MSc Wildlife & Conservation Management

The effect of bio-based fertilisers on soil carbon, soil macrofauna and soil microbial composition: a case-scenario in Irish agricultural soils

By

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Abstract

As the earth's population increases, the demand for a sustainable food source has become a priority in the agricultural research of recent years. The global food demand has led to the development of efficient, yet invasive and intensive agricultural practices over the last century. These detrimental practices have led to a decline in soil health and ecosystem functioning. The use of chemical fertilisers on Irish agricultural soils had been increasing in recent years. This study aimed to investigate the effects of chemical fertilizer use of soil macrofauna and the carbon cycle, while also surveying the potential benefits of organic-based fertilisers to these variables of soil health. Earthworm sampling and analyses were conducted alongside analysis of soil organic carbon (SOC) and soil organic matter (SOM) content of soil plots at Johnstown Castle Research Centre, Wexford, Ireland. Earthworm biomass and density were overall unaffected by bio-based fertilizer application, however the cattle slurry application and struvite application individually led to an increase in average earthworm biomass ($p = 2.2 \times 10^{-6}$ and $p = 0.0127$ respectively). The use of bio-based fertilisers also resulted in plots with a greater diversity of species ($p = 0.00415$). While the SOC and SOM stocks were largely unaffected by bio-based fertilizer application, there was a positive correlation between increased organic matter and increased SOC and SOM stocks ($p = 0.0043$ and $p = 0.0114$ respectively). This could point to a potential increase in SOM and SOC stocks with continued application of organic fertilisers. Conclusively, the use of bio-based fertiliser, particularly cattle slurry and struvite, show the potential of improving the overall health of the soil ecosystem.

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Abbreviations

FAO	Food and Agriculture Organisation of the United Nations
SOC	Soil organic carbon
SOM	Soil organic matter
GHG	Greenhouse gases
TOC	Total organic carbon
DOM	Dissolved organic matter
C	Carbon
N	Nitrogen
P	Phosphorous
K	Potassium
S	Sulphur
ANOVA	Analysis of variance

Section 1: Introduction

1.1 Global population growth and demands for food

A healthy soil ecosystem is essential to all forms of life on earth and vital for ensuring the continuing growth of both plant and animal life. Sustainable management of soil is becoming increasingly important with the advancement of climate change in recent decades. While soil habitats can mitigate many of the effects of climate change, this ecosystem is easily disrupted, and in-depth research must be carried out to ensure a healthy future for soil. With the world's population constantly expanding, the demand for sustainable food sources is influencing current and future research. The Food and Agriculture Organisation of the United Nations (FAO) has observed food consumption in the last two decades increasing at a faster rate than population, as a result of increased urbanisation and a higher concentration of people in cities (European Commission, 2019). The rise of consumption per capita has had a large impact on the food production system, resulting in the development of time and labour-saving methods of farming which can be detrimental to the environment such as deforestation, irrigation and the chemical additions to soil (Cervantes-Godoy *et al.,* 2014). With the world's population estimated to increase from 7.9 billion in 2021 to 9.73 billion in 2050, global food production would need to increase by 50% from 2012 levels (Cervantes-Godoy *et al.,* 2014). To reach this target while preventing harm to the environment, sustainable methods of farming and food production much be achieved.

1.2 The environmental costs of conventional farming

For agriculture to keep up with the food demands of the growing population, soil enrichment with fertilisation approaches has become an essential tool in farming. The use of conventional inorganic fertilisation on agricultural soil has become a popular practice among farmers across the world in the last century as it increases yield of crops even on nutrient-poor soils. However, the addition of certain chemicals to soil can have a detrimental effect on both soil microorganisms and edaphic fauna, including the ecosystem services they provide (Addison *et al.* , 2021). The use of mineral fertiliser can also affect soil macroaggregates which reduces soil stability (Zhao *et al.* , 2021). In addition to chemical fertiliser use, many other invasive agricultural practices present a threat to soil ecosystems. Tillage and ploughing disturbs the upper layers of the soil, drastically decreasing earthworm biomass and population structure (Briones and Schmidt, 2017). Deforestation has been a popular subject in recent years, with forests being cleared to facilitate crop growth. This rapid change in land cover can affect soil pH and alter soil structure, which in turn can have negative effects on water filtration and soil resilience (Garrard and Bekessy, 2014).

1.3 Modern agriculture and carbon cycling

Soil carbon stocks and carbon (C) sequestration have been popular topics of research in recent decades, with many research teams investigating the impact of different anthropogenic factors, as well as the impacts of climate change, on the carbon stocks in soil (Valarini *et al.* , 2003; Plaza *et al.* , 2013; Qiu *et al.* , 2015; Dębska *et al.* , 2016; Xavier *et al.* , 2019). Many soil chemistry studies undertaken in recent years demonstrate the ability of bio-based fertilisers to induce microbial activity which in turn increases soil organic carbon (SOC) content (Valarini *et al.* , 2003; Plaza *et al.* , 2013; Saarnio, Heimonen and Kettunen, 2013; Dębska *et al.* , 2016). SOC facilitates the sequestration of carbon from the atmosphere, thus playing a vital role in modern agriculture, a sector which is often reprimanded for its high carbon output (Dębska *et al.* , 2016). Greenhouse gas (GHG) outputs from agriculture in Ireland were measured at 32.6% of total Irish GHG outputs in 2020 (Environmental Protection Agency, 2020a). The use of synthetic or chemical fertiliser increased by 15.7% in the Irish agricultural system since 2013, which in turn increased yearly CO2 emissions by an average of 10% (Environmental Sciences Committee, 2015; Environmental Protection Agency, 2020a). While some of this GHG output is offset by carbon sequestration in Irish agricultural soils, much improvement is needed to increase the carbon sequestration potential of soils, increasing this offsetting ability. There is a possible reduction of 12.4% in the agriculture sector's GHG emissions, if potential improvements on current farming methods are implemented (Environmental Protection Agency, 2020b). Increasing the organic matter of soil through organic fertilisation or no-till practices could have a widespread positive impact, including improving soil quality (Valarini *et al.*, 2003). This increase in soil quality will provide benefits to the surrounding ecosystems, from soil microorganisms to soil macrofauna, and more widely affecting humans and animals through crop production.

The direct relationship between bio-based fertilisers and soil carbon sequestration is an area that has not been widely studied, however, related studies prove optimistic for potential improvement in carbon stocks with a change to modern fertilisation. Dębska *et al.* (2016) found positive evidence that the total organic carbon (TOC) content of soil can be increased with the application of an organic fertiliser. They found that the organic fertiliser induced an increase in microbial activity which resulted in a 6.3% increase in TOC of several samples when compared to control soils over a period of three years. This microbial activity increased the rate of humification of the new organic matter introduced into the soil. This study also attributed any soil depletion and carbon loss observed in several samples to a high level of dissolved organic matter (DOM). This is due to the formation of mobile components that caused soil carbon loss. Qiu *et al.* (2015) also noted the possible impact of high levels of DOM. In this case, samples treated with DOM resulted in increased CO2 emissions and a decreased rate of C sequestration. DOM leads a priming effect on the SOM which then accelerates the mineralization of SOC, leading to the rate of C output exceeding the rate of C input.

These studies suggest that alterations in the processes involved in the soil C cycle can have a dramatic impact on the amount of C stored and the rate of C sequestration. An increase in the humification rate of new organic matter can result in a net increase in the amount of C stored by soil (Dębska *et al.* , 2016). Xavier *et al.* (2019) also explored this idea using sugarcane processing by-products as a biological fertiliser, resulting in an overall higher level of C and N stored in the soil which was attributed to an increase in the rate of humification of organic matter. The stability of organic matter plays an important role in carbon storage, with a higher stability allowing for an increased amount of carbon being stored. Plaza *et al.* (2013) observed an increase of 16% in soil carbon storage as a result of a no-till approach to land management. This increased stability was attributed to the higher rate of organic matter available for degradation. This resulted in the formation of organo-mineral complexes, which provides additional stability in soil.

While both organic and chemical fertilisers are the topic of many studies relating to the soil carbon cycle, a combination of these fertiliser types also shows a promising future. Combined organic-chemical fertilisers can have a wide range of effects on soil functions. Ibrahim *et al.* (2021) observed a mixture of biochar and chemical fertiliser increased plant yield and uptake of nutrients in addition to reducing soil carbon mineralisation, which resulted in reduced production of CO_2. As this area of research is novel, there has been little study on the relationship between combined fertiliser and soil carbon.

1.4 The value of soil fauna for ecosystem services

Earthworms have long been used by farmers as an ecological indicator of soil health, with the idea being initially proposed in an academic setting by Ghilarov in 1949 (Römbke, Jänsch and Didden, 2005). Earthworm population abundance and the diversity of species present depends on soil management practices and background physiochemical traits, where the presence of a higher richness and generally high numbers of animals indicates a healthy soil system (Bartz, Pasini and Brown, 2013). Soil benefits from a range of ecosystem services supported by earthworms, which supports the historic association of a healthy soil to the presence of these animals. A high density of earthworms will therefore indicate high functional activity (Curry *et al.* , 2008; Hoeffner, Santonja, *et al.* , 2021).

