

KEVIN R. BUTT¹, PHILIP D. PUTWAIN², DONATA DADÒ³

¹ Ecological Engineering, University of Lancashire, Preston, PR1 2HE, UK; e-mail: krbutt@lancashire.ac.uk, ORCID: 0000-0003-0886-7795; ² School of Environmental Sciences, University of Liverpool, Liverpool L69 3BX; E-mail: philp@liverpool.ac.uk; ³ Ecological Engineering, University of Lancashire, Preston, PR1 2HE, UK; E-mail: ddado@lancashire.ac.uk, ORCID: 0009-0008-5953-5284

EARTHWORM COMMUNITY DEVELOPMENT IN RECLAIMED COLLIERY SPOIL AMENDED WITH ORGANIC MATTER

Abstract: Earthworms were sampled after two and eleven years following experimental addition of organic matter to a semi-restored colliery spoil site at Chisnall Hall in Lancashire. Organic matter (OM) in the form of anaerobic digestate (AD) and/or organic growth medium (OGM) was incorporated into 200 m² plots on site using a mechanical digger. Six treatments, including a control with no amendment were replicated 3 times. A quarter of each plot was planted with ash, cherry, willow or reed canary grass. After 2 years, results showed that addition of OM gave rise to significantly higher community densities of earthworms with the greatest (638 m⁻²) in a high (1875 t ha⁻¹) digestate application treatment, compared with 192 m⁻² in the unamended control ($p < 0.05$). Of the nine species found, *Allolobophora chlorotica* and *Aporrectodea caliginosa*, both shallow-working, and *Aporrectodea longa*, a deep burrowing earthworm, contributed to greatest numbers. Eleven years after OM addition, the density of earthworms had fallen significantly in most of the OM-amended plots (maximum of 428 m⁻² in high anaerobic digestate) with no change in the control. Overall, earthworm community biomasses had also fallen but not significantly. Species composition on site had changed during the intervening period with less epigeic species present (e.g., *Bimastos rubidus* no longer present after eleven years) and a change in dominance of endogeic species to *A. rosea*. Tree-related species, e.g., *Dendrobaena octaedra* were also found more recently. Experimental plant species used did not affect earthworm abundance or biomass. Addition of organic matter to a colliery spoil site initially enhanced earthworm community density, most likely through a combination of immigration from surrounding areas and through increased reproduction associated with the abundance of food. After eleven years, the reduction in earthworm numbers was most likely a function of nutrient depletion.

Keywords: anaerobic digestate, colliery spoil, earthworms, organic growth medium

**ROZWÓJ POPULACJI DŹDŹOWNIC W WYROBISKU POKOPALNIA NYM
REKULTYWOWANYM MATERIAŁ ORGANICZNĄ**

Abstrakt: Dżdżownice pobrano po dwóch i jedenastu latach od eksperymentalnego dodania materii organicznej do częściowo zrehabilitowanego składowiska odpadów kopalnianych w Chisnall Hall w Lancashire. Materię organiczną (OM) w postaci beztlenowego pofermentu (AD) i/lub podłoża organicznego (OGM) wprowadzono na poletka o powierzchni 200 m² za pomocą koparki mechanicznej. Sześć zabiegów, w tym kontrolę bez modyfikacji, powtórzono 3 razy. Jedną czwartą każdej działki obsiano jesionem, wiśnią, wierzbą lub mózgową trzcinową. Po 2 latach wyniki

wykazały, że dodanie OM spowodowało istotnie wyższe zagęszczenie populacji dżdżownic, z największą (638 m^{-2}) wartością w przypadku zastosowania dużej ilości (1875 t ha^{-1}) pofermentu, w porównaniu ze 192 m^{-2} w kontroli, bez modyfikacji ($p < 0,05$). Spośród dziewięciu znalezionych gatunków, *Allolobophora chlorotica* i *Aporrectodea caliginosa*, oba płytko kopiące, oraz *Aporrectodea longa*, głęboko kopiąca dżdżownica, osiągnęły najwyższą liczebność. Jedenaście lat po dodaniu OM zagęszczenie dżdżownic znacznie spadło na większości działek, na których zastosowano OM (maksymalnie 428 m^{-2} w beztlenowym pofermencie), bez zmian w kontroli. Ogólnie rzecz biorąc, biomasa zbiorowisk dżdżownic również spadła, ale nieznacznie. Skład gatunkowy na miejscu zmienił się w okresie badań, z mniejszą liczbą gatunków epigeicznych (np. *Bimastos rubidus* nieobecny już po jedenastu latach) i zmianą dominacji gatunków endogeicznych na *A. rosea*. Gatunki związane z drzewami, np. *Dendrobaena octaedra*, zostały również znalezione niedawno. Eksperymentalne gatunki roślin nie wpłynęły na liczebność ani biomasę dżdżownic. Dodanie materii organicznej początkowo zwiększyło zagęszczenie populacji dżdżownic, najprawdopodobniej poprzez połączenie imigracji z okolicznych terenów oraz wzmocnionej reprodukcji związanej z obfitością pożywienia. Po jedenastu latach spadek liczebności dżdżownic był najprawdopodobniej funkcją wyczerpywania się składników odżywczych.

