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The effect of field application of food-based anaerobic digestate on earthworm populations

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Abstract
In general, farming systems that provide the greatest organic matter returns to the soil support the highest earthworm populations and typically the application of organic materials will increase earthworm numbers because of an abundance of food. There has been limited work on the effects of digestate applications on earthworm populations with most focus on the short-term effects of digestate from manure or crop-based feedstocks, not from food wastes. To address this gap in current knowledge, the objective of this study was to assess the impact of repeated food-based digestate applications on earthworm populations/biomass in both the short term (*c.* 6 months after digestate application) and longer term (2 years later) in comparison with other commonly used inputs (manufactured nitrogen-N fertilizer, compost and livestock manures). This multi-site field experiment has shown that the application of food-based digestate at application rates above current good practice can have a short-term, negative impact on earthworm numbers under certain conditions. Six months after the final digestate addition, earthworm numbers were 32%–60% lower than following the additions of other organic materials at four of the seven sites investigated. A combination of high ammonium/low organic matter loadings and soil compaction most likely explained the observed effects.

KEYWORDS
ammonia, ammonium, livestock manure, organic materials, soil organic matter

1 | INTRODUCTION

As well as generating renewable energy (‘biogas’), anaerobic digestion (AD) produces large quantities of nutrient-rich digestate (or ‘biofertilizer’). The increasing number of biogas plants in operation in the UK, up from 17 in 2009 to 259 in 2015, has led to a concurrent increase in digestate production, with more than 2 million tonnes applied to UK agricultural land in 2013 (WRAP, 2017). In 2015, food waste made up 35% of digestate feedstock and further growth is predicted such that around 5 of the 7 million tonnes of food waste currently sent to landfill each year could be available for digestion by 2020 (DECC & Defra, 2011). Whilst digestate is recognized as a valuable source of plant nutrients, there is uncertainty about its nutrient supply characteristics and effects on the wider environment. Recent work has shown that digestate applications increased soil nutrient supply within a short timescale (<3 years) (Bhogal et al., 2016) but did not improve overall soil quality (Bhogal et al., 2018). Nicholson et al. (2017) reported that ammonia emissions from food-based digestate were higher than from livestock slurry which was attributed to its higher ammonium-N content and pH, but that nitrous oxide emissions were low.

Earthworms (Order: Oligochaeta) significantly influence soil quality, with populations widely promoted as a simple indicator of the overall health of a soil (e.g. Pérès et al., 2011) as well as a bio-indicator of potential soil contamination (Fründ, Graefe, & Tischer, 2010). In general, farming systems that provide the greatest organic matter returns to the soil support the highest earthworm populations (Scullion, Neale, & Philipps, 2002). Most livestock manures are highly palatable and nutritious food sources for earthworms (e.g. Marhan & Scheu, 2005). However, field studies have reported inconsistent effects of other organic manures on earthworm populations, albeit most report a positive response in the longer term. Kinney et al. (2012) suggested that this was a result of variations between the applied materials (in both nutrient and potentially toxic compound concentrations), as well as differences in soil characteristics. For example, Marhan and Scheu (2005) reported an increase of 20% in earthworm biomass following farmyard manure (FYM) additions and Leroy, Bommele, Reheul, Moens, and De Neve (2007) and Griffiths et al. (2010) also found increases in earthworm populations where compost or slurry had been applied, compared with the control treatment (no compost/slurry). In contrast, Whitmore et al. (2017) found that neither OM type (digestate, compost, FYM or straw) nor rate had a consistently significant effect upon earthworm numbers. Moreover, various authors (e.g. Hansen, 1996; Van Vliet & De Goede, 2006; Whitmore et al., 2017) have reported short-term negative effects of slurry applications, attributed to ammonia toxicity, waterlogging (potentially restricting oxygen supply) or exposure to high salt concentrations.

