbon content following biosolids application. It is possible however, to rapidly alter soil properties from changes in management practices. Melero *et al.*, (2011) found that ploughing soil that had been no-tillage for 8-years resulted in a significant decrease of total organic carbon (by 23%), and water-soluble carbon (by 27%), among other measures compared to the control after only 6 months. Significant changes in soil properties due to land use change were seen in Chapter 3, with the reduction of MWD from the LEY to the LEY-CT at almost 50%.

The LEY treatment was in the same paired fields and on identical soil type, as the LTA, and up until 3 years before sampling had experience identical tillage and cropping. However, the 3 years of ley growth, with no-tillage, caused these soils to function completely differently, showing major improvements in soil structure and health. In Figure 6-1, this is represented by the increase in earthworm numbers, particularly larger deep burrowing earthworms, and improved drainage. This is additional evidence for the beneficial effects of temporary leys for regenerative agriculture described and reviewed in several studies (Ball et al., 2005; Knox et al., 2011). Particularly Berdeni *et al.* (2021), who found that periods of ley (in the same field site as used in this experiment), as short as 18 months, can significantly benefit soil function on severely degraded soils. This included a significant decrease in bulk density, increase in infiltration rate through 1-6 mm sized pores, and increases in above ground biomass of the following crop, compared to the long-term arable control. Comparing the range of management practices investigated in this thesis, periods of ley followed by reduced tillage or conventional tillage still retained approximately two-thirds of the increased MWD of the LEY, maintaining them above that of the degraded LTA and producing approximately 2 times the grain yield. Puerta *et al.* (2018) drawn similar conclusions, finding soil structural improvements were most improved through reduced tillage, but that including leys in intensive tillage rotations provided an effective means to improve soil structure by increasing aggregate stability.

In this thesis, there was no evidence for additional improvements to soil properties when combining biosolids and reduced tillage management. This is likely due to the single application and shorter length of the monolith study, of only one cropping cycle and less than the maximum permissible application rate. Similar has been reported in the literature; after 2 years, Puerta *et al.* (2018) found the combination of reduced tillage and organic material amendment (cattle manure slurry) to have the most improvement on soil structure, when comparing the organic amended intensive tillage with the organic amended reduced tillage plots there was no significant difference but an increase of approximately 5% higher MWD in the reduced tillage treatments.

Although the basis for the hypothesis that there may be combined benefits from the application of biosolids and reduced tillage was sound, given the breadth of evidence for both the benefits of reduced tillage and organic material application (Dicks et al., 2019; Morris et al., 2010; B. Sharma et al., 2017). Nevertheless, evidence from this thesis suggests that in the short-term (< 1 year), there are no additive benefits of biosolids and reduced tillage for some measures, including the proportion of macroaggregates. There could even be a possible negative effect of biosolids, as most treatments observed a decline in MWD of up to 20% compared to the control. Referring specifically to a reduction in macroaggregates, this is supported by Jin *et al.* (2015), who found macroaggregation in the 1-2 mm fraction to decrease with an increasing application rate of biosolids after 8 years, with reductions from 5% up to 40%.

Taking this evidence into account, farmers and land managers should be mindful that the applications of biosolids as a source of organic material for a "quick fix" to help improve soil quality is not the case. Instead, thoughtful and appropriate soil management should be considered first.

6.2.2 Biotic and abiotic factors

6.2.2.1 Rainfall

Practically the effect of soil management on biosolid-soil interactions is driven by the associated changes in biotic and abiotic profile of each soil management. As illustrated in Figure 6-1, Soils with good coverage by perennial vegetation had a big effect on how rainfall interacted with the soil surface, compared to bare ploughed arable soil, softening the impact of rainfall, and providing protection from erosion. In a review of the role of cover crops towards sustainable soil health, Sharma *et al.* (2018) highlighted one of the main benefits of improving soil health through controlling erosion. Hence, when applying organic materials to the surface, cover crops should reduce their loss too. Although this may be the case, as seen in this thesis there was a quantity of biosolid material left on the soil surface under the layer of vegetation in both the LTP and LEY treatments at harvest. The vegetative cover provided a buffer for rainfall, but at the same time reduced the opportunity for rainfall-driven incorporation of the biosolids, where bare soil in the LTA, LEY-NoT and LEY-CT provided the best opportunity for this.