Burns *et al.* (2006) described a good soil health indicator as having several distinct properties. These include the availability of methods applied in measurement, ease of measurement interpretation, and its reliability. Earthworms fulfil the above properties in terms of availability and ease of measurements, with their reliability as a biological indicator the focus of many studies over a range of soil types and climates (Singh, 1997; Burns *et al.* , 2006; Guéi and Tondoh, 2012; Al-Maliki, Al-Taey and Al-Mammori, 2021). Singh (1997) noticed that earthworm biomass declined when soil moisture, acidity and food source palatability declined in their study on worm-soil interactions in an arid landscape in India. Al-Maliki, Al-Taey and Al-Mammori (2021) found that temperature affects soil moisture content and soil pH which in turn affects earthworm biomass. They concluded that earthworms can be used effectively as a bio-indicator for soil quality as previously suggested (Römbke, Jänsch and Didden, 2005; Burns *et al.* , 2006; Bartz, Pasini and Brown, 2013). Guéi and Tondoh (2012) went into further detail in their study in forested areas of the Ivory Coast aiming to distinguish ecosystem preferences for different earthworm species. They observed how different species can act as specialist indicators, such as *Millsonia sp*., which they associated to forests and populated areas rich in soil organic matter.

Little study has been carried out on earthworm species as soil quality indicators on Irish soils. Of the few studies on the relationship between earthworms and Irish soils in recent years, two observed details of the preferences of different species for various soil conditions and land use type (Muldowney *et al.* , 2003; Curry *et al.* , 2008). Muldowney *et al.* (2003) noted that deeper dwelling anecic species increased in biomass in more fertile soils, possibly due to an increase in food supply, while earthworm density was much less affected by land use. Curry *et al.* (2008) emphasised the importance of high earthworm populations for soil rejuvenation on intensive farms, observing that the soil compacting damage incurred by a high stocking rate can be countered by high earthworm biomass. Concurrently, while a high stocking rate can induce soil damage, the increase in organic matter through higher levels of dung production is greatly beneficial to earthworm populations, leading to an increase in the recovery of soil condition (Guo *et al.* , 2016).

In addition to soil rejuvenation, a high earthworm population will provide many other ecosystem services to a soil habitat. Several species of earthworm such as *Lumbricus terrestris* are described as ecosystem engineers due to the wide range of processes through which they can alter the composition of their biome (Römbke, Jänsch and Didden, 2005). These ecosystem services include soil aeration, organic matter breakdown, nutrient mineralisation, carbon sequestration, pest control and restoration of degraded soils (Pfiffner, 2014). Soil aeration occurs through the movement of worms through the soil, particularly by endogeic and anecic species, which dwell in the body of the soil as opposed to the litter dwelling epigeic species (Römbke, Jänsch and Didden, 2005). The process of soil aeration also benefits water infiltration, with the deep burrowing anecic species breaking through compacted soil and allowing surface water run-off through their burrows (Pfiffner, 2014). Epigeic species such as *Lumbricus rubellus* and *Eisenia fetida* are primarily responsible for carbon cycling (though all species contribute to this process to some extent) through their burrowing, feeding and casting activities (Zheng *et al.* , 2018). Earthworms fulfil their nutrient requirements through ingestion of organic residues of carbon (C) and nitrogen (N), converting the residues to a lower C/N ratio through this nutrient acquisition (Pfiffner, 2014). Organic matter breakdown and the associated nutrient mineralisation occur through earthworm feeding. This process results in the production of worm casts, which contain concentrated levels of nutrients that are deposited on the soil surface and within the soil, readily available to provide enriched nutrients to plants (Pfiffner, 2014). When the land is suitably managed, these casts can act as fertiliser for cover crops, increasing plant yield through the availability of nitrogen and phosphorous (P) in organic form as opposed to synthetic fertiliser (Butt and Nuutinen, 2021).

1.4.2 The effects of conventional agriculture practices on earthworms

The effect of intensive agriculture, particularly the use of synthetic fertilisers, on earthworms has been an interesting topic of studies in recent years (Timmerman *et al.,* 2006; Curry *et al.,* 2008; Iordache and Borza, 2010; Schreck *et al.,* 2012; Cai *et al.,* 2020, 2021; Hoeffner, Santonja, *et al.* , 2021). While earthworms require nutrients in order to thrive, it is debated whether or not they benefit from nutrients from a synthetic source. Muldowney *et al.* (2003) mention that while mineral fertilisers can benefit earthworm abundance by improving litter quality, the use of nitrogen-rich fertilisers can diminish earthworm populations. Their study found that earthworm biomass was generally increased by the use of synthetic fertiliser, while earthworm density showed little response. These results were prominent in anecic species, with the additional fertilisation providing more food source, resulting in larger anecic earthworms. Hoeffner *et al.* (2021a) reinforced these results with the observation that mineral fertiliser again increased earthworm biomass, particularly in anecic species. Their study differed, however, with less intensive use of nitrogen fertiliser, and as a result did not observe population decrease due to nitrogen saturation. Hoeffner *et al.* (2021b) also produced similar results to this, with mineral fertilisers positively affecting the biomass and density of epigeic earthworms when compared to organic fertilisation. They also attributed this result to improved food availability for these worms. Conversely, Schreck *et al.* (2012) noticed that the use of chemical weeding methods negatively impacted earthworm populations, decreasing both biomass and density. There is also evidence to suggest that synthetic fertilisation may inhibit the earthworms ability to perform ecosystem services. Cai *et al.* (2020) observed increased abundance and diversity of the soil’s bacterial community with earthworm application, however when earthworm application was paired with synthetic nitrogen fertiliser the microbial abundance and diversity showed no significant change. This suggests that while earthworm biomass and density may not appear to be affected by the application of synthetic fertiliser, there are other parameters that must be studied in greater detail, such as the earthworm's ability to fulfil its role in the ecosystem under the effects of synthetic fertiliser.

Although there are few studies dedicated to investigating the effects of biological fertilisers on earthworm community composition, there are mixed conclusions from existing studies. Many of the studies observing a positive relationship between earthworm abundance and bio-based fertilisers note that the positive responses become clear in the long term as opposed to short term application of fertiliser. The short-term effects of any fertiliser, not only bio-based fertiliser, can have a different outcome to its long-term effects; these effects must be considered before selection of fertiliser. This point was markedly demonstrated by Moinard *et al.* (2021) in their investigation of anaerobic digestate as an organic fertiliser. Initial application of this fertiliser proved toxic to the earthworms for a short period of a few hours. However, upon observation over two years, this fertiliser displayed a positive effect on the earthworm community structure, with abundance increasing by 150% when compared to soil treated with chemical fertilisers. The initial toxicity of the fertiliser was attributed to the worms surfacing and coming into contact with the liquid organic residue, which contained high levels of ammonia, proving lethal to the worms. The long-term benefits were possible due to the increased food availability from the input of additional organic matter in the form of fertiliser. These results contrast the results in a study by Timmerman *et al.* (2006), which investigated the changes in earthworm abundance over 23 years in The Netherlands. They observed no positive change in earthworm abundance when comparing plots treated with cattle slurry and zero fertiliser. They attributed these unexpected results to soil hydrology, as there was no other observable reason for the decline in abundance. However, comparing the results of these two studies indicates that, while comparisons between zero fertiliser and organic fertiliser alone may not show a positive change, there is a possible detrimental effect of chemical fertiliser on soil macrofauna.

The relationship between earthworms and the physiochemical properties of the soil has been extensively studied, with many studies concluding that the chemical input to the soil can affect earthworm abundance, biomass and function. One example of this relationship is the role the earthworm plays in the carbon-nitrogen (C/N) ratio of the soil. Earthworms play an important role in the C/N cycle through decomposition of plant matter and releasing plant-available N. This in turn increases microbial activity which aids mineralisation of C. Zhou *et al.*

(2021) observed that low levels of C in the soil coupled with high levels of N was detrimental to earthworm population and growth, reducing the benefits provided to the soil by the worms. There is also evidence to suggest that the earthworms enhancement of the C/N cycle increases plant yield (Butt and Nuutinen, 2021), when the fertiliser applied is organic, due to improved functionality of the earthworms ecosystem services. Similar to C and N, phosphorous has been noted as a chemical element of interest in relation to earthworms. The processes by which earthworms ingest and mineralise phosphorous have been widely studied (Kuczak *et al.,* 2006; Ros *et al.,* 2017), however the effects of added phosphorous through fertilisation on earthworm populations is a topic that has not been extensively studied.

1.4.3 The relationship between earthworms and the soil microbiome

Earthworms and soil microbes co-exist and co-evolve to provide mutualistic benefits to soil. This highly organised interaction of invertebrates and microorganisms make soil more resilient even in harsh environmental conditions (Lavelle *et al.* , 2006). However, soil management practices which damage these populations can affect their beneficial services to soil health. A possible reason for the alteration in the earthworm's ecosystem engineering abilities could be the changes that occur to the earthworms gut due to ingestion of fertilised matter. As the gut plays an integral role in the worm's many biome services, any changes in the gut microbiota could affect the outcome of these services. Bi *et al.* (2021) investigated the effect of inorganic fertiliser compared to organic-inorganic fertiliser on the gut microbiota of different species of earthworm. They found that the use of organic-inorganic fertiliser altered the gut microbiome of the earthworms to the extent that services such as nutrient cycling and facilitating soil carbon storage were enhanced when compared with the inorganic fertiliser.

