



Landspreading with co-digested cattle slurry, with or without pasteurisation, as a mitigation strategy against pathogen, nutrient and metal contamination associated with untreated slurry

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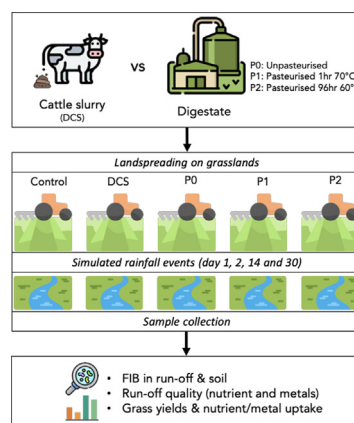
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HIGHLIGHTS

- Co-digesting cattle slurry with food processing waste mitigates environmental impacts.
- Lower microbial, nutrient and metal concentrations in runoff from digestate compared with slurry.
- Reduced microbial runoff from digestate was the most prominent difference compared with slurry.
- Pasteurisation further improved the environmental benefits of amending soils with digestate.

GRAPHICAL ABSTRACT



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ABSTRACT

North Atlantic European grassland systems have a low nutrient use efficiency and high rainfall. This grassland is typically amended with unprocessed slurry, which counteracts soil organic matter depletion and provides essential plant micronutrients but can be mobilised during rainfall events thereby contributing to pathogen, nutrient and metal incidental losses. Co-digesting slurry with waste from food processing mitigates agriculture-associated environmental impacts but may alter microbial, nutrient and metal profiles and their transmission to watercourses, and/or soil persistence, grass yield and uptake. The impact of EU and alternative pasteurisation regimes on transmission potential of these various pollutants is not clearly understood, particularly in pasture-based agricultural systems. This study utilized simulated rainfall (Amsterdam drip-type) at a high intensity indicative of a worst-case scenario of $\sim 11 \text{ mm hr}^{-1}$ applied to plots 1, 2, 15 and 30 days after grassland application of slurry, unpasteurised digestate, pasteurised digestate (two conditions) and untreated controls. Runoff and soil samples were collected and analysed for a suite of potential pollutants including bacteria, nutrients and metals

Abbreviations: ABP, Animal by-products; AD, Anaerobic Digestion; °C, Celsius; DAFM, Department of Agriculture, Food and the Marine (Ireland); DCS, Dairy cattle slurry; EU, European Union; FIB, Faecal indicator bacteria; K, Potassium; MPN, Most probable numbers; N, Nitrogen; P, Phosphorus; TC, Total Carbon; XRF, X-ray fluorescence.

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following rainfall simulation. Grass samples were collected for three months following application to assess yield as well as nutrient and metal uptake. For each environmental parameter tested: microbial, nutrient and metal runoff losses; accumulation in soil and uptake in grass, digestate from anaerobic co-digestion of slurry with food processing waste resulted in lower pollution potential than traditional landspreading of slurry without treatment. Reduced microbial runoff from digestate was the most prominent advantage of digestate application. Pasteurisation of the digestate further augmented those environmental benefits, without impacting grass output. Anaerobic co-digestion of slurry is therefore a multi-beneficial circular approach to reducing impacts of livestock production on the environment.

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1. Introduction

In North Atlantic Europe, grassland systems have a low nutrient use efficiency (~23% for N; Buckley et al., 2013) and high rainfall (>800 mm per annum). Historically, these grassland systems have received both inorganic and organic fertilizers. The use of cattle slurry over inorganic fertilizer has many advantages e.g. it counteracts soil organic matter depletion (Bhattacharya et al., 2016), thereby enhancing soil health (Larkin, 2015) and also provides essential plant micronutrients (Nikoli and Matsi, 2011; Slepetiene et al., 2020). However, land applied cattle slurry can become temporarily mobilised during rainfall events thereby contributing to pathogen, nutrient (nitrogen (N) and phosphorus (P)) and metal incidental losses along surface and subsurface pathways (Clagnan et al., 2019, 2018; Misselbrook et al., 1995; Peyton et al., 2016; Roberts et al., 2017).

Pre-treatment of organic fertilizers before land application attempts to mitigate such transmission of pollutants to the environment. For example, anaerobic digestion (AD) of slurry captures methane that would otherwise be emitted during storage and landspreading, thereby reducing overall greenhouse gas emissions (Amon et al., 2006). However, as slurry has a relatively low biomethane potential, it is typically necessary to co-digest with energy crops, and/or to take in the organic fraction of municipal waste, food waste or wastes arising from the processing of food (fats, oils and grease; belly grass; fish offal etc.) to ensure feasibility (Clemens et al., 2006). Utilization of externally sourced waste streams may introduce pathogens not typically found in agriculture. Digestate resulting from AD of these waste streams is landspread, potentially increasing the risk to human and animal health. Pathogenic microorganisms may survive the AD process, depending on the type of organism, the initial concentration of the organism and AD conditions, particularly temperature (Jiang et al., 2020; Sahlström, 2003; Strauch, 1991). Retention time and feedstock composition also play a role (Chen et al., 2012; Jiang et al., 2020; Nolan et al., 2018; Smith et al., 2005) along with mixing efficacy and the extent of bypass flow (Smith et al., 2005). Moreover, further pathogen die-off may occur during digestate storage before spreading (Luo et al., 2017; Paavola and Rintala, 2008).

Although digestate from AD consistently demonstrates reduced pathogen load compared with undigested slurry, the additional risk of cross-contamination between food production facilities and farms prompted European Union (EU) legislation requiring a pasteurisation step (70 °C for 1 h) in AD systems importing animal by-products (ABP) (Directive No. 142/2011). An additional allowance was made for Member States to introduce national legislation, provided that it achieved the same reductions in faecal indicator bacteria (FIB) numbers. To this end, the Department of Agriculture, Food and the Marine (DAFM) in Ireland, introduced the National Transformation parameter of pasteurisation at 60 °C for 96 h (DAFM, 2014), which was historically applied to composting. Thus, Irish AD plants handling ABP may use either the EU or national pasteurisation standard.

AD of slurry breaks down complex organic compounds, converting carbon to biogas, and mineralising N compounds to NH_4^+ -N, potentially enhancing N-availability (Weiland, 2010) and soil N_{org} -mineralisation when compared with unprocessed slurry (Möller and Müller, 2012). The impact of pasteurisation on the nutrient concentration and risk of

runoff following landspreading must also be considered. While Ware and Power (2016) demonstrated increased bioavailability of soil organic matter following pasteurisation of slaughterhouse waste, the impact of digestate pasteurisation on nutrient concentration and availability has not been clearly established.

In an effort to characterise bio-based fertilizers and their impact on the environment and human health, a toolbox of techniques must be deployed during field experiments. The first of these tools are mobile, field rainfall simulators, which can be deployed to simulate heavy rainfall and attendant runoff from which to examine edge of field runoff losses of faecal indicator bacteria, nutrients and metals at several time-points (24 h, 48 h etc.) after application (Peyton et al., 2016). Such losses represent “worst case” scenarios and do not factor in attenuation further along the transfer continuum. FIB are non-pathogenic indicators of faecal contamination and as such can be more safely and easily monitored to assess risks of pathogenic infection to humans and animals in field environments (Kay et al., 2008; Oliver et al., 2009). Another tool is temporal soil and crop sampling with x-ray fluorescence (XRF) analysis to determine metal uptake in soil and plant tissues (Daly and Fenelon, 2017).

The combined positive impacts of AD (energy, carbon capture etc.) may suggest the need to mandate processing of slurry in an AD plant prior to landspreading, in line with European Union (EU) Circular Economy and European Green Deal goals of improved environmental and climate performance (COM, 2019). However, as studies to date have focused on individual environmental impacts of grassland soil amendment with unprocessed slurry compared with digestate (typically either microbial or nutrients or metals), a comprehensive approach considering multiple possible emission sources together is essential to facilitate drafting of appropriate, informed policies. Furthermore, the impact of mandatory pasteurisation to EU or Member State alternative standards on concentration of pollutants in runoff, soil persistence or grass crop is not generally understood, particularly in digestate from the same source.

Thus, the aim of this study was to undertake a comprehensive examination of FIB, nutrients and metal concentration in soil, transmission in runoff and uptake in grass after land application of a) unprocessed slurry and b) slurry co-digested with FW in an anaerobic digester, without pasteurisation and with pasteurisation at c) 70 °C for 1 h (EU Standard) and d) 60 °C for 96 h (DAFM Standard), with all of these treatments being compared with e) untreated controls. To achieve this aim, 20 micro plots were established and examined for: microbial, nutrient and metal load in runoff resulting from simulated rainfall; microbial, nutrient and metal retention in soil; nutrient and metal uptake in grass.

Hypothesis tested: microbial, nutrient and metal concentrations are lower in runoff, soil and grass following application of unpasteurised and pasteurised digestate (2 conditions) from co-digestion of slurry with FW compared with unprocessed slurry, without negatively impacting grass yield.

2. Materials and methods

2.1. Field site characterisation

The study site was a 0.6-ha mid-slope, non-grazed plot located on the beef farm at Teagasc, Johnstown Castle Environment Research

Centre, Co. Wexford, in the southeast of Ireland (latitude 52.293415, longitude -6.518497). The area has a cool, maritime climate, with an average temperature of 10.1 °C and mean annual precipitation of 879 mm. The site has been used as a grassland sward for over 25 years with organic and inorganic nutrient inputs applied as necessitated by routine soil testing. The site has undulating topography with average slopes of 6.7% along the length of the site and 3.6% across the width. The field is moderately drained with a soil texture gradient of clay loam to sand silt loam, as classified by Brennan et al. (2012). As phosphorus (P) index is used as the limiting factor in organic fertilizer amendment, to determine treatment loading rate, composite 10 cm soil cores from each section ($n = 20$) were analysed for Morgan's P (Pm) using Morgan's reagent (Morgan, 1941; Table 4).

2.2. Micro-plot installation

Micro-plots have been used in several field studies to facilitate precise rainfall simulation and collection of all runoff from each plot (Bochet et al., 2006; Brennan et al., 2012; Gillingham and Gray, 2006; Healy et al., 2017; McConnell et al., 2013; Peyton et al., 2016). Micro-plots represent edge of field runoff losses in worst case scenarios, and results from micro-plots have been validated as proxies for field-scale trials (Larsbo et al., 2008). Twenty grassland micro-plots were isolated using stainless steel frames, hammered into the soil to a depth of 50 mm (Fig. 1). Each micro-plot was 0.4 m in width and 0.9 m in length (0.36 m²), oriented with the longer dimension in the direction of the slope. The frames isolate each plot at the back and sides, and include a runoff channel at the front with a spout for runoff to drain into sample cups (Fig. 1). Once installed, the front rim was sealed to avoid by-pass flow, and any soil disturbed during construction was washed away.