6.2.2.2 Earthworms

Considering soil functioning, adequate infiltration rates provided by good soil structure and micro- and macro-pores are important for reducing run-off under high rainfall rates. Hence, biological agents like earthworms, that both physically incorporate biosolids into the soil and improve soil surface macroporosity, are of particular importance. Earthworms help to increase infiltration through the creation of large macropores through which the majority of fast-infiltration into soil occurs (Hallam et al., 2020), and their increases in macroaggregation and macroporosity improve soil water storage capacity for the infiltrated water and provide channels through which water may drain into subsoil and groundwater. Soils with a higher number of earthworms, particularly deep burrowing species, increase soil drainage and has been illustrated in Figure 6-1.

Earthworm improvements to soil structure, as well as physically moving biosolids materials through ingestion and casting gives complementary benefits. The earthworm exclusion part of the monolith experiment in Chapter 3 provided additional evidence for the importance of these organisms in soil functioning and in crop growth. This was particularly evident for the LTA and LTA-e, which showed that even in degraded and intensively managed soils with low earthworm populations and low species richness, the earthworms that are present are playing a vital role in soil functioning. The role of leys in helping to restore earthworm populations is yet another reason to support their introduction into rotations. Significant increases in earthworm abundance were seen by Berdeni *et al.* (2021) after only 18 months using soil monoliths of the same sizes as in Chapter 3. At the same field sites used in this thesis and by Berdeni *et al.*, (2021), Hallam *et al.* (2020) used monoliths of the same size incubated in the ground of the fields and showed clear evidence of the importance of earthworms in enhancing soil structure and hydrological functioning within new leys. In a bioassay experiment, Hallam *et al.* (2020) found the growth of wheat was improved significantly with an increase of 20% with treatments containing earthworms compared to those without. The findings in this thesis support these results, observing approximately a 10% decrease in above ground biomass between LEY to LEY-e and LTA to LTA-e treatments.

In this thesis, the combination of increased soil structural and hydrological functioning of the herbicide-treated leys and their increased biological activity, meant that when biosolids were applied to the LEY-NoT treatment they disappeared from the soil surface in the shortest time frame. There was some evidence in Chapter 3 for biosolids having a positive effect and boosting earthworm biomass in the most degraded soil, LTA. However, as this effect was not seen in the other treatments, it may only be effective on soils with low numbers of earthworms to start with. This increase in earthworm biomass was also seen by Nicholson *et al.* (2018) in long term field studies under conventionally tilled biosolids applied plots. However, other evidence suggests that regional and site-specific differences may have more influence, as other studies showed no overall conclusive effects of biosolids on earthworms (Bhogal *et al.*, 2018; Kiss, 2019; Waterhouse *et al.*, 2014).

6.2.2.3 Flooding

In Chapter 5, the flooded and non-flooded treatments showed significant differences in soil aggregation, providing evidence that the flooded treatment had a major influence on how the biosolids interacted with the soil. In the non-flooded treatment, the ADTH and the ADLS had an increasing effect on soil macroaggregate proportions, even after only a short time. However, treatments experiencing a flood event showed no effect of either biosolid type on soil aggregation compared to the control. Interestingly, there is evidence to suggest a similar trend towards the disaggregation of soil macroaggregates in Chapter 3, which was discussed as possibly caused by saturation in the monoliths due to inadequate drainage over the winter, which was also seen in Chapter 5 in the flooded treatments. A slight trend suggesting that in the biosolids treated pots with a flood event there was a reduction in MWD, compared to the unflooded treatment, by a higher proportion compared to the unamended control. Whilst the findings of Chapter 5 on the effects of biosolids on soil aggregation are somewhat equivocal, it is important to recognise that the pot experiment was run for a much shorter-term than the monolith study, and due to the Covid-19 imposed lockdown, this experiment was curtailed early. Longer-term replicated studies on a broader range of soil types are needed to further resolve the extent to which biosolids impact positively or negatively on soil structure, and time-frames over which these effects manifest.