1.5 The influence of soil organic matter and soil carbon on earthworm populations

There have been many studies into the effects of earthworms on soil organic matter distribution and soil carbon in soils (Amossé *et al.* , 2015; Frazão *et al.* , 2019; Hong *et al.* , 2011; van Vliet *et al.* , 2007). Earthworms promote the sequestration of soil carbon through the stabilisation of SOM. Earthworms ingest SOM in the form of plant litter, breaking it down and reorganising soil aggregates as this SOM passes through their guts (Hong *et al.* , 2011). These soil aggregates then influence the dynamics of SOM. With soil carbon being mineralised through this process, the SOM with a higher C/N ratio will result in a higher rate of C sequestration (Hong *et al.* , 2011). While it is generally accepted that a high SOM level in soil will result in high earthworm activity and abundance, the literature presents some conflicting insights. Van Vliet *et al.* (2007) produced a variation of results due to changing weather conditions, however they concluded that plots treated with slurry manure had a higher organic matter content and resulted in a higher density of earthworms. They attributed this result with the higher rates of organic matter providing a greater food source and larger, healthier habitats for earthworms. Conversely, Frazão *et al.* (2019) observed that the addition of organic matter through crop residues resulted in reduced macroaggregate formation and a lower earthworm biomass, despite an observed increase in soil porosity through earthworm activity. These results were explained by a possible trade-off between the earthworms contribution to soil porosity and their fitness, with the increase in activity negatively affecting their biomass. Amossé *et al.* (2015) produced interesting results, concluding that soils with a high organic matter can negatively affect earthworm activity but positively affect earthworm growth and biomass. These results were attributed to the organic matter providing a greater food source, resulting in higher biomass and reducing the need for a higher rate of foraging for food, which in turn decreased the overall earthworm activity. These studies provide an interesting insight into the exchange between earthworm populations and SOM, a relationship which further influences the SOC dynamics of soil.

1.6 Aims of this study

This study aims to investigate the effects of both bio-based (organic fertiliser combined with mineral fertiliser) and inorganic fertiliser on three variables of the soil ecosystem: earthworm populations, organic matter and carbon storage. Each of these components of the ecosystem play important roles in food production and mitigation of the effects of climate change. As such, understanding of possible effects of various forms of fertilisation on these variables is vital to sustaining the health of the soil ecosystem.

This study was conducted under the following objectives:

1. To examine the effects of bio-based fertiliser application on soil organic carbon fractions and soil organic matter fractions in Irish agricultural soils, hypothesizing that bio-based fertilisers will result in increased rates of SOC and SOM fractions.

2. To compare the effects of bio-based fertilisers and chemical fertilisers on earthworm biomass, density and diversity, hypothesising that organic-based fertiliser will benefit these variables.

3. To investigate the relationship between earthworm populations and soil organic matter and soil organic carbon, under the hypothesis that there will be a positive correlation between these factors.

Section 2: Methods

2.1 Site Description

The study site consists of 115 2m x 6m perennial ryegrass plots with sandy loam soil covering 1748 m^2 at Teagasc, Johnstown Castle Research Centre, County Wexford, Ireland (52°17' N 6°29' W, 48m elevation). These plots have been used for a bio-based fertiliser and phosphorus fertiliser experiment since April 2019. The bulk density of the soil was calculated at 1.3 g/cm^3. Soil pH was on average 5.64 at the beginning of the fertiliser treatment in 2019 and 6.36 in 2021 (Table 1).

Table 1: Average soil pH for each fertiliser application plot in 2019, 2020 and 2021.

Fertiliser Treatment	pH 2019	pH 2020	pH 2021
P0	5.7	5.8	6.4
Min N, P, K, S	5.7	5.7	6.3
Cattle Slurry	5.7	6	6.6
Activated Sludge	5.5	5.8	6.2
VEV Struvite	5.6	5.9	6.3

2.2 Experimental Design

Twenty-five of the grassland plots were sampled in this study. Bio-based fertilisers were applied to each plot after each harvest (March, May and August) since 2019. Each fertiliser application had five replicates, located randomly across the site (Fig. 1).

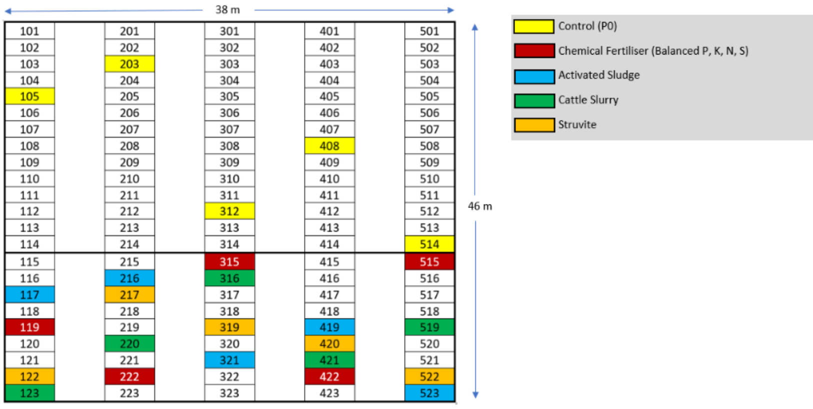

Figure 1: Plot plan of fertiliser applications at Johnstown Castle Research Centre. Colour coded plots were samples in this study, blank plots are involved in separate Teagasc study.

The applications investigated in this study included two chemical fertilisers: balanced P, K, N, S; and zero phosphorus (K, N, S only), while the three bio-based applications were: waste-water treatment sludge (activated sludge); cattle slurry; struvite (from pig manure). The bio-based fertilisers were mixed with mineral fertiliser to ensure sufficient nutrient composition. Each fertiliser provided N, P, K and S from organic sources in different ratios, and nutrient concentration was varied at each application (see appendix, Table X). The VEV struvite fertiliser was changed to Cartif struvite in 2021, however nutrient concentrations remained similar.

2.3 Carbon analysis

Soil samples were collected from the 0-10cm soil increment of each plot after each harvest (March, May and August) since 2019. Samples were dried in the oven at 40°C for 72 hours before being ground to <2mm in the soil sieving machine. Soil samples were prepared for pH analysis by mixing 10mL of dried soil with 20mL of deionised water. The pH of the soil was found using a Metler-Toledo pH electrode (see Table 1). Total carbon values for each soil sample were determined using high temperature combustion method with LECO TruSpec CN analyser. The averages of the soil organic carbon levels for each application were calculated and mapped over time to observe changes in levels with fertiliser application. These averages were analysed using a one-way ANOVA in R. Similar methods were used to analyse the soil organic matter averages for each application. All results were considered significant at $p < 0.05$. Statistical analyses packages used were “ggplot2” (Wickham, 2016), “ggpubr” (Kassambara, 2020), “tidyverse” (Wickham *et al.,* 2019), “broom” (Robinson, Hayes and Couch, 2021), and “AICcmodavg” (Mazerolle, 2020).

2.4 Earthworm Sampling

Two quantitative earthworm samplings were carried out six weeks apart, directly following the first and second harvesting cuts of the year. Earthworm samplings took place after harvests for ease of access to the soil and to ensure little disturbance is incurred on the grass yield. The first earthworm sampling took place on 10th June 2021 with the month prior to this recording 129.8 mm of rainfall and a mean temperature of 9.8°C (The Irish Meteorological Service, 2021). The second sampling took place on 30th July 2021 after a dry month with 78.3 mm of rainfall and a mean temperature of 16.4°C (The Irish Meteorological Service, 2021). A soil monolith (20cm x 20cm x 20cm) was dug in the centre of each of the 25 sample plots and earthworms were collected by hand sorting. Collected specimens from each plot were sorted in labelled bags and transferred to the laboratory. Specimens were washed in distilled water and total wet weight of worms per plot was obtained before preserving the worms in 98% ethanol. Both wet weights and ethanol weights were obtained for analysis, with the assumption that the weight loss in the preservative solution has no consequence for comparisons when preservation is done in the same way across all treatments. The earthworms were divided into developmental stage (adult, juvenile) and counted to obtain values for total density, total adult density and total juvenile density. The adult worms for each plot were then identified and sorted into epigeic, endogeic and anecic ecological groups. These were counted and weighted again (after preservation in 70% ethanol for approximately 20 days). Biomass (kg) and density (number of individuals) per hectare were obtained for each treatment. Analysis of variance (ANOVA) was used to compare biomass and density across the different treatments using R software. A nested ANOVA tested species diversity for each fertiliser application, with nested factors being considered fertiliser type and ecological group. All results were considered significant at $p < 0.05$. Linear regression analysis was performed on the combined soil organic matter, soil organic carbon, and earthworm biomass and density to investigate correlation between these variables. Statistical analyses packages used were "ggplot2" (Wickham, 2016), "ggpubr" (Kassambara, 2020), "tidyverse" (Wickham *et al.,* 2019), "broom" (Robinson, Hayes and Couch, 2021), and "AICcmodavg" (Mazerolle, 2020).

Section 3: Results

3.1 Carbon Analyses

3.1.1 Soil Organic Carbon Analysis

Soil carbon levels showed a variety of increases and declines over the fertiliser application period (Fig. 2). The average SOC levels across each fertiliser application show a good variance, with the bio-based fertilisers resulting in a higher C level over the chemical-based fertilisers, particularly the N, P, K, S fertiliser (Fig. 3).