2.3. Soil characterisation

Composite soil core (10 cm) samples were taken from the four corners outside each plot prior to treatment (t_0), and within each plot post-treatment (Day 15 and Day 30), prior to the rainfall simulation on those days. Representative subsamples for each plot were used for soil physico-chemical characterisation including dry matter (105 °C for 24 h) and soil pH, which was determined using a 2:1 ratio of deionised water to soil (Peyton et al., 2016). Samples were ground to 2 mm before being analysed for total P (TP) using the microwave-assisted acid digestion method (US EPA, 1996). Total nitrogen (TN) and total carbon (TC) were determined using the high-temperature combustion method by a LECO

TruSpec CN analyser (Table 4). Soil concentrations of Al, Fe, Ca and trace metals (cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn)) were determined using an Agilent 5100 synchronous vertical dual-view inductively coupled plasma optical emission spectrometer (Agilent 5100 ICP-OES) following the microwave-assisted acid digestion method (US EPA, 1996; Supplementary Table 1).

Additionally, composite samples were tested for the presence and enumeration of FIB, namely total coliforms, *E. coli* and enterococci. Samples were suspended in sterile deionised water (1:9 w/vol), vortexed briefly and shaken in an end-over-end shaker for 30 min. Following serial dilution, most probable numbers (MPN) of total coliforms and *Escherichia coli* were quantified using IDEXX Colisure with Quanti-Tray/2000 incubated at 35 °C for 24 h. MPN of enterococci were determined using IDEXX Enterolert kit with Quanti-Tray/2000 incubated at 41 °C for 24 h (Table 4).

2.4. Treatment characterisation

Five treatments were examined in this study: untreated controls; dairy cattle slurry (DCS); and three types of AD digestate, namely unpasteurised (P0); pasteurised for 1 h at 70 °C (P1) and; pasteurised, 96 h at 60 °C (P2). All digestates were sourced from the same semi-continuously fed, mesophilic, continuously stirred tank bioreactors, which were co-digesting DCS with waste from a food processing facility (FW). DCS was collected from a dairy farm in Co. Galway, Ireland, following mechanical agitation of the underground slurry tank. Fresh DCS and digestates were collected in sealed, 10 L-capacity plastic storage containers and transported to the field site location where they were briefly stored at 4 °C prior to application.

Dry matter was determined by drying fresh samples in an oven at 105 °C for 24 h, after which samples were placed at 550 °C for 2 h in a furnace to determine organic matter (loss on ignition). Treatment pH was determined with a pH meter (Mettler-Toledo Inlab Routine). Following freeze drying and microwave-assisted acid digestion (US EPA, 1996), samples ($n = 3$) from the four treatments were analysed for concentrations of nutrients (phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), sodium (Na), and calcium (Ca)), and metals (arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), aluminium (Al), iron (Fe), cobalt (Co), molybdenum (Mo) and manganese (Mn)) using an Agilent 5100 synchronous vertical dual view inductively coupled plasma optical emission spectrometer (Agilent 5100 ICP-OES). Freeze dried samples were also analysed for TC and N using a LECO TruSpec CN analyser. The FIB numbers in the



Fig. 1. Depiction of experimental set-up, using micro-plots (40 × 90 cm) to which different treatments (slurry or digestate) were applied before plots were subjected to a number of rainfall simulation events, where specialized frames allowed collection of runoff water.

four treatments were determined as outlined for soil (Table 1). Prior to application, all treatments were thoroughly mixed to re-suspend solids.

2.5. Treatment application and replication in micro-plots

The five treatments (untreated control, DCS and three digestates; P0, P1 and P2), used in this study were each replicated four times (5×4) through assignment to 20 micro-plots. These were divided into four 'replicate blocks' each containing five micro-plots to which one of the five treatments was randomly assigned. To aid logistics of rainfall simulation, sample collection and processing, replication was performed over time, with one week between the application of treatments to each of the four treatment blocks.

Application of DCS and digestate to the micro-plots was governed by the P content of the treatments and the P index of the soil. For comparable results, all micro-plots were classified into Index 2 P soil, which meant that all treatments were applied to all plots at a rate of 40 kg P ha^{-1} (Wall and Plunkett, 2016). As a result of the P content (highest in P2 treatment) and the DM of each individual digestate, application rates per individual plot were 1644 g of P0, 1547 g of P1 and 1440 g of P2. The DCS was spread at 3830 g per individual plot.

DCS and digestate were surface applied in rows to each micro-plot using a watering can to replicate normal trailing shoe application (Fig. 1). To ensure even distribution, each micro-plot was divided into four quadrants (each 0.09 m^2 in area) and a proportionate amount of treatment was applied in each quadrant.

2.6. Rainfall event simulation and application

As replication was performed over time (one week between each), 'rainout' shelters and a rainfall simulator were used to ensure each replicate run received the same rainfall (Fig. 1). This also allowed regulated runoff from the plots for comparative assessment of nutrient, metal and FIB load in runoff. In order to simulate rainfall events with controlled intensity and duration, an Amsterdam drip-type rainfall simulator, similar to that described by Bowyer-Bower and Burt (1989) was used, with the addition of wheels for easier movement. It was designed to form droplets with a median diameter of 2.3 mm, spaced 30 mm apart in a $1000 \text{ mm} \times 500 \text{ mm} \times 8 \text{ mm}$ Perspex plate over a 0.5 m^2 simulator area. The rainfall simulator was calibrated to deliver a rainfall intensity of 11 mm hr^{-1} , as was the case in other studies such as Peyton et al. (2016).

For better control of rainfall simulations and to prevent runoff losses caused by natural rainfall events, individual micro-plots were covered from the time of treatment application to the end of the third rainfall event by 'rainout' shelters (large plastic shelters on steel frames that prevent direct rainfall onto soil, while allowing air circulation). The first rainfall simulation event (RS1) occurred 24 h after treatment application, so as to demonstrate losses representative of a worst-case breach of regulations which stipulate that spreading of organic manure should not be carried out within 48 h of forecast heavy rain. The second rainfall event (RS2) was performed 48 h after initial application, which was representative of current legislation, the third (RS3) after 15 days and the fourth (RS4) 30 days after initial application, representing normal animal exclusion time from treated fields.

Volumetric moisture content (MC) of the soil in each plot ($n = 3$) was measured immediately prior to and after each rainfall event using a time domain reflectometry device (Delta-T Devices Ltd., Cambridge, UK), which was calibrated to measure resistivity in the upper 50 mm of the soil in each plot.

2.7. Runoff sample collection and analysis

Surface runoff was deemed to occur once 50 mL of water was collected in the sample collection cup. The collection of the first 50 mL ($t = 0$) was used to indicate time to runoff (TR), and was used for part of the microbial

analysis. Samples for nutrient and metal analysis were collected every 10 min ($t = 10, t = 20, t = 30$) from TR to allow for the flow weighted mean concentration (FWMC) to be calculated (Brennan et al., 2012; Peyton et al., 2016). Following this, another 50 mL of surface runoff water was collected so that it could be combined with the first 50 mL of runoff to create a 100 mL composite sample for microbial analysis. The rainfall simulator was then switched off and a final sample was collected until no runoff occurred, to determine the final runoff ratio. Immediately after collection, all samples were stored in cool boxes with ice until they were returned to the laboratory for analysis.

The 100 mL composite samples designated for FIB analysis were serially diluted with sterile water and analysed using kits as described for soil (Section 2.3). An aliquot of each runoff water sample was filtered through $0.45 \mu\text{m}$ filter paper and a sub-sample was analysed calorimetrically for P, nitrite ($\text{NO}_2^- \text{N}$), dissolved organic N (DON) and ammonium ($\text{NH}_4^+ \text{N}$) using a nutrient analyser (Aquachem Labmedics Analytics, Thermo Clinical Labsystems, Finland). Unfiltered runoff water samples were analysed for total P with an acid persulphate digestion as well as P, total C, total N and total organic carbon (TOC) using the Aquachem Analyser. Metal and nutrient analysis (Al, Ca, Cd, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) was carried out on the filtered samples using inductively coupled plasma optical emission spectroscopy (ICP-OES). All samples were tested in accordance with the Standard Methods (APHA, 2005).

2.8. Grass sample collection and analysis

Prior to treatment application, the grass on all micro-plots was cut to 50 mm. Nitrile gloves were worn during sample collection, and were changed between plots to avoid cross-contamination. Thereafter, grass was collected on days 14, 30, 57, 85 and 112 to determine yield as well as metals and nutrient uptake. Collected grass was weighed, then dried in an oven at 60°C for 48 h to determine solids content. Dried samples were ground and analysed using energy-dispersive X-ray fluorescence (EDXRF) spectroscopy as described by Daly and Fenelon (2017), using a Rigaku NEX CG EDXRF spectrometer equipped with a nine-place sample changer with spin function using slow and steady spinning mode. Grass samples were analysed for % N, P, K, S, Ca, Na, Mg, as well as mg/kg of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, and Zn using EDXRF as described by (Daly and Fenelon, 2017, 2018). As higher levels of Mo were detected as function of some treatments, the ratio of Cu:Mo was calculated in order to determine if these levels were toxic to animals.

2.9. Data analysis

The data was a blocked one-way classification (5 treatments) with repeated measures over the course of experimental time (corresponding to rainfall simulation events for runoff and soil data) (Peyton et al., 2016). Variables measured for each sample type collected, and those grouped together for statistical analyses and graphical representation are detailed in Supplementary Table 2. Data were tested for normality (Shapiro Wilk test), and some variables were identified as non-normally distributed. Therefore, for each dataset a Friedman test (repeated measures for non-parametric data) was performed, to determine if differences were seen as a function of treatment. If differences were seen, then for each variable, at each sample day, statistical differences in means as a function of treatment were tested using the non-parametric Kruskal-Wallis test. Where statistical differences were seen ($p < 0.01$), a post-hoc test was performed using Fisher's Least Significant Difference (LSD). Where multiple variables were presented on the same figure, p -values were corrected for multiple comparisons using the false discovery rate (FDR) approach. Statistical tests were performed as implemented in the agricolae package (de Mendiburu, 2020), while data was plotted using ggplot2 (Wickham, 2016) in R (R Core Team, 2019). Points on all plots represent the mean of each variable

Table 1

FIB ($\log_{10}$ cfu/g), organic matter, N, P, K and S analysis of four treatments, with standard error ($n = 4$). For each variable, shared letters denote no difference while different letters denote statistically significant differences ($p < 0.05$) as a function of treatment.