6.2.2.4 Seasonality

A further consideration for the effect of biotic and abiotic factors on soil-biosolid interactions and integration is seasonality. Changes in atmospheric temperatures and rainfall patterns cause the soil environment to change throughout the year. This gives rise to dynamic changes in key components such as earthworm populations, which are highly responsive to soil moisture. In this thesis, Chapter 3 simulated autumn application of biosolids, whereas Chapter 5 simulated spring application. Although both experiments focused on different aspects of biosolid-soil interactions, the small effects seen in Chapter 3 when compared to Chapter 5, could be somewhat driven by differences in soil temperature and rainfall patterns. Earthworm activity is known to vary throughout the year; Kiss (2019) found earthworm abundance and biomass to be higher in the autumn compared to other times throughout the year. Similarly, microbial activity also has strong seasonality and is effected by crop and land use (Kaiser & Heinemeyer, 1993). Hence, differences observed between the experiments in Chapter 3 and 5, other than the inherent differenced in design, may have been affected by these seasonal differences in earthworm and microbial activity. Additionally, seasonal changes in weather patterns are likely to have also influenced biosolid-soil interactions. Even though in Chapter 5, the experiment was manually watered, the soil was maintained at field capacity, compared to the almost saturated monoliths over the winter in Chapter 3 and substantial decline over the summer period. The average soil moisture between experiments was on average 10% higher in the monoliths over the year compared to the pots in Chapter 5. Biological and chemical components of the soil exhibit different activity levels and properties when soil moisture and temperature is changed and the soil misture and temperature at the time of biosolid application may significantly affect the biological and chemical properties of biosolid-soil interactions. Unfortunately, the extent to which these factors influence biosolid-soil interactions is hard to determine without a focused experiment assessing exactly that.

6.2.3 Biosolid processing method

There are numerous types of biosolid, and these differences come about through the treatment processes they undergo for land application. The AHDB nutrient management guide (AHDB, 2019) splits biosolids into digested cake, thermally-dried, lime-stabilised and composted. However, in practice, a biosolid cake may have also undergone lime stabilisation or other further treatment. Classifying biosolids into these broad categories, without full details of their prior-processing method, prevents differences in effects due to particular treatment combinations from being drawn out and reported in the literature. The results from Chapter 5 highlight the differences in the magnitude of effect that the two differently treated biosolid cakes, ADTH and ADLS, had on the soil structure and aggregate proportional distribution. The 7-day and 45-day treatments showed different proportions of the effects of each biosolid. However, the flood treatment effect had sufficient impact to overcome any effects the biosolids may have had previously and for the ADTH treatment reduced the soil MWD to below that seen in the ADTH 7-day treatment. The overall trends of the ADTH and ADLS biosolids were similar, in that in this short-term study, there was an increase in MWD compared to the control. However, the proportion to which each increased the MWD was different, with ADLS triggering the greatest increase.

6.2.4 Biosolids: surface applied, ploughed in or an alternative approach?

The initial research questions posed how surface applied biosolids affect soils. However, to perform a short evaluation in Chapter 5, they were incorporated to increase soil-biosolid interactions. The effects of surface application vs incorporation by ploughing (in order to minimise risk to the environment) has both risks and benefits in each case. Whilst a universal requirement to incorporate biosolids by ploughing may reduce risks of unintended dispersal of biosolids into non-target environments and natural habitats by surface run-off or wind erosion. With the increasing adoption of less intensive tillage management, this may limit the land-areas that can receive these inputs (in accordance with the current regulations) and concentrate the inputs on some of the most intensively cultivated and structurally degraded soils, where run-off and soil erosion risks may be greatest. However, this thesis has shown that when applied to soils under no-tillage agriculture with sufficient infiltration rate, stubble, earthworm populations and low slope angle, the biosolids can become incorporated/disappear from the soil surface in a shorter period than more degraded soils. The less intensive management systems, and rotations that include leys and direct drilling, deliver benefits to soil structure and function that are far superior to the effects of adding biosolids, as shown in this thesis, so the adverse effects of ploughing need to be better respected.

It is worth noting that other issues surround the incorporation or ploughing of biosolids as well as other organic materials, including the rate of nutrient mineralisation for crop uptake, which would consequently be affected by the timing of applications. Autumn applications of biosolids provide the longest time frame for nutrient mineralisation producing a soil nutrient stock ready for crops when they need it the most, during the spring and summer. However biosolids would spend longer on potentially bare soil surfaces over winter with greater chances of losses during inclamental weather, with greater chances of nutrient losses to water. In comparison spring appllications of biooslids are likely to be incorporated in a shorter time scale due to the greter biological activity with the warmer weather in the spring, but the nutrients may not be availiable in the quantities required to make a difference in crop yield. Losses to water will still be likely with ‘spring showers’ as well as warmer temperatures increasing amonium volatilisation and loss.