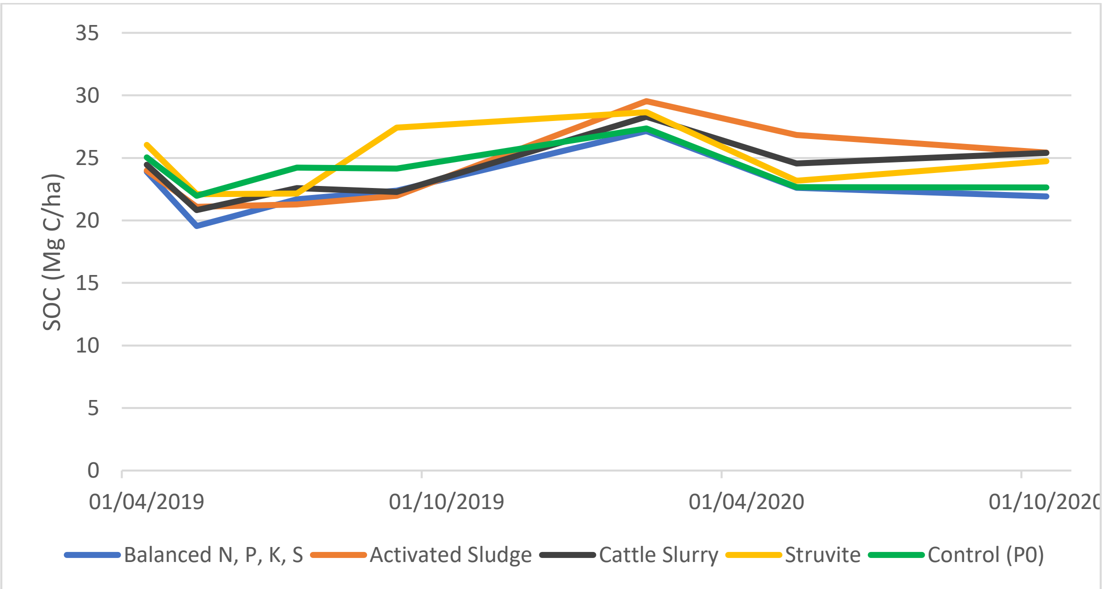

Figure 2: Progression of average soil organic carbon (SOC) stock for each fertiliser application over the course of the experiment. SOC is measured in Mg C/ha. Date is presented in six-month increments.

There was no significant effect on the C levels due to fertiliser application, however a substantial change is observed in the C levels across applications over the period of the trial. The activated sludge application shows the largest positive change of 6%, while the N, K, S fertiliser shows a 9.5% decrease in C levels.

Figure 3: Percentage change in the average soil organic carbon (SOC) stocks in each plot for each fertiliser application during the experiment. SOC was measured in Mg C/ha.

3.1.2 Soil Organic Matter Analysis

The soil organic matter levels of the soil initially displayed less variation than the SOC levels, with a smaller change in levels between over the period of the trial. Cattle slurry had the greatest increase in organic matter over the trial at 8%, while the largest decrease was in the activated sludge plots at just 1.8% decrease (Fig. 4). Overall, bio-based fertilisers did not favour an increase in SOM to a significant extent.

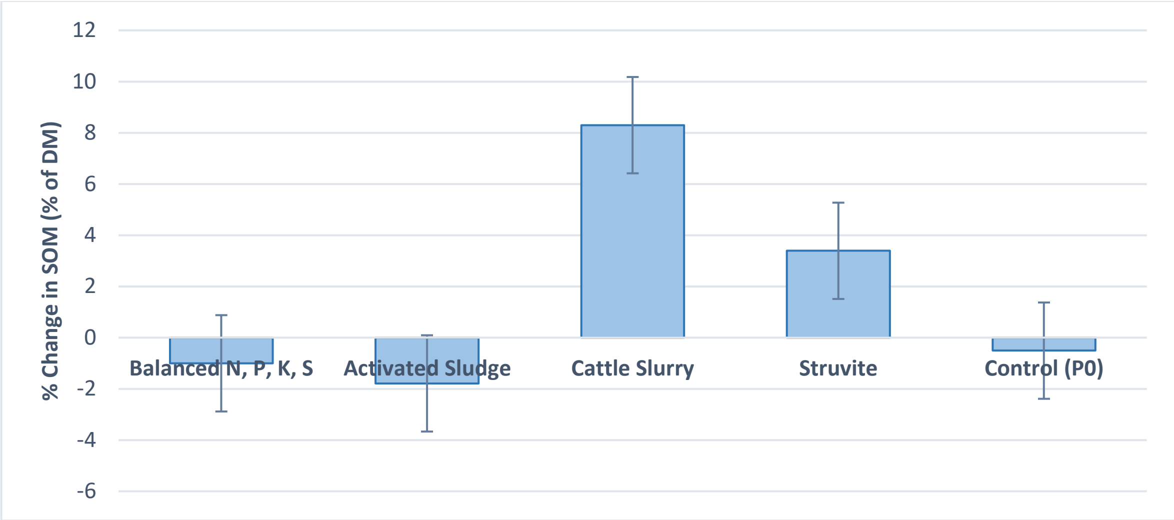

Figure 4: Percentage change in the average SOM level in plots with each fertiliser application during the experiment. SOM was measured in % of dry matter.

3.2 Earthworm Analyses

3.2.1 Earthworm Biomass Analysis

Average earthworm biomass for each fertiliser appears to be higher for the bio-based fertilisers (Activated Sludge, Cattle Slurry, Struvite) when compared with the overall mean for chemical-based fertilisers (N, P, K, S and N, K, S) (Fig. 5).

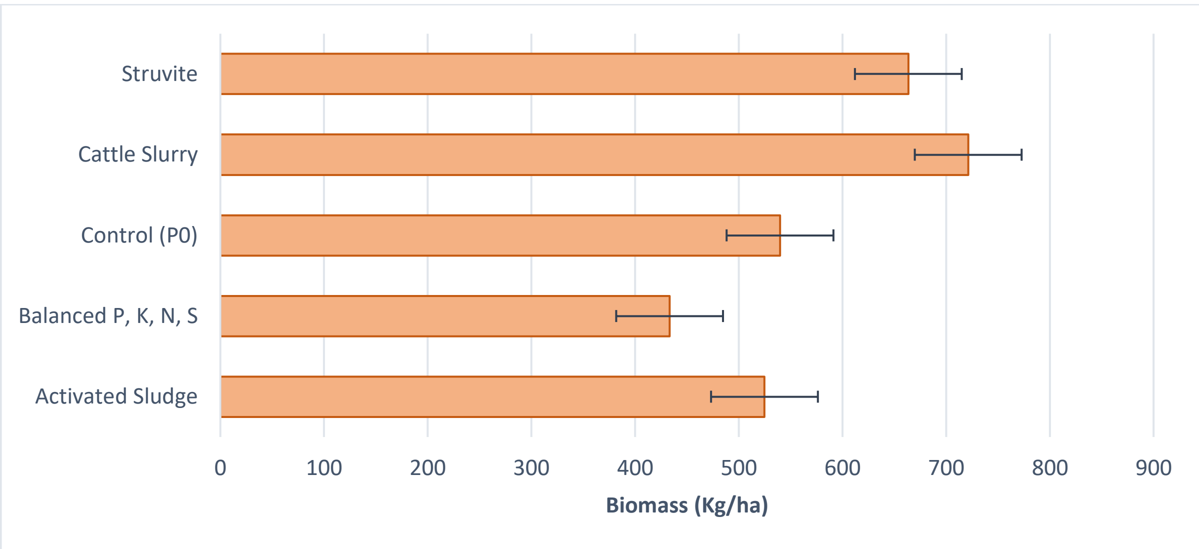

Figure 5: Average earthworm biomass for each fertiliser application from both sampling dates combined. Biomass values are for the 0-20cm soil increment, measured in Kg/ha.

The first earthworm count in June 2021 displayed low variance in biomass outcomes (CV = 0.5), despite having a wide range in results with the mean biomass of cattle slurry at 41.5 kg/ha and mean biomass of N, P, K, S fertiliser at 18 kg/ha (Fig. 6). Biomass values showed higher variance in the second count in July 2021 (CV = 0.75), which is attributed to unusually hot and dry weather in the weeks prior to this count affecting some plots more than others (The Irish Meteorological Service, 2021).