Digestate properties are influenced by the feedstock (e.g. waste food, purpose-grown crops and livestock manures) and the digestion process. Typically, the total nitrogen (N) content in digestate is not different from the feedstock although the ammonium-N to total N ratio will be higher (Möller & Müller, 2012). Degradation of proteins, with increases of *c*.15%–30% ammonium-N (relative to feedstock), has been reported (e.g. Möller, Stinner, Deuker, & Leithold, 2008; Sørensen, Mejnertsen, & Møller, 2011). Total ammonium-N consists of both un-ionized ammonia (NH_3) and the ammonium ion (NH_4^+) which are at a pH and temperature dependant equilibrium. The higher the pH and temperature during digestion, the higher the ratio of NH_3: NH_4^+ (Angelidaki & Ahring, 1994). Below pH 7.0, a greater proportion of NH_3 is bound as NH_4 with the dissociation of NH_3 from NH_4 more likely to occur in alkaline conditions (Panetta, Powers, & Lorimor, 2005). NH_3 is more toxic to earthworms than NH_4^+, and hence, food-based digestate might be expected to be more harmful to earthworms than slurry due to the higher pH and ammonium-N content (digestate: *c*. 4 kg t^{-1} fw, pH 8.5; livestock slurry: 1.5 kg t^{-1} fw, pH 7.4. Bhogal et al., 2018) such that the ratio of NH_3: NH_4^+ will be greater.

To date, there has been limited work on the effects of digestate applications on earthworm populations with most of the reported work short-term and on manure/crop-based digestate. However, there is no published data on the effect of food-based digestate applications on earthworm populations/biomass. To address this gap in current knowledge, this study aimed to assess the impact of food-based digestate applications on earthworm populations in both the short-term (*c*. 6 months after digestate application) and longer term (2 years later) in comparison with other commonly used inputs (manufactured N fertilizer, compost and livestock manures), which were used as reference levels. Of the comparator materials livestock slurry had the most similar profile to food-based digestate, albeit with a lower pH and ammonium-N content. As a result, it was hypothesized that food-based digestate

TABLE 1 Characteristics and cropping at the seven experimental sites

	Soil properties			Average annual rainfall (mm)[a]	Cropping rotation		
Site (Grid reference)	Texture	% Clay	SOM (% dm)[b]		2010–11	2011–12	2012–13
Aberdeen (NJ870117)	Sandy loam	16	8.3	815	SB	WB	WOSR
Ayr (NS382227)	Sandy clay loam	19	4.0	939	G	G	G
Devizes (SU153558)	Silty clay loam	20	8.4	770	Lin	WW	WW
Faringdon (SU252940)	Clay	62	5.8	685	WW	WW	WC
Harper Adams (SJ714203)	Sandy loam	11	3.4	805	POT	SB	WW
Lampeter (SN542614)	Clay loam	26	7.8	1,217	G	G	G
Terrington (TF548186)	Silty clay loam	28	2.3	653	WW	WW	WOSR

Abbreviations: G, permanent (cut) grassland; Lin, linseed; POT, potatoes; SB, spring barley; WB, winter barley; WC, whole crop oats/peas; WOSR, winter oilseed rape; WW, winter wheat.

[a]30-year average weather Met Office data for 1981–2010.

[b]Organic carbon % dry matter × 1.724 (MAFF, 1986).

would have a similar effect on earthworm mortality to livestock slurry (transient toxicity due to the presence of ammonium-N) and that any short-term effects would be reversed in the longer term.

2 | MATERIALS AND METHODS

2.1 | Experimental sites

In autumn 2010, a network of seven experimental sites (two permanent cut grassland, five in arable rotation) was established on a range of soil types and agro-climatic zones in the UK (Table 1) as part of a wider programme of work to establish a robust scientific evidence base on the nutrient supply properties of digestate and compost applications to land (Bhogal et al., 2018).

At each site, 18 experimental plots (60–160 m^2, depending on the site) were laid out in a randomized block design (six treatments with three replicates, Table 2), with treatments applied for three consecutive years. In the first cropping year, organic materials were applied in autumn 2010 at Ayr and Terrington, and at the other sites (Aberdeen, Devizes, Faringdon, Harper Adams and Lampeter) in spring 2011. Organic material applications were repeated in autumn 2011 at Aberdeen, Devizes, Faringdon, Lampeter and Terrington and in spring 2012 at Ayr and Harper Adams, with a final application in autumn 2012 at all seven sites. Treatments were hand applied to the soil surface; autumn applications to the arable sites were incorporated soon after application. To comply with the nitrate vulnerable zone (NVZ) field limit (SI, 2009), all solid organic materials were applied at a target rate of *c*. 250 kg total nitrogen (N) ha^{-1}, and the liquid materials were applied at rates between 120 and 250 kg total N ha^{-1}, depending on the volume (restricted to ≤80 m^3 ha^{-1}). The average annual N loading for the food-based digestate was 220 kg N ha^{-1} compared with average N loadings for the other materials of 192–261 kg N ha^{-1} (Table 3). It should be noted that current good practice guidance suggests that digestate applications should aim to apply 50%–60% of crop nitrogen requirement so that application rates used in this study were greater than present-day guidelines (WRAP, 2016).