Other considerations include biosolids being stored in stockpiles all year round on farms regardless of spreading regulations, the workability of soils in wetter areas is reduced in the spring after prolongued wet winters. Additionally spring crops are often sewn if winter crops fail by drilling/sewing straight into the ground with little ground preperation to incorporate biosolids. In surface applied systems, a balance between timing of application and level of incorporation might be needed to reduce the risk of nutrient losses while maximising availiable nutrients for crop growth at critical times in the crop's development. An argument could be made that even if biosolids are incorporated into degraded soils to maximise nutrient mineralisation, a high rainfall event may lead to erosion of the topsoil, which contained the biosolids as well as soil.

Evaluating the regulations and assurance schemes that require biosolids to be incorporated, the evidence from this thesis suggests that there are circumstances where this might not need to be the case. If regulations can be changed, surface applications of biosolids on soils with low slope angle and high biological activity could be permitted but would have to be monitored. If regulations are not changed and, therefore, biosolids cannot be used in no-tillage arable systems, perhaps biosolids would be better suited to root crops that already disturb the soil (potatoes, sugar beet etc.). These typically cannot be grown very often as the soil needs to recover from the extensive biological and physical disturbance, and the build-up of pathogens avoided. Ploughing in biosolids as part of the soil preperation for root crops to be sown provides an alternative option for their use, rather than just on cereals. However guidelines at present (as mentioned in Chapter 1) have a 10 month harvesting interval for fruit, salads and vegetables (ADAS, 2001) which creates a time barrier for most of these crops for application of biosolids in the spring or autum as fruits and vegetables are typically harvested in the summer and autumn. An alternative approach would be ploughing in biosolids as part of the rotations used for soil restoration after root crops in a rotation could be more beneficial and less environmentally damaging to earthworms, structure and soil organic matter accumulation than ploughing alone or indeed rather than direct drilling leys.

To maximise the benefits from using biosolids in agriculture this thesis has provided evidence that surface application of biosolids onto soils under reduced tillage that are sufficiently biologically active, of good quality and with adequate crop residue/stubble can lead to the incorporation of biosolids in a timely manner. The crop residue provides a rough surface to help slow surface water flow and reduce runoff, while increasing the time for nutrients to mineralise and become plant availiable for spring and summer growth. This approach also uses less field passes reducing the use of fuel while reusing waste materials and closing nutrient cycle. All in all creating a more sustainiable farming approach to the reuse of biosolids. To achieve this agronomy and policy need to change to consider the quality and health of soils on a field by field basis to determine if the soil meets minimum requirements in order to reduce the chance of possible adverse effects, perticularly runoff and pollution. The change in farming subsidies with the transition to the Environmental Land Management Scheme (ELMS) is a step in the right direction to considering soil health and provision of ecosystem services in subsidising farmers, which may be the catalyst which accellerats farming towards a greater uptake of more sustainable practices, however policy will need to keep up with the advancements in the field.

6.3 Final conclusions

This thesis has evaluated the evidence for current best practice, assurance schemes and regulations in relation to the restrictions of biosolids use with reduced tillage operations in agriculture in England. Concerning trends pointing to possible disaggregating effects of biosolids seen in Chapter 3 were followed up in Chapter 5, and a potential cause was determined as flooding or extended saturation of soils in the presence of biosolids exacerbating soil disaggregation. A novel method was tested to trace the physical movement of biosolids through a soil profile. Although the results were not as robust as initially hoped, the method has set the groundwork for follow on experiments that could provide useful insight into the movement of biosolids within soil systems. The main conclusions of this thesis are as follows:

- One time surface application of biosolids was not sufficient to significantly change soil properties.
- Soil management plays a more superior role in the functioning of soil systems than the addition of biosolids. As such, soil management should be the primary consideration for improving soil health.
- The effect of biosolid amendments was minimal in comparison to the inherent properties of the soil due to soil management, but there was no evidence to suggest a serious negative effect of their combination.
- Incorporating leys into arable rotations has substantial long-term benefits for soil health and earthworm populations.
- Earthworms play a vital role in biosolid-soil interactions by providing significant changes to the soil environment, whereby abiotic factors interact with the soil differently.
- Biosolids that have been processed in different ways do affect soil structure differently. This should be considered and investigated further.
- The role of flooding in the disaggregation of soil structures has been supported here, and the influence of flooding on biosolid-soil interactions showed a concerning trend towards elevated disaggregation, which should be investigated further.