Słowa kluczowe: beztlenowy poferment, wyrobisko pokopalniane, dżdżownice, podłoże organiczne

I. INTRODUCTION

Site Information

The former Chisnall Hall colliery waste site near Coppull, Lancashire (53.606994, -2.6803930) was reclaimed in 1976 to agricultural pasture and woodland end-uses. Much of the site was covered with a thin (0.2 m) layer of topsoil. The original coal waste was acidic (pH 3.5 - 4.0) and therefore 100 t ha^{-1} of ground limestone was ripped in, to depths of 0.5 m, to raise the pH above neutral and 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, 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 & Resources Action Programme (WRAP, 2015) were instrumental with setting up a trial to investigate the potential effects of organic matter amendments on plant growth within the reclaimed Chisnall Hall soil. This trial used BSI PAS 110 anaerobic digestate (AD) (WRAP n.d.) in addition to organic growth medium (OGM) produced by Global Renewables Lancashire from domestic organic waste, as a contribution to the Lancashire Woodlands from Waste Programme (WFW n.d.), see table 1.

Six organic matter treatments (fresh materials) were utilised in the trial:

- i) AD Low rate + OGM ($937.5 \text{ t ha}^{-1} + 1500 \text{ t ha}^{-1}$); ii) AD Low rate only (937.5 t ha^{-1});
- iii) AD High rate + OGM ($1875 \text{ t ha}^{-1} + 1500 \text{ t ha}^{-1}$); iv) AD High rate only (1875 t ha^{-1});
- v) OGM only (3000 t ha^{-1}) and vi) Control (no addition).

Delivery of organic matter to Chisnall Hall and incorporation took place in April 2012, as materials were mixed into designated experimental plots. Before mixing, each plot (20 x 10 m) was dug to 1 m using a 13 tonne 360° excavator to relieve compaction. The appropriate organic material was then applied and dug into the soil with the excavator (WRAP, 2015). Selected analyses of the blended spoil and amendment are provided in table 1.

Each replicated ($n=3$) plot of the organic matter treatment and control was planted with 4 species. These were two woodland trees: ash (*Fraxinus excelsior*) and cherry (*Prunus avium*), cell-grown in size 20-40 cm, supplied by 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 (4 years) with the major plant-related results presented in a report (WRAP, 2015). The trial was set up specifically to look at plant growth on an OM-amended brown field site, but it was felt that additional data could be determined on soil fauna, namely earthworms. See fig. 1 for extent of vegetation cover when earthworms were monitored.

Table 1 – Tabela 1

Selected chemical and physical analysis of soil blend treatments comprising colliery waste mixed with AD and/or OGM in 2012 [adapted from WRAP 2015] / Wybrane analizy chemiczne i fizyczne mieszanek glebowych zawierających odpady węglowe zmieszane z AD i/lub OGM w 2012 r. [zaadaptowano z WRAP 2015]

Soil Property <i>Właściwości</i>	Units <i>Jednostka</i>	AD Low rate / Niska dawka+ OGM	AD Low rate / Niska dawka	AD High rate / Wysoka dawka+ OGM	AD High rate / Wysoka dawka	OGM Only Tylko	Control <i>Kontrola</i>
pH		7.83	7.95	8.07	8.25	7.57	7.30
Electrical Conductivity	$\mu\text{S cm}^{-1}$	4100	2900	4366	4600	2867	2100
Organic Matter	%	18.3	14.0	14.1	17.5	15.0	10.6
Total N	mg kg^{-1}	13333	9000	15666	19250	3133	2633
Total P	mg kg^{-1}	2533	1165	1113	2250	1600	530
Total K	mg kg^{-1}	3767	2900	3433	3950	3467	2467
Extractable P	mg kg^{-1}	124.0	70.5	42.0	227.5	40.0	11.7
Extractable K	mg l^{-1}	1410.0	555.0	880.0	1285.0	713.3	114.3
NH ₄ (Water soluble)	g l^{-1}	1193.3	745.0	1266.7	1950.0	163.3	92.3
Nitrate (Water soluble)	mg l^{-1}	<1.0	17.0	<1.0	<1.0	<1.0	8.4



Fig. 1. An example of an organically amended plot at Chisnall Hall, showing cherry in the foreground and willow behind after (a) 2 years, (b) 11 years