The control treatment received the recommended amount of inorganic N fertilizer as ammonium nitrate for the crop grown (Defra, 2010; SAC, 2010a), with individual application rates not exceeding 120 kg N ha^{-1} (60 kg ammonium-N). For the organic material treatments, supplementary applications of inorganic N fertilizer (as ammonium nitrate) were made to match the N supplied, by the control treatment, using MANNER-*NPK* predictions of organic material crop available N supply (Nicholson et al., 2013). Phosphorus, potassium and sulphur were applied at a single rate across all treatments, based on the requirements of the untreated control (Defra, 2010; SAC, 2010b). Mineral fertilizer applications aimed to ensure (as far as was practically possible) that no major nutrient limited crop growth and that crop yields and residue returns were the same on all treatments (i.e. the only difference in OM inputs was from the organic materials).

TABLE 2 Treatment details

No	Treatment
1	Control (no organic material application; recommended rates of inorganic fertilizer only)
2	Green compost equivalent to 250 kg N ha^{-1}
3	Green/food compost equivalent to 250 kg N ha^{-1}
4	Food-based digestate equivalent to 120–250 kg N ha^{-1} [a]
5	Cattle farmyard manure equivalent to 250 kg N ha^{-1}
6	Cattle slurry equivalent to 120–250 kg N ha^{-1} [a,b]

[a]Rate depended on the volume that could be applied (restricted to ≤80 m^3 ha^{-1}).

[b]Pig FYM and pig slurry at Terrington at the same N rates.

2.2 | First earthworm sampling—spring 2013

Earthworms were sampled in spring 2013 when the soil was close to field capacity, approximately 6 months after the third and final treatment. Sampling consisted of hand sorting (Schmidt, 2001) to a depth of *c*. 0.25 m. A 0.3 m × 0.3 m area in the centre of the plot was delineated before a block of soil 0.25 m deep was removed and searched for earthworms. Each block was searched for a period of 5 min (10 min for very heavy or wet soils), and earthworms were placed in vented containers containing moist moss to prevent desiccation. The process was repeated three times in each plot and total earthworm numbers (individuals/m^2) and biomass (g m^{-2}) were recorded.

2.3 | Second earthworm sampling—autumn 2014

Earthworms were sampled again in autumn 2014 at four sites (Ayr, Lampeter, Faringdon and Terrington) approximately 2 years after final treatments. In addition to hand sorting as described above, extraction of deeper living earthworms using the 'mustard method' (Gunn, 1992) was made. 'Hot' mustard powder/water mix (15 g L^{-1}–0.75 L) was poured into the excavated hole, and emerging earthworms were collected, washed and then placed in a vented container. After 10 min, the extraction was repeated and earthworms collected for another 10 min. The process was repeated for three replicates per plot. For each plot, adult earthworm species (with clitellum) were identified according to Sims and Gerard (1999); for each species, the number of individuals and biomass were

TABLE 3 Mean organic material application rates, total N, ammonium-N and organic matter loadings and chemical properties ($n = 21$, mean of samples taken from seven sites in three seasons with three replicates). Standard errors in parenthesis

	Food-based digestate	Livestock slurry	Green compost	Green/food compost	Farmyard manure
Application rate (m^3 ha^{-1})	47	72	27	18	37
Annual loading rates					
Total N (kg ha^{-1} $year^{-1}$)	220 (13.5)	192 (12.6)	261 (12.3)	211 (9.5)	247 (18.1)
Ammonium-N (kg ha^{-1} $year^{-1}$)[a]	139–237	61–148	1–8	1–18	2–16
Organic matter (t ha^{-1} $year^{-1}$) [total for 2010–2013 (t ha^{-1})][b]	0.4–0.7 [1.2–2.2]	0.7–3.9 [2.1–11.7]	5.3–7.5 [15.9–22.4]	2.9–4.3 [8.8–12.9]	4.5–6.4 [13.6–19.1]
Properties					
Dry matter (%)	2.16 (0.18)	4.60 (0.47)	70 (3.00)	66 (1.66)	27 (2.25)
pH	8.50 (0.06)	7.37 (0.09)	8.26 (0.09)	7.91 (0.10)	8.16 (0.13)
Total N (kg t^{-1} fw)	4.67 (0.18)	2.67 (0.14)	9.59 (0.49)	11.8 (0.63)	6.67 (0.53)
Ammonium-N (kg t^{-1} fw)	3.78 (0.17)	1.44 (0.12)	0.09 (0.02)	0.38 (0.13)	0.25 (0.07)
Nitrate-N (kg t^{-1} fw)	<0.01 (0.0003)	<0.01 (0.0002)	0.15 (0.03)	0.42 (0.07)	0.21 (0.06)
RAN (kg t^{-1} fw)[c]	3.78 (0.17)	1.44 (0.12)	0.24 (0.03)	0.81 (0.10)	0.46 (0.09)
Zinc (mg kg^{-1} dm)	136 (8.32)	257 (41.2)	234 (13.5)	278 (21.0)	280 (49.5)
Copper (mg kg^{-1} dm)	45.5 (5.04)	152 (36.0)	66.1 (5.80)	74.1 (6.36)	65.3 (9.55)
Lead (mg kg^{-1} dm)	7.14 (0.96)	9.13 (2.79)	120 (11.3)	91.8 (4.91)	10.7 (2.27)