To further the work of this thesis, follow on investigations into the effects of biosolid-soil interactions, particularly when surface applied, should focus on confirming the findings of this thesis in the field. Field investigations should have a particular focus on slope angle, rainfall events and erosion and flood risk, particularly for soil types prone to flooding and erosion. These were all things that were not in the scope of this thesis but would provide additional insight into its themes. Building on the method development in Chapter 4, utilisation of the tracer on the soil surface, in combination with field experiments, would provide an insight into the timely manner of the incorporation/disappearance of the biosolids from the soil surface. But would also provide additional insight into the risk of erosion in the field. Finally, as highlighted by the flooded and unflooded treatments in Chapter 5, additional investigations should be made into the effects of a wider variety of biosolids on soil properties and whether any negative impacts not found here may exist.

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Appendix A: Chapter 3 additional results

A.1 Soil solution and leachate chemistry

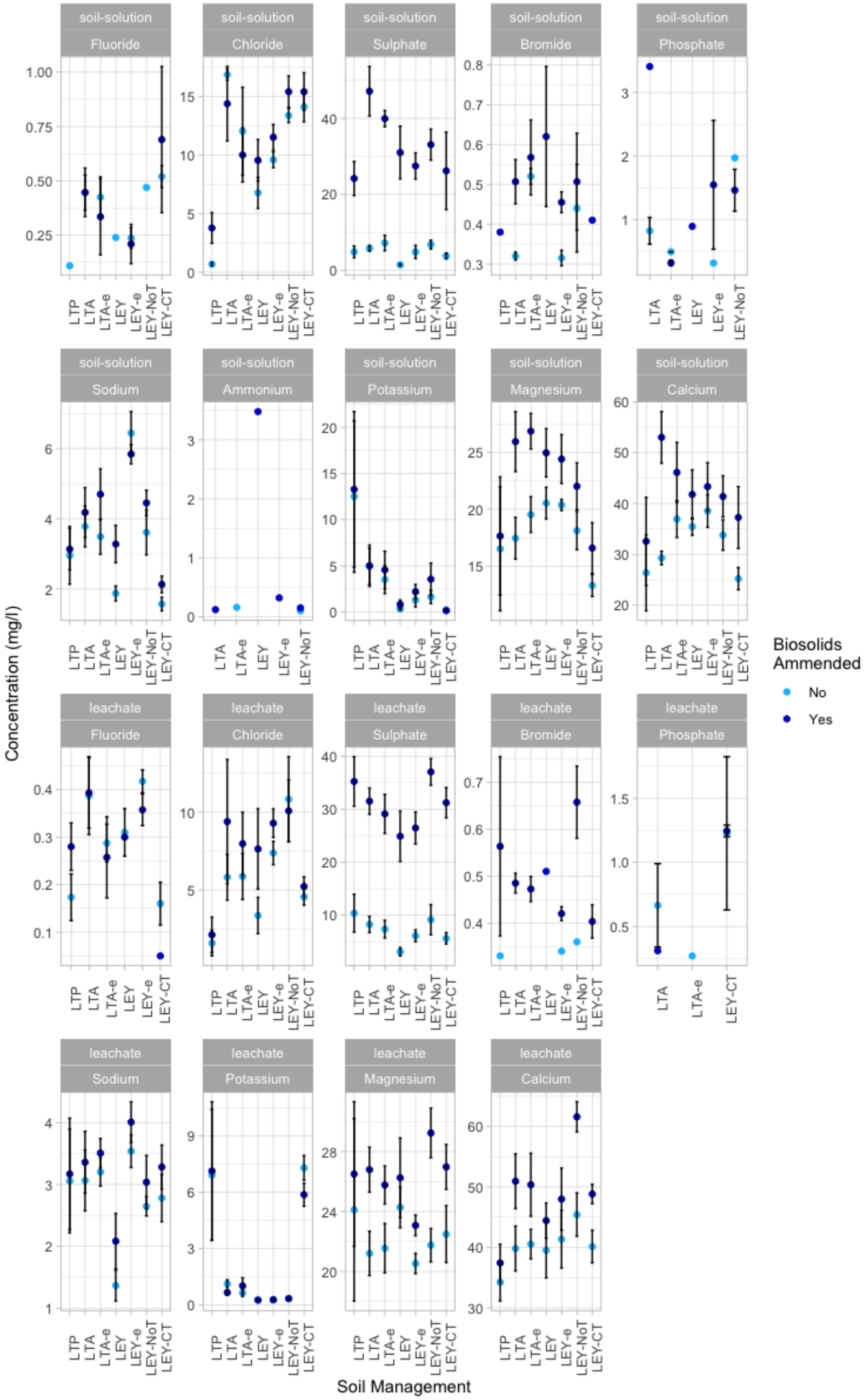

Figure A-2: Additional analytes for soil solution and leachate chemistry.