Rys. 1. Przykład organicznie zmienionej działki w Chisnall Hall, na której na pierwszym planie widać wiśnię, a na drugim wierzbę po (a) 2 latach, (b) 11 latach

Earthworm ecological groups and influence of organic matter

Earthworms form 3 general ecological categories, first proposed by Bouché (1977), refined over time (Bottinelli et al., 2020), but essentially epigeic, endogeic and anecic. Epigeic earthworms (such as *Lumbricus rubellus*) live in the litter layer, normally above the mineral soil

or totally within OM deposits. They are highly pigmented, small and have high fecundity. Endogeic species (such as *Allolobophora chlorotica* – the green worm) 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., *Lumbricus terrestris* – the dew worm) tend to inhabit deep, semi-permanent burrows, feed at the soil surface on dead organic matter and tend to be K-selected (Satchell, 1980).

Earthworms obtain their nutrition in a variety of ways from sources of OM (Curry & Schmidt, 2007). Degradation of OM 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 sampling

Earthworm collection can take numerous forms (Butt & Grigoropoulou, 2010), but to have a realistic opportunity of extracting all species from a given area, all ecological categories of earthworms need to be targeted. Epigeic, endogeic and juvenile 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 (Nuutinen & Butt, 2003). 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 can be used (Gunn, 1992), or Allyl isothiocyanate (AITC) (Zaborski, 2003) - the active ingredient of mustard powder. Therefore, a combination of methods (hand sorting and vermifuge expellant) is now seen as best practice (Butt 2000; Pelosi et al., 2009; Butt et al., 2010).

Experimental Aims and Objectives of the study

The aim of the current work was to establish if there were any discernible effects of organic matter amendments to colliery spoil and types of planting on the development of earthworm communities on the reclaimed Chisnall Hall site. Specific objectives were to record i) earthworm species present, ii) ecological groupings, iii) community density (ind. m⁻²) and iv) community biomass (g m⁻²). Two surveys were undertaken in autumn 2014 and 2023, 2 and 11 years after OM addition, to assess changes that may have arisen over the given timeframes. The results from sampling in 2014 were previously made available by Butt & Putwain (2017).

II. METHODOLOGY

Sampling for earthworms was undertaken twice. First in November 2014, two years after organic matter application and planting up of the experimental plots and then 11 years after planting (October 2023). At both times, soils were still moist and earthworms had not entered resting states associated with adverse soil conditions (Edwards & Arancon, 2022) so results obtained were considered representative. In 2014, earthworms were collected from each of the 4 plant species sub-plots (ash; cherry; willow; reed canary grass) within each of the 6 treatments (details above) at Chisnall Hall. At the second sampling – nine years after the first – the same procedure for earthworm sampling was followed, but ash was excluded. This was due to ash dieback disease (*Hymenoscyphus fraxineus*) (Woodland Trust, n.d.). All ash trees had been cut down in the intervening period and chippings (accidentally) incorporated across the ash tree plots, so no realistic earthworm sampling for comparative purposes could be undertaken. The reed canary grass had all died away by 2023,

and the willow had grown tall (>10 m) and begun to fall over, as no coppicing had occurred. Nevertheless, earthworm sampling was possible in these plots.

At sampling, soil was dug to approximately 0.2 m from within an area of 0.1 m² (delineated by a quadrat) and hand-sorted for earthworms in the field on plastic sheeting. Then (in 2014) a mustard powder vermifuge (50 g in 10 litres water) was poured into the hole previously created by digging (Butt, 2000), but in 2023, AITC was utilised (Zaborski, 2003). 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 Sherlock (2018) with recent updates (specifically re. *Dendrodrilus rubidus*, which is now called *Bimastos rubidus*) from the Earthworm Society of Britain (ESB n.d.), counted and had preserved wet masses determined.

Data analyses

Earthworm community densities and community biomasses were determined. The number of replicates, when separated for organic matter treatment and for plant species were small (n=3), so data were combined, as appropriate, to permit more robust statistical analyses. Descriptive statistics were applied, as was one way ANOVA for earthworm densities and for earthworm biomasses across organic matter treatments (R 4.5.2) with Tukey pairwise comparisons for effects of specific treatments and for the effect of year (2014 and 2023) in R (R Core Team, 2025). A similar analysis was also undertaken to assess any effects of plant species across the combined OM treatments.