[a]Range of annual loading rates at seven sites.

[b]Range of annual loading rates at seven sites/total for 2010–2013, calculated from Table 5, Bhogal et al., (2018) (organic carbon × 1.724).

[c]RAN: readily available nitrogen (i.e. ammonium-N + nitrate–N).

measured. In addition, total numbers of juvenile earthworms were recorded. Juvenile earthworms could not be identified to species because of incomplete development of the distinguishing features used for identification.

2.4 | Statistical analysis

2.4.1 | Spring 2013

At each experimental site, the effect of the different organic material treatments on earthworm numbers and biomass was assessed using analysis of variance (ANOVA). In addition, a cross-site analysis of variance was performed, with separate models carried out for the five arable sites and the two grassland sites. Post hoc testing was undertaken to evaluate which treatment means were different from each other using Duncan's multiple range test (using Genstat version 12; VSN International Ltd, 2010).

2.4.2 | Autumn 2014

Individual site ANOVAs were undertaken, as detailed above, for total, adult and juvenile earthworm numbers/biomass. In addition, for each site, the number/biomass of endogeic (living in the topsoil, making horizontal tunnels), epigeic (living in the surface humus layer) and anecic (building permanent vertical burrows) earthworms for each treatment was also compared.

3 | RESULTS

3.1 | Spring 2013

There were treatment differences in earthworm numbers at four of the sites—Ayr, Faringdon, Lampeter and Terrington ($p < .05$) (Table 4).

At Ayr, a grassland site with the greatest number of earthworms, food-based digestate reduced earthworm numbers in comparison with all other treatments ($p = .001$). Earthworm numbers were also reduced on the food-based digestate treatments in comparison with the FYM and slurry treatments at Faringdon, the FYM, slurry and green/food compost treatments at Lampeter, and the FYM and green compost treatments at Terrington ($p < .05$). Significant differences in biomass were noted at Faringdon and Terrington only (Table 4) where earthworm biomass was reduced on the food-based digestate in comparison with the FYM treatment.

Aggregating data across both grassland sites (Ayr and Lampeter), overall earthworm numbers on the food-based

TABLE 4 Earthworm counts (number of earthworms/m^2) and biomass (earthworms g m^{-2}) to 25 cm depth at seven sites in spring 2013. Rows within a column labelled with different letters differ significantly according to post hoc testing with Duncan's multiple range test ($p < .05$)

Site	Aberdeen (arable)		Ayr (grass)		Devizes (arable)		Faringdon (arable)		Harper Adams (arable)		Lampeter (grass)		Terrington (arable)	
Treatment	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}
Control	301	68.7	607[a]	273	33	7.60	158[a]	*37.1*[ab]	10	2.91	222[ab]	130	119[ab]	29.9[ab]
Green compost	326	86.3	691[a]	287	53	19.0	205[ab]	49.8[abc]	58	8.20	351[abc]	107	151[bc]	29.6[ab]
Green/food compost	281	79.9	760[a]	328	53	18.6	147[a]	33.5[a]	49	12.4	420[bc]	130	86[ab]	15.1[a]
Food-based digestate	232	81.4	291[b]	181	58	26.6	147[a]	45.1[ab]	44	18.2	173[a]	103	75[a]	11.5[a]
FYM	370	92.0	825[a]	363	44	12.2	270[b]	74.2[c]	69	18.0	553[c]	124	195[c]	47.9[b]
Slurry	242	52.0	691[a]	316	88	34.3	244[b]	65.4[bc]	25	10.7	400[bc]	128	146[abc]	27.3[a]
SED	96.8	34.5	117	53.1	19.7	9.58	32.7	12.1	17.3	10.4	88.2	28.2	30.4	8.22
p Value*	NS (.72)	NS (.87)	.01	NS (.08)	NS (.21)	NS (.16)	.01	.04	NS (.06)	NS (.67)	.02	NS (.86)	.02	.02

SED, standard error of difference of the means.

