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Earthworm community development in organic matter-amended plots on reclaimed colliery spoil

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Abstract:

Earthworms were sampled at a semi-restored colliery spoil site at Chisnall Hall in Lancashire, two and a half years after the site had been experimentally treated with a number of organic matter applications of anaerobic digestate and compost-like output, in isolation and in combinations. This gave six treatments including a control with no amendment. The material was mechanically dug into the site into replicated 20 x 10 m plots. Within each plot, four types of plant, ash, cherry, willow and reed canary grass, were introduced. Results showed that all organic treatments gave rise to significantly higher community densities of earthworms, with the greatest (638 earthworms m^{-2}) in the high digestate application (1875 t ha^{-1}) treatment, compared with 192 earthworms m^{-2} in the unamended control ($p<0.05$). Species that contributed to greatest numbers were *Allolobophora chlorotica* (the green worm) and *Aporrectodea caliginosa* (the grey worm), both shallow-working, and *Aporrectodea longa* (the black-headed worm), a deep burrower. Nine earthworm species were encountered in total. Planting type had no significant effect on earthworm density. Addition of organic matter to a colliery spoil site greatly enhanced earthworm community density, through a combination of immigration from surrounding areas and increased reproduction.

Key words:

Anaerobic digestate, Colliery spoil, Compost-like output, Earthworms.

Introduction

The former Chisnall Hall colliery waste tip near Coppull, Lancashire (SD 55078 12486) was reclaimed in 1976 to agricultural pasture and woodland end-uses. The majority of the site was covered with a thin (200 mm) layer of topsoil. The original coal waste was acidic (pH 3.5 to 4.0) and therefore 100 t ha^{-1} ground limestone was ripped in to depths of 50 cm, to correct the existing pH to above neutral and to contain future acid generation from residual iron pyrites (Costigan *et al*, 1981). The site was also treated with a high rate of phosphate fertiliser. Although these treatments appeared relatively successful for agricultural grassland, but general tree growth across the site was considerably reduced compared to similar areas with less disturbed soils, possibly due to a lack of organic matter in the compacted reclaimed soils.

More recently, Waste & Resouces Action Programme (WRAP, 2015) was involved with the setting up of a trial to investigate the potential effects of organic matter amendments on plant growth within the reclaimed soil at Chisnall Hall. This trial used BSI PAS 110 anaerobic digestate (DG) in addition to compost-like output (CLO) produced by Global Renewables Lancashire (GRL), as a contribution to the Lancashire Woodlands from Waste Programme (WFW, n d). Six organic matter treatments (fresh materials) were utilised in the trial:

i) DG Low rate + CLO (937.5 t ha^{-1} + 1500 t ha^{-1});
ii) DG Low rate only (937.5 t ha^{-1});
iii) DG High rate + CLO (1875 t ha^{-1} + 1500 t ha^{-1});
iv) DG High rate only (1875 t ha^{-1});
v) CLO only (3000 t ha^{-1});
vi) Control (no addition).

The digestate was sourced from Biffa Waste Services Ltd, Cannock and the CLO from GRL in Leyland, Lancashire. Delivery of organic matter to Chisnall Hall and incorporation took place in April 2012, as materials were mixed into designated experimental plots (see Figure 1). Before mixing, each plot (20 x 10 m) was dug to 1 m using a 13 tonne 360° excavator in order to relieve compaction. The appropriate organic material was then applied and then dug into the soil with the excavator (WRAP 2015). Analyses of the blended spoil and amendment are provided in Table 1.

Each replicated (n=3) plot of the organic matter treatment was planted with four species (Figure 2). These were two woodland trees: ash (*Fraxinus excelsior*) and cherry (*Prunus avium*), cell-grown in size 20-40 cm, supplied by