	Coliforms $\log_{10}$ cfu/g	<i>E. coli</i> $\log_{10}$ cfu/g	Enterococci $\log_{10}$ cfu/g	OM %	N %	P %	K %	S %
Slurry	6.7 $\pm$ 0.57 ^a	6.6 $\pm$ 0.61 ^a	6.0 $\pm$ 0.11 ^a	8.61 $\pm$ 0.3 ^a	2.53 $\pm$ 0.05 ^a	0.4 $\pm$ 0.01 ^a	6.12 $\pm$ 0.05 ^a	0.47 $\pm$ 0.01 ^a
Digestate P0	3.2 $\pm$ 0.76 ^b	2.8 $\pm$ 1.73 ^b	3.3 $\pm$ 0.13 ^b	6.41 $\pm$ 0.04 ^b	2.62 $\pm$ 0.1 ^a	1.34 $\pm$ 0.16 ^b	3.39 $\pm$ 0.5 ^b	0.60 $\pm$ 0.02 ^b
Digestate P1	0.5 $\pm$ 0.50 ^c	0.0 $\pm$ 0.00 ^c	2.8 $\pm$ 0.59 ^c	6.35 $\pm$ 0.08 ^b	2.48 $\pm$ 0.02 ^a	1.53 $\pm$ 0.01 ^c	2.87 $\pm$ 0.02 ^b	0.63 $\pm$ 0.01 ^c
Digestate P2	0.0 $\pm$ 0.00 ^c	0.0 $\pm$ 0.00 ^c	1.7 $\pm$ 1.18 ^d	6.26 $\pm$ 0.07 ^c	2.35 $\pm$ 0.08 ^a	1.47 $\pm$ 0.01 ^b	2.75 $\pm$ 0.01 ^c	0.62 $\pm$ 0.01 ^b

over the 4 repeated rainfall simulations performed and error bars show standard error of the mean ($n = 4$). Letters within the points illustrate statistical differences as a function of treatments at a given time point, where shared letters denote no difference ($p > 0.05$), and unshared letters denote a statistical difference within a group ($p < 0.05$).

3. Results and discussion

3.1. Microbial load of the four organic amendments tested

The unprocessed DCS contained significantly higher numbers of all three FIB tested prior to application (Table 1). Processing in AD without pasteurisation resulted in $>4 \log_{10}$ reduction of coliforms and *E. coli*, as well as a $2 \log_{10}$ reduction in enterococci numbers. The results above are in line with previously reported sanitization effects of AD on slurry (Nag et al., 2019; Sahlström, 2003). This reduction in FIB partially results from a dilution effect, whereby slurry with high FIB is mixed with FW with low FIB, and then fed into an anaerobic digester with low background FIB numbers, but this does not account for the total FIB decrease (Nolan et al., 2018). Furthermore, studies of pathogen survival in mesophilic anaerobic digestion, have consistently identified a significant sanitization effect beyond that of dilution, attributed to free volatile fatty acid and free ammonia concentration and competition for resources (Jiang et al., 2020; Nolan et al., 2018; Sahlström, 2003; Smith et al., 2005; Zhao and Liu, 2019). Protozoan grazing and fungal antimicrobial activity are also factors that have not received much attention (Avery et al., 2014), while the major role played by bacteriophage in other bacterial spheres makes it a good candidate for further research with a view to manipulation for improving sanitization effect. However, the extent of pathogen inactivation is affected by a variety of physical factors as outlined previously, and hence a precautionary pasteurisation step has been deemed necessary. Pasteurisation at both the EU and Irish standards (P1 and P2 respectively) resulted in a further reduction of coliforms and *E. coli* below the limit of detection (100 cfu g^{-1}) with no significant difference between pasteurisation conditions. Enterococci were reduced by both pasteurisation conditions below the 1000 cfu g^{-1} required for landspreading, but proved to be more resilient to pasteurisation than *E. coli*, and hence a more conservative indicator bacteria where pathogen persistence is a concern, as previously highlighted in the literature (Nolan et al., 2018; Sahlström, 2003).

3.2. Microbial load in runoff and persistence in soil

At every time point post-application (24 h and 2, 15 and 30 days), all FIB tested were highest from the plots treated with DCS (Fig. 2). Coliform and *E. coli* numbers were significantly higher in runoff from slurry treated plots ($6.43 \pm \log_{10}$ per 100 mL) than plots treated with pasteurised digestate (both conditions) and untreated controls after 24 h. Coliform numbers in runoff from rainfall simulation 24 h post-treatment displayed a lower trend for unpasteurised digestate (P0) than slurry-amended plots. High mobilization of FIB during the first rainfall event may be attributed to solubilization of unattached cells in the organic amendments (Chadwick et al., 2008), and likely would

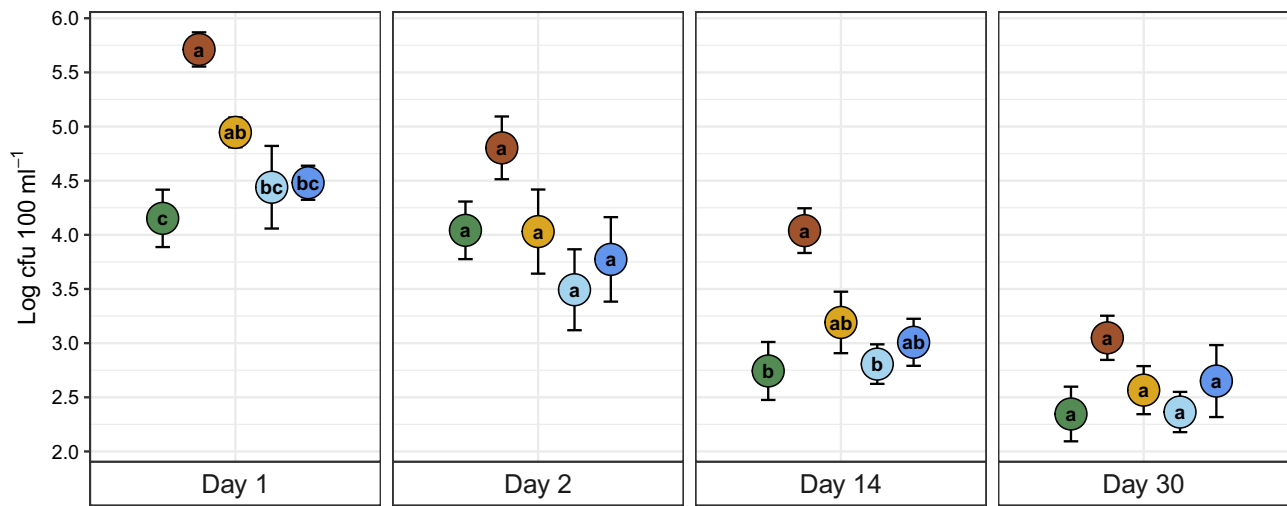
have been lower had FIB been exposed to sunlight for more than one day (Oladeinde et al., 2014). By the second rainfall simulation (2 days post-application), coliform and *E. coli* numbers in all digestate treatments aligned with the untreated controls, while *E. coli* numbers from DCS-treated plots were significantly higher. These results agree with those observed by (Peyton et al., 2016), who reported more resilient coliform survival in DCS than in biosolids, resulting in higher numbers in runoff from rainfall simulations 15 days post application. Although *E. coli* numbers in runoff declined steadily over time, by Day 30 runoff from slurry-treated plots still contained higher *E. coli* numbers than those recorded from untreated controls after the first rainfall simulation (465 versus 188 cfu per 100 mL). Background coliform and *E. coli* in the untreated control plots decreased over time, indicating that heavy rainfall events could wash out residual low-level indicator organisms.

The difference between treatments was most stark for enterococci (Fig. 2, panel 3), which were significantly higher ($2\text{--}3 \log_{10} \text{ cfu } 100 \text{ mL}^{-1}$) in runoff from slurry-treated plots than all other treatments, which would be expected given the $>2 \log_{10}$ higher starting numbers in slurry (Table 1). Amendment with digestate (pasteurised or unpasteurised) did not increase enterococci levels in runoff above those recorded for the untreated controls. The high ($>5 \log_{10} \text{ cfu } 100 \text{ mL}^{-1}$) enterococci numbers observed in runoff from Day 1 rainfall simulation highlights the importance of adherence to regulations around proximity of landspreading with forecast heavy rainfall to prevent incidental losses (Chadwick et al., 2008). Furthermore, the elevated enterococci numbers in Day 30 runoff from slurry-treated plots indicate detachment of residual FIB (Tyrrel and Quinton, 2003), and the prolonged risk requiring remedial action to reduce agricultural impact on the environment through contamination of watercourses resulting from landspreading of raw slurry. Processing slurry in AD would significantly reduce the risk of microbial pollution of watercourses, while pasteurisation may further reduce that risk for bacteria susceptible to heat treatment.