*NS, not significant.

digestate treatments were lower than all other treatments ($p < .001$; Figure 1a). In contrast, earthworm numbers across the five arable sites were similar on most treatments, except that the FYM treatment numbers were higher than on the control, green/food compost and food-based digestate treatments ($p < .01$; Figure 1b).

At the two grassland sites (Ayr and Lampeter), where earthworm numbers were, as expected, generally higher than the arable sites, differences in OM loadings were significantly positively associated with earthworm numbers (Figure 2), albeit with only 60% of the variation in numbers explained by differences in OM loading, suggesting other factors were also involved. A significant positive relationship was also noted at Harper Adams but not at the other arable sites.

3.2 | Autumn 2014

3.2.1 | Ayr

Total earthworm biomass at the Ayr grassland site, remained lower where digestate had been applied, relative to the fertilizer only control 2 years after the last digestate application. This was largely as a result of a reduction in the biomass of juvenile earthworms ($p < .01$—Table 5), which comprised the majority (88%–97%) of the earthworm population. There was also a numeric difference in juvenile earthworm numbers with digestate (at 432/m^2) having 150 earthworms/m^2 less than the control (at 584/m^2). A total of ten earthworm species were found on the site, of which the majority (66%) were endogeic species (living in the topsoil, making horizontal burrows) (Table S1).

3.2.2 | Faringdon

At the Faringdon arable site, juvenile earthworm numbers (which made up 67%–77% of the earthworm population) were also numerically lower where food-based digestate had been applied, although the only significant difference was between digestate and both FYM and slurry ($p < .05$—Table 5). A total of seven earthworm species were found on the site, of which the majority (86%) were endogeic species (Table S1).

3.2.3 | Lampeter and Terrington

There were no differences in earthworm numbers or biomass at these two sites ($p > .05$). The majority of the earthworms at each site (59%–78% at Lampeter, 66%–81% at Terrington) were juveniles, Table 5. A total of nine and seven earthworm

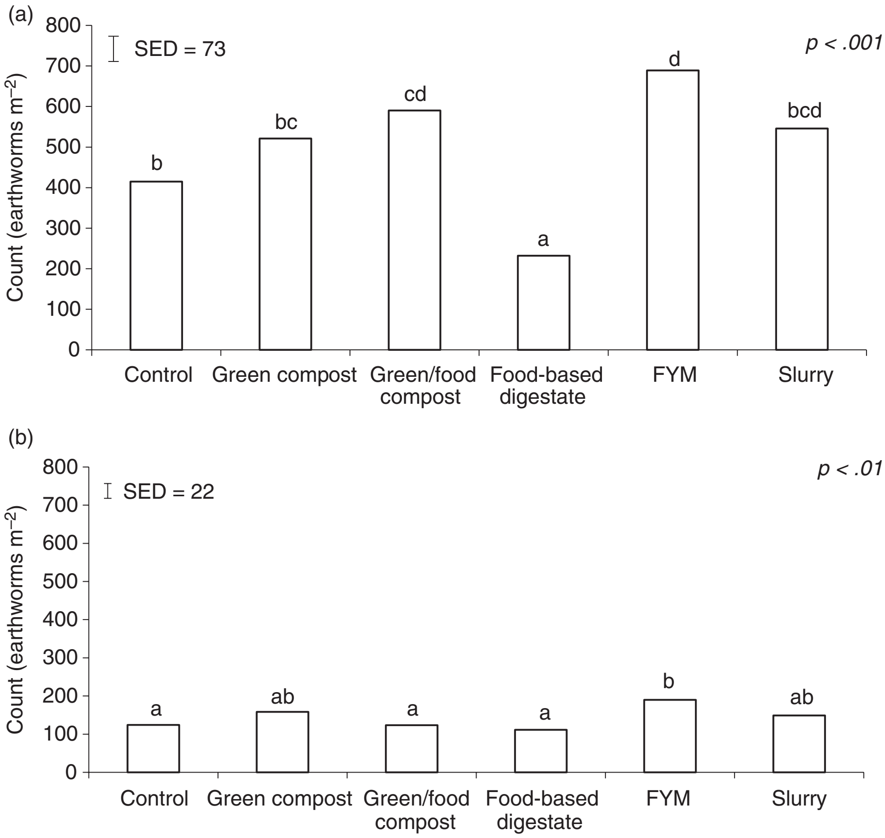


FIGURE 1 Aggregated earthworm numbers in spring 2013 at (a) the two grassland sites and (b) the five arable sites. Standard error of difference (SED) between means; bars labelled with different letters differ significantly according to post hoc testing with Duncan's multiple range test ($p < .05$)

species were found at Lampeter and Terrington, respectively, of which the majority (≥83%) were endogeic species (Table S1).