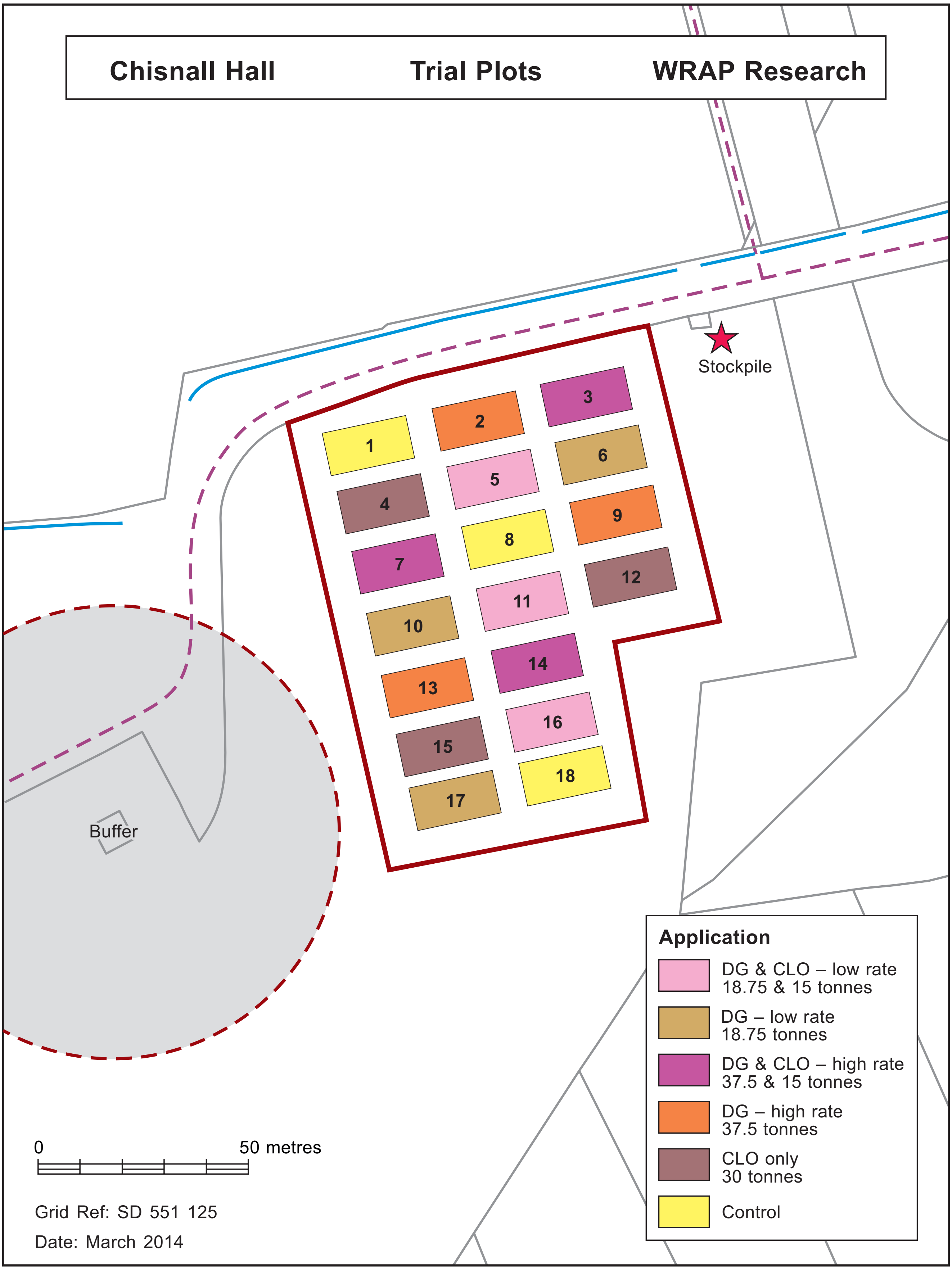

Figure 1: Randomised arrangements of plots at Chisnall Hall with given organic matter treatments (adapted from WRAP 2015).

Table 1: Chemical and physical analysis of soil blend treatments comprising colliery waste mixed with Digestate (DG) or Compost-like output (CLO) or DG and CLO together, from May 2012 (adapted from WRAP 2015).