III. RESULTS

General findings

During sampling in 2014, a total of 3,230 earthworms were collected, with nine species present (not shown). By contrast, 2023 saw 1,491 earthworms collected (from ³/₄ the number of plots as for 2014; as ash was not sampled), with a total of eight species (with six species in common to 2014). *Bimastos rubidus*, found in each treatment in 2014 except the control, had the highest mean density of 83 ind. m⁻² in the AD High rate + OGM treatment, whilst in 2023 this epigeic species was completely absent. Moreover, in 2023 *Lumbricus rubellus* and *Aporrectodea limicola* were also no longer found, but the endogeic species, *Octolasion cyaneum*, was recorded at low density (<2% overall) (table 2).

Mean community density across the site in 2014 was 475 ind. m⁻² with a biomass of 167 g m⁻², while in 2023 these metrics were 300 ind. m⁻² and 159 g m⁻², respectively. For density in 2023, a significant difference was found only between the control and the high anaerobic digestate treatment, while mean community biomass showed no significant difference among organic matter treatments. Endogeic, epigeic and anecic earthworms represented 50%, 12% and 38% by number in 2014, and 56%, 4%, 39% in 2023, respectively, showing a community decrease in epigeic and increase in anecics. In 2014, species that contributed to greatest numbers were *Allolobophora chlorotica* and *Aporrectodea caliginosa*, both endogeic, and *Aporrectodea longa*, an anecic species. Similar trends were recorded in 2023, but the endogeic species *Aporrectodea rosea* increased in number on site, while *A. chlorotica* decreased. *A. longa* contributed most significantly to overall biomass in both years.

OM Treatment influences in earthworm communities between 2014 and 2023

For earthworm community density (2014-2023), five OM treatments showed a significant decrease (p<0.05) (fig. 2), the exception was the earthworm-rich AD High rate only. Moreover, there were no significant differences between years for earthworm abundance in the control. Earthworm communities revealed an overall biomass and density decrease, in each ecological group. Anecic species exhibited a significant density reduction for each treatment, anaerobic digestate High rate excluded (table 3). Furthermore, the AD High rate + OGM showed the most significant density decrease in the epigeic ecological group. The OGM treatment displayed a significant

density decrease between sampling years for the endogeic group. For epigeic species, there was a significant decrease in biomass for AD High rate only, AD High rate + OGM and AD Low rate + OGM. Regarding differences between communities within year, the highest density generally occurred when treatments had the highest amount of anaerobic digestate applied (1875 tonnes ha⁻¹ instead of 937.5 tonnes ha⁻¹), although these differences were not significant.

Table 2 – Tabela 2

Mean density (ind. m⁻²) of earthworm species, separated and totalled for ecological group, collected at Chisnall Hall (October 2023) below Organic Matter Treatments. Relative proportions (%) of each species and ecological group, column-wise, in parenthesis. Total mean earthworm density and biomass also displayed / Średnie zagęszczenie (ind. m⁻²) gatunków dżdżownic, odnalezionych w grupach ekologicznych, w Chisnall Hall (październik 2023 r.) zależnie od dodatku materii organicznej. W nawiasach podano względne proporcje (%) poszczególnych gatunków i grup ekologicznych. Pokazano również całkowite średnie zagęszczenie dżdżownic i biomasę

Earthworm Species <i>Gatunek dżdżownicy</i>	AD low rate / niska dawka + OGM	AD only low rate tylko niska dawka	AD high rate / wysoka dawka + OGM	AD only high rate tylko wysoka dawka	OGM only / tylko	Control <i>Kontrola</i>
ENDOGEIC:						
<i>Allolobophora chlorotica</i>	6.67 (2.40)	8.89 (2.94)	27.78 (8.62)	35.00 (8.17)	6.67 (2.41)	4.44 (2.31)
<i>Aporrectodea caliginosa</i>	95.56 (34.40)	133.33 (44.12)	131.11 (40.69)	190.00 (44.36)	115.56 (41.77)	50.00 (26.01)
<i>Aporrectodea rosea</i>	54.44 (19.60)	16.67 (5.51)	40.00 (12.41)	36.67 (8.56)	25.56 (9.24)	37.78 (19.65)
<i>Octolasion cyaneum</i>	5.56 (2.00)	4.44 (1.47)	2.22 (0.69)	3.33 (0.78)	2.22 (0.80)	0.00 (0.00)
Group total / <i>Suma grupy</i>	162.22 (58.40)	163.33 (54.04)	201.11 (62.41)	265.00 (61.87)	150.00 (54.22)	92.22 (47.98)
EPIGEIC:						
<i>Dendrobaena octaedra</i>	7.78 (2.80)	3.33 (1.10)	8.89 (2.76)	8.33 (1.95)	6.67 (2.41)	2.22 (1.16)
<i>Lumbricus castaneus</i>	8.89 (3.20)	4.44 (1.47)	5.56 (1.72)	5.00 (1.17)	3.33 (1.20)	2.22 (1.16)
Group total / <i>Suma grupy</i>	16.67 (6.00)	7.78 (2.57)	14.44 (4.48)	13.33 (3.11)	10.00 (3.61)	4.44 (2.31)
ANECIC:						
<i>Aporrectodea longa</i>	88.89 (32.00)	118.89 (39.34)	85.56 (26.55)	123.33 (28.79)	103.33 (37.35)	84.44 (43.93)
<i>Lumbricus terrestris</i>	10.00 (3.60)	12.22 (4.04)	21.11 (6.55)	23.33 (5.45)	12.22 (4.42)	8.89 (4.62)
Group total / <i>Suma grupy</i>	98.89 (35.60)	131.11 (43.38)	106.67 (33.10)	146.67 (34.24)	115.56 (41.77)	93.33 (48.55)
<i>L. spp</i>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.33 (0.78)	1.11 (0.40)	2.22 (1.16)
Mean earthworm density <i>Średnie zagęszczenie dżdżownic / (ind. m⁻²)</i>	277.78	302.22	322.22	428.33	276.67	192.22
Mean earthworm biomass <i>Średnia biomasa dżdżownic / (g m⁻²)</i>	146.91	176.54	167.21	196.97	178.35	86.80