Overall, there was no treatment effect on individual earthworm species numbers or biomass at any of the four sites sampled in autumn 2014. In addition, there were no statistical treatment differences ($p > .05$) between the numbers and biomass of different functional earthworm groups (i.e. endogeic, epigeic and anecic) or species at any of the sites.

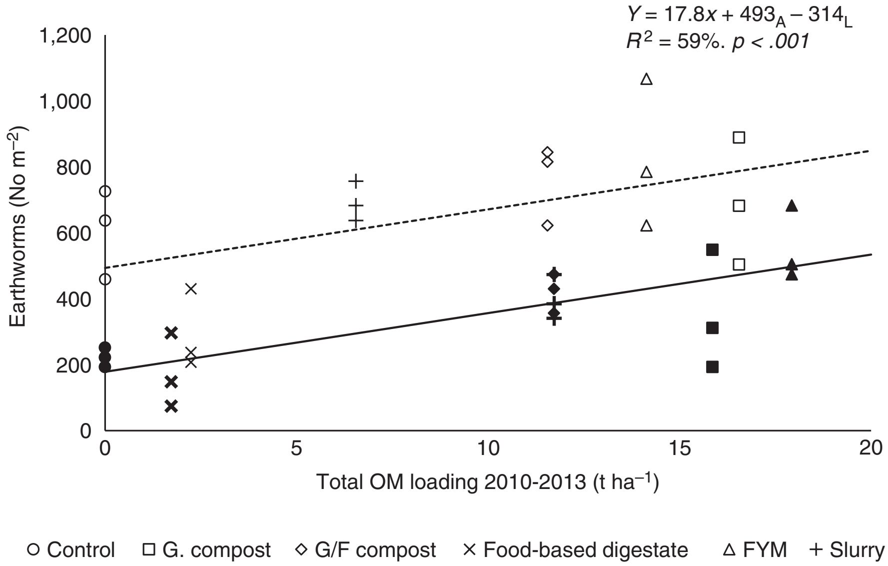
4 | DISCUSSION

Inter-site differences in overall numbers of earthworms, which ranged from *c*. 50 earthworms/m^2 at Harper Adams and Devizes (arable), to >600 earthworms/m^2 at Ayr (grassland) is likely to relate to the soil characteristics and cropping at each site. Typically, earthworm numbers are greater in grassland (permanent pasture and leys) than in tillage land that is disturbed annually (for seedbed preparation, crop planting, etc.). Tillage can either physically damage or kill earthworms or expose them to predation, as well as decreasing soil organic matter levels as a result of oxidation (Briones & Schmidt, 2017). However, intra-site differences in earthworm numbers are likely to be related to the different experimental treatments.

In general, the application of organic materials will increase earthworm numbers because of an abundance of food (Scullion et al., 2002). However, the results from this multi-site field experiment have shown that food-based digestate applications can have a negative impact on earthworm numbers under some conditions. Six months after the final food-based digestate applications at the grassland sites, earthworm numbers were lower than for all other treatments. In contrast, earthworm numbers at the arable sites were similar across treatments except that numbers on the FYM treatment were higher than on the control, green/food compost and digestate treatments ($p < .01$). Earthworms have low migration rates, with observed natural dispersal rates of only 1.4–9 m per year (Hale, Frelich, & Reich, 2005; Ligthart & Peek, 1997). Hence, the reductions in earthworm numbers at the grassland sites *c*. 6 months after application of the digestate is most likely to be attributed to direct effects on earthworm mortality rather than dispersal. The higher earthworm numbers at grassland sites made direct contact with the applied digestate (and other materials) more likely, than at the arable sites.