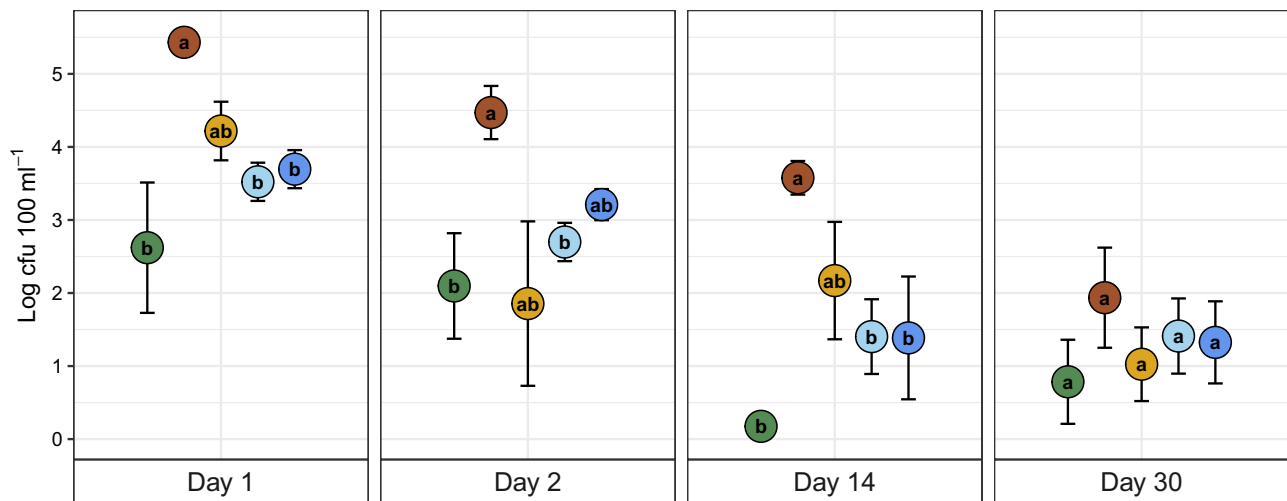
Soil samples were taken prior to application of the treatments, as well as 14 days and 30 days after application. Background FIB numbers in the soil prior to application were higher in some plots, affecting the significance of the results. This was possibly due to historical slurry spreading, as *E. coli* have been found to persist in soil for more than nine years following a single manure application (Brennan et al., 2010). Despite this variability, soil from slurry-amended plots had higher *E. coli* numbers than the digestate-amended plots (pasteurised and unpasteurised: Supplementary Fig. 2 panel B). After 14 days the number of coliforms in the soil was $2 \log_{10} \text{ cfu g}^{-1}$ higher in slurry-treated soil compared with untreated controls, and $1 \log_{10}$ higher than soil amended with digestate (Supplementary Fig. 2 panel A). There was no significant difference in coliform or *E. coli* levels in soil treated with unpasteurised or pasteurised digestate after 14 days. By Day 30, plots treated with unpasteurised and pasteurised digestate had similar faecal coliform numbers to untreated plots, while slurry-treated plots had at least $1 \log_{10} \text{ cfu g}^{-1}$ higher survival (Table 3). However, modeling of *E. coli* survival after landspreading of DCS indicates that an additional 10 days (40 day exclusion) would be sufficient to reduce risk to grazing animals (Ashkuzzaman et al., 2018). The results support the findings of Goberna et al. (2011), who similarly observed lower *E. coli* numbers in

● Untreated control ● Slurry (DCS) ● Digestate P0 (Unpasteurised) ● Digestate P1 (Pasteurised 1hr 70°C) ● Digestate P2 (Pasteurised 96hr 60°C)

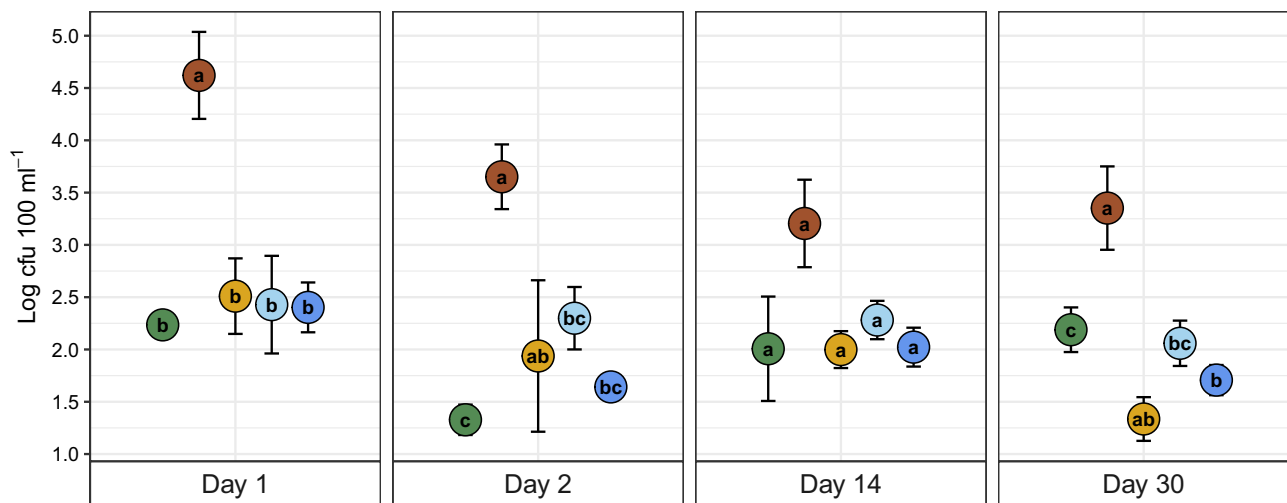
Coliforms



E. coli



Enterococci



Day post application

soil treated with digestate than manure 30 days after application, which they attributed to the activity of the indigenous soil microbial community. The results may however also indicate that coliforms and *E. coli* did not infiltrate the soil and were predominantly washed off in runoff during the rainfall simulations. As the main purpose of this study was the runoff pathway, the slope was required. If flat land and well-drained soil were used for the trial the DCS in particular would likely have remained on the surface (due to high solids). In that case as surface application was used, exposure to ultraviolet radiation would likely have reduced FIB numbers in the treatments (Oladeinde et al., 2014), with similar studies finding a decline to background numbers within 17 days (Hodgson et al., 2016).

Enterococci in the soil were higher for slurry and unpasteurised digestate-treated soils on Day 14 than on Day 0, and lower for both pasteurised digestate treatments (Supplementary Fig. 2 panel C). By Day 30 however, enterococci numbers in soil treated with unpasteurised digestate were similar to those for pasteurised digestate and untreated controls. Slurry application resulted in a steady increase in soil-borne enterococci over time, so that by Day 30 enterococci numbers remained higher than all other treatments (Table 3). Desiccation and ultraviolet radiation are the primary factors in microbial survival following landspreading (Lu et al., 2012) and as each plot was subjected to similarly intensive rainfall, the likely differentiating factor contributing to the observed higher survival in DCS is that higher fibrous solids shielded bacteria from UV radiation (Table 1 and Fig. 1),

3.3. Fate of metals and nutrients – soil and runoff concentrations

As hypothesised, nutrient and metal concentrations were found to be lower in runoff and soil following application of unpasteurised and pasteurised digestate (2 conditions) from co-digestion of slurry with FW compared with unprocessed slurry. Application of DCS or digestate (all pasteurisation conditions) did not result in samples exceeding metal limit values in soil tested 14 and 30 days post-application (EU Directive 86/278/EEC), with no substantial differences in soil concentrations observed in that timeframe (Supplementary Table 1). These results are in line with a recent examination of metal accumulation in grassland soils amended with wastewater treatment sludge (Ashkuzzaman et al., 2019). Some metals are required in the AD process (Fermoso et al., 2015: Ca, Co, Cu, Fe, Mo), and the reduced levels of some of those (Ca, Cu, Fe, Mo) found in the digestate used in this trial compared with DCS indicate a reduced risk of accumulation of those elements in the environment following landspreading of digestate compared with slurry. A recent study of cattle slurry and digestate from four full scale Irish AD plants recorded results of elemental analysis over two years and similarly found reduced Cu levels in the AD plant co-digesting slurry with food waste, but no significant difference in Ca or Fe between digestate from that AD plant and slurry (Coelho et al., 2020). Similarly to Coelho et al. (2020) however, some elements (Al, Cr, Ni, Pb, S, Zn) had higher levels in the digestate used in this study compared with DCS, and although there was no significant soil accumulation compared with controls in this trial, elevated Cd, Cr, Ni and Pb soil results from other studies indicate that it may be necessary to incorporate metals into the analysis suite for regular (5 year) soil tests (Dragicevic et al., 2018; Tang et al., 2020). This may be particularly necessary for digestate from plants co-digesting municipal wastewater treatment sludge with food waste, given the comparatively high results for Al, Ca, Cr, Cu, Fe, Pb and Zn reported by Coelho et al. (2020).

Iron (Fe; $200 \mu\text{g L}^{-1}$) and manganese (Mn; $50 \mu\text{g L}^{-1}$) in runoff samples from DCS, P0 and P2 exceeded EU drinking water limits (Council Directive 98/83/EC; Statutory Instrument, 2014) during the first rainfall simulation. However, landspreading within 24 h of forecast heavy rain constitutes a breach of best practice, and hence an uncommon, worst-

case scenario. Fe and Mn limits were again exceeded only in runoff from DCS during RS2 (48 h post-application), while metals in runoff from all other treatments satisfied maximum acceptable concentrations (MACs) or environmental quality standards (EQS) established by regulations such as the Drinking Water Directive and Surface Waters Regulations (EC, 1998; EPA, 2001; EU, 2013; S.I. No. 272/2009; Table 5). Although Peyton et al. (2016) observed no breach of metal limits in runoff from DCS-amended plots, they did not report Fe or Mn results.

Slurry application resulted in significantly elevated Mn in runoff for 15 days post-treatment. Mn in runoff from digestate-amended plots (all pasteurisation conditions) was higher than untreated controls at RS1 (1 day post application), but had returned to the same level as untreated controls 2 days post application (RS2). Digestate application resulted in lower Mn runoff than slurry (69% lower for RS1 and 91% lower for RS2), and on day 14 (RS3) Mn concentrations in runoff from slurry treated plots were still 4 times higher than those from digestate treated and untreated control plots (4.7 ± 2 vs $1.1 \pm 0.5 \mu\text{g L}^{-1}$). Despite breaching EU limits during RS1 and RS2 (DCS), Mn concentrations from all treatments did not come close to WHO health-based values ($400 \mu\text{g L}^{-1}$) at any point throughout the trial (WHO, 2006). Although a 2018 survey identified 73 of 104 countries with tighter Mn standards than the WHO, this significant discrepancy may be cause for re-evaluating WHO standards, particularly in agricultural regions where landspreading of unprocessed slurry is practiced, given the breaches observed in the present work (WHO, 2018).

Application of all organic amendments resulted in higher Zn in runoff during RS1 (1 day post application) compared with the untreated control (Fig. 3). The Zn runoff from the slurry-treated plots remained elevated during RS2 (2 days post application) compared with all digestate treatments (21 ± 9 vs $5-8 \pm 0.86 \mu\text{g L}^{-1}$). By Day 30 (RS4), Zn runoff from all treatments had reached background levels seen in the untreated control plots (Fig. 3).