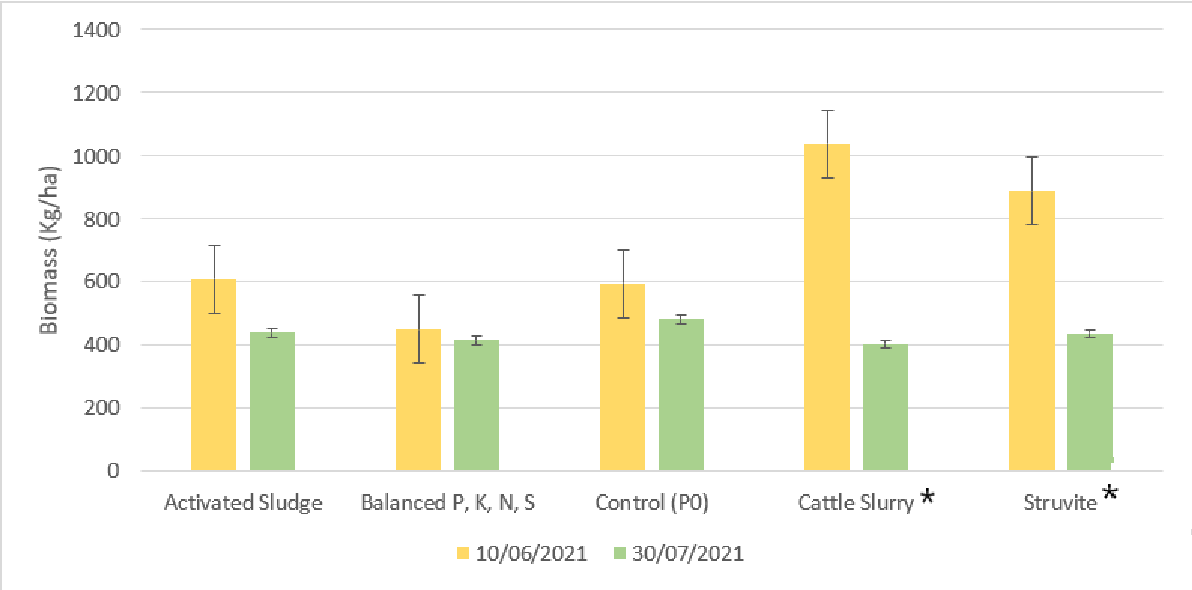

Figure 6: Average biomass values per fertiliser application on each sampling date. Biomass values are for the 0-20cm soil increment, measured in Kg/ha. Starred applications observed a statistically significant effect of fertiliser on earthworm biomass (Cattle Slurry ($p = 2.2 \times 10^{-6}$), Struvite ($p = 0.0127$)).

Overall, earthworm biomass was not significantly affected by fertiliser application, however, cattle slurry showed a significant increase in earthworm biomass [$F(1,38) = 31.01$, $p = 2.2 \times 10^{-6}$], as did the application of struvite [$F(1,38) = 6.842$, $p = 0.0127$].

3.2.2 Earthworm Density Analysis

Earthworm density values displayed a higher variation across both counts, despite the difference in weather at the times of the counts (Fig. 7).

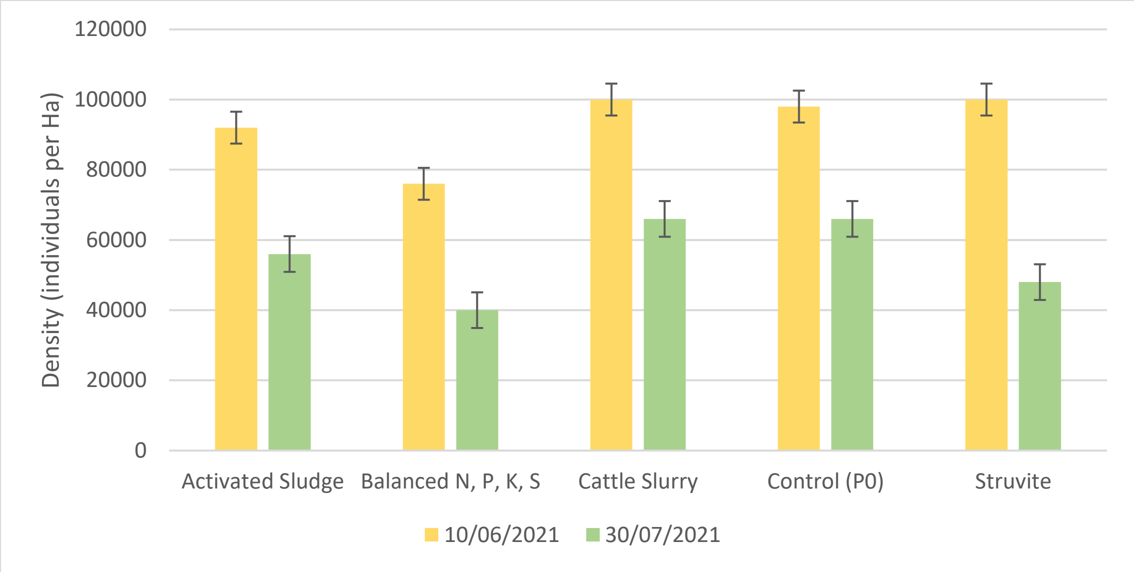

Figure 7: Average earthworm density per hectare for each fertiliser application on each sampling date. Earthworm density is measured in individuals per hectare.

Overall, there was a less significant difference across values when compared with the variance of the biomass analysis values, however, a similar pattern was observed with higher earthworm density values across the bio-based fertilisers. The N, K, S fertiliser also displayed a high density of earthworms, which was unexpected considering the low biomass values for this fertiliser. A high proportion of juveniles were present across all fertiliser applications (Fig. 8), as was expected.

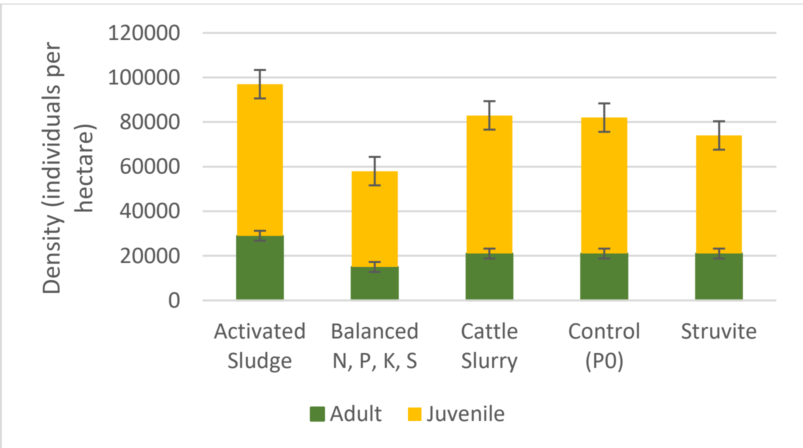

Figure 8: Total average density with adult and juvenile earthworm densities. Densities include results for both sampling dates.

3.2.3 Earthworm Diversity Analysis

A high diversity between epigeic and endogeic earthworm species was observed across all fertiliser application (Fig. 9). No adult anecic species were catalogued, however several juvenile anecic earthworms such as *Lumbricus rubellus* were found in the bio-based fertiliser plots.

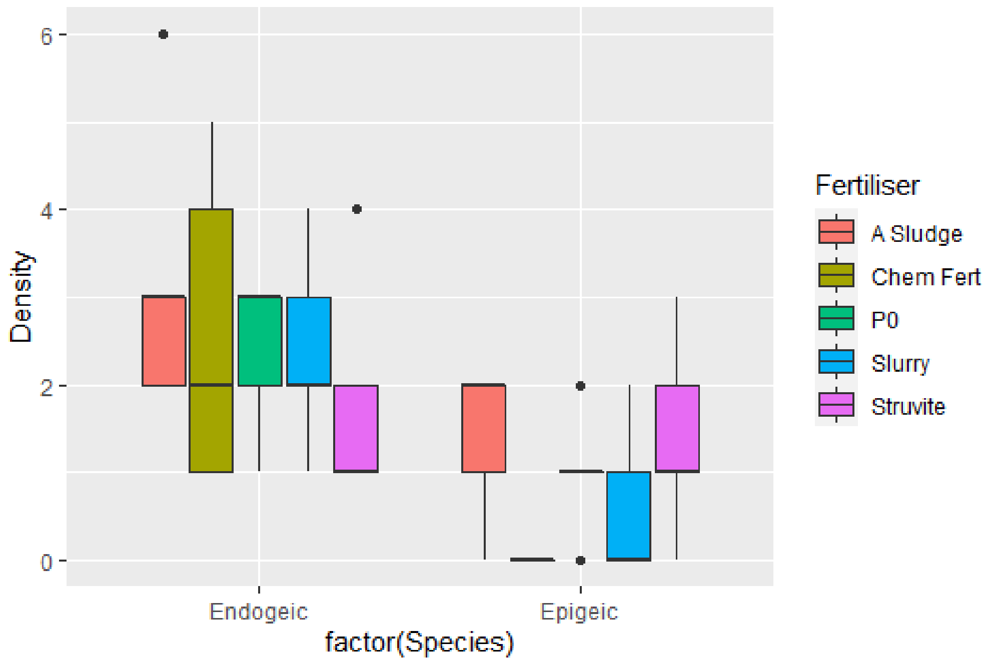

Figure 9: Diversity of endogeic and epigeic species across each fertiliser type. Fertilisers include activated sludge ("A Sludge), balanced N, P, K, S ("Chem Fert"), Control ("P0"), cattle slurry ("Slurry"), and struvite. Values were tested in a nested ANOVA with nested factors being fertiliser type and ecological group (p=0.00415).

Endogeic species were more prevalent across all plots, which is expected for the 0-20cm soil increment being analysed. No epigeic species were observed for any of the N, P, K, S plots. There was a significantly higher diversity of earthworm species for each of the bio-based fertiliser plots when compared to the N, P, K, S plots [F (5,90) = 3.721, p = 0.00415], suggesting that fertiliser can affect earthworm diversity.

3.3 Earthworm populations and carbon

There was a positive correlation between earthworm biomass when compared against both SOM and SOC (Fig. 10).