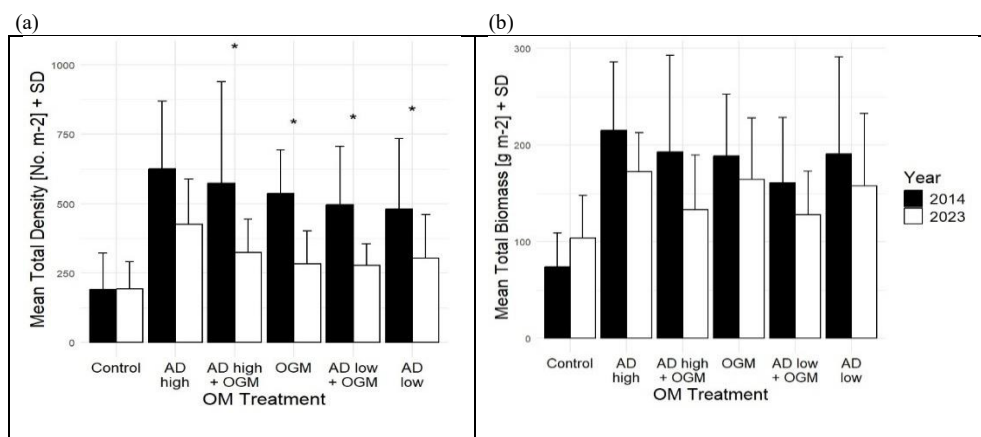


Fig. 2. (a) Mean earthworm community density (ind. m⁻²) + SD and (b) mean biomass (g m⁻²) + SD for organic matter treatments by sampling year (2014 and 2023) at Chisnall Hall

Rys. 2. (a) Średnie zagęszczenie zbiorowiska dżdżownic (ind. m⁻²) + SD i (b) średnia biomasa (g m⁻²) + SD dla dodatku materii organicznej według roku pobrania próbek (2014 i 2023) w Chisnall Hall

Table 3 - Tabela 3

Mean earthworm density (ind. m⁻²) and mean biomass (g m⁻²) p-values for differences in the three ecological groups between 2014 and 2023, for Organic Matter Treatments at Chisnall Hall / Wartości p dla średniego zagęszczenia dżdżownic (szt. m⁻²) i średniej biomasy (g m⁻²) w trzech grupach ekologicznych w latach 2014–2023 dla dodatku materii organicznej w Chisnall Hall

OM Treatment Dawka materii organicznej	AD low rate / niska dawka + OGM	AD only low rate tylko niska dawka	AD high rate / wysoka dawka + OGM	AD only high rate tylko wysoka dawka	OGM only / tylko	Control Kontrola
Mean Density / Liczebność Anecic	0.004 (**)	0.034 (*)	0.006 (**)	0.074	0.007 (**)	0.343
Mean Density / Liczebność Epigeic	0.431	0.906	0.007 (**)	0.083	0.871	0.751
Mean Density / Liczebność Endogeic	0.065 (.)	0.147	0.357	0.476	0.001 (***)	0.69
Mean Biomass / Biomasa Anecic	0.515	0.344	0.138	0.422	0.778	0.25
Mean Biomass / Biomasa Epigeic	0.035 (*)	0.076	0.002 (**)	0.005 (**)	0.521	0.621
Mean Biomass / Biomasa Endogeic	0.476	0.935	0.485	0.972	0.067	0.765

Plants species influence on earthworm communities between 2014 and 2023

With respect to plant species, earthworm community metrics showed an overall decrease in density and biomass between 2014 and 2023 (fig. 3). Total earthworm density exhibited a significant reduction for each of the three plant treatments sampled each time. Particularly, anecic and endogeic ecological groups displayed a significant reduction in density (table 4). For biomass, reed canary grass and willow showed a significant decrease for epigeic earthworms, and cherry for endogeic earthworms.