Concentrations of Mg and Ca in runoff were higher from slurry than digestate or untreated controls during RS1, but there was no significant difference thereafter (Fig. 3). Na runoff was higher for all organic amendments during RS1, but returned to the same level as the untreated controls for digestate (P1) by RS2, while slurry-associated Na remained higher in RS2 and RS3. By Day 30 Na concentration in runoff was still significantly higher for slurry, although maximum acceptable concentrations (MAC) were not exceeded from any treatment throughout the trial. Although higher Al was detected in runoff from unpasteurised digestate plots than for slurry and untreated control plots, the results were skewed significantly by high readings in one of the four treatment blocks, as evidenced by the large error bars. This may be associated with the ~15% higher than average Al concentration in soil from that plot prior to treatment application (15.2 ± 0.29 vs 17.7 g/kg). By 14 days post application (RS3), only runoff from slurry-amended plots contained significantly elevated Al concentrations, and by Day 30 all treatments had fallen to the same level seen in runoff from untreated control levels (Fig. 3). No organic fertilizer used in this trial resulted in Al runoff concentrations exceeding typical limits (WHO, 2018; $200 \mu\text{g L}^{-1}$).

For the elements detected in higher concentrations in digestates used for plot amendments, namely Al ($3\times$), Cr ($>2\times$), Ni ($1.5\times$) and Zn ($1.5\times$), digestate application resulted in levels of these elements similar to that seen in runoff from DCS at RS1 (1 day after application; Table 2). However, while they remained relatively high in DCS-derived runoff, their levels fell rapidly in runoff from digestate (P0, P1 and P2) treated plots (Fig. 3). Peyton et al. (2016) also reported higher runoff of Cr from DCS than from digestate, similarly peaking in runoff during RS1, although their reported peak doubled that of the present work (3.89 vs $1.6 \mu\text{g L}^{-1}$). DCS application resulted in significantly

Fig. 2. Faecal indicator bacteria ($\log_{10} \text{cfu mL}^{-1}$) in runoff from five treatments from rainfall simulation 1, 2, 14 and 30 days after application. Error bars indicate standard error of the mean ($n = 4$). Numbers of Coliforms, *E. coli* and Enterococci detected in runoff ($\log_{10} \text{cfu/mL}^{-1}$). Statistically significant differences ($p < 0.05$) are represented below each panel, where coloured dots correspond to the treatment against which differences in FIB were significant.

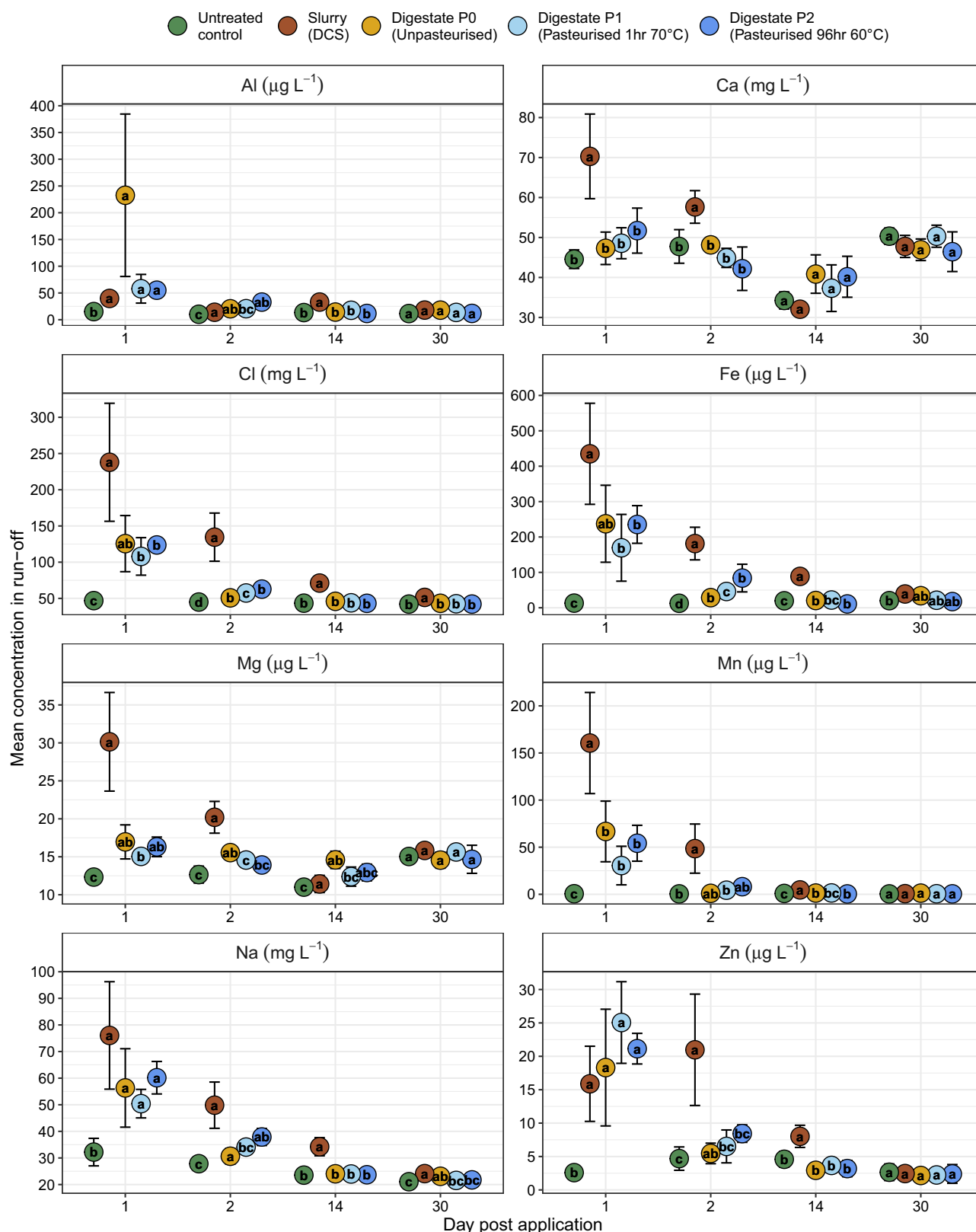


Fig. 3. Al, Fe, Mg, Mn, Zn ($\mu\text{g L}^{-1}$) and Ca, Cl, Na (mg/L) following five treatments (T1 = untreated control (no organic amendment); T2 = DCS; T3 = unpasteurised digestate; T4 = pasteurised digestate (70 °C); T5 = pasteurised digestate (60 °C)) from rainfall simulation 1, 2, 14 and 30 days after application. Error bars indicate standard error of the mean (n = 4).

higher and sustained runoff of Na, Mn, Mg, Cl, Fe, Ca, than application of digestate with or without pasteurisation. By Day 14 concentrations of metals and nutrients in runoff from all treatments began to equilibrate (with DCS still highest), and by Day 30 there was no significant

difference between treatments with the exception of higher Fe from DCS. Dry matter content has been identified as the main determining factor affecting infiltration of slurry into soil (Misselbrook et al., 2006). The reduction of dry matter by anaerobic digestion (Table 1) logically

Table 2

Nutrient and metal analysis of four treatments, with standard error ($n = 4$). For each element, shared letters denote no difference while different letters denote statistically significant differences ($p < 0.05$) as a function of treatment.

	Al	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Mo	Ni	Pb	Zn
	g/kg	%	mg/kg	mg/kg	mg/kg	mg/kg	g/kg	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Slurry	4.1 ± 0.1 ^a	4.6 ± 0.07 ^a	0.37 ± 0.02 ^a	1.39 ± 0.06 ^a	9.8 ± 0.90 ^a	106 ± 1.3 ^a	3.74 ± 0.3 ^a	0.69 ± 0.01 ^a	249 ± 2.6 ^a	9.49 ± 0.15 ^a	9.66 ± 1.0 ^a	3.01 ± 0.05 ^a	106 ± 1.3 ^a
Digestate P0	11.6 ± 1.3 ^b	4.0 ± 0.06 ^b	0.47 ± 0.04 ^a	1.92 ± 0.12 ^a	23.2 ± 2.60 ^b	70.7 ± 5.8 ^b	3.08 ± 0.1 ^a	0.74 ± 0.01 ^b	245 ± 0.48 ^a	7.77 ± 0.28 ^b	15.1 ± 1.2 ^b	4.12 ± 0.09 ^b	143 ± 6.2 ^{bc}
Digestate P1	12.9 ± 0.1 ^b	4.0 ± 0.02 ^b	0.51 ± 0.02 ^a	2.03 ± 0.04 ^a	26.5 ± 0.17 ^c	65.0 ± 0.5 ^b	3.17 ± 0.01 ^a	0.75 ± 0.01 ^b	246 ± 0.78 ^b	7.48 ± 0.02 ^b	16.9 ± 0.16 ^c	4.11 ± 0.15 ^a	150 ± 0.67 ^c
Digestate P2	12.7 ± 0.1 ^{ab}	3.8 ± 0.03 ^c	0.49 ± 0.01 ^a	1.98 ± 0.02 ^a	24.6 ± 0.02 ^b	62.6 ± 0.2 ^c	3.04 ± 0.01 ^a	0.71 ± 0.01 ^a	235 ± 0.66 ^{ab}	7.18 ± 0.05 ^c	15.6 ± 0.19 ^b	3.86 ± 0.09 ^{ab}	145 ± 1.1 ^b

Table 3

FIB averages ($\log_{10}$ cfu g^{-1}) in soil pre-application of organic fertilizers, 14 days post-application and 30 days post-application ($n = 4$). For each FIB, on the 3 different sample days, shared letters denote no difference while different letters denote statistically significant differences ($p < 0.05$) as a function of treatment.