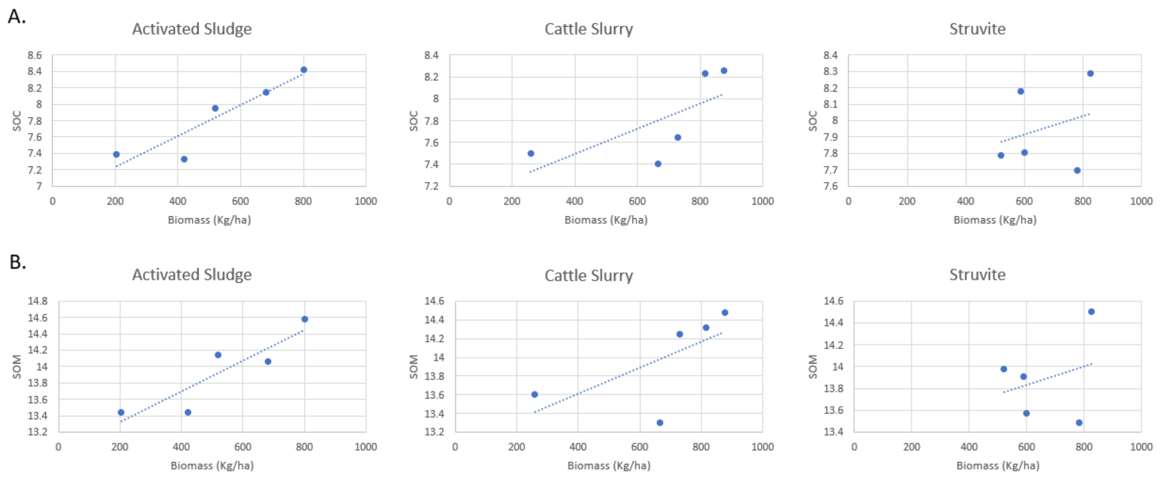

Figure 10: Linear correlation between earthworm biomass, SOM and SOC for the three bio-based fertilisers. SOM and SOC were transformed using an ArcSine transformation to produce a proportionate dataset. Biomass is measured in Kg/ha. Row A depicts the earthworm biomass and SOC relationship while row B depicts the biomass and SOM relationships.

Earthworm biomass and SOC proved to have a positive linear relationship when these parameters for each plot were compared for the bio-based fertilisers [$F(1,13) = 11.94$, $p = 0.0043$]. This positive linear relationship was not observed between earthworm density and SOC values. Similar results were observed between earthworm biomass and SOM values for each bio-based fertiliser plot [$F(1, 13) = 8.658$, $p = 0.0114$]. Again, this relationship was not observed between earthworm density and SOM values. These observations among the bio-based fertiliser plots were not apparent in the mineral-based fertiliser plots.

Section 4: Discussion

4.1 Carbon Analyses

4.1.1 Changes in Soil Organic Carbon

The use of bio-based fertilisers did not have a significant impact on SOC over the trial period of 18 months. While there are observable differences in the average SOC levels between the bio-based fertiliser treatments and the mineral fertiliser treatments (Fig. 2), these variances were not sufficient to conclude a positive impact from bio-based fertilisers. Despite this, there are positive trends in the change in SOC stocks in bio-based fertiliser plots over the 18-month period (Fig. 3). Plots treated with both cattle slurry and activated sludge showed a higher SOC content at the end of the trial, which was expected due to the additional input of SOM. The application of activated sludge resulted in the highest increase in SOC stocks in the experimental plots by the end of the trial period, with a 6% increase in SOC. The cattle slurry plots also experienced a 4% increase in SOC stocks during the trial period. These bio-based fertiliser applications displayed increased C storage in the soils on which they were applied, a result similar to many studies in the literature (Dębska *et al.* , 2016; Xavier *et al.* , 2019; Ibrahim *et al.* , 2021). Both mineral-based fertilisers, P0 and N, P, K, S lost 9.5% and 8% of their SOC stocks respectively. This was generally unexpected, as previous studies have shown that mineral fertilisation results in increased C storage (Poeplau *et al.* , 2018; Cai *et al.* , 2021). Interestingly, plots containing struvite experienced a 5% loss in SOC stocks, despite having the highest average SOC stocks of 24.9 Mg C/ha throughout the trial period. While the struvite plots had the highest average SOC stock, they also experienced much variance in stocks over the trial period, with particularly large changes in stocks between September 2019 and October 2020 (Fig. 2). While studies on the relationship between struvite and SOC are limited, this result was unexpected, as bio-based fertilisers are generally thought to increase C storage (Dębska *et al.* , 2016; Ibrahim *et al.* , 2021). This loss of SOC could be associated with the low N input from organic sources in the VEV Struvite applications (Appendix, Table. 2). A low C:N ratio leads to the organic matter being quickly and easily decomposed by microorganisms, resulting in the loss of soil C (Xavier *et al.* , 2019). However, further study is necessary to examine this parameter and determine the reason for loss of SOC, and to study the different effect of organically sourced N compared with synthetically sourced N. This study was limited in the time frame necessary to monitor the changes to parameters and the influences of external factors. Future research should monitor the long-term effects of bio-based fertilisers and mineral fertilisers on the SOC stocks of soil, as this trial period of 18 months was not sufficient to provide a conclusive answer to the research question.

An increase in SOC stocks provide many benefits to farmers, such as increased soil stability, higher crop yield and restoration of degraded soils (Robert, 2001). Methods such as bio-fertilisation can increase SOC stocks, given that the sufficient nutrient levels are reached. As such, these methods can be adopted by farmers to both improve soil quality and increase the soil's C sequestration potential. Carbon sequestration in turn provides many benefits to the environment such as counteracting desertification through soil stability and improved air quality (Robert, 2001).

4.1.2 Changes in Soil Organic Matter

Soil organic matter levels were largely unaffected by the use of bio-based fertilisers. There was less variance in SOM content of the plots when compared to the SOC stock changes. However, there was a large change in SOM of the cattle slurry plots over the duration of the experiment. Between April 2019 and October 2020, the SOM content of the plots treated with cattle slurry increased by 8%. This is similar to studies from the literature, where cattle slurry resulted in an increase in SOM, and even alleviated the degradation of SOM by chemical fertiliser (Guo *et al.* , 2016). While struvite also experienced a 3.4% change, the other three fertilisers decreased in SOM over the trial period. These negative changes were much smaller than the changes in SOC content (Fig. 4). The plots treated with activated sludge had the greatest loss of SOM, with a 1.8% decrease. While small, this decrease was unexpected as the literature suggests the use of bio-based fertilisers will increase SOM content of soils (Yagüe *et al.* , 2012; Plaza *et al.* , 2013; Dębska *et al.* , 2016; Guo *et al.* , 2016). Although this seems to be the case for many bio-based fertilisers, there has been very little study on the relationship between the use of activated sludge and SOM content of soil. Future research should focus on the variables involved in loss of OM, particularly with the use of novel bio-fertilisers such as dairy processing sludge. This study was limited in the monitoring of such variables. Soil organic matter has been observed to provide many benefits to soil such as soil structure, soil aggregate stability and promotion of earthworm populations, resulting in its use as an indicator of soil quality (Robert, 2001; Guo *et al.* , 2016). Practices for the preservation of SOM include bio-fertilisation and low/no tillage and must be employed to promote soil health without the harmful effects of mineral fertilisers. These practices have the additional benefit to farmers of being low cost and require little working time. Improvements in SOM content also provide benefits to the wider environment in terms of water filtration, soil stability, and detoxifying pollutants (Robert, 2001).

4.1.3 Relationship between soil organic matter and soil organic carbon

The differences between SOC and SOM changes were largely unexpected, as high levels of SOM is generally associated with high C stocks in soil (Robert, 2001). This was not the case for struvite or activated sludge, both of which had conflicting increases and decreases in these parameters. The expected positive relationship was only observed in the cattle slurry plots, which had an increase of 8% in SOM and an increase of 4% in SOC stocks (Fig. 3, Fig. 4). Cattle slurry has been observed to be beneficial to both SOC and SOM contents, improving the overall health of the soil (Guo *et al.* , 2016). The reasons for the varying changes in the plots utilizing struvite and activated sludge were not clear. As stated above, the high N levels in struvite could be associated with its loss of SOC, while its SOM content increased as expected with bio-based fertilisers. In contrast, activated sludge experienced a net loss of SOM despite showing a 6% increase in SOC stocks throughout the experiment. A low content of OM is associated with low activity of soil microorganisms and soil fauna (Robert, 2001). As stated above, the plots treated with activated sludge displayed a generally low biomass of earthworms in comparison with struvite or cattle slurry. The low levels of SOM in these plots could be a result of low soil fauna biomass.

With the mutually beneficial relationship between SOC and SOM, efforts must be taken to preserve both of these parameters in agricultural soil. High levels of both SOC and SOM have been shown to provide numerous benefits to agricultural yields, soil health and the wider environment.