		Treatments					
Soil Property	**Units**	**DG Low + CLO**	**DG Low**	**DG High + CLO**	**DG High**	**CLO Only**	**Control**
pH		7.83	7.95	8.07	8.25	7.57	7.30
Electric Conductivity	μS/cm	4100	2900	4366	4600	2867	2100
Organic Matter	%	18.3	14.0	14.1	17.5	15.0	10.6
Total N	mg/kg	13333	9000	15666	19250	3133	2633
Total P	mg/kg	2533	1165	1113	2250	1600	530
Total K	mg/kg	3767	2900	3433	3950	3467	2467
Total Mg	mg/kg	4333.3	3500.0	4566.7	4600.0	5666.7	3866.7
Extractable P	mg/l	124.0	70.5	42.0	227.5	40.0	11.7
Extractable K	mg/l	1410.0	555.0	880.0	1285.0	713.3	114.3
Extractable Mg	mg/l	393.3	155.0	200.0	200.0	240.0	114.0
Extractable Ca	mg/l	4366.7	3150.0	3066.7	3100.0	3866.7	3633.3
NH4 (Water Soluble)	g/l	1193.3	745.0	1266.7	1950.0	163.3	92.3
Nitrate (Water Soluble)	mg/l	<1.0	17.0	<1.0	<1.0	<1.0	8.4
Sulphate	mg/kg	34.3	46.0	57.3	4.0	600.0	350.0
Dry Density	g/cm^3	0.6	0.5	0.4	0.4	0.7	1.0
Clay	%	14.0	15.0	14.7	13.5	19.0	25.3
Sand	%	65.7	68.5	67.0	68.5	62.7	55.3
Silt	%	20.3	16.5	18.3	18.0	18.3	19.3
Total As	mg/kg	19.3	26.0	21.3	31.5	20.0	26.0
Total Cd	mg/kg	<1	<1	<1	<1	<1	<1
Total Cr	mg/kg	29.0	19.5	22.7	21.0	31.0	21.0
Total Cu	mg/kg	156.7	135.0	129.7	205.0	160.0	105.0
Total Pb	mg/kg	121.7	115.0	131.3	85.5	126.7	66.3
Total Hg	mg/kg	<1	<1	<1	<1	<1	<1
Total Mo	mg/kg	4.7	5.0	4.0	5.0	5.7	5.0
Total Ni	mg/kg	38.0	38.5	33.3	39.0	47.7	42.3
Total Se	mg/kg	<3	<3	<3	<3	<3	<3
Total Zn	mg/kg	283.3	140.0	164.0	135.0	310.0	100.7

Alba Trees, East Lothian, Scotland; short rotation coppice willow (*Salix* hybrids) cuttings of 20 cm, from Murray Carter, Yorkshire, and the energy crop, reed canary grass (*Phalaris arundinacea*) as seed from DLF Trifolium, Worcestershire. All trees were planted and seeds sown in early May 2012. Monitoring of the trees, grasses and soils took place over the duration of the project (four years) with the major plant-related results presented in a report (WRAP, 2015).

The aim of the current work was to establish if there were any discernible effects of organic matter amendments to soil on the development of earthworm communities on the reclaimed colliery spoil at Chisnall Hall. Specific objectives recorded earthworm species present, ecological groupings, community density (no m^{-2}) and community biomass (g m^{-2}).

Influence of organic matter on earthworms

Earthworms obtain their nutrition in a variety of ways from sources of organic matter (Curry and Schmidt, 2007). This can be from direct ingestion e.g. *Lumbricus terrstris* (the dew worm) which pulls leaf litter into its burrow from the soil surface; through the action of eating soil (geophagy) as demonstrated by the green worm (*Allolobophora chlorotica*); or by ingestion of organic waste material such as animal dung, as shown e.g. by *Lumbricus rubellus*. Degradation of organic matter by heterotrophic earthworms increases the surface area by comminution, which either permits direct digestion or increases the activity of microorganisms, giving rise to smaller organic compounds and mineral nutrients. Earthworms therefore increase nitrogen mineralisation and make this element more available to plants, and are responsible for additional processes in the soil and provision of numerous ecosystem services (Blouin *et al*, 2013).

Earthworm ecological groups and earthworm sampling

Earthworms can generally be grouped into three ecological categories. These groupings were first proposed by Bouché (1977), have been refined over time, but are essentially epigeic, endogeic and anecic. Epigeic earthworms (such as *L. rubellus*) live in the litter layer, normally above the mineral soil or totally within organic matter deposits e.g. dung. A sub-group are often referred to as "composting worms" and may be used to process household organic wastes. Epigeic earthworms are highly pigmented, small in size and have high fecundity. Endogeic species (such as *A. chlorotica*) live in temporary, horizontal burrows within the upper layers of the mineral soil. They tend to be geophagous, are generally unpigmented and have a moderate reproductive rate. Anecic earthworms (e.g. *L. terrestris*) tend to inhabit deep, semi-permanent burrows, feed at the soil surface on dead organic matter and tend to be K-selected (Satchell, 1980).