Day	Coliforms			<i>E. coli</i>			Enterococci		
	0	14	30	0	14	30	0	14	30
Untreated Ctrl	2.7 ± 1.06 ^a	1.6 ± 0.22 ^a	1.9 ± 0.52 ^a	1.5 ± 0.91 ^a	0.7 ± 0.24 ^a	0.3 ± 0.25 ^a	3.2 ± 0.20 ^a	3.5 ± 0.21 ^a	3.1 ± 0.22 ^a
Slurry (DCS)	3.8 ± 0.50 ^a	2.7 ± 0.28 ^a	3.1 ± 0.42 ^a	2.2 ± 0.80 ^a	2.4 ± 0.17 ^{ab}	2.2 ± 0.22 ^{ab}	3.1 ± 0.24 ^a	3.4 ± 0.07 ^a	3.5 ± 0.14 ^a
Digestate (P0)	4.1 ± 0.28 ^a	1.8 ± 0.47 ^a	1.7 ± 0.62 ^a	2.5 ± 0.93 ^a	1.5 ± 0.36 ^{ab}	1.1 ± 0.59 ^{ab}	3.5 ± 0.20 ^a	3.6 ± 0.28 ^a	3.1 ± 0.17 ^a
Digestate (P1)	3.5 ± 0.61 ^a	1.7 ± 0.55 ^a	2.0 ± 0.61 ^a	2.8 ± 0.60 ^a	0.8 ± 0.55 ^b	0.3 ± 0.25 ^b	3.6 ± 0.22 ^a	3.3 ± 0.09 ^a	3.1 ± 0.04 ^a
Digestate (P2)	3.7 ± 0.49 ^a	1.7 ± 0.30 ^a	2.1 ± 0.56 ^a	1.7 ± 0.55 ^a	1.5 ± 0.21 ^b	0.9 ± 0.33 ^b	3.5 ± 0.18 ^a	3.2 ± 0.20 ^a	3.0 ± 0.17 ^a

facilitates improved infiltration, reducing risk of surface runoff. Although this increased infiltration did not result in statistically significant differences in soil metal concentrations in this study, repeated application may result in accumulation and necessitates regular soil testing, as previously discussed (Nkoa, 2014).

DCS contained 17.5, 18.8 and 15.3% more Fe than unpasteurised, P1 and P2 digestates, respectively (Table 2), yet runoff from DCS-amended plots had twice as much Fe than digestate-amended plots at RS1, 3.4 times more at RS2 and 5 times more at RS3 (88.63 ± 20.4 vs $17.7 \pm 3.3 \mu g L^{-1}$) (Fig. 3). By Day 15 there was no difference between digestate (all pasteurisation conditions) and untreated controls. By Day 30 there were no significant differences between organic amendments and untreated controls (Fig. 3). A study examining alum amendment of poultry litter for reduction of incidental P losses observed an attendant significant reduction of Fe in runoff (Moore et al., 1998). The elevated levels of Al in digestate may be in part responsible for the significantly lower Fe in runoff observed from digestate in these trials compared with DCS.

Table 4

C, N, P, K and pH data of soil prior to (Day 0) and following application of DCS, unpasteurised digestate (P0) and pasteurised digestate (two conditions), ± standard error of the mean ($n = 4$). No statistically significant differences were seen as a function of treatment for any variable on any testing day.

Day	Tmt	C %	N %	P mg/kg	K g/kg	pH
0	Ctrl	3.47 ± 0.15	0.330 ± 0.02	639 ± 22.6	2.44 ± 0.49	5.55 ± 0.02
	DCS	3.68 ± 0.08	0.366 ± 0.01	570 ± 58.0	2.21 ± 0.31	5.51 ± 0.02
	P0	3.52 ± 0.20	0.317 ± 0.02	603 ± 63.3	2.47 ± 0.40	5.54 ± 0.07
	P1	3.64 ± 0.22	0.349 ± 0.02	575 ± 41.3	2.22 ± 0.27	5.49 ± 0.05
	P2	3.68 ± 0.05	0.340 ± 0.00	567 ± 27.8	2.60 ± 0.30	5.54 ± 0.05
14	Ctrl	3.63 ± 0.11	0.348 ± 0.01	634 ± 34.1	2.50 ± 0.26	5.70 ± 0.06
	DCS	3.56 ± 0.18	0.339 ± 0.02	605 ± 45.5	2.76 ± 0.30	5.74 ± 0.06
	P0	3.76 ± 0.24	0.349 ± 0.03	603 ± 49.4	2.55 ± 0.28	5.77 ± 0.08
	P1	3.68 ± 0.14	0.350 ± 0.02	616 ± 37.5	2.51 ± 0.50	5.71 ± 0.08
	P2	3.63 ± 0.16	0.338 ± 0.02	620 ± 34.5	2.80 ± 0.30	5.73 ± 0.10
30	Ctrl	3.69 ± 0.22	0.347 ± 0.02	601 ± 37.8	2.21 ± 0.21	5.75 ± 0.05
	DCS	3.72 ± 0.10	0.346 ± 0.02	582 ± 49.4	2.47 ± 0.42	5.84 ± 0.06
	P0	3.78 ± 0.34	0.344 ± 0.03	598 ± 70.0	2.36 ± 0.28	5.86 ± 0.03
	P1	3.56 ± 0.15	0.337 ± 0.02	601 ± 51.0	2.51 ± 0.27	5.83 ± 0.06
	P2	3.47 ± 0.20	0.311 ± 0.01	560 ± 22.7	2.40 ± 0.42	5.88 ± 0.11

Elemental analysis of runoff from the five treatments showed no significant differences between any treatments for Cd, Cr or Pb in the runoff and these data are therefore not shown. The effect of mandatory digestate pasteurisation on nutrient and metal runoff potential has not been considered in the literature, and the present work found no significant impact on nutrient and metal runoff.

Incidental P losses account for between 50 and 90% of all P losses from soil to water (Withers et al., 2003) and occur when slurry is spread in close proximity to a heavy rainfall event, allowing insufficient time for slurry to infiltrate soil (Brennan et al., 2011). Digestate (all pasteurisation conditions) resulted in lower incidental P losses than slurry in runoff from all rainfall simulations (Fig. 4), despite having significantly higher P in the starting substrate ($3\times$ higher; Table 2). TP in runoff from digestate for RS1 was on average 25% lower than slurry, 28% lower for RS2 and 47% lower for RS3. Two factors may account for the lower incidental P losses from digestate; firstly, the faster assimilation of P into the soil, due to the lower solids and viscosity, is visible in the soil test results from Day 14 (Table 4), where soils treated with digestate have 32% higher P, compared with 25% for slurry and – 11% for untreated controls. A second factor may be the higher levels of Al in digestate (Table 2). Al (in alum form) has been used as a slurry amendment to form stable Al-P precipitates (Brennan et al., 2011), reducing P solubility and by extension, incidental P losses, particularly in soils with high background P (Kalbasi and Karthikeyan, 2004). Additional amendment of digestate with alum may further reduce incidental P losses. As with Peyton et al. (2016), the concentration of nutrients in runoff decreased across successive rainfall events (Fig. 4).

3.4. Grass yield and elemental accumulation following application of slurry compared with unpasteurised and pasteurised digestate

Grass yield was significantly higher for all treated plots than the untreated controls (Fig. 5), with the treatment effect still evident 85 days post-treatment application. By Day 112, grass yields across treatments were no longer significantly different to untreated controls, although grass growth generally had by then declined. Yields at Day 14 post-application suggest that all three digestates supported a more rapid growth response than DCS, with average yield of $814 \text{ kg DM ha}^{-1}$ from digestates comparing favourably with 662 and $480 \text{ kg DM ha}^{-1}$

Table 5

Summary of FIB, metal and nutrient concentrations in water, soil and grass following five treatments (T1 = untreated control (no organic amendment); T2 = slurry; T3 = unpasteurised digestate; T4 = pasteurised digestate (70°C); T5 = pasteurised digestate (60°C)).

		Untreated Controls				Slurry DCS				Digestate P0				Digestate P1				Digestate P2					
Rainfall Sim		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4		
FIB ^e	Coliforms	✗	✗	✓	✓	✗	✗	✗	✗	✗	✗	✓	✓	✗	✗	✓	✓	✗	✗	✓	✓		
	<i>E. coli</i>	✓	✓	✓	✓	✗	✗	✗	✗	✗	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	✓		
	Enterococci	✓	✓	✓	✓	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Nutrients	N ^a	✓	✗	✓	✗	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✗	✓		
	N-NO ₂ ^d	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	N-NH ₄ ^d	✓	✓	✓	✓	✗	✗	✗	✓	✗	✗	✓	✓	✗	✗	✓	✓	✗	✗	✓	✓		
	TP	✓	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	✓		
	TOC ^b	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Elements/Metals	Al ^d	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	Cd ^c	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	Cl ^d	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	Cr ^d	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	Cu ^d	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	Fe ^d	✓	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓		
	Mn ^d	✓	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓		
	Na ^d	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	Ni ^{c (i)}	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
	Pb ^c	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Zn	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
		Untreated Controls				Slurry DCS				Digestate P0				Digestate P1				Digestate P2					
Soil	Day	0	14	30		0	14	30		0	14	30		0	14	30		0	14	30			
	Cd	✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓			
	Cu	✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓			
	Ni	✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓			
	Pb	✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓			
	Zn	✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓			
		Untreated Controls				Slurry DCS				Digestate P0				Digestate P1				Digestate P2					
Grass	Day	0	14	30	57	85	112			0	14	30	57	85	112			0	14	30	57	85	112
	Cu	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓
	Ni	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓
	Pb	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓
	Zn	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓

^aN: 1 mg L⁻¹ is limit for A1, 2 for A2 and 3 for A3 (European Communities Environmental Objectives (Surface Waters) Regulations, 2009 (S.I. No. 272 of 2009)), threshold set at >3 mg L⁻¹ for this table as worst case scenario.

^bNo abnormal change" – (European Drinking Water Directive 98/83/EC).

^cCd, Ni, Pb: Max admissible concentration (MAC) Environmental Quality Standard (EQS) as per Directive 2013/39/EU.

⁽ⁱ⁾Exceeds annual average (AA) on RS1 for all organic amendments but not MAC.

^dEuropean Drinking Water Directive 98/83/EC.