A.2 Earthworm data post identification

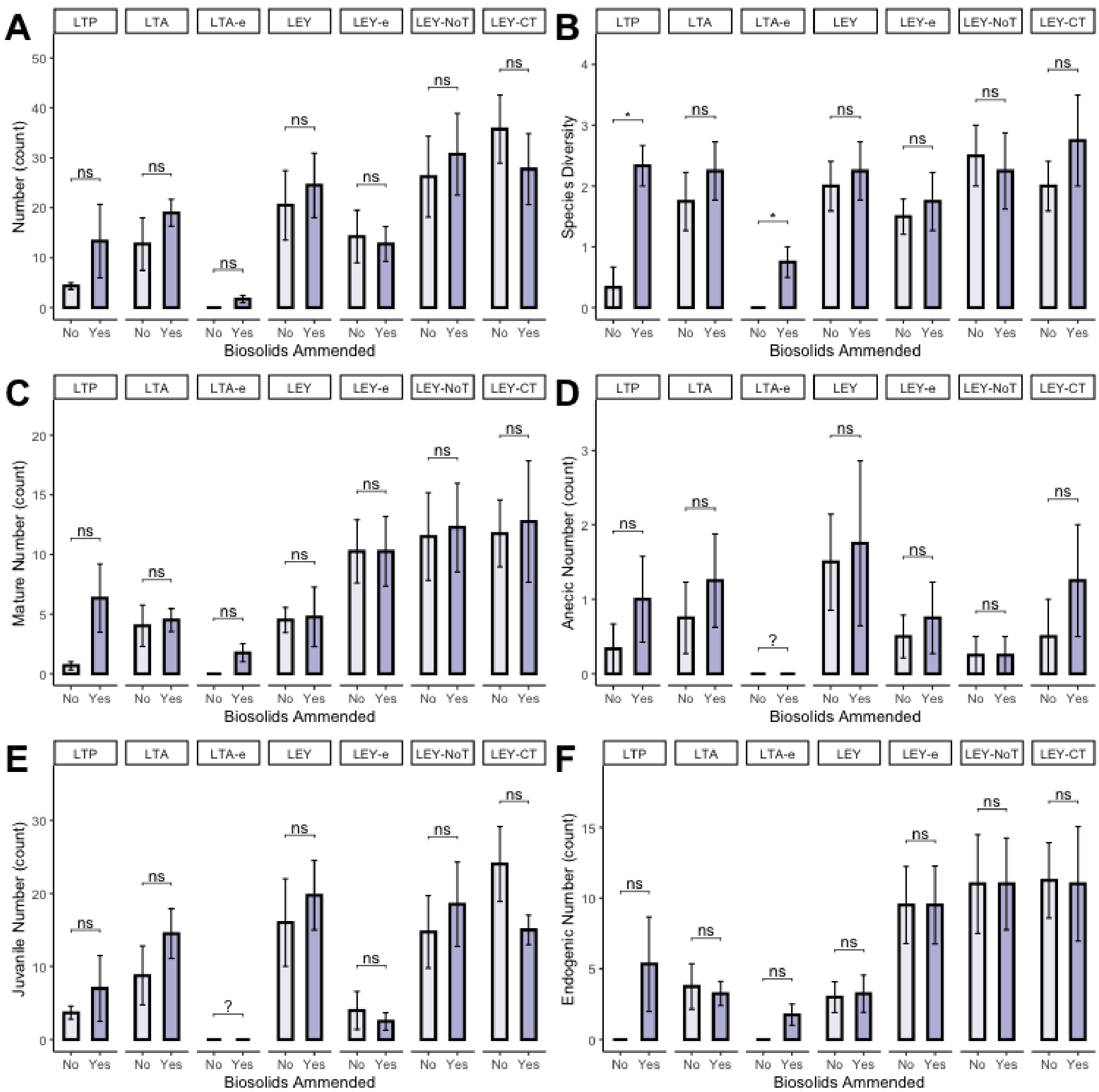

Figure A-3: Monolith earthworm results post identification. (A) total number, (B) species diversity, (C) mature worm count, (D) anecic ecotype count, (E) juvenile count, (F) endogenic ecotype count.