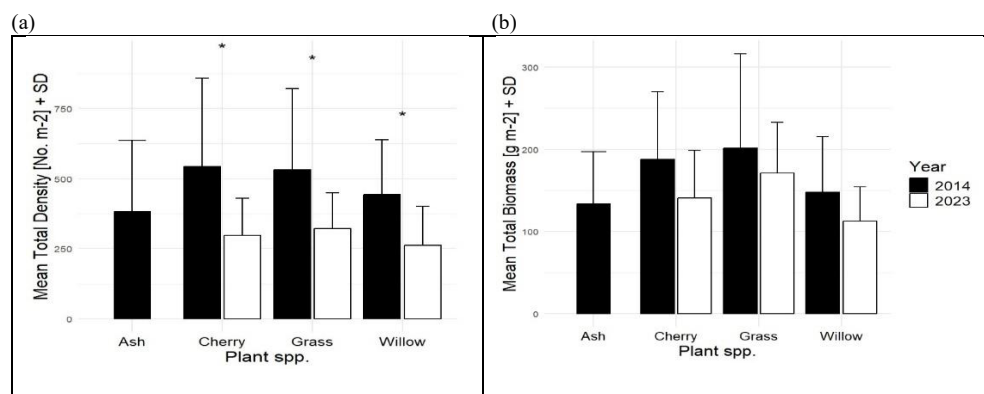


Fig. 3. (a) Mean total density (ind. m⁻²) + SD and (b) mean total biomass (g m⁻²) + SD per plant species separated by year (2014 and 2023) at Chisnall Hall

Rys. 3. (a) Średnia całkowita gęstość (szt. m⁻²) + SD i (b) średnia całkowita biomasa (g m⁻²) + SD dla każdego gatunku rośliny, rozdzielona według roku (2014 i 2023) w Chisnall Hall

Table 4 - Tabela 4

Mean earthworm density (ind. m⁻²) and mean biomass (g m⁻²), p-values for difference in the three ecological groups between 2014 and 2023 / Wartości p dla średniego zagęszczenia dżdżownic (ind. m⁻²) i średniej biomasy (g m⁻²) w trzech grupach ekologicznych w latach 2014-2023

Plant spp ^a / Gatunek rośliny	Cherry / Wiśnia	Reed Canary Grass / Trawa	Willow / Wierzba
Mean Density / Liczebność Anecic	0.015 (*)	0.008 (**)	0.003 (**)
Mean Density / Liczebność Epigeic	0.093	0.104	0.173
Mean Density / Liczebność Endogeic	0.003 (**)	0.046 (*)	0.182
Mean Biomass / Biomasa Anecic	0.178	0.678	0.189
Mean Biomass / Biomasa Epigeic	0.103	<0.001 (***)	0.008 (**)
Mean Biomass / Biomasa Endogeic	0.035 (*)	0.261	0.765

^abelow plant species at Chisnall Hall / poniżej gatunków roślin w Chisnall Hall

IV. DISCUSSION

Influence of OM treatments on earthworms between 2014 and 2023

Across the OM treatments, a dominance of endogeic earthworms in 2023 (table 2) was similar to that reported by Butt & Putwain (2017) for 2014, which reflects the advanced development of the soil on site after 2 and 11 years of the experiment. Epigeic earthworms are early colonisers and move rapidly over the soil surface and tend to be drawn to organic resources in the soil (Addison, 2009; Bouché, 1977; Butt & Putwain, 2017). After such colonisation, soil conditions stabilise and endogeics colonise the area, with an associated reduction in epigeic numbers. Consequently, by 2014 and 2023, the incorporation of blended spoil and amendment in soil had permitted the development of good condition for endogeic domination. Moreover, in 2023 the biomasses of epigeics had decreased, principally due to the disappearance of *Bimastos rubidus*, a species with high OM preferences (Sims & Gerard, 1999; Sherlock, 2018). Interestingly, *Aporrectodea rosea* increased in preference on site by 2023, while *Allolobophora chlorotica* decreased (tab. 2). This finding highlights a likely negative interaction (competition) between these two species. Furthermore, a study by Natalio et al. (2021) suggests that *A. chlorotica* is negatively impacted by anaerobic digestate,

possibly through osmotic stress. This may explain the reduction in density of this species between periods of monitoring, particularly in AD-related treatments.