Reductions (relative to the control) in earthworm biomass (weight) in response to digestate were less consistent than those for abundance (numbers). A cross-site analysis of average live weight per earthworm (6 months post-application) showed that earthworms from the digestate treatment (*c*. 0.42 g) were heavier ($p < .01$) than from the other treatments (*c*. 0.30 g average of all other treatments). Soil moisture is

FIGURE 2 Relationship between total organic matter loading (2010–2013) and earthworm numbers at Ayr (A) ---- and Lampeter (L) —— experimental sites. Ayr: open symbols; Lampeter: closed/bold symbols



an important determinant of earthworm weight (Fründ, Butt, et al., 2010), although it is unlikely to have caused treatment differences in earthworm weight. Earthworms were sampled in spring when soils were close to field capacity and although soil moisture varied between sites, reflecting differences in soil types, the digestate treatment had no effect on soil moisture. An increase in average individual earthworm live weight is more likely to be due to a population shift towards a greater abundance of adults, an increase in the proportion of larger species within the population or a combination of these factors. Any negative impact of food-based digestate applications is liable to be more pronounced for smaller species and juvenile earthworms due to their greater surface area to mass ratio meaning that smaller individuals were more likely to be affected, regardless of species or age.

Two years after the last food-based digestate application, most of the differences in earthworm numbers and biomass had disappeared and there were few observed longer term effects of any of the treatments on earthworm populations. However, at Ayr, earthworm biomass on the food-based digestate treatment was lower than all of the other treatments. In addition, there were numerically fewer juvenile earthworms compared with the control plots; juvenile earthworms made up 88% and 95% of the population on the digestate and control treatments, respectively. The reduced earthworm biomass and numbers suggest that the negative effects of the digestate applications had persisted for up to 2 years after application only at the Ayr site.

Earthworms need organic matter as a source of food and when all other factors are equal the availability of organic matter will be the most important factor in determining earthworm numbers. However, other factors may override the importance of organic matter including tillage, crop rotations and residue management. Previous, field

TABLE 5 Juvenile earthworm mean number (no m^{-2}) and weight (g m^{-2}) in autumn 2014. Rows within a column labelled with different letters differ significantly according to post hoc testing with Duncan's multiple range test ($p < .05$)

	Ayr (grass)		Faringdon (arable)		Lampeter (grass)		Terrington (arable)	
Treatment	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}	no m^{-2}	g m^{-2}
Control	584	267^{b}	185^{ab}	22.4^{a}	125	74	94	13.1
Green compost	535	290^{b}	178^{ab}	26.1^{ab}	147	57	114	19.3
Green/food compost	612	293^{b}	214^{abc}	31.6^{abc}	186	88	75	11.7
Food-based digestate	432	177^{a}	165^{a}	33.2^{abc}	180	100	88	15.8
FYM	721	274^{b}	265^{c}	47.5^{c}	137	57	153	29.1
Slurry	686	311^{b}	241^{bc}	40.0^{bc}	144	82	140	22.4
SED	88.6	29.1	29.6	6.68	44.4	21.0	38.7	7.59
p Value*	NS (.08)	.01	.04	.04	NS (.69)	NS (.32)	NS (.35)	NS (.28)

Abbreviation: SED, standard error of difference of the means.

*NS: not significant, that is *p* value is > .05

studies have provided inconsistent results with respect to the effects of organic manures on earthworm populations. Kinney et al. (2012) suggested that this was a result of variations between the applied materials (in both nutrient and potentially toxic element (PTE) concentrations), as well as differences in soil characteristics. In this experiment, PTE concentrations (i.e. zinc, copper and lead; Table 3) were lower in the digestate than the comparator materials and hence are unlikely to be responsible for the negative effect on earthworm numbers. However, the digestate treatment provided less OM than the other organic material treatments. Although the food-based digestate had a similar OM content (58% dm) to slurry (66% dm) the higher total N content (4.67 kg t^{-1} fresh weight) compared with the slurry (2.67 kg t^{-1} fresh weight) meant that the application rate of digestate was lower (to comply with NVZ regulations) and less OM was applied. At the grassland sites (Ayr and Lampeter), where digestate effects on earthworm numbers were reported, differences in OM loadings were significantly positively associated with earthworm numbers. However, at the arable sites, there was no significant effect of organic matter loading on earthworm numbers ($p > .05$). This suggests that the observed results could not be explained simply as a result of OM additions but that other factors were also important, which may relate to the ammonium-N content of the digestate.