^eEU Surface Water Regulations (1989) as outlined in EPA (2001).

for DCS and untreated plots respectively. The impact of the rapid and highly available N in digestate aligns with the findings of Albuquerque et al. (2012a) examining the fertilizer potential of digestate for horticulture, while also flagging the need to ensure that this highly available N is not lost to the environment. In a study comparing digestates with mineral fertilizer for growing tomatoes, Barzee et al. (2019) also demonstrated that digestate is as good as or better than mineral N fertilizer for horticulture crops, while Walsh et al. (2012) and Coelho et al. (2019) observed a similar finding for grasslands. Coelho et al. (2019) did however note that digestate from an AD plant processing sewage sludge did not perform as well in terms of grass growth as digestate from co-digestion of animal slurry with FW. Similarly, after examining 12 agriculture based digestates, Albuquerque et al. (2012b) concluded that agriculture-based AD digestate has good fertilizer potential, which they attributed to the high NH₄-N content. The concern raised in that study about the potential need for post-treatment of the digestate prior to application has been addressed in the present work, with results indicating that post-AD pasteurisation

does not negatively affect the fertilizer potential of the digestate. In fact, the opposite may be hinted at by the slightly higher grass yield from the digestate pasteurised to EU Standard (70 °C for 1 h) compared with unpasteurised, particularly evident on Day 30 (1147 vs 968 kg DM ha⁻¹). It may be the case that this short heat treatment alters the digestate nutrient bioavailability (Ware and Power, 2016), improving fertilizer potential, although as none of the nutrient or metal parameters examined are obviously different across treatments, this theory would require further examination. The results of the present research indicate that as hypothesised, digestate from co-digestion of slurry with FW, whether pasteurised or not, is at least as good as untreated slurry as an organic fertilizer for growing grass (Figs. 5 and 6).

Albuquerque et al. (2012b) raised a further concern about the higher Zn content typical of agriculture-based AD, citing disease-prevention additives in animal diets as the source. Although the levels of Zn in the digestates used in this trial were within acceptable limits (WRAP PAS110), digestate from AD plants processing pig slurry may have higher concentrations, given the more widespread use of Zn in

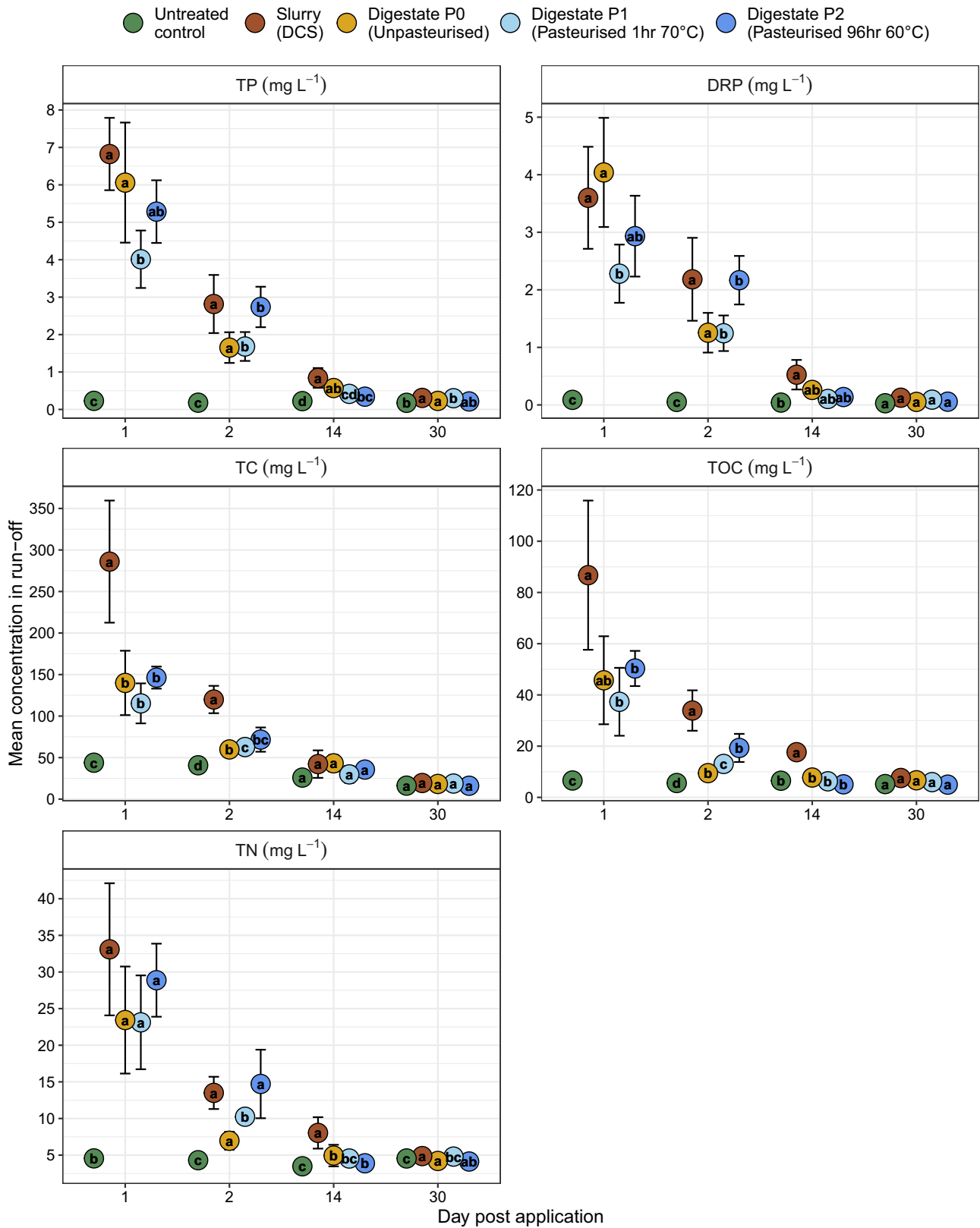


Fig. 4. Nutrients (TP, DRP, TC, TOC and TN) in unfiltered runoff (mg/L) following five treatments (T1 = untreated control (no organic amendment); T2 = slurry; T3 = unpasteurised digestate; T4 = pasteurised digestate (70 °C); T5 = pasteurised digestate (60 °C)) from rainfall simulation 1, 2, 14 and 30 days after application. Error bars indicate standard error of the mean (n = 4).

that industry. There was no significant difference between slurry and digestate treated plots in terms of Zn uptake in grass, nor were any treatments significantly different from untreated plots.

Mo concentration in grass from the DCS and P0 treatments was higher than pasteurised digestate (P1 and P2) and the untreated control after 14 days (Fig. 7). While Mo concentration in grass from all three

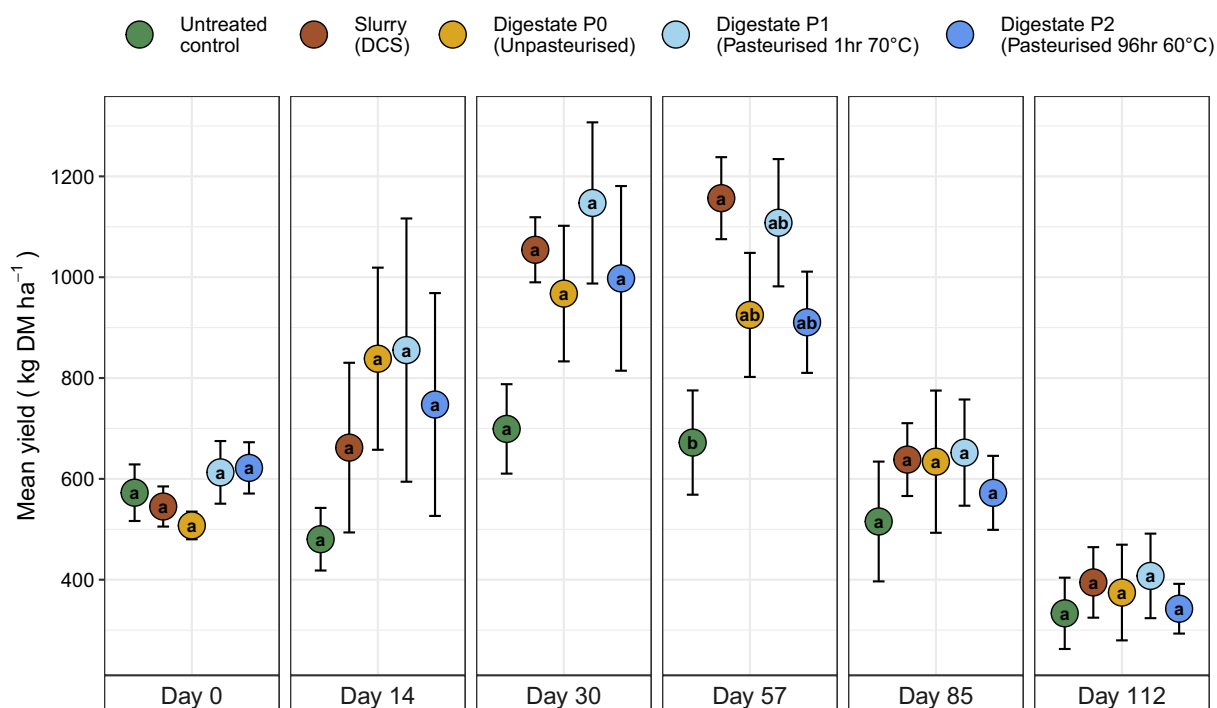


Fig. 5. Grass yield following application of DCS, unpasteurised digestate and pasteurised digestate (two conditions) and an untreated control. Error bars indicate standard error of the mean (n = 4).

digestates declined over time, grass from DCS plots saw a steady Mo increase in concentration for two months after treatment, stabilizing at 2.2–2.3 mg/kg throughout the remainder of the trial, a level 3–4 times higher than the untreated plots. Molybdenum is an essential

element for nitrate reductase (Kaiser et al., 2005), but can be problematic at concentrations above 2 mg/kg (Brogan et al., 1973). At these concentrations, Mo reduces Cu availability in the rumen by forming Mo-S compounds called thiomolybdates which pass through the rumen wall