4.2 Earthworm Analyses

4.2.1 The influence of fertilisers on earthworm biomass

Overall, fertiliser application did not have a significant impact on earthworm biomass; however, there are some observable differences in the results, particularly in the cattle slurry and struvite applications (Fig. 6). All applications had a low earthworm biomass on the second application date in July 2021. This can be explained by the unusually hot and dry weather in the weeks leading up to the sampling. As a result of the lack of moisture in the soil, earthworms migrated deeper into the soil profile. This was also observed by Al-Maliki, Al-Taey and Al-Mammori (2021), who notes that the surface dwelling epigeic species are the most affected by drought conditions. These species feed off plant litter, which declines in abundance in dry weather, resulting in a decline in earthworm weight or biomass. As a result, long periods of dry weather led to a decrease in abundance and biomass of earthworms in this study, specifically epigeic species. The application of cattle slurry to the soil plots led to the highest overall biomass of earthworms, with 41.54 kg/ha on the first sampling date. Compared with the plots containing both mineral fertilisers, there is a significant difference in earthworm biomass with cattle slurry application [$F (1,38) = 31.01$, $p = 2.2 \times 10^{-6}$]. This was the expected result, as the literature observes an increase in earthworm biomass with the addition of cattle slurry (Estevez, N'Dayegamiye and Coderre, 1996; Timmerman *et al.* , 2006; Curry *et al.* , 2008; Guo *et al.* , 2016; Butt and Nuutinen, 2021). This result can be associated with the cattle slurry providing an additional food source to earthworms (Estevez, N'Dayegamiye and Coderre, 1996). As cattle slurry has been observed to result in a high yield of cover crops, the additional food and nutrients provided by this plant matter also played a role in a high biomass of earthworms (Curry *et al.* , 2008). Guo *et al.* (2016) combined mineral fertiliser with organic fertiliser, a method which was also used in this study. This mixture of fertilisers resulted in a large increase in earthworm biomass and could provide a suitable alternative to farmers where the use of organic fertiliser alone does not satisfy the nutritional needs of the soil, while still providing benefits to the soil fauna and surrounding environment. The application of struvite to the experimental plots also conveyed significant results with an average earthworm biomass of 35.5 kg/ha [$F (1,38) = 6.842$, $p = 0.0127$]. This was generally expected as the literature suggests the use of organic fertilisers is beneficial to earthworms (Timmerman *et al.* , 2006; Arai *et al.* , 2018; Butt and Nuutinen, 2021; Moinard *et al.* , 2021), though very little research has examined the effects of struvite on earthworm biomass and densities. Zhou *et al.* (2021) noted that the addition of organic matter with a high nitrogen concentration, such as struvite, can have a negative effect on earthworm population. The struvite application in this study had a relatively low nitrogen concentration from organic sources (Appendix, Table 2). This could account for the high biomass of earthworms being unaffected by the nitrogen levels in the organic fertiliser. Further study is required to examine the effect of nitrogen concentrations from organic and mineral sources on earthworm biomass in different soil with different nutrient levels. Activated sludge was the only organic fertiliser in this experiment which did not affect earthworm biomass significantly. This was unexpected when considering the benefits of organic fertilisers generally seen in the literature, however additional study is required to determine the reason for this lower biomass. Nutritional imbalance could be a possible reason for this result as activated sludge has different chemical properties to cattle slurry or struvite, with a high phosphorous concentration and low potassium concentration. The relatively low SOM input of activated sludge could also result in a diminished food source for earthworms, concluding in no significant increase in earthworm abundance.

These results highlight the importance of monitoring soil nutrients and determining the sufficient nutritional concentrations required when selecting an appropriate fertiliser. Although the benefits of bio-based fertilisers are observed in this study, a prior knowledge of soil nutrient requirements is essential in choosing the most suitable bio-fertiliser.

4.2.2 The influence of fertilisers on earthworm density

Earthworm density was not as drastically affected by fertiliser application as earthworm biomass (Fig. 7). While there was variation among results with bio-based fertiliser resulting in slightly higher density of earthworms, there was no significant difference. This is consistent with many studies which observed an increase in earthworm biomass when treated with organic fertiliser yet no increase in earthworm densities (Muldowney *et al.* , 2003; Timmerman *et al.* , 2006). This could be due to organic fertilisers increasing the food source for earthworms, resulting in larger worms, but not affecting earthworm reproduction. This observation was not seen in all similar studies, however. Moinard *et al.* (2021) applied anaerobic digestate as organic fertiliser and observed a 150% increase in earthworm density over two years when compared to plots treated with chemical fertiliser. This highlights a limitation in this study, as the initial earthworm density prior to fertiliser application in 2019 is unknown. Future study would monitor the changes in earthworm population over the course of the experiment, as this study only observed density differences across applications.

While the overall earthworm density is important to monitor, the density of adult earthworms is the area of focus. On the first sampling date, 64% of earthworms were juvenile, while 83% were juvenile on the second sampling date. The higher number of juvenile earthworms on the second sampling date could be associated with the dry weather leading up to this sampling. Juvenile earthworms may not have the ability to burrow deeper into the soil profile during dry weather when the soil is difficult to penetrate (Muldowney *et al.* , 2003). Overall, the density of adult earthworm was not significantly affected by fertiliser application, though they may have been affected by seasonal weather changes. This may also account for the little change in earthworm density on the second sampling date when compared to the drastic change in earthworm biomass. Though the earthworm density was still relatively high, 83% of this density consisted of juvenile earthworms, resulting in the lower biomass results for the second sampling date. This is consistent with results by Muldowney *et al.* (2003), who noted the decrease in adult earthworm activity in dry weather conditions. The relatively high earthworm density in the P0 mineral fertiliser was unexpected, as previous studies claim mineral fertilisers reduces earthworm density (Moinard *et al.* , 2021). The P0 fertiliser displayed similar earthworm density results to all three bio-based fertilisers, despite having a much lower earthworm biomass (Fig. 8). The P0 applications had similar juvenile/adult earthworm ratios to the other plots, with 65% of earthworms in the first sampling being juvenile and 87% in the second sampling. Therefore, the low biomass and high-density results for P0 could be attributed to a low diversity of species with a large body mass. The absence of phosphorous in this fertiliser could be a possible reason for this result, which highlights a knowledge gap in the study of earthworms and fertiliser. The impact of phosphorous input to the soil would be an interesting topic of future study which would connect this knowledge gap.

4.2.3 Earthworm diversity impacts from fertilisers

A high diversity of earthworm species was prevalent during both samplings, with both epigeic and endogeic species observed across bio-based fertiliser plots and P0 plots. There were no epigeic species present in any of the plots treated with N, P, K, S mineral fertiliser. No anecic species were present in any of the plots as expected; the soil plots sampled were not deep enough to penetrate the habitat of the deep-burrowing anecic species. The application of bio-based fertiliser appeared to affect the diversity of earthworms present in the soil plots [$F_{(5,90)} = 3.721$, $p = 0.00415$]. This is consistent with previous studies observations that organic fertilisers were beneficial to earthworm diversity (Estevez, N'Dayegamiye and Coderre, 1996; Mba, 1996; Butt and Nuutinen, 2021). Although there are few studies on this topic, the literature points to an increase in soil organic matter as a food source to be a key aspect of increased earthworm diversity. The plots treated with struvite had the highest earthworm diversity (Fig. 9), with an epigeic/endogeic ratio of 44/56 on the first sampling date and 40/60 on the second sampling date. While the plots containing struvite had a low SOM average when compared to the other bio-based fertilisers (5.8 % of dry matter), these plots also had a 3.4% increase in SOM levels over the trial period, possibly leading to this high diversity of earthworms. All plots treated with bio-based fertilisers had a reasonably good diversity of species, with the plots treated with N, P, K, S chemical fertiliser showing low diversity (only epigeic species). This is concurrent with Hoeffner, Hotte, *et al.* (2021) where the use of mineral N fertiliser had no positive effect on earthworm species diversity.

A high diversity of earthworms is beneficial to soil, as each ecological group plays a role in maintaining soil health. The presence of each ecological group is therefore essential to ensure all ecosystem services provided by earthworms are available. Epigeic species break down plant litter and organic matter, which benefits carbon cycling (Stroud and Bennett, 2018). These species were present in plots treated with bio-based fertilisers and P0 mineral fertiliser. Endogeic species were present in all fertiliser treatment plots, benefitting the mineralisation of plant nutrients and soil aggregation (Stroud and Bennett, 2018). Interestingly, plots treated with the P0 mineral fertiliser had good earthworm diversity (Fig. 9). This was not expected as previous studies observed no positive change in diversity when mineral fertiliser was used (Hoeffner, Hotte, *et al.* , 2021). These plots also had a good SOM content (5.9 % of dry matter), which could account for this high diversity. The absence of phosphorous in the mineral fertiliser appears to benefit earthworm populations, as these plots also showed a high density (Fig. 8) and reasonably high biomass (Fig. 5). An interesting topic of further research on this topic would be similar to Kuczak *et al.* (2006), who explored the phosphorous pools in earthworm casts. This approach to the study may help determine the role of phosphorous in determining earthworm diversity and abundance.

While there was a large difference in earthworm biomass and density on each of the sampling dates due to dry weather, the diversity of earthworms was largely unaffected by this variable (Fig. 9). However, there was a small decrease in epigeic species across most plots, which can be attributed to the dry weather mostly affecting the top layer of soil in which these species inhabit. Al-Maliki, Al-Taey and Al-Mammori (2021) had a similar observation with periods of drought decreasing the abundance of epigeic species. This can have detrimental consequences to soil as these species provide valuable soil ecosystem services. As a result, the carbon cycle can be affected, reducing the amount of carbon stored in the soil in dry weather conditions (Al-Maliki, Al-Taey and Al-Mammori, 2021). The only outliers to this observation were the plots treated with cattle slurry which saw a larger number of epigeic species on the second sampling date. This could possibly be attributed to the high levels of SOM provided by cattle slurry which provided an essential food source for epigeic earthworms during this period of dry weather. This observation is valuable as it appears that soil treated with cattle slurry leads to a more resilient population of earthworms, being beneficial to agricultural soil in time of harsh weather conditions. Further study would observe the soil quality of slurry-treated soil in harsh weather conditions, to investigate if the earthworms resilience benefits the soil ecosystem and extends this resilience to the entire ecosystem.