High concentrations of ammonium-N (>100 kg t^{-1}) are known to impact negatively on earthworm populations (e.g. Edwards & Lofty, 1982; Hansen, 1996). For example, cattle slurry and urine have been shown to be transiently toxic to earthworm populations as a result of ammonia (and benzoic acid and sodium sulphide) contents over 7–8 weeks in laboratory experiments (Curry, 1976). In contrast, compost and FYM have a low ammonium-N concentration (around 0.5–1 kg m^{-3}), which would not have a negative impact on earthworms. Additionally, both FYM and compost supply more organic matter than digestate and slurry so are potentially better sources of food for earthworms. Notably, the reductions in earthworm populations following digestate application (compared with the untreated control, compost, slurry and FYM) were most marked at the Ayr grassland site which had one of the highest ammonium-N loadings (*c*. 225 kg ammonium-N ha^{-1}). The food-based digestate used in these experiments also had a higher pH than the comparator livestock slurry so that a greater proportion of the ammonium-N in the digestate would have been present as free ammonia (NH_3). For example, at pH <6.0, the proportion of ammonia is very low, whereas at pH *c*. 9 the ratio is 50:50 NH_3- to ammonium-N (Fangueiro, Hjorth, & Gioelli, 2015). This difference in the ammonium/NH_3 equilibrium is potentially responsible for the measured reductions in earthworm populations where loading rates were high.

Other research undertaken to date has not reported any adverse effects of digestate on earthworm populations. For example, Clements, Salter, Banks, and Poppy (2012) measured earthworm numbers following applications of cattle slurry and manure-based digestate in comparison with an untreated control on earthworm populations in ley grassland. Six weeks after application, there were no differences in earthworm numbers between any of the treatments. In addition, Koblenz, Tischer, Rücknagel, and Christen (2015) assessed the effect of digestate (from crop and manure feedstocks) applying 150–160 kg ammonium-N ha^{-1} on earthworm populations at two field sites growing maize. At both sites, there was no difference between the slurry and the digestate in terms of total earthworm numbers or biomass. Importantly, both these experiments used digestate from crop or manure-based feedstocks, which had a lower ammonium-N content than the food-based digestate used in our experiments.

In this study, topsoil compaction was significantly greater on the digestate treatment at the Ayr grassland site (as measured by bulk density and penetration resistance) although the reasons for this are unclear (Bhogal et al., 2018). Soil compaction is often observed where livestock slurries have been applied due to heavy trafficking, particularly if conducted under wet conditions. However, all organic materials (including the livestock slurries and digestates) were applied by hand, so it is unlikely that soil compaction occurred as a result of the application method. The immediate toxicity of slurry applications (high in ammonium-N) on earthworm numbers has reported to be more pronounced in compacted soil where earthworm movements will be restricted. For example, Hansen (1996) reported that many dead earthworms (not quantified) were observed on the soil (sandy loam) surface a few hours after slurry application to a grass ley, especially after 'heavy' dressings (*c*. 75 m^3 ha^{-1} applying *c*. 102 kg ammonium-N ha^{-1}) to compacted soil. In addition, both D'Hose et al. (2014) and van Eekeren, Bokhorst, and Brussaard (2010) have observed negative correlations between earthworm abundance and soil compaction, as measured by penetration resistance at 0–15 cm.

5 | CONCLUSIONS

This multi-site field experiment has shown that the application of food-based digestate can have a short-term impact on earthworm numbers under some conditions. Six months after the final food-based digestate applications, earthworm numbers were lower than following additions of other organic materials at four of the seven sites. At the grassland sites, OM loadings were significantly positively associated with earthworm numbers. However, at the other sites, there was no significant effect of organic matter loading on earthworm numbers suggesting that the observed results could not be explained simply as a result of differences in OM additions but that other factors were also important. High concentrations of ammonia/ammonium have negative impacts on earthworms. The ammonium-N loading rate from digestate was higher than from the other organic materials and the high pH would have resulted in a greater proportion of the ammonium-N in the digestate being present as ammonia, which is more toxic to earthworms than ammonium-N. At one site, the negative effect persisted 2 years after food-based digestate was applied, most likely due to a combination of high ammonium-N/low organic matter loading, possibly exacerbated by compaction on digestate plots soil where earthworm movements would be restricted and oxygen supply may have been limited. In conclusion, our research suggests that whilst food-based digestate is an effective renewable fertilizer it is important to manage application rates and timings to limit impacts on the wider environment. Following best practice guidance and accounting for nutrients supplied by digestate in fertilizer planning will minimize the risk of excessive nutrient applications. Whilst the observed effects on earthworms were most notable on sites where application rates were greater than those suggested in present-day best practice guidance, further field and laboratory investigations are required to improve guidance on the sustainable use of digestate in agricultural systems.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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