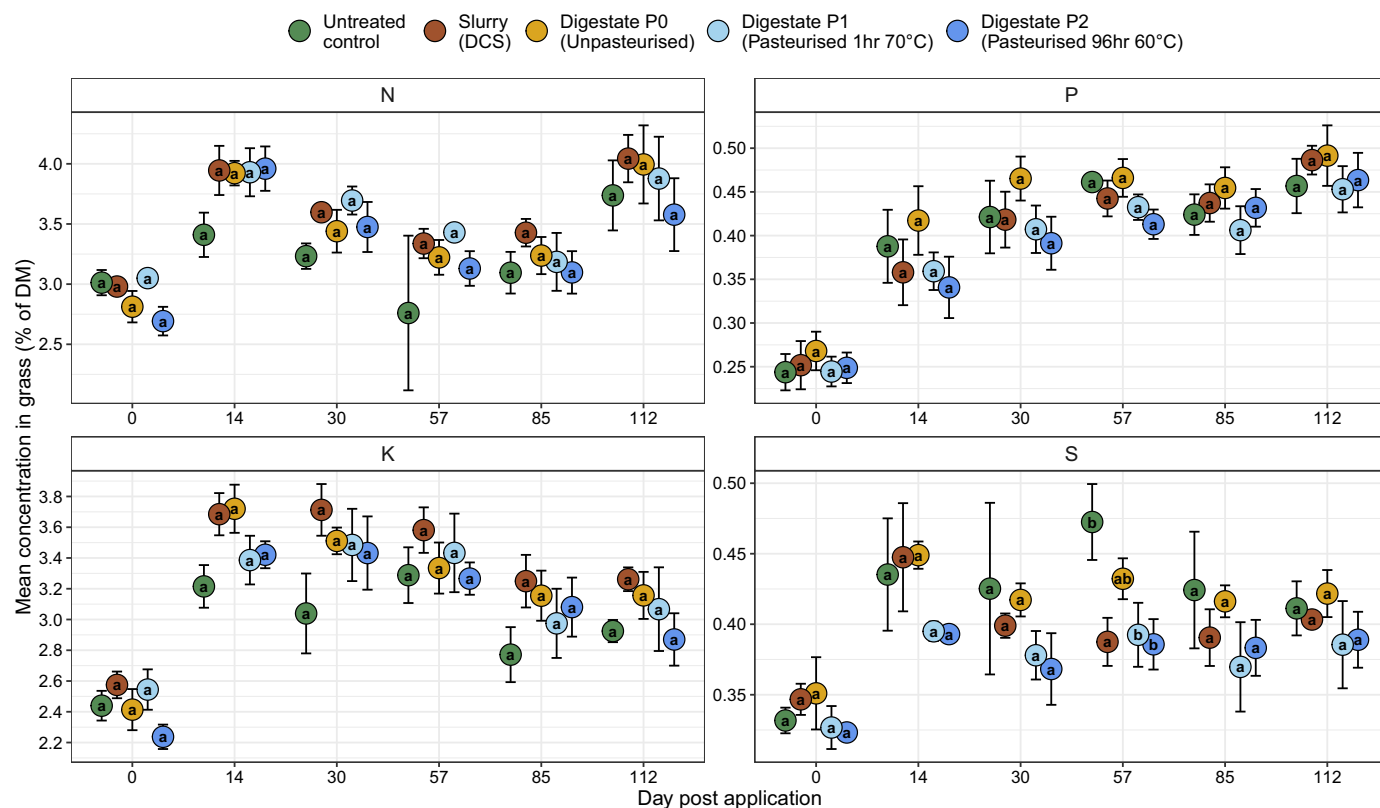


Fig. 6. N, P, K and S content in grass harvested before (Day 0) and following application of DCS, unpasteurised digestate and pasteurised digestate (two conditions), and an untreated control plot. Error bars indicate standard error of the mean (n = 4).

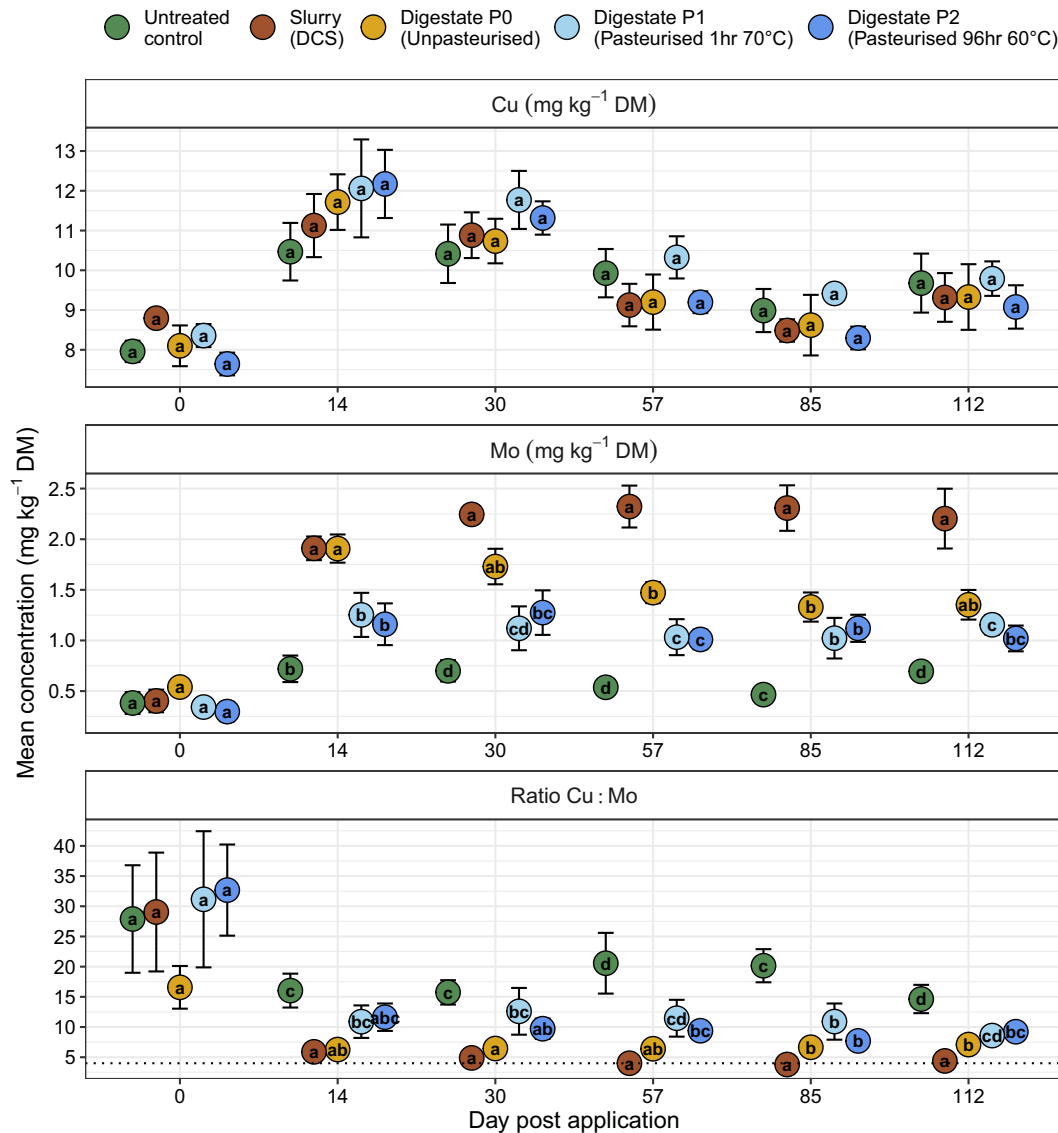


Fig. 7. Cu and Mo content and Cu:Mo ratio in grass harvested before (Day 0) and following application of DCS, unpasteurised digestate and pasteurised digestate (two conditions), and an untreated control plot. Error bars indicate standard error of the mean ($n = 4$).

and readily bind to Cu, causing toxicity with clinical signs similar to Cu deficiency (Gould and Kendall, 2011). This negative affect is compounded if the ratio of 4:1 Cu:Mo, considered safe, is breached (Pitt, 1976), with acute Mo toxicosis possible from a ratio below 2:1 (Miltimore and Mason, 1971). Although none of the samples tested dropped below the critical 2:1 ratio, forage collected from the DCS plots breached the 'safe' 4:1 ratio on Day 57 and Day 85 (3.9 and 3.7 respectively; Fig. 7), likely owing to the higher initial Mo concentration in DCS compared with all three digestates tested (Table 2).

The lower initial Mo concentrations in digestate compared with DCS may be explained in part by experiments reporting benefits of Mo supplementation for biogas production (59% increase), indicating that Mo in slurry is utilized by anaerobic microbial consortia (Cai et al., 2018). It may also indicate an additional benefit of processing slurry in AD, given the reduced risk of Mo-induced hypocupraemia.

3.5. Summary table of treatments

For ease of comparison results are tabulated with a red cross indicating a breach of regulatory or recommended limits and a green tick indicating satisfactory levels (Table 5). The most significant difference was

visible between treatments for runoff of FIB, particularly enterococci, which remained high from slurry-treated plots beyond 30 days. Pasteurisation did not significantly alter runoff of FIB, accumulation or uptake of metals and nutrients, but one pasteurisation condition (P2: 60 °C for 96 h) did result in higher P and N runoff on Day 2, similar to that recorded from slurry. Clearly anaerobic co-digestion of slurry with FW is an approach that facilitates shifting the focus "from compliance to performance", improving water quality and significantly reducing use of chemical fertilizers in line with European Green Deal goals (COM, 2019). However, to prevent the risk of 'pollution swapping' a holistic assessment that includes gaseous emissions and fertilizer replacement value is necessary. Finally, the data from these trials should be incorporated into models that assess benefits and risks to human health from improved water and air quality in order to inform synergistic manure and waste management policies,

4. Conclusions

This study compared the traditional practice of landspreading untreated slurry with landspreading of unpasteurised and pasteurised digestate from agriculture-based AD. Our results indicate that for each

environmental parameter tested: microbial, nutrient and metal runoff losses; accumulation in soil and uptake in grass, digestate from anaerobic co-digestion of slurry with FW resulted in reduced potential for pollutant transmission to watercourses, soil and grass than traditional landspreading of slurry without treatment. Reduced microbial runoff from digestate was the most prominent advantage of digestate application. Pasteurisation of the digestate further augmented those environmental benefits, without impacting grass output. Anaerobic co-digestion of slurry is therefore a multi-beneficial circular approach to reducing impacts of livestock production on the environment.

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CRediT authorship contribution statement

S. Nolan: Investigation, Data curation, Writing - original draft. **C. Thorn:** Validation, Formal analysis, Data curation, Visualization. **S.M. Ashkuzzaman:** Methodology, Resources. **I. Kavanagh:** Investigation. **R. Nag:** Conceptualization, Investigation. **D. Bolton:** Project administration, Funding acquisition. **E. Cummins:** Writing - review & editing. **V. O'Flaherty:** Funding acquisition, Writing - review & editing. **F. Abram:** Writing - review & editing. **K. Richards:** Supervision, Funding acquisition. **O. Fenton:** Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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