Figure 2: Example of one organically-amended plot at Chisnall Hall, showing ash, reed canary grass, cherry and willow growth after 30 months.

Earthworm collection can take a number of forms (Butt and Grigoropoulou, 2010), but to have a realistic opportunity of extracting all species from a given area, all of the ecological categories of earthworms need to be targeted. Epigeic, endogeic and juveniles of anecic earthworms can be extracted from the soil by digging out the upper (e.g. 0.2 m) layers and searching through this material in the field (hand-sorting). However, any human activity in the field will cause vibrations in the soil that may induce larger, anecic earthworm to withdraw to the depths (1 m or more) of their burrows. To extract these animals, it is necessary to use a vermifuge – a substance which will drive the earthworms from their burrows, by causing irritation to the skin. To avoid carcinogens and chemicals that can damage the environment, a suspension of mustard powder in water is now commonly used (Gunn, 1992). Therefore, a combination of methods (hand sorting and vermifuge expellant) is now seen as best practice (Butt, 2000; Pelosi *et al*, 2009).

Earthworms at Chisnall Hall

During investigation of trees and soils, samples for earthworms were taken within the organic matter treatment plots (WRAP, 2015). Results showed differences between the treatments, (not examined statistically due to small sample size: n=2), but with a suggestion that CLO provided a food resource that favoured earthworms (an average of 278 earthworm m^{-2} recorded). This compared, for example, with 138 and 95 m^{-2} for the high digestate treatment and the control (no organic amendment) respectively, although only hand-sorting extraction was used. A later, more comprehensive investigation of the earthworms at Chisnall Hall was undertaken as a part of a UCLan undergraduate project in November 2013 under the supervision of one of the authors. This involved collecting earthworms systematically using a combined extraction method (Butt, 2000) from all of the OM treatment plots and plantings and resulted in an unpublished dissertation (Carter, 2014). Here, earthworm densities exceeded those reported by WRAP (2015) with mean value across all treatments of 361 m^{-2}. However, on a later, closer inspection of the preserved earthworms, the identity of some species was shown to be questionable. To this end, the detailed data from 2013 has been re-interpreted from the original dissertation, and have been utilised for comparative purposes with the current work, with respect to earthworm community density (no m^{-2}) and ecological groupings.

Materials and Methods

Sampling for earthworms was undertaken on 10th November 2014, thirty months after organic matter application and planting up of the experimental plots. At this time, soils were still moist and earthworms had not entered resting states associated with adverse soil conditions (Edwards and Bohlen, 1996) so results obtained could be considered representative. Earthworms were collected from each of the four plant species sub-plots (ash; cherry; willow; reed canary grass) within each of the six organic matter treatments (details above and in Figure 1) at Chisnall Hall. This gave a total of 72 potential samples, although only 68 were collected due to exclusion of plot 9 which was not created in accordance with experimental design (see WRAP 2015 and Figure 1).

Each sample for earthworms used a standard procedure; soil was dug to approximately 0.2 m from within an area of 0.1 m^2 (delineated by a quadrat) and hand-sorted for earthworms in the field on plastic sheeting (Figure 3). Then a mustard powder vermifuge (50 g in 10 litres water) was poured into the hole previously created by digging (Butt, 2000). This meant that all ecological groups of earthworm within the sample area could be extracted. On collection, all earthworms were preserved in 4% formaldehyde in labelled bottles and returned to the laboratory. Individual earthworms were identified to species, using the nomenclature of Sims and Gerard (1999) and preserved wet masses determined. Earthworm community densities and community biomasses could then be determined.

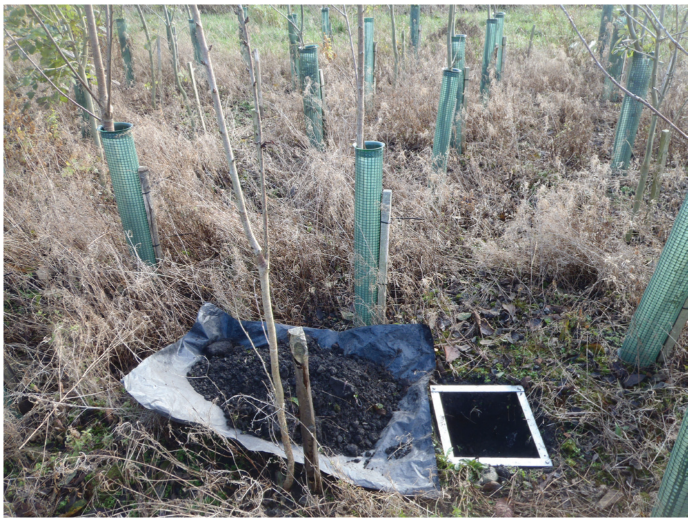

Figure 3: Sampling for earthworms at Chisnall Hall from within a 0.1 m^2 area.

The number of replicates, when separated for organic matter treatment and for plant species were small (n=3), so data were combined to permit more robust statistical analyses. Descriptive statistics were applied, as was one-way ANOVA for earthworm numbers and for earthworm biomasses across organic matter treatments (Minitab 17) with Tukey pairwise comparisons for effects of specific treatments. A separate analysis was also undertaken to assess any effects of plant type across the combined OM treatments.

Results

During sampling in 2014, a total of 3,230 earthworms were collected, represented by nine species from all three earthworm ecological groups (see Table 2). Of these, more than 97% were identified to species level (some smaller juveniles were not). Mean community density across the site was 475 earthworms m^{-2} with a biomass of 167 g m^{-2}. Endogeic, epigeic and anecic earthworms represented 50%, 12% and 38% by number and 21%, 7% and 72% by mass, respectively. Species that contributed to greatest numbers were *Allolobophora chlorotica* (the green worm) and *Aporrectodea caliginosa* (the grey worm), both endogeic, and *Aporrectodea longa* (the black-headed worm), an anecic species. The other species had only a small effect on numbers (Table 2). *A. longa* contributed most significantly to overall biomass on site. Two individual epigeic species, *Lumbricus rubellus* and particularly *Dendrodrilus rubidus*, had higher mean population densities where high levels of digestate were applied. *Aporecctodea rosea*, *L. terrestris* and *Lumbricus castaneus* all recorded low population densities across the treatments and *Aporrectodea limicola* was only recorded from two of the treatments (Table 2).

Table 2: Density (number m^{-2}) of earthworm species, separated and totalled for ecological category, collected at Chisnall Hall (November 2014) below Organic Matter Treatments, with relative proportions (%) of each species, column-wise, in parenthesis. For both Mean Earthworm density and for Mean Earthworm Biomass (community total data only), different letters show a significant difference (p<0.05) between treatments.

Earthworm Species	DG Low rate + CLO		DG only Low rate		DG High rate + CLO		DG only High rate		CLO only		Control	
ENDOGEIC:												
Allolobophora chlorotica	88	(18)	52	(11)	130	(23)	131	(21)	113	(21)	38	(20)
Aporrectodea caliginosa	152	(30)	161	(33)	111	(19)	184	(29)	192	(36)	65	(34)
Aporrectodea limicola	0	(0)	0	(0)	3	(<1)	0	(0)	0	(0)	1	(<1)
Aporrectodea rosea	11	(2)	7	(1)	2	(<1)	5	(1)	5	(1)	4	(2)
Group total	**251**		**220**		**246**		**320**		**310**		**108**	
EPIGEIC:												
Dendrodrilus rubidus	6	(1)	4	(1)	83	(15)	68	(11)	1	(<1)	0	(0)
Lumbricus castaneus	1	(<1)	2	(<1)	2	(<1)	1	(<1)	2	(<1)	7	(3)
Lumbricus rubellus	30	(6)	8	(1)	27	(5)	19	(3)	8	(1)	9	(5)
Group total	**37**		**14**		**112**		**88**		**11**		**16**	
ANECIC:												
Aporrectodea longa	197	(40)	201	(42)	198	(35)	214	(34)	212	(39)	61	(32)
Lumbricus terrestris	3	(<1)	1	(<1)	3	(<1)	1	(<1)	1	(<1)	1	(<1)
Group total	**200**		**202**		**201**		**215**		**213**		**62**	
L. spp	10	(2)	45	(9)	17	(3)	15	(2)	7	(1)	6	(3)
Mean Earthworm density (No m^{-2})	**498**	**(a)**	**481**	**(a)**	**576**	**(a)**	**638**	**(a)**	**541**	**(a)**	**192**	**(b)**
Mean Earthworm biomass (g m^{-2})	**161**	**(AB)**	**191**	**(A)**	**193**	**(A)**	**215**	**(A)**	**188**	**(A)**	**73**	**(B)**

(DG – Anaerobic digestate; CLO – Compost-like Output; see text for details of application rates)

The effect of organic matter amendments was found to be significant (p<0.05) but these differences were only present between the five treatments and the unamended control (Figure 4). The four plant species sampled (combined for organic matter amendments) had no significant effect on the number of earthworms present (Figure 5). By comparison with earthworm numbers collected in 2013, a general increase was found in 2014 (Figure 6).

Discussion

Earthworm species and ecological groupings

Of the nine earthworm species collected at Chisnall Hall, most can be considered as typical of grassland soils. The three species with the greatest contribution to both community density and biomass, *A. chlorotica*, *A. caliginosa* and *A. longa*, are common in Britain being found across a range of habitats (Sims and Gerard, 1999). Given the numbers collected, it is likely that *A. longa* was responsible for most of the surface casting seen on site. Seven of the earthworm species found in 2014 at Chisnall Hall were the same as those confirmed as collected by Carter (2014) in the previous year. However, *Eiseniella tetraedra* and *Octolasion tyrtaeum*, found in 2013, were not collected in 2014. *E. tetraedra* is a semi-aquatic species (Sims and Gerard, 1999), so maturation of the Chisnall Hall site, e.g. through stabilisation of the organic matter, may have led to generally drier soil conditions that became less suitable to this species. This may also account for relatively few records of *A. limicola* in 2014 (not recorded in 2013) as it is another species that is only common in particularly wet soils. *O. tyrtaeum* accounted for only 5 records of 2,455 earthworms collected in 2013, so no presence in 2014 was not unexpected. Although this species is widespread in Britain, it is often uncommon (Sims and Gerard 1999).

The dominance of endogeic species may also reflect the developmental stage of the site, sampled 30 months

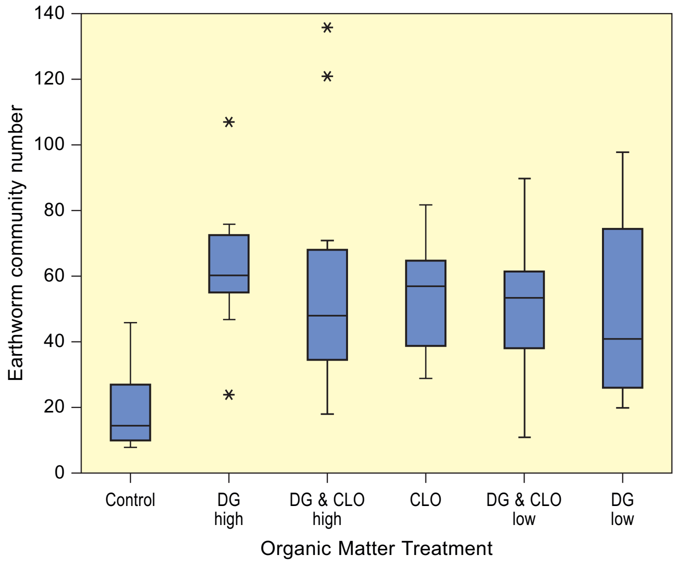

Figure 4: Box and whisker plots of earthworm community numbers (per sampled quadrat) with associated organic matter soil amendments (see text for treatment details).

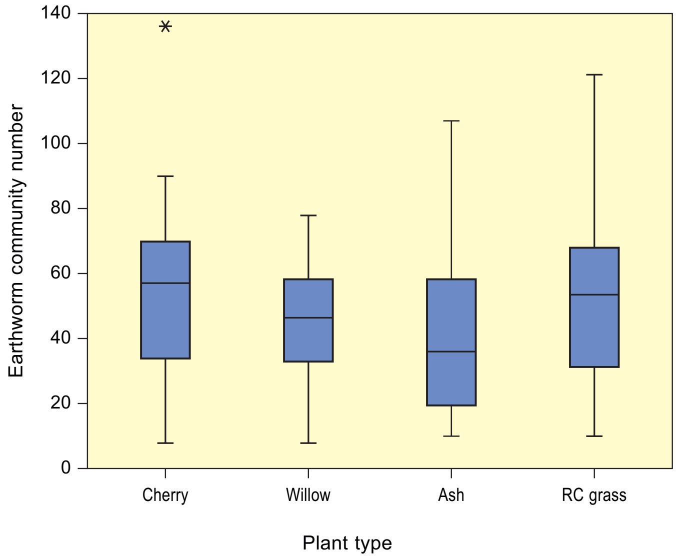

Figure 5: Box and whisker plots of earthworm community numbers below given plant species (OM treatments combined).

after organic matter incorporation. It is likely that epigeic species would have been present in highest number at the beginning of the trial, as these species tend to be drawn to organic resources in the soil and are more mobile over the soil surface (Bouché, 1977). Growth of endogeic populations would then tend to follow with stabilisation of soil conditions with an associated reduction in epigeic numbers in a successional pattern. It is perhaps surprising that so few *Lumbricus rubellus* were collected, as this species exhibits more of an epi-endogeic behaviour and ought to have been suited to the given soil conditions. Numbers of *D. rubidus* were high in the high digestate treatments, indicating that this organic material in higher quantity appeared to have a longer lasting effect than the others with respect to this epigeic species. This is in contrast to the earlier results from WRAP (2015) which suggested that CLO contained most earthworms (likely to have been epigeic) in the early stages of site development.

Earthworm numbers

The community densities recorded for the given organic treatments were in excess of those reported from earlier sampling on site (WRAP, 2015; Carter, 2014). This is likely a function of site development as earthworm immigration and population development occurred. Densities of 481-638 earthworms m^{-2} recorded from treated plots in 2014 are within the bounds recorded from numerous grassland sites in temperate regions (Edwards and Bohlen, 1996), but would be considered high for recently reclaimed soils (Butt, 1999). This is undoubtedly a function of the organic applications, as shown by the significantly smaller numbers found in control plots. Increased earthworm numbers (in excess of 1,000 m^{-2}) have previously been recorded in pasture where excessive inputs of cow manure occur naturally (Butt *et al*, 1993) and movement of earthworms, particularly *A. chlorotica*, have been demonstrated into such high organic matter areas through field experimentation (Curry and Cotton, 1983; Butt *et al*, 2004).

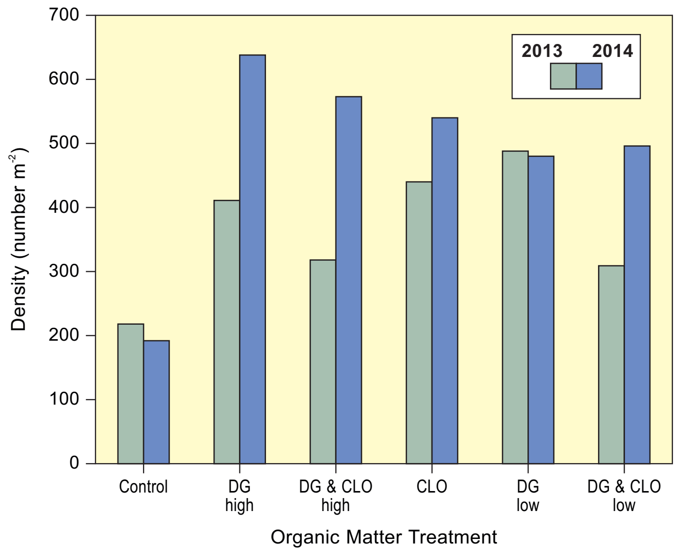

Figure 6: Earthworm community density within organic matter treatments at Chisnall Hall (2013 and 2014).

It was not unexpected to find no significant differences between earthworm communities and the type of planting that had taken place, as thirty months after the trial began may have been too early to seek such information. As organic inputs to the soil were relatively large, their legacy will remain for some years. Only after this material has been utilised by soil fauna and the plants themselves will the more natural re-use of organic matter on site through leaf litter production and incorporation begin to be felt. Had this trial been specifically set up to look at soil fauna, then unvegetated plots would also have been incorporated into the experimental design. However, earthworm monitoring of the Chisnall Hall site in future years may prove to be valuable to observe further community development in association with changes in soil properties.

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