In general, results displayed an overall decrease in earthworm densities and biomasses between 2014 and 2023 (fig. 2), across all ecological groups (table 3). The principal factor that may explain our findings is nutrient depletion in plots, since no additional OM sources were directly added over 9 years, except from natural leaf fall. A large number of studies have demonstrated the importance of organic matter quality and quantity for earthworms, particularly in woodland settings (e.g., Ashwood et al., 2017; Ganault et al., 2021; Muys et al., 1992; Rajapaksha et al., 2013). Moreover, in a study in post-mining, afforested colliery spoil heaps, Frouz et al. (2007) found that differences in the humus layer were a predictor of soil fauna shift, with a combined interaction between earthworms and vegetation on shaping litter. This conclusion is also supported by the observation that OM treatments with the highest non-significant total earthworm density were those with the highest amount of AD-added. Finally, an observation of note — linked with nutrients in anaerobic digestate — was the only non-significant difference in total earthworm density between 2014 and 2023 in the AD High-rate application. In the final WRAP report (2015) concerning Chisnall Hall, it was suggested that 18 months after application, soil values of available N and P were greater in the digestate treatments than in the OGM blended with colliery spoil. Consequently, the nutrient depletion was probably minor for AD only applied treatments, particularly for the higher application rate of AD.

Influences of plants species between 2014 and 2023

Earthworm species recorded at Chisnall Hall are mostly considered as typical of grassland soils (Butt & Putwain, 2017), however by 2023 two new woodland species *Dendrobaena octaedra* and *Octolasion cyaneum* (Sims & Gerard, 1999; Sherlock, 2018), had colonised the site. This highlights the species shift during the 9 years between earthworm monitoring, with an increase of specialised earthworm species occupying the new niches created by the presence of trees. Overall, total earthworm density and biomass recorded in 2023 for different plant plots followed the same decreasing trend as reported for OM treatments (fig. 3), with significant differences particularly for anecic and endogeic species (table 4). As for OM treatment, this result is likely related to the nutrient depletion of anaerobic digestate and organic growth medium by plants and soil organisms (see above). For findings within year, either 2014 or 2023, we found non-significant differences between plant species (fig. 3). This may be due to the high mortality of tree species already recorded after 2 years of the experiment (WRAP, 2015). Here, a high electrical conductivity was recorded in the anaerobic digestate, which suggested a high rate of salts such as sulphates, chlorides and ammonia. Moreover, by 2023 all the reed canary grass had died, and as indicated above, all ash had been removed after detection of ash dieback disease. Consequently, the high plant mortality rate caused a homogenisation of earthworm communities present on site.

V. CONCLUSION

Results from the organically enriched colliery spoil at Chisnall Hall showed an overall decrease in earthworm density and biomass between 2014 and 2023 for each ecological group. The principal factor was likely nutrient depletion of the organic soil layer, as no additional amendments were added during the 9 years between monitoring. Additional soil and litter analyses would be necessary to determine if this reduction is driven by soil nutrient content. Moreover, extended monitoring of the Chisnall Hall site in future years may highlight further earthworm community dynamics as the experimentally amended site further equilibrates with the surrounding restored ecosystem.

Acknowledgements: Thanks to Pete Bentley, Jackie Gilbert and Siobhan Quigg for assistance with earthworm collection in 2014. WRAP provided financial support for setting up of the organic matter amendment experiment.