4.3 The relationship between soil organic matter, soil carbon, and earthworms

Earthworm biomass was influenced by the organic matter inputs of the bio-based fertilisers [$F(1, 13) = 8.658$, $p = 0.0114$]. There was a clear positive correlation between the SOM content of each plot with the corresponding earthworm biomass in that plot (Fig. 10). This was the expected result, as the literature also suggests this correlation (Amossé *et al.* , 2015; Hong *et al.* , 2011; van Vliet *et al.* , 2007). Previous studies attribute this positive relationship to the organic matter acting as an additional food source for earthworms. This then results in larger earthworms with a higher overall biomass (Amossé *et al.* , 2015). The biomass of earthworms in each bio-based fertiliser plot also correlated positively with soil carbon stocks [$F(1,13) = 11.94$, $p = 0.0043$]. It appears that the plots with a high SOC content also produced a high biomass of earthworms (Fig. 10). This again is related to an increase in SOM resulting in an increase in mineralisation of soil C, and thus an increase in SOC. These three variables, SOC, SOM and earthworm biomass are positively correlated through mutually beneficial relationships. An increase in SOM increases both SOC and earthworm biomass at a similar rate, which in turn will possibly benefit the soil ecosystem services attributed to high C content and high earthworm populations. This study could be further extended by investigation into a possible correlation between earthworm biomass and earthworm activity, as some previous studies observed an increase in earthworm activity due to the addition of high rates of organic matter (Frazão *et al.* , 2019). Interestingly, the SOM and SOC content of the soil plots was not correlated to earthworm density. This is in contrast with many studies which observe an increase in earthworm numbers due to the addition of high levels of organic matter (van Vliet *et al.* , 2007). The reason for this anomaly is not clear, however it could be attributed to the earthworms feeding on the additional organic matter, thus increasing their biomass, yet not resulting in a higher level of reproductive activity, and as a result no observed increase in density. Additionally, the positive correlation between earthworm biomass and SOM seen in plots treated with bio-based fertiliser was not seen in plots treated with the mineral based fertiliser (P0 Control and Balanced N, P, K, S). This is possibly due to the low levels of organic matter input in these fertilisers resulting in a decreased food source for the earthworms. This study would benefit from an analysis of earthworm activity in response to varying SOM and SOC levels, as the literature has produced a wide range of results on this topic (Amossé *et al.* , 2015; Fonte *et al.* , 2010; Frazão *et al.* , 2019; van Vliet *et al.* , 2007). Additionally, future research would benefit from a temporal study on the long-term addition of various SOM levels and earthworm abundance, as this study was limited in terms of long-term study.

Section 5: Conclusion

This study aimed to bridge the knowledge gap between the use of different types of fertilisers and their effects on soil macrofauna and carbon sequestration potential, investigating the dynamics of the relationship between these variables and the mutual benefits afforded to each. Earthworm samplings were carried out alongside analysis of SOC and SOM stocks in the soil plots since 2019 with the addition of bio-based fertilisers. The bio-based fertiliser generally provided greater benefits to the overall soil ecosystem than synthetic based fertiliser, reflecting results found in the literature. The addition of organic matter to soil in the form of organic fertiliser benefits the earthworm populations, which in turn benefits the mineralisation of soil C, increasing the C/N ratio and allowing for an increase in soil C sequestration. In particular, the application of a cattle slurry-based fertiliser produced the most significant overall results, with an increase in earthworm biomass and species diversity apparent in these plots. Significantly, the plots treated with cattle slurry observed a greater resilience of earthworm populations when compared with all other plots. The cattle slurry treated plots had a consistent biomass and density of earthworms throughout the experimental period despite harsh weather conditions. A resilient population of earthworms will likely benefit the resilience of the soil ecosystem, possibly with ecosystem services remaining constant throughout a diverse range of weather. This observation raises an interesting topic for future study; an investigation into a possible correlation between resilient earthworm population and the consistency of ecosystem services would be of benefit to modern agricultural research, as the developing effects of climate change will likely lead to adverse weather conditions in the future. The overall constraint of this study was the time period involved. Each variable examined in this study would benefit from a long-term study on the temporal changes in earthworm population and soil carbon stocks in response to continued use of bio-based fertilisers. However, this study observed impactful results from organic fertiliser application in the short-term. The addition of organic fertiliser or a combination of organo-chemical fertilisers is recommended to ensure long-term health and resilience of soil macrofauna populations, soil C stocks and overall soil ecosystem health.

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Section 7: Appendix

Table 2: Nutrient input for fertiliser applications from mineral and organic sources.

			Mineral Fertiliser Source (Kg/ha)				Organic Fertiliser Source (Kg/ha				Total Applied (Kg/ha)			
Application (	Treatmen	Fertilisers	N	P	K	S	N	P	K	S	N	P	K	S
12/04/2019	15	Balanced I	125	40	155	20	0	0	0	0	125	40	155	20
	17	Activated :	72	0	149	13	53	40	6	7	125	40	155	20
	18	Cattle Slur	45	23.5	39	10	80	16.5	116	10	125	40	155	20
	19	CTSruvite1	106	0	151	20	19	40	4	0.0	125	40	155	20
	20	VEVStruvit	105	0	155	20	20	40	0	0.0	125	40	155	20
29/05/2019	15	Balanced I	100	10	75	20	0	0	0	0.0	100	10	75	20
	17	Activated :	99	0	74	18.3	13	10	1	1.7	112	10	75	20
	18	Cattle Slur	86	0	5	13.9	48	10	70	6.1	134	10	75	20
	19	CTSruvite1	95	0	74	20.0	5	10	1	0.0	100	10	75	20
	20	VEVStruvit	95	0	75	20.0	5	10	0	0.0	100	10	75	20
24/07/2019	15	Balanced I	100	10	75	20	0	0	0	0.0	100	10	75	20
	17	Activated :	99	0	74	18.3	13	10	1	1.7	112	10	75	20
	18	Cattle Slur	86	0	5	13.9	48	10	70	6.1	134	10	75	20
	19	CTSruvite1	95	0	74	20.0	5	10	1	0.0	100	10	75	20
	20	VEVStruvit	95	0	75	20.0	5	10	0	0.0	100	10	75	20
20/03/2020	15	Balanced I	125	30	125	20	0	0	0	0.0	125	30	125	20
	17	Activated :	121	0	115	16.3	39	30	10	3.7	160	30	125	20
	18	Cattle Slur	95	12	-4	7.1	99	18	129	12.9	194	30	125	20
	19	CTSruvite1	111	0	122	20.0	14	30	3	0.0	125	30	125	20
	20	VEVStruvit	110	0	125	20.0	15	30	0	0.0	125	30	125	20
21/05/2020	15	Balanced I	100	10	75	20	0	0	0	0.0	100	10	75	20
	17	Activated :	99	0	72	18.8	13	10	3	1.2	112	10	75	20
	18	Cattle Slur	84	0	3	12.8	55	10	72	7.2	139	10	75	20
	19	CTSruvite1	95	0	74	20.0	5	10	1	0.0	100	10	75	20
	20	VEVStruvit	95	0	75	20.0	5	10	0	0.0	100	10	75	20
09/07/2020	15	Balanced I	100	10	75	20	0	0	0	0.0	100	10	75	20
	17	Activated :	99	0	72	18.8	13	10	3	1.2	112	10	75	20
	18	Cattle Slur	81	0	15	13.2	63	10	60	6.8	144	10	75	20
	19	CTSruvite1	95	0	74	20.0	5	10	1	0.0	100	10	75	20
	20	VEVStruvit	95	0	75	20.0	5	10	0	0.0	100	10	75	20
27/04/2021	15	Balanced I	125	20	155	20	0	0	0	0.0	125	20	155	20
	17	Activated :	122	0	150	16.7	23	20	5	3.3	145	20	155	20
	18	Cattle Slur	92	0	37	6.1	110	20	118	13.9	202	20	155	20
	19	CTSruvite1	115	0	153	20.0	10	20	2	0.0	125	20	155	20
	20	CartifStruv	116	0	154	20.0	9	20	1	0.0	125	20	155	20
14/06/2021	15	Balanced I	100	10	75	20	0	0	0	0.0	100	10	75	20
	17	Activated :	99		72	18.4	11	10	3	1.6	110	10	75	20
	18	Cattle Slur	84		16	13.1	55	10	59	6.9	138	10	75	20
	19	CTSruvite1	95		74	20.0	5	10	1	0.0	100	10	75	20
	20	CartifStruv	96		75	20.0	4	10	0	0.0	100	10	75	20
03/08/2021	15	Balanced I	100	10	75	20	0	0	0	0.0	100	10	75	20
	17	Activated :	99		72	18.4	11	10	3	1.6	110	10	75	20
	18	Cattle Slur	84		16	13.1	55	10	59	6.9	138	10	75	20
	19	CTSruvite1	95		74	20.0	5	10	1	0.0	100	10	75	20
	20	CartifStruv	96		75	20.0	4	10	0	0.0	100	10	75	20