REFERENCES

- Addison J.A. (2009). Distribution and Impacts of Invasive Earthworms in Canadian Forest Ecosystems. *Biological Invasions*. 11(1). 59-79.
- Ashwood F., Butt K.R., Doick K.J., Vanguelova E.I. (2017). Investigating tree foliar preference by the earthworms *Aporrectodea longa* and *Allolobophora chlorotica* in reclaimed and loam soil. *Applied Soil Ecology*. 110. 109-117.
- Blouin M., Hodson M.E., Aranda Delgado E., Baker G., Brussaard L., Butt K.R., Dai J., Dendooven L., Pérès G., Tondoh J., Cluzeau J., Brun J-J. (2013). A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*. 64. 161-182.
- Bottinelli N., Hedde M., Jouquet P., Capowiez Y. (2020). An explicit definition of earthworm ecological categories - Marcel Bouché's triangle revisited. *Geoderma*. <https://doi.org/10.1016/j.geoderma.2020.114361>
- Bouché M.B. (1977). Stratégies lombriciennes in (Lohm U. and Persson T. Eds.) *Soil Organisms as components of Ecosystems*. Biological Bulletin (Stockholm). 25. 122-132.
- Butt K.R. (2000). Earthworms of the Malham Tarn Estate (Yorkshire Dales National Park). *Field Studies*. 9. 701-710.
- Butt K.R., Grigoropoulou N. (2010). Basic Research Tools for Earthworm Ecology. *Applied and Environmental Soil Science*. 562816. <http://www.hindawi.com/journals/aess/2010/562816.html>
- Butt K.R., Kostecka J., Lowe C.N. (2010). Field collection of earthworms: Comparisons of commonly used techniques. *Zesz. Prob. Post. Nauk Roln.* 547. s. 67-75.
- Butt K.R., Putwain P.D. (2017). Earthworm community development in organic matter-amended plots on reclaimed colliery spoil. *North-West Geography*. 17 (2). 1-8.
- Costigan P.A., Bradshaw A.D., Gemmell R.P. (1981). The Reclamation of Acidic Colliery Spoil. I. Acid Production Potential. *Journal of Applied Ecology*. 18. 865-878.
- Curry J.P., Schmidt O. (2007). The feeding ecology of earthworms – a review. *Pedobiologia*. 50. 463-47.
- Earthworm Society of Britain (ESB). No date. British Isles Earthworm Species Checklist: Natural Environments | Earthworm Society of Britain (accessed 01/04/26)
- Edwards C.A., Arancon N.Q. (2022). *Biology and ecology of earthworms*. Springer. New York.
- Frouz J., Pižl V., Tajovský K. (2007). The effect of earthworms and other saprophagous macrofauna on soil microstructure in reclaimed and un-reclaimed post-mining sites in Central Europe. *European Journal of Soil Biology*. 43. S184-189. doi: 10.1016/j.ejsobi.2007.08.033
- Ganault P., Nahmani J., Hättenschwiler S., Gillespie L.M., David J-F., Henneron L., Iorio E., Mazzia C., Muys B., Pasquet A., Prada-Salcedo L.D., Wambsganss J., Decaëns T. (2021). Relative Importance of Tree Species Richness, Tree Functional Type, and Microenvironment for Soil Macrofauna Communities in European Forests. *Oecologia*. 196(2). 455-68. doi:10.1007/s00442-021-04931-w
- Gunn A. (1992). The use of mustard to estimate earthworm populations. *Pedobiologia*. 36. 65-67.
- Muys B., Lust N., Granval Ph. (1992). Effects of Grassland Afforestation with Different Tree Species on Earthworm Communities, Litter Decomposition and Nutrient Status. *Soil Biology and Biochemistry*. 24(12).1459–66. doi:10.1016/0038-0717(92)90133-I
- Natalio A.I.M., Back M., Richards A., Jeffery S. (2021). The Effects of Saline Toxicity and Food-Based AD Digestate on the Earthworm *Allolobophora chlorotica*. *Geoderma*. 393.115005. doi: 10.1016/j.geoderma.2021.115005
- Nuutinen V., Butt K.R. (2003). Interaction of *Lumbricus terrestris* L. burrows with field subdrains. *Pedobiologia*. 47. 578-581.
- Pelosi C., Bertrand M., Capowiez Y., Boizard H., Roger-Estrade J. (2009). Earthworm collection from agricultural fields: comparisons of selected expellants in presence/absence of hand-sorting. *European Journal of Soil Biology*. 45. 176-183.

- R Core Team. (2025). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rajapaksha N.S.S., Butt K.R., Vanguelova E., Moffat A.J. (2013). Earthworm selection of Short Rotation Forestry leaf litter through preference testing and direct observation. *Soil Biology and Biochemistry*. 67. 12-19. doi: 10.1016/j.soilbio.2013.08.006
- Satchell J.E. (1980). R worms and K worms: A basis for classifying lumbricid earthworm strategies, in *Soil Biology as related to land use practices* (ed. Dindal D.L.). Proc 7th Int Colloquium on Soil Zoology. EPA Washington DC. pp 848-854.
- Sherlock E. (2018). Key to the Earthworms of the UK and Ireland. Telford. Field Studies Council.
- Sims R.W., Gerard B.M. (1999). Earthworms: Synopses of the British Fauna No. 31 revised. The Linnean Society and the Brackish-Water Sciences Association. Shrewsbury.
- Woodlands from Waste (WFW). No date. Lancashire County Council <http://www3.lancashire.gov.uk/corporate/web/?siteid=5592&pageid=30717> (accessed 24 May 2017).
- Woodland Trust. No date. Ash Dieback (*Hymenoscyphus fraxineus*) - Woodland Trust (accessed 12 April 2026).
- WRAP. (2015). Compost-like output and digestate enhance the growth of woodland trees and the biomass yield of SRC Willow. Report April 2015.
- WRAP. No date. BSI PAS 110: Producing Quality Anaerobic Digestate | WRAP - The Waste and Resources Action Programme (accessed 12 April 2026).
- Zaborski E.R. (2003). Allyl isothiocyanate: an alternative chemical expellant for sampling earthworms. *Applied Soil Ecology*. 22. 